BBGuy Essentials 088:
Warm Autoantibody Best Practices with Alyssa Ziman and Meghan Delaney
Released January 27, 2021

Alyssa: Hi, I'm Alyssa Ziman from UCLA.

Meghan: And I'm Meghan Delaney from Children's National in DC, and this is the Blood Bank Guy Essentials Podcast.

Joe: Hi everyone, I want to welcome you to Blood Bank Guy Essentials, the podcast made to help you learn the essentials of Transfusion Medicine. This is episode 088, and it is the first episode of 2021. My name is Joe Chaffin, and I am your host. I've got an interview for you today where I get really practical with the authors of two of my favorite recent papers on dealing with warm autoantibodies, which is one of my favorite topics.

But first, you should know that this is NOT a continuing education episode. You can find tons of other episodes where physicians and laboratorians can get free continuing education credits at BBGuy.org/podcast (they're labeled cleverly with the letters "CE"), or at wileyhealthlearning.com/transfusionnews. The continuing education episodes are brought to you courtesy of transfusionnews.com, and Transfusion News is brought to you by Bio-Rad, who has no editorial input into this podcast.

One of the more challenging things that those of us who work in transfusion services and reference labs have to deal with is the patient with warm autoantibodies. As you might recall from a recent podcast I did with Dr. Jill Storry called "What to do When Everything is Incompatible" (and that's episode 085CE, by the way, and you should check it out if you haven't), "warm autos," as we call them, react against pretty much all red cells from any other human, and they make all crossmatches incompatible. And that can be tough. When you have to deal with one and the patient is in urgent need of blood, they can really scare you. Plus there's so many funky words and phrases that get tossed around like "autoadsorption" and "alloadsorption" and "elution," and my least favorite and the most *galactically stupid* one of all (in my opinion): "Least incompatible." Don't get me started...

[Chuckles] My guests today, Drs Alyssa Ziman from UCLA and Meghan Delaney from Children's National in DC, they recognized the struggle a few years back and they decided to help us all out. They've published two incredibly great papers in conjunction with the BEST Collaborative that outline, really, best practices in the laboratory management and workup of warm autoantibodies. And I can't recommend these papers any higher. You can find links to both of them at BBGuy.org/088, which is the show page for this episode. And you SHOULD get them.

Now, the first paper was published in the journal Transfusion in February, 2017, and it was called "Warm-reactive (immunoglobulin G) autoantibodies

and laboratory testing best practices: review of the literature and survey of current practice" [Linked HERE]. Now, Alyssa Ziman is the lead author on that one. And I've referred to this paper numerous times over the past several years as a reference for strategies for both the lab workup and management of patients with warm autoantibodies. It is pure gold. Now, the second paper just came out near the end of 2020 in the journal Vox Sanguinis, and it is called "Red-blood-cell alloimmunization and prophylactic antigen matching for transfusion in patients with warm autoantibodies" [Linked HERE]. Again, it is pure gold for understanding what does and does not seem to work when choosing blood for these patients. And Meghan Delaney, my other guest today, was the primary or lead author on that paper.

Before we start, a quick word about my guests. You can find more complete bios for both on the show page for this episode at BBGuy.org/088, but here are the basics for both of them.

Dr. Alyssa Ziman is Division Chief of Transfusion Medicine at Ronald Reagan UCLA Medical Center, and she's also the Chief of Laboratory Medicine and Professor of Pathology at David Geffen School of Medicine at UCLA. Dr. Meghan Delaney is Chief of the Department of Pathology and Laboratory Medicine at Children's National Health System in Washington, DC. Both of these two amazing physicians are well-published, highly respected, and generally, they're just awesome. I am really privileged to know both of them.

So let's roll, no more waiting, with today's episode, Warm Autoantibody Best Practices.

****

Joe: Hi Alyssa! welcome to the Blood Bank Guy Essentials Podcast.

Alyssa: Good morning, Joe. It's a pleasure to be here.

Joe: Thank you so much for doing it. And I'm so grateful to have Meghan back. Thank you for coming back, Meghan.

Meghan: Thanks so much for having me again, Joe. It's great to be here.

Joe: Well, I am incredibly excited to have both of you guys here to talk about some best practices in warm autoantibodies and warm autoimmune hemolytic anemia. You guys have been very heavily involved in writing and dreaming up two of my absolute favorite papers on warm autoantibody workups and best practices. Everyone listening, I will have links to both of these on the show page for this episode. So please get these papers. They're fantastic. As we start, I wanted to talk just a little bit first about how these suckers came about. How did you guys decide there's a gap here? There's something that people need to know about warm autoantibodies. What drove you guys to co-lead these two papers?

Meghan: So the papers and the ideas came from collaboration through a group called "BEST Collaborative," where Alyssa and I are both members. In that group, there are many different countries and institutions represented, and we do projects together that really benefit improvements in transfusion safety and visibility to what different practices might be better or maybe not optimal practices. And so in the topic of warm autoantibodies, Alyssa and I both felt that there was so much variation in practice. This was our initial hunch, just based on all of us being together at these meetings and talking about how we handle these problems in our labs, that we felt like it was a great topic to begin to study. And these papers are essentially the years of study over time as we've dove deeper and deeper into them.

Alyssa: And if I can just add to Meghan's comment, from my perspective, I was really interested in pheno-matching units for red cell transfusion, which we'll get to later on in the talk, and we kind of moved backwards starting with that concept. Like why do we provide this? Does it really help? And from that, we then moved back to starting with, how do we even get there? How do we make this determination that there's a warm auto present? And then what do we do?

Joe: Your work has really contributed heavily to my more recent understanding of where we are and where we're going with this. So we're going to get into both of these papers, but I thought we would start with just a real quick overview of warm autoantibodies and the challenges that they bring about. So Meghan, let's talk a little bit with you about that. Those that listen to this podcast will know I just did an episode, episode 085 with Jill Storry on how to work up things and what to do when everything is incompatible, and there's a lot of serologic detail there. So I hope everyone takes at least a little bit of time to listen to that episode. But Meghan, let's just take this high level. Let's just overview this. What does it mean when we say that someone has a warm autoantibody?

Meghan: So a warm autoantibody is a type of antibody that we find in the tube or in the test system that is IgG that has a maximal reactivity at 37 degrees of body temperature. The other way that we sometimes call these is that if you are doing an elution, you isolate this antibody and then you react it with a panel of cells again, it reacts with all cells. So you might hear people also call it a "panagglutinin." These antibodies get in our way of our serological workup. I'm sure Dr. Storry went into great detail about that. And medically, they can be seen with or without autoimmune hemolytic anemia, which is an autoimmune process where the red cells are being destroyed by the patient's own immune system.

Joe: I think there is some misconception out there that warm autoantibodies equal warm autoimmune hemolytic anemia. What are your general thoughts on that? Is it always correlated? Is there ANY correlation?

Meghan: So that is one of the very things that Alyssa and I wanted to understand better with these papers. And so we'll talk about it throughout this podcast, but the bottom line is they're not always equal. That someone can have a warm autoantibody and will not have autoimmune hemolytic anemia. However, on the flip side, typically, if someone has autoimmune hemolytic anemia, often they're associated with warm autoantibodies, but again, not 100% of the time.

Joe: Just a couple other things before we move on to the 2017 paper that I think are important for us to get out of the way. And you're right, Jill went into great detail on the serologic workup, but let's just kind of thumbnail this for the back of the envelope discussion here real quickly. What's the big challenge? So if you have someone who has a warm autoantibody, why is that difficult and why does that cause problems for us in the transfusion service?

Meghan: So what's really difficult as it boils down to is, "can you crossmatch the cells?" is one thing, and that's more of what I believe Dr. Storry covered. But then the other piece that we're really worried about is because of this interfering antibody, will that patient make red cell alloantibodies? And is there any way to prevent that with something that we can do in the transfusion service? As transfusion professionals, we always want to pick the absolute safest red cell for that patient when they need transfusion. And we really felt like this was a question is that we do different things in the laboratory to try to pick the best red cell, but we didn't know what was the most efficacious.

And just for instance, the alloimmunization rate in different patient groups is very variable. So there's papers that cite patients with autoimmune hemolytic anemia have an alloimmunization rate that ranges from 12% to 40%. Other papers that are just looking at it from the perspective of just having a warm auto, not necessarily with the autoimmune hemolytic anemia, and that range is 8% to 39%. So, similar, but those are pretty broad ranges and there's probably more going on there that would help us make that more finesse to those numbers and figure out what the real risk is.

Alyssa: I think what I didn't appreciate was the ability to how to simplify the workup. And then a lot of what people did was really to remove the challenges of a warm auto, whether that was providing pheno-matched red cells or doing a phenotype, but it was to get rid of the time consuming and expensive nature of warm autoantibody workups.

Joe: So Meghan, one more thing before we leave the general overview, and that's, for a blood banker when they see someone coming in with a warm autoantibody and they're trying to get blood out to them, in your view, what's the biggest or one of the biggest things that that serologist needs to be thinking of?

Meghan: I believe that it is to determine if there are underlying alloantibodies. If you think about it at the time, you know that the warm auto, if it's strong enough, will make your crossmatch incompatible, but yet you also want to know this

other piece of information is that have I made sure that there's nothing else there that's going to impact the patient? And so that's a real challenge and often a challenge that has to be undertaken with a time constraint. And that's one thing that we really focused on in the 2017 paper is really trying to pragmatically find what is the best practice for when you have time, and then what if you don't have time and then what do you do?

Joe: Yeah, such a big and important thing. And so let's get into that. Alyssa, I would love to have you take us through the highlights as best we can of the 2017 paper [NOTE: Ziman A et al. Warm-reactive (immunoglobulin G) autoantibodies and laboratory testing best practices: review of the literature and survey of current practice. Transfusion 2017;57:463-477]. And just, everyone listening, I want you to know there is SO MUCH information in this paper. There is no possibility that we can do anything but hit the highlights. Please get that paper. There's so much to discuss. But high level, Alyssa: First, tell us a little bit about what you did in terms of getting this information to answer the questions that you guys have already talked about. So what did you do?

Alyssa: So we approached this, Joe, in kind of two phases. We wanted to understand what was available in the literature. What had people described over the last few decades, as well as what people are currently doing. So we did an extensive literature review and then tried to cull everything down into what we felt was most essential. And then we also surveyed several or all of the members of our BEST Collaborative as well as some additional individuals representing AABB, CAP, ISBT to really understand what's actually happening in the laboratory, not just what's published as best practice.

Joe: As I look at this paper, you started off with a massive amount of papers in the literature. Holy cow, how did you sort through that?

Alyssa: [Laughs] Well, Meghan and I started, we really tried to attack this and logically go through one, what was written in English, what was pertinent to humans. There are animal studies about this. So we really tried to distill it down to what was most relevant and practical.

Joe: It was a pretty massive task. So I salute you for that effort. Holy moly! Also, if you could talk us through a little bit of the detail on the places that were surveyed in the BEST Collaborative. Did they lean towards one part of blood bank or another? In other words, were you serving only reference labs or with these hospital transfusion services as well? What was the kind of the general breakdown there?

Alyssa: Our survey turned out to be a mix of hospital transfusion services as well as reference labs, as we also included a couple of centralized transfusion services, but the hospital-based transfusion services tended towards large academic medical centers.

Joe: I'm struck by just the quantity of data that you guys were able to acquire. There were enormous numbers of different opinions and different approaches. And before we get into what you guys determined were the best practices, as you talked about in the paper, how do you make those judgements on what is best practice when you've got so many varying opinions?

Alyssa: So, Joe, I think it was something that we struggled with. We had a project team, so it really was a collaborative effort in understanding what resources were available and really being able to define "urgent" versus "routine" need and just trying to understand what people are doing and what you really need. You spoke to it earlier. If patients urgently need to be transfused, you need to find a product that is safe for them with the least amount of testing at that time, and then kind of make up the testing after the fact.

Meghan: I think another thing, if I can jump in, is that a lot of immunohematology papers suffer from small sample size, and Alyssa and I both wanted to break that mold with these projects and really try to be as broadly generalizable as possible. And so if there was a study of just a few patients published a long time ago, it wasn't as highly considered as the bigger papers. We did weigh amongst the author group, which all worked together, the validity or the power of the evidence, even though it's not really possible in these types of studies to really look at them with levels of evidence because they're usually observational studies or retrospective studies.

Alyssa: I think the other thing that we found in doing the literature review is there are, as Meghan said, cases or small case series that are done in the laboratory, and then there are reports of clinical outcomes, but bridging the gap from laboratory to clinical, there's very few studies that really accomplish that and the numbers are small.

Joe: That I think to me is one of the biggest strengths of what you guys did. Our wonderful technologists that do all this work at the bench, it's massively important what they do, but it's also important how this fits in with clinical reality. So marrying those two I'm sure was a massive challenge. And I, again, salute you guys for taking that on.

So, Alyssa, let's come back to, as I said, there's so much in this paper. I think we have to focus in on the synthesis of what you guys found. And there is an incredibly wonderful table in this paper in the February 2017 paper in Transfusion. It's table 3. If you have the paper and you're following along, it's on pages 471 and 472 of that publication. So this is really the nuts and bolts. This is the core of what you guys did in terms of synthesizing that data. So, Alyssa, let's take a few minutes to just walk through that. What are the categories of things that you guys looked at when you were summarizing the approaches to warm autoantibodies?

Alyssa: Certainly. So as we started to do the literature review, we found that the literature really fit into four broad categories: [1]Testing methodology (how do

you detect underlying alloantibodies?); [2] Performance of a DAT, and then if positive, performance of elution studies; [3] Phenotyping and genotyping of the patient to understand what alloantibodies could be formed; and then [4] Selecting red cell units for transfusion.

Joe: In as much or little detail as you feel works for us, Alyssa, let's talk through first, the test methodologies. What were you finding from this in-depth look in terms of how people were detecting those underlying alloantibodies? And we talked about that before. That should be at or near the top of our priority. So how are people doing that? And what did you determine were the implications for our everyday practice?

Alyssa: So first we found both in the literature and the survey that there are numerous ways to test for detecting underlying alloantibodies or just basic antibody screens. And the practice across laboratories varies tremendously, and that individual laboratories have multiple methodologies available to do that testing. We started there. We started appreciating that there were multiple test methodologies and then the challenge of trying to do autoadsorption or alloadsorption studies came into the picture. And what really people needed in the urgent setting is a quick or simple method to understand whether there's underlying alloantibodies. And so that pointed us towards dilution studies or using saline IgG in tube as an opportunity to detect those underlying alloantibodies as a first step.

Joe: Most of our listeners are very well aware that in most immunohematology, both reference labs and transfusion services, that things like gel, solid-phase, and in reference labs a lot of times the use of polyethylene glycol to potentiate the reactions is used. But when you use saline IgG, you're deliberately choosing something that's less sensitive. And again, I don't want to beat this to death, but I think I was a little surprised that that was something that was so widely used in reference labs, at least. So can you talk through a little bit about the logic behind that?

Alyssa: Absolutely. So I have to admit when I first started, I was surprised that that was a technique that was utilized and the immunohematology specialists at UCLA both felt really strongly that this was a powerful tool, particularly in an urgent setting and they rarely, if ever, saw an alloantibody that was weaker than the autoantibody. And when we reached out and spoke to our friends at the reference laboratory, they use this routine or this technique routinely.

Joe: So the general idea is simply being that if you are running it in saline, you're very unlikely to miss an alloantibody, but the autoantibody would generally be weaker. Is that kind of the logic?

Alyssa: Yes, that's the logic. And so at UCLA, we use that as a first step and if the warm autoantibody is removed from the results and we can rule out underlying alloantibodies, we move on. If not, then we would move on to auto- or alloadsorption depending upon the patient's history.

Joe: That's a big thing because as you're talking about this, Alyssa, you're talking about a scenario, especially when someone urgently needs blood, we all know autoadsorptions take a while. And sometimes with strong autoantibodies in particular and you have to multiply adsorb, they can take a LONG while. When this paper came out in fact, I went to my reference lab staff and I'm like, "What do you think of this?" And they're like, "Joe, we use it all the time." "Oh, great. Yeah, I'm on top of things." [Laughs] Let me bring Meghan in. Meghan, what's your experience with this? Were you surprised by this as well or...You're probably way ahead of me, I'm guessing?

Meghan: [Chuckles] So before I moved to Children's National, I was the medical director of the Immunohematology Reference Lab at Bloodworks in Seattle, and so I wasn't surprised. We think of how do you back away from enhancing antibodies that you don't want to see? And so I wasn't at all surprised, although I will add that linkage to the clinical is that in my practice now, which is all children, if we get an unfortunate little kid in with a really strong warm auto that as we're trying to move to saline and you still get reactivity, you still have to make this judgment call and say, "You know what? This four year old child has never been transfused before." So it's okay. We don't have to be... They need blood now and we don't have to keep hunting because for instance, my laboratory now is more like other transfusion services that doesn't have the capacity to do an autoadsorption on-site.

Alyssa: And I think that's an important point. What capabilities do you have on-site, where the patient is, versus what you have to send out to have performed, and how much does sending that testing out to a reference laboratory delay patient care? So our practice at UCLA is that if saline IgG will rule out underlying alloantibodies, we do not proceed with adsorption studies. So we use that as a very powerful tool in terms of simplifying the workup and it has worked.

Meghan: And I would say back to thinking about all different hospitals and all different transfusion services, I completely agree with what Alyssa just said, is that, say you're a blood bank that only has gel and you have a panagglutinin and you have time. You might end up sending it out because you actually might not even have saline in your lab. There's just so many different variations of transfusion services. There's not exactly one "right" way to always do it, and that is okay. However, I do think that with those strong warm autos that the child I just explained that has not been transfused. I need blood now, I'm going to give out incompatible blood without clearly being able to rule out underlying allos, I will send that for an autoadsorption with that really, the strong one. It's just the answer will come back in a day or two. So it doesn't help me for today.

Joe: [Laughs] Yes, I understand completely. Alyssa, before we leave this topic, there's an important point that you guys make when you were talking about routine transfusion implications. And that's something that I think people that are learning often have trouble distinguishing between, and that's when you should do an alloadsorption....if you're going to do an adsorption, when you

should do an alloadsorption versus doing an autoadsorption? Could you illuminate that for us?

Alyssa: Absolutely. So if saline IgG does not remove the reactivity of the warm auto in the case like Meghan just described, you need to proceed with adsorption studies. And so then the question becomes, one, do you have enough red cells in your specimen? Which in cases with little kids may be the driving factor, and whether or not the patient has a recent history of transfusion or pregnancy. And three months is the cutoff that laboratories use. And then that would switch you from doing an autoadsorption if they DON'T have that history and you have enough red cells from the patient, versus an alloadsorption. And the complexities are different because you can take the patient's cells with an autoadsorption to remove that antibody from the serum and then test, versus with an alloadsorption, you're taking a mix of three different phenotype cells to try and elucidate what underlying alloantibodies remain after the adsorption.

Joe: I think that's a super-important point. And I assume that the distinction with the recent transfusion is that the cells that you would be using aren't necessarily all the patient's cells. Is that the deal?

Alyssa: Yes, that would be the case. You'd have a mix of both patient and transfused cells. So then you don't really know what you're adsorbing out and you could risk adsorbing out an alloantibody.

Joe: I think we need to move on and talk about doing the direct antiglobulin test and/or potentially doing elution studies.

Alyssa: Absolutely. So if you have a positive DAT, particularly for IgG, elution allows you to a study of an "eluate," is the study of the IgG that was coating the red cell. So you remove that IgG and then you test it to see what the specificity is.

Joe: What typically do you find when you do an elution?

Alyssa: You find a panagglutinin. So the warm autoantibody not only is it in the serum, but it is also coating the patient's red cells.

Joe: So with that being said, why don't you talk us through what you guys were able to discover and find in your literature search and survey regarding the use of the DAT and elution studies.

Alyssa: So we found that a DAT is a very common tool in the workup of a warm autoantibody. And when we surveyed our group, almost 70% performed a DAT every time the IAT was positive or the antibody screen was positive, and then a significant portion performed it on the first time they identified a warm auto. So seeing that panagglutinin and DAT was important. And then again, like I said, some do it every single time, others, once they've identified the warm autoantibodies, subsequent workups, they don't repeat that testing.

I think once you establish that there's a warm autoantibody in the plasma and on the red cells through elution studies, particularly if the patient does not have any intervening transfusion between testing points, that repeat elutions are not necessary, but it's really that DAT that's performed every time. And then along the comments that you were just making, I do wonder why it has to be repeated every single time, right? Once you have the warm auto and if it's present, do you really need a DAT again?

At UCLA, we do repeat it and we look at the strength of that DAT result and that guides whether or not we need to do additional studies or another elution, particularly in patients with recent histories of transfusion, right? So if they've been transfused, the DAT increases in strength, could there be something else coating the red cells? And therefore the new elution studies would be indicated.

Joe: So a tool kind of to suggest a potential change or the development of something new that you want to work up further? Am I accurately summarizing your position there?

Alyssa: Yes, for subsequent testing. And that's what we described also in this table 3 from our review. So it's important at the initial detection to tie the picture together. And then for subsequent testing, with a history of transfusion, you're looking to find a new alloantibody or evidence of a delayed hemolytic transfusion reaction.

Joe: Before we leave the DAT though, you guys make a very strong statement that I 1,000% agree with. And I've had this discussion many times with clinicians, in terms of using the DAT as a tool for monitoring hemolysis. You make a very clear statement that DAT is not useful for monitoring hemolysis. Talk us through that argument.

Alyssa: Both in the literature as well as in my own practice, I have seen patients who have a weakly positive DAT and are hemolyzing like crazy and patients that have a really strong DAT and there's no evidence of hemolysis. And even though we have some clinicians, I've have had the same conversations you just alluded to, that continuously follow the DAT, we've found that it really is NOT a useful tool. That following a patient's hemoglobin is much more effective to see if there's hemolysis, and that people's immune systems work differently in terms of how much IgG is coating the red cells and whether that red cell is removed from circulation. So the literature doesn't support it, our practice doesn't support it. And some people in the survey had found the same thing. A lot of our survey results or respondents didn't know how their clinicians use the DAT, and that may be because they were from reference laboratories. So the survey wasn't great at giving information there.

Joe: [Laughs] Wait, are you saying that reference laboratories live in a little bubble sometimes, Alyssa? Come on.

Alyssa: [Laughs] No, we all live in our own little bubbles, right? Some of the bubbles are just farther removed and they never bumped into a clinician's bubble.

Joe: Yeah, you are not wrong about that. That is for sure. Okay, so anything to add on DAT and eluate before we move on?

Alyssa: I think the only other thing would be that if the DAT is NOT positive for IgG, if it's only positive for compliment, there's not value or much value in doing an elution unless you're really hunting for something because the patient is hemolyzing like crazy.

Joe: That's a big point. Okay, let's move on and talk about phenotyping and genotyping. And I don't want to spend a ton of time on this. I think the guidance is relatively uncontroversial, I would think. But if you could, summarize this for us what you found in terms of that.

Alyssa: From the survey perspective, 75% of our respondents provide phenotype- or genotype-matched units. And so they use this tool both for understanding what alloantibodies patients may make as well as to select units for transfusion. So it is a widely used tool. At a minimum, people are phenotyping for Rh antigens and Kell antigen, but then many are doing full phenotypes that extend beyond that.

But genotyping plays a role in patients who are heavily transfused where you can't get a phenotype, predominantly. We found in the survey as well as in the literature, there are sites that routinely use genotyping as a tool. And I think Meghan can probably provide more information in regards to that and her reference lab history. It's not something that we use routinely at UCLA. We don't have ready access to genotyping, and so the turnaround time is really slow. And so it doesn't help with providing units for transfusion, but really it is a tool that people use if it's readily available, I found, or in reference laboratories, but more often people rely on phenotyping if that's possible.

Joe: And Meghan, you and I have had conversations about genotyping in the past. In fact, we did a full episode on the use of genotyping and how it works [NOTE: See BBGuy.org/029]. So do you have anything to add to what Alyssa just said before we go to the last one?

Meghan: Well, I really agree about that if genotyping is available, the places to use it become easier to see. There's not that barrier to send it out. And I think that just in the context of warm auto alone in my practice, sometimes a warm auto can actually be so strong that if you're trying to phenotype the red cells, you cannot even do that because you can't get it off of the red cells through your laboratory procedures. And so in those situations, when you have access to it, it's a nice way to get an extended phenotype, but you're really doing a geno-match because the warm auto is making the phenotyping technically impossible or very difficult.

Joe: As we transition from this wonderful paper in 2017 into the wonderful paper you guys are having come out in 2020, let's focus a little bit on a couple aspects of the selecting red cell units for transfusion in patients with warm autoantibodies. And I want to talk about antigen matching briefly with you, Alyssa, because obviously there's more to come with that. But before we get to that, I wonder one of the questions that I often get asked in my role in a blood center and reference lab from hospitals that are transfusing patients with warm autos is, "How do I crossmatch these people? If we've done the work, if we've done all the stuff that we've talked about in the previous things and we've ruled out underlying alloantibodies, for example, we know the crossmatch is going to be incompatible. Can I use any shortcuts? Technically, how do I do that crossmatch?" So I wonder if you'd take on that first before we get to the antigen matching part.

Alyssa: Of course. So I think there is, the literature has at least one study, if not some other examples, in case reports of limiting crossmatch testing to either immediate spin or use of electronic crossmatch if there are no underlying alloantibodies. And at our institution, we do have electronic crossmatch. And so if there is a warm auto with no underlying alloantibodies, we move on to electronic crossmatch. If we do identify underlying alloantibodies, and that has either been through saline IgG or doing adsorption studies, we will use the adsorbed plasma to do the crossmatch or that desensitized method as well to do the crossmatch.

Joe: Practically speaking, my assumption would be that that would lead to fewer, and correct me if I'm wrong here, but that might lead to fewer conversations with clinicians. And, "soapbox time" for just a second: One of the things I've talked, again, a lot with people about is the use of the so-called "waiver," where clinicians are asked to sign some draconian horrible thing saying that "I absolve the blood bank of all responsibility for this incompatible crossmatch," which is absurd. We all know that. But by doing it that way, is that one practical benefit that you don't have to have as many of those awkward and weird conversations about incompatible crossmatches?

Alyssa: So that does help if we provide electronically crossmatched units because there is no underlying alloantibodies, or if we use the adsorbed plasma, the crossmatch is compatible. And so yes, the unit goes out that way. In addition, we've switched over to the term "most compatible" as opposed to "least incompatible," because we truly are providing the most compatible blood for these patients. And I think that that slight difference rests a little better in the clinicians when we talk to them about that. We don't have them at UCLA sign that piece of paper. We take on that responsibility and we tell them, "We've selected the best units for your patient. These are the most compatible red cells."

Meghan: So, Joe, for all your listeners, and I know that a lot of them are newer to the field and trying to learn about blood banking, if you're new to a blood banking call schedule, I would suggest that you practice your speech for how you're

going to talk to a transfusing clinician about what this means, because I can tell you that if you get very, very complicated, it's harder to understand. And so by listening to what, how Alyssa just described it, which was really drilling it down to as simple as possible, that those words really do matter, because doctors are taught to be scared of incompatible blood transfusions for good reason, but yet it means that they think it happens a lot more than we all know that it does. And so I think it's a really good thing to really get comfortable with, because one of those common calls that they're really listening to every word you're saying.

Alyssa: I think we need to instill trust in our clinicians that we are taking responsibility for selecting blood products and we have the patient's best interests in mind and that we are doing everything we can, but in those cases, just like Meghan alluded to, the warm autoantibody IS going to interfere with our testing. There is really no way around it, but once you've ruled out underlying alloantibodies, that takes away the risk.

Joe: So what did you find, Alyssa, in terms of how people are matching red cells or choosing red cells for transfusion to these patients with warm autoantibodies?

Alyssa: We found that people were providing pheno-matched red cells or genotype-matched if they had that testing capability. And as I mentioned earlier, about 75% of our respondents provided at a minimum Rh- and Kell-matched red cells in patients who had warm autoantibodies.

Joe: And did that change if someone already had an alloantibody?

Alyssa: It varied. In some institutions, if the patient had made an alloantibody, they extended the phenotyping, but not at every institution. So we recommend if you have that ability to extend the phenotyping, certainly to do the phenotyping on your patient, the extended phenotyping, so you're aware of what other antibodies that patient could make, and then to do pretty much the best that you could to provide antigen-matched red cells.

Joe: I want to take what you just told us about matching and transition Meghan into a discussion of the paper that we've mentioned that's coming out in Vox Sanguinis in 2020 [NOTE: Delaney M et al. Red-blood-cell alloimmunization and prophylactic antigen matching for transfusion in patients with warm autoantibodies. Vox Sanguinis 2020;115;515–524], that tried to answer the question of, do we need to do that? Or what effect does it have when we do that matching? So, Meghan, take us through what you were trying to determine and if that was a direct attempt to answer that question from the 2017 paper.

Meghan: Joe, I really want to thank you for covering these papers as an arc, because when you first start on a topic, you don't know where the path will lead, but each part that we've already talked about and then what I'm going to talk about going forward is just trying more and more to get at these questions that I think

a lot of people wonder about. And so they come from a very pragmatic place of why and what should we do?

So this study was trying to move away from reporting about, okay, now we understand what people do right now from the survey and what people have reported in the literature from our literature review. Well, now let's actually go look at patient records and find out what is happening to the best of our abilities.

So we did a... It was a retrospective cohort, but essentially to find out several things around patients that have warm autos. One question was, how many actually have alloimmunization? And then if you were to select red cells that were antigen-matched, meaning more than ABO and RhD, does that have any impact? I start everything from the place of everything we're trying to do here is the best thing for the patient, but at the same time, are we doing things that we don't need to do is also important to know, or are we doing things that are very high value and we should keep doing them?

Joe: Again, everyone, there is a MOUNTAIN of information in this paper. And Meghan, I'm going to give you a little bit more free rein to talk us through some of the highlights, but those questions that you are asking in terms of the overall prevalence of these and how often they're alloimmunized. Let's start there and then get at the end to, is the matching effective?

Meghan: Sure. So I just want to start by telling the listeners what the study was. So it was over 10 years. We included all patients that were older than one year or equal to one year that had had an antibody detection test, and then those that had evidence of a warm autoantibody. And just again, the paper describes... We had to define warm autoantibody one way because different centers might not exactly define it the same. And so it was a panagglutinin in the serum and/or eluate and a positive autocontrol and/or a positive DAT with IgG. We also excluded patients that had known drug or known daratumumab, as well as we excluded sickle cell disease patients. We can circle back to that.

So it ended up being eight centers from five countries. And I really want to say the thing that I'm most tickled about with the whole study, was just so thrilled about, is how big it is, because as I said at the top of the piece, a lot of what we saw in the literature review is a lot of small studies and single center studies. And those are hard to generalize, as you heard our discussion. And so here, we were able to get over 1 million patients. And so that's the denominator and it's all about the denominator. We did have one very large IRL that we had to remove because we felt that that skewed the data. And so when we'd look at just hospital transfusion services, we had 734,000 patients.

Joe: That's massive, Meghan! As you said, we're used to seeing studies with 50 people, but that's an ENORMOUS number of patients that were involved in this.

Meghan: Absolutely. And so then what is the punchline? Right? [Laughs] Because that's the best part (and you can hear me smiling because it's so interesting), is that so of those 734,000 patients, the prevalence, so the number that had our study definition of a warm autoantibody was 0.17%, which I just think we all... If you're listening to this podcast, you KNOW that in the blood bank, we spend a LOT of time on warm autoantibodies, and that number really just tells us that it's on the vast minority of patients that have this. And so, it kind of fits the rules of medicine and science is that there's the unique things that are special like this, you spend a lot of time and focus, but the vast majority of patients do not have warm autoantibodies.

Joe: And I have to admit, Meghan, when I saw that, I thought it was a typo or I thought I'd read it wrong. I was like, "Oh, 17%. Sure. That makes sense." And I was like, "Wait 0.17%? Are you kidding me?" I was astonished by that number! I have to tell you the truth.

Meghan: Yeah, I know. I think we all were, right Alyssa? We were like, "Oh my God." [Laughs] But the whole study, like just that number, it's like I was done. I was like, "Wow, we got this number."

Alyssa: [Laughs] That's right. Absolutely. But we do spend a TON of time on this TINY little proportion of patients.

Joe: And in reference lab world where I live, it feels like every other case is a warm auto. We could wax eloquent on that for a while. So let's move on to what else you found in this rich trove of data in terms of the previous questions that we've asked with alloantibodies, for example.

Meghan: Right. So then what we were wondering about, and I'm glad we did this study so big because it still only ended up yielding a little over 1,000 patients with warm auto, right? And we wanted to try to look for differences amongst that population in particular. And when I say differences, I mean differences in diseases and other easily determined risk factors, and then as well as the way that they were transfused and then what happened to them subsequently. The study design is really important here is that we put people in successively smaller and smaller buckets as we categorized them to say, well, first, it's a little bit over 1,000 people with warm auto. Well, then how many of them were transfused? Because we can't look at the end point of alloimmunization unless they had transfusion. Of course, pregnancy is also a factor which comes out in our data.

And then we wanted to look at transfusion strategy, so how many got what we called "PAM" or "prospective antigen-matched" red cells and then how many did not. And I want to also make sure that we're clear about how we defined it. When you're doing a big study like this, you have to pick your definition. And so we said that a center was using the PAM approach if they provided either partial antigen matching, so essentially the extended Rh and Kell, or

considered fully antigen-matched, including Kidd, Duffy, and MNS. And we just had to bucket them into PAM or no PAM.

So then going further, you can see how it starts to get smaller. And we have an image in the paper that shows this branching logic. Then we said, "Well, how many had serological follow-up?" Because we wanted to try to isolate patients that had a new alloantibody or as best as we could perceive it to be a new alloantibody, and those that did not. So we used the cutoff of 30 days. So we wanted to know, was there a difference between patients that at presentation had warm auto only, (this means that their first antibody screen had warm auto only) or had warm auto with a concurrent alloantibody? And that was about two thirds had warm auto only and about one third had concurrent allo.

We tried to categorize these into patient diagnoses that we thought would be associated with warm autoantibodies, which tend to be disorders that impact the immune system or pregnancy, and we did find that it was statistically significant that most patients that either had actually had autoimmune hemolytic anemia or other immune disorders such as connective tissue diseases or leukemia or lymphoma were more likely to present with warm autoantibody only. And that's interesting because it's more of like an epidemiological question and answer. It's saying these autoimmune diseases can concurrently have a warm auto that we pick up in the blood bank, and it doesn't necessarily have anything to do with transfusion.

Joe: The argument that so many of these patients that have antibodies at the time of presentation, has been used to say that we need to consider matching these patients like we do patients with sickle cell. Is that argument something that resonates with you, Meghan, in terms of justifying people's position that we need to do that prophylactic matching?

Meghan: I do think so. I think it's one of the justifications for why prophylactic matching is something that laboratories might want to do. Just as we've talked about different hospitals have different capabilities, they also have different patient groups. And I can tell you in Washington, DC, we have a very high rate of people in the population that have sickle cell disease. And so the decisions I might make for my transfusion service when I have a new warm auto in a patient with a new sickle cell patient, I might put policy in place in my blood bank to do something different depending on my capabilities in what I can do.

Joe: Since we're running a little bit short on time, I do want to cut to the chase of the effectiveness of that prophylactic antigen matching or PAM strategy that you guys talked about. Let's just nitty-gritty that: What did you guys find in terms of, does this work? Is it an effective strategy?

Meghan: So to talk about the alloimmunization, what we had to talk about first was how many were actually transfused. Those ranged from 59% in the patients with warm auto only to 73% in the patients with those that presented with concurrent allo. So again, epidemiologically, it tells you that while that patient

probably was transfused elsewhere, is my guess. They have a disorder that requires transfusion, to simplify.

So when we broke it down amongst those groups between those that were receiving PAM red cells versus not PAM red cells, even though that number I started with was so big, it gets small because of all these different parameters you had to go through to biologically get to this end point we were trying to look at. And so, when you boil it all down, the patients that have warm autoantibodies comparing those that received PAM and those that did not receive PAM, there was no statistically significant difference in future alloimmunization. In addition, when you look at the patients that already made allos, and so there's that idea of once you've made an allo, you're potentially more at risk for more, and there's other nice papers out to talk about that, again, the same thing was found, that the patients that got PAM or no PAM transfusion strategies did not differ in their rate of additional alloimmunization. So that was a really big finding of the paper.

Joe: That's huge. Massive. And so, if you were sitting here and looking at your paper, if you were trying to poke holes in that logic [chuckles]...I'm asking you to poke holes in your own paper. That's not really very fair. What would you describe as the strengths and weaknesses of those conclusions?

Meghan: One thing I would say is that from study design, you do the lit search, you do a survey, you do an observational or retrospective type of study, and then you do a randomized controlled trial. And just these numbers, when you start to see how big we started and how small we got, it's a challenging study to do. They have done some randomized controlled trials in the Netherlands about prospective matching (this was not in the context of warm auto), and did show that if you can match, you can prevent, but it's very hard to pull off.

So back to your question is that for the purposes of not making another antibody, I think it's very difficult to show that that should be the reason to do a PAM approach. However, there are other more laboratory-based reasons that sort of hearken back to what Alyssa was talking about before by doing PAM, that maybe you don't have to do repeated adsorptions, which was, we were just talking about how difficult that can be. And in fact, there's a really nice paper from Hopkins in 2002 that shows that there's about 4.2 additional adsorptions that you can avoid if you just use prospective antigen matching [NOTE: Shirey RS et al. Prophylactic antigen-matched donor blood for patients with warm autoantibodies: an algorithm for transfusion management. Transfusion 2002;42:1435-1441].

So I think it ends up being a judgment call, but I think our paper shows pretty clearly that there's a lot going on with the immune system and that if you provide prospective antigen matching, don't think that the patient's not going to make an antibody. And those antibodies could be a mixture of things they've seen before. That's also a very difficult thing to control for in

immunohematology studies is, was it a recall antibody or a delayed amnestic response?

Joe: The practical aspect of it is that it could save you doing additional work, it could allow a patient to get blood faster, but also on the other hand, in some cases, trying to prophylactically match someone can be really challenging and it doesn't necessarily prevent them from making new antibodies. Is that a fair way to put it?

Meghan: I think so. The other thing I think our paper showed, besides the 0.17% of the overall prevalence is that about 63% of these patients, of a warm auto patient, require transfusion, and that the new alloimmunization rate in that group, remember at the beginning I said there's big range of like 8% to 40%. Well, we actually found it's more like 8% to 16%. So it's a little bit tighter. And that's a nice number to know, right? Because we like to say the general population, the risk of alloimmunization is probably less than 5%, even smaller, whereas in sickle cell patients or thalassemia patients, it's quite a bit higher. All comers have warm autos, which is a mixed group of patients, it's not all the same, that somewhere in there, it's about 8% to 16% of those patients if you put them together will form a new alloantibody.

Joe: Great, great, great, great info. Based on everything that we've talked about today, all the work that you guys have done, how has this, practically speaking, informed your practice at the facility where you work?

Alyssa: I have to say we have not made any changes yet. So we at UCLA continue to provide Rh- and Kell-matched units for all patients who have warm autoantibodies, based on the fact that it does simplify our transfusion workup. So it really simplifies testing. Theoretically, we still believe that it could provide some protection in patients that come to our institution for transfusion and don't go elsewhere. Perhaps that is also a component. Kind of next steps, I would love to be able to identify which patients with warm autos go on to require transfusion, so we could focus this practice on those patients that will benefit most from it, as opposed to those who just have a warm autoantibody that kind of waxes and wanes but doesn't provide any, or doesn't have any clinical implications.

Joe: Meghan, same question to you.

Meghan: What we found as well is that females and those that had more red cell transfusion requirements were at higher risk of forming alloantibodies. And so that kind of goes back to patients without antibodies, right? Or without warm autos. So I think that it's another piece of the puzzle of when you're making your policies and looking at the resources you have available, what is your antigen-negative supply like on-site? How are you able to support these patients? But always know, especially from the first paper we described, that all of these complicated things about selection are really important, but that we never want to withhold transfusion for someone who comes in very anemic.

So I haven't really changed my practice either. I think this has very much informed and made me feel more comfortable and confident about all the things that we do. I think the blood bank world does a great job with something really complicated, frankly. There's so much thought and people think about and really train our up and coming people about this.

And then I just want to say, and Alyssa might say, "Don't say that live," but we have plans to look at additional patient groups. We haven't looked at our stem cell transplant patients. That's another really interesting group to think about warm autos. And then of course we really want to think about our sickle cell patients with warm autos, but we also know that those were going to be very hard, both of those groups. So we basically did these projects first.

Alyssa: [Laughs] Yes, don't say that now!

Meghan: [Laughs] Yeah.

Alyssa: But I think, just to kind of tie up, I think it just adds more information. And really to reiterate Meghan's point is that the laboratories are doing everything they can in the best interest of patient care. And so, using this information to support the decisions you make with the resources you have, particularly in talking to clinicians about the safety of transfusion, I think can be really helpful.

Joe: I think that's a wonderful place to end, you guys. Thank you both so much for being here. It has been a pleasure. I think this'll be super helpful for people. So thank you guys so much.

Alyssa: Thank you. It's been a pleasure, Joe.

Meghan: Thank you so much.

****

Joe: Hi everyone, it's Joe with just a couple of closing thoughts. As I said several times, please check BBGuy.org/088 for direct links to the two articles we discussed. I promise they will be really, really useful to you.

If you have a chance, I'd appreciate you giving the podcast a rating and review at Apple Podcasts. And the reason is that you're doing so helps other learners find Blood Bank Guy Essentials, which is all I'm trying to do.

I'll be back in two weeks with my first Continuing Education episode of 2021, an interview with Dr. Christine Cserti-Gazdewich from the University of Toronto called "Earth, Wind and Fire!" (I love that title), where Christine takes us through the sometimes challenging distinction between transfusion-related acute lung injury and transfusion-associated circulatory overload. I'm very excited to share that one with you as well as the many other fun episodes that are coming your way in 2021.

But until then my friends, I hope that you smile, and have fun, tell the ones that you love that you do, and above all, never, EVER stop learning. Thanks so much for listening. I'll catch you next time on the Blood Bank Guy Essentials Podcast.