



**Joe Chaffin:** Hi, everybody! Welcome to the Blood Bank Guy Essentials Podcast! My name is Joe Chaffin, and I am your host. I am delighted to have as my guest today Patricia, or “Pat” Arndt, from the American Red Cross, Southern California. Pat, welcome to the podcast!

**Pat Arndt:** Thank you very much for inviting me.

**Joe:** It is my pleasure! I would like, if you don’t mind, to tell people a little bit about you, as if people don’t know you! I think you are very widely known, for good reason. Pat is an SBB who received her BA in Biological Sciences from the University of California, Santa Barbara, she got her MS in medical technology at Cal State University, Dominguez Hills, and continuing on with her training, she did her medical technologist internship, and five wonderful years later, I’m assuming, she did her specialist in blood banking internship at the American Red Cross in Southern California. That facility has been a constant in her life for quite a long time. She was a research associate in Dr. George Garratty’s research lab at the American Red Cross in Southern California from 1983 all the way until Dr. Garratty’s untimely death in 2014. Pat still works for the Red Cross. She occasionally, I guess, performs laboratory tests related to the topic we are going to talk about today, but her job title is now “Lead Technologist,” and the lab is now called the “Special Immunohematology Laboratory.” Whew, Pat, that’s a mouthful, you’ve got a lot going on!

Further, she’s on the editorial boards for “Immunohematology” and “Transfusion,” and she is secretary for the Invitational Conference of Investigative Immunohematologists group (say that ten times fast, I dare you!)

**Pat:** That’s why they call it “ICII!”

**Joe:** ICII! See, I should have known that! That’s much better! She has also received numerous awards, including the Kay Beattie Lectureship at the Michigan Association of Blood Banks in 2009, and the AABB Sally Frank Memorial Award and Lectureship in 2012. Pat, you obviously have a very long and rich history in blood banking, and you are a very widely known and very widely respected individual, and like I said, I’m just really honored that you would be on the podcast today!

**Pat:** Thank you.

**Joe:** So, I always start the podcast out the same way, Pat; it’s something that I’m really interested in, and I think, as I’ve mentioned to you before, a lot of students and people that are just figuring out what they want to do with their lives are listening to these podcasts. So, I was wondering if you would mind telling us a little bit about...I mean, I’ve just kind of summarized your journey in blood banking, but I haven’t really talked, because I don’t know, about what really kind of made blood banking seem like the right

fit for you? What was it about blood banking, when you were in your training, when you were learning to be a medical technologist, that made you say, “Wow! Blood banking, now that’s for me!”?

**Pat:** Well, partly, it’s just the puzzle-solving part of it. It’s just so interesting to try to figure out what these antibodies are, and with drug antibodies, it’s so interesting to try to figure out how they are going to work, how you can detect them. But also, I’m kind of not really crazy about machines, so you know, chemistry and (laughs) didn’t appeal to me that much. So, you know, just the puzzle-solving part of it.

**Joe:** I see. Well, if you want to say something bad about microbiology right now, I’m right there with you!

**Pat:** OK!

**Joe:** (Laughs) Maybe we should stay away from that, because some people listening might like Micro! But I’ve also heard you talk about, I believe you said this in the Sally Frank Lectureship, you described your journey a little bit as “being in the right place at the right time...”

**Pat:** Absolutely!

**Joe:** I think that you obviously found yourself at the crossroads of some amazing, brilliant minds doing some incredible work when you got to the the ARC, so I know people would be really interested in hearing as much as you are willing to share about your journey with Dr. Garratty at the ARC lab in Southern California.

**Pat:** Well, it’s just really hard to describe! He was such a great person and such a great boss, and so intelligent, and it was just a wonderful experience to be able to spend 30 years in his lab. We learned so much, and were always learning...I mean, he was a great mentor because he realized as he got older, the older he got, the less he knew, and the less he was sure of things, and he wasn’t afraid to say that.

**Joe:** That’s a rare gift! For someone who obviously knew as much as he did to be able to say that...astonishing! He and I did not cross paths, unfortunately, although I was at Cedars-Sinai for the last couple of years, from 2012-2014, so I was aware of the work that you guys were doing, obviously, on behalf of that hospital when I was there, but unfortunately, I never got the opportunity to meet him, and I’m very sad about that. So, anyway, forgive me, I know that’s an emotional topic for you, and forgive me for prying, but like I said, I know that people are aware of your time with him, and I just wanted to give you the chance to tell me how you felt about it. It’s obvious that’s a very deeply personal and great time for you.

**Pat:** Yeah, it was. And education was so important to him, and so I think that he would truly be impressed by your web site and what you are doing to educate people, and we are trying to keep on the education.

**Joe:** Agreed, and that's why we are here today! Thank you for saying that, by the way. That is exactly why we are here today, because Pat and I, everyone, are going to talk about one of her very favorite entities, and that is "Drug-induced Immune Hemolytic Anemia!" (cue the cheers!)

**Pat and Joe:** Yayyyyyyy!!!

**Joe:** I have to tell you, Pat, this is full disclosure time, it's one of *your* favorite topics; it's not necessarily one of *my* favorite topics, and don't get mad at me! Honestly, what I've felt over the years is that...I mean, I'm not as hostile to it as I am to microbiology, for example, but I've just found over the years that it's been somewhat hard to keep track of how things are categorized, how things are characterized. I think one of the things that you and Dr. Garratty have done, obviously, the work that you guys have put out from your laboratory is pretty much the international authority on this, but I think that many people still find this challenging! Have you seen that in your experience?

**Pat:** Yes, it is a difficult concept to come to grips with, and all the drugs are so different, and all the antibodies that you have to deal with with each drug is very different, so we're constantly on a learning curve with these drug antibodies. But that's what makes it interesting to me!

**Joe:** Laughs. You are a puzzle-solver, that is for sure! You are a natural blood banker, obviously!

So why don't we, if you don't mind...we've thrown out the term: "Drug-induced Immune Hemolytic Anemia." So just for the sake of everyone listening to us today, let's do a couple of definitions. Are you good with that?

**Pat:** OK. Yes!

**Joe:** Let's just start with the most basic one of that phrase: "Anemia." How would you define anemia?

**Pat:** Anemia is where you have a deficiency of red blood cells; they are below the normal level.

**Joe:** OK, and further, we said, "*Hemolytic* anemia." Now, that's a specific subset of anemia, correct?

**Pat:** Right, I mean because you can be anemic from bleeding, but hemolytic anemia means that your red cells are having shortened lifespan. Normally, they live up to about 120 days, and they are not living that long.

**Joe:** OK, so hemolytic anemia, destruction of the red cells, and you said another word (and we're kind of building backwards here), we're going to "Immune." What about *immune* hemolytic anemia, what does that mean?

**Pat:** OK, well that's when the red cells are having shortened red cell survival, directly or indirectly due to antibodies.

**Joe:** Ahh, OK. I'm assuming that would mean there are other types, perhaps *non-immune* hemolytic anemia?

**Pat:** Yes, like sickle cell anemia would be non-immune, anything that has something to do with the red cells not living their normal lifespan for other reasons other than antibodies.

**Joe:** Got it. OK, today we are going to talk about a deficiency of red cells that is caused by shortened red cell life (in other words, the red cells being destroyed), and specifically, in terms of the immune type, where that destruction is being caused by antibodies.

**Pat:** Yes!

**Joe:** Alright, so we're building here! Let's build on that for a second, though. When we talk about immune hemolytic anemia, obviously, we are going to talk about drug-induced hemolytic anemia, but that's not the only thing in that category. So why don't we start by talking a little bit about the mechanisms? How do these antibodies destroy the red cells, and how do we categorize that immune hemolytic anemia, generally speaking?

**Pat:** OK, well there's two main mechanisms. In one mechanism, the antibodies can bind a protein called "complement," and the complement can go through a very complex "cascade" with various complement proteins to get to a point where it punches a hole in the red cell membrane, and then due to changes in the ionicity of the inside of the red cell, it bursts open and releases the free hemoglobin into the blood stream. That's called "**intravascular hemolysis**." That is very damaging to the body, to have that type of hemolysis, so luckily that doesn't happen too often, maybe that's ABO-type of incompatibilities will lead to that. Rare autoimmune hemolytic anemias like "Paroxysmal Cold Hemoglobinuria" and a few types of drug-induced immune hemolytic anemia will cause that devastating type of hemolysis.

The other type of hemolysis, which happens more commonly, is "**extravascular**," where in your organs like your liver and your spleen, you have macrophages, tissue macrophages, and they have receptors that will recognize either IgG or complement on those coated red cells, and then those macrophages will engulf those red cells, or maybe secrete enzymes that will destroy those red cells. That's not *quite* as devastating, it doesn't happen quite as fast as the intravascular type. Most alloantibodies work by that mechanism; most autoantibodies and many drug-induced autoantibodies work by that extravascular mechanism.

**Joe:** And you said that extravascular is, generally speaking, more common, and generally less clinically severe, right?

**Pat:** I mean, it can be severe, but not as bad as the intravascular.

**Joe:** And just for the students that are listening: One of the things that people like to throw at you on exams, when they talk about intravascular vs. extravascular hemolysis is the appearance of the red cells depending on whether the hemolysis is intravascular or extravascular. So just a free tip for all you guys listening: Intravascular hemolysis, the classic red cell abnormality is the “schistocyte,” whereas extravascular, it’s the “spherocyte.” So, sorry for that digression, Pat...

**Pat:** No problem!

**Joe:** I like saying “schistocyte!” It makes me happy!

**Pat:** Ok, yeah, that’s a fun word!

**Joe:** OK, so we’ve got these two main mechanisms, two main ways that immune hemolytic anemias occur, or immune red cell destruction occurs. So with that being said, let’s now actually, and I jumped the gun on this a little bit, let’s categorize the immune hemolytic anemias a little bit, and talk about the different ways that we can get to immune hemolytic anemia.

**Pat:** Well, there’s **alloimmune hemolytic anemia**, where a person makes an alloantibody against an antigen that is foreign to them, and that can cause hemolytic transfusion reactions or hemolytic disease of the fetus and newborn. There’s **autoimmune hemolytic anemia**, where a person makes an antibody to their own antigens on their own red cells. Luckily, this is not too common. It has a frequency of, it’s been estimated to be about 1 in 80,000. And then there’s **drug-induced immune hemolytic anemia**, where the immune hemolytic anemia is related to a drug. It’s been estimated that this is somewhere like 1 in a million, but it’s really not known for sure, that’s just a guesstimate.

**Joe:** Dr. Garratty made that guesstimate, is that correct?

**Pat:** Yes, Dr. Garratty and Dr. Petz.

**Joe:** I actually wanted to ask you about that, because, you know, when we talk about (and I’m throwing you a curve here, Pat, I apologize in advance), but when we talk about things like HIV from blood transfusion, just to throw an example out, we talk about that with a risk of 1 in 1.5 million or so, HCV in the range of 1 in a million. It seems to me, though, that drug-induced immune hemolytic anemia happens! I mean, you guys are seeing those in your lab. I realize that you are THE LAB for this, so you see more than most places do, I’m sure, but by comparison, the risk of 1 in a million for HCV, for example, from transfusion, as compared to this; it seems like drug-induced hemolytic anemia is more common.

**Pat:** It does, I guess it seems maybe what the denominator is, how many doses of these drugs are being given? In comparison to how many transfusions?

**Joe:** Ah, I see. That makes sense. Sure. You're right. Obviously, they are potentially orders of magnitude different there, so I guess that makes sense. OK. So, I was trying to throw the curve at you, and you hit it out of the park. Good job!

**Pat:** Thank you.

**Joe:** OK, we've got these three types. Again, just to summarize where we are: We are **categorizing "Immune Hemolytic Anemias"** into those caused by alloantibodies (antibodies against someone else), autoantibodies (antibodies against yourself), and then drug-induced immune hemolytic anemias. We are going to get deep in the weeds on that in just a second, but Pat, before we move on from that, you had mentioned the mechanisms, extravascular and intravascular. Is it fair to say that those mechanisms aren't necessarily clearly associated with any one of those three categories, that either one of them could happen in any category?

**Pat:** I think there are examples of intravascular in each of those, and I think that's fair to say, yes.

**Joe:** So, we are finally, Pat, at your favorite topic! We have laid some groundwork, and we are now at drug-induced hemolytic anemia. So, let's talk about that just a little bit. When we say that, what kind of drugs are we talking about? What is the history of this? How did we realize that this was happening, and what do we know about it now in terms of drugs associated with it?

**Pat:** Well, the history is that it was first noted back in the 1950's with a drug called "stibophen," and they noted that the patient appeared to be hemolyzing, and it seemed to be related to this drug, and they were actually able to show an antibody to that drug, that was reacting with that drug. Since then, there have been a number of different mechanisms and a number of different drugs that have been described, so that now the list is probably about 140 or more different drugs that have been described causing drug-induced immune hemolytic anemia. Many of them have only been described once. There's a few that are kind of the main offenders that you see over and over and over again. It tends to be the antibiotics, the non-steroidal anti-inflammatories, and some of the cancer drugs are causing some of this problem.

**Joe:** So, does any one of those predominate?

**Pat:** The antibiotics, for sure. And among those, **we see cefotetan, ceftriaxone, and piperacillin are the top three offenders**. If you'd asked back in the 70's, it would have been penicillin, but high-dose intravenous penicillin is not used as much as it used to be. So it really has a lot to do with what drugs are commonly being used.

**Joe:** And that makes total sense. The more medications are being used, the more the potential for something like this happens. Though, I'm very interested in having you take us through the mechanisms, but I'd like before we do that... People that read older textbooks, people that look at articles that were published ten-fifteen years ago, many times will see a categorization that is a little bit different than it is today. So, you've been

on the forefront of this, and I know that you've talked about some of this in your Sally Frank lecture actually, you talked through some of the history of it, so we don't want to take a ton of time with this, so could you just take us through how our thought process has changed a little bit on these mechanisms, before we get to the specific mechanisms that we understand today?

**Pat:** Well, some of the changes were like that the penicillin and the cephalosporin drugs, we always thought that they were going to react by coating cells with the drug; that was the only way to detect those, and they were always going to cause extravascular cell destruction. And then, some of these newer cephalosporins came along, and these newer penicillins came along, and all of a sudden, they are breaking these rules! You *can't* coat cells with them, or maybe you can detect them by a *couple of methods*; one of them is coating cells, some of them are causing intravascular red cell destruction, some of them are binding complement, some of them you can't even detect in an eluate. So they are kind of breaking the rules of what we thought we knew when we had studied penicillin so well back in the '60s.

**Joe:** So new drugs, new discoveries, new instances of drug-induced immune hemolytic anemia led you to figure out that the rules weren't as hard and fast as everyone had thought?

**Pat:** Exactly! We used to kinda think, "OK, if it's this kind of drug, we would test it this way, and if it's that kind of drug, we would test it a different way." And now, basically we test everything both ways until we know differently.

**Joe:** That makes sense, and we'll get to that when we talk a little bit about how to approach these things, an approach your laboratory has used for years on this, but before we get to that, let's actually start to break these down into what we think today, in terms of the modern mechanisms of drug-induced immune hemolytic anemia. Let's do them one by one. What would be the first mechanism of drug-induced immune hemolytic anemia, Pat?

**Pat:** OK, the first mechanism is the mechanism that was first discovered, and that's the **drug-dependent antibody**, where the patient gets a drug and they make an antibody directed against the drug, and there's two subtypes of that. Sometimes, drugs will bond to red cells very firmly, they will form covalent bonds, and you actually get drug-coated red cells both inside the patient and in the laboratory you can make those. But sometimes the drugs *do not* form covalent bonds, and so you do not get drug-coated cells. So those are two different ways of testing for antibodies. One is to make drug-coated cells, the other one is to mix together the drug, the patient's serum, and the red cells in a test tube so they are all together at the same time. Those are two different subtypes we use to test for those drug-dependent antibodies.

**Joe:** And that's a really important distinction that I think a lot of students miss, so let's just make sure that everybody's clear on that. So the first subtype, as you said, is where the drug actually binds to the red cell, so you can in the laboratory you can bind that

drug to the red cell and then see the antibody-antigen reaction. Is that a fair way to put it? Is that correct?

**Pat:** Correct, yes.

**Joe:** So, without the drug binding to that red cell first, you don't get any interaction.

**Pat:** Correct.

**Joe:** What's the classic drug that's associated with that?

**Pat:** **Penicillin** is the *classic* drug.

**Joe:** The classic one, and as you mentioned, we are seeing different things...

**Pat:** The one we are seeing most often now is **cefotetan**.

**Joe:** Cefotetan, ok. So, classically penicillin, when they used to nail people with *millions* of units of penicillin IV, right?

**Pat:** For days and days and days. The patient would get penicillin-coated red cells in vivo, and then when they made that antibody, it would attack their red cells.

**Joe:** I realize that our thought process has changed somewhat on this in terms of the classic type of hemolysis associated with this, but historically, anyway, we tended to think the type of hemolysis associated with this was extravascular, is that correct?

**Pat:** Correct, yes.

**Joe:** OK, so you've got the first mechanism, the drug binds tightly to the red cell. And then the second mechanism where the drug actually has to bind to the antibody *first*, before the red cell, is that correct?

**Pat:** That's one *theory*, that's one theory that's seems to be predominant for platelets, and people thought that was also true for red cells, but there hasn't been any proof of that. But it's kind of a nice way to think about it, that you have the drug and the antibody, and that they form a complex, and they attach to the red cell, and they bind complement (because drugs that work this way do tend to bind complement very well). But there's no proof for that, and so it could be that maybe the drug is binding loosely to the red cell and forming some sort of neoantigen, some sort of drug-membrane combination antigen that the antibody recognizes and binds complement. Nobody really knows; it could be a combination of both. It's sort of out there for people to work on.

**Joe:** I'm betraying myself there, because as I mentioned, I sometimes have trouble keeping the old...what I was describing was kind of the classic old "immune complex" theory, where the antibody has to bind to the drug first, but I have read exactly what you are saying (I may have read it from you, as a matter of fact!), that we don't have proof



that it happens that way. It's just a convenient way to think about it, but it may not actually happen like that. Again, classically speaking (let's talk classically and then what we are seeing today), classically, what drug or drugs was associated with this type of mechanism?

**Pat:** Classically, that would be quinine was a drug that worked that way. Currently the drug that we're seeing work that way is ceftriaxone.

**Joe:** Ceftriaxone, ah yes. Okay. So again, and you mentioned this, because the drugs that seem to interact this way that in the laboratory, anyway, they react when serum and red cells and drugs are mixed at the same time, they tend to fix complement well, so I'm assuming, they would again, classically be more associated with intravascular hemolysis or at least, more at risk for that?

**Pat:** Exactly. Ceftriaxone, definitely. Well, ceftriaxone antibodies tend to be IgM, and they're really good at binding complement so those patients typically have very severe intravascular hemolysis.

**Joe:** I haven't been a clinician for a while. I was for a little while—I haven't been a clinician for a while, but my memory of ceftriaxone was that it was not an uncommon drug, used in pneumonias and osteomyelitis and really serious infections, as I recall. Is that what you guys are seeing?

**Pat:** We're seeing it a lot in little kids.

**Joe:** Ahhh— —okay. Kids with sepsis and the like?

**Pat:** Yeah, or kids with sickle cell anemia, kids that get infections. Unfortunately, they have severe reactions! Their hemoglobins sometimes go down to 1 or 2 g/dL, in like less than an hour.

**Joe:** Whoa! Hang on! Less than an hour?!? After the first dose or do they require....

**Pat:** No, no, no. They have to have several doses, build up their antibody, and they might have some subclinical hemolysis going on with some of those previous doses. And then all of a sudden they get a dose and BOOM! They crash! It's very sad.

**Joe:** I'm sure there are fatalities associated with it.

**Pat:** Yes, quite a few.

**Joe:** That's bad news. That's obviously one to be scared about. So again, the two scenarios: 1) The drug binding directly to the red cell 2) And the second mechanism, the drug, red cell, serum, all together and in some way, figuring out how to bind. How is that? Did I say that better that time?

**Pat:** Sure, sure!

**Joe:** (laughs) And both fall into the “drug-dependent antibodies.”

**Pat:** Right, because those are definitely antibodies that are reacting with the drug.

**Joe:** Got it. Now, I’m going to take a wild guess here, because I like those. I like wild guesses, so let me do that. Since we’ve learned so much over the years, I’m going to take a guess and say that you have seen some drug antibodies that are reacting by more than one of those methods. Are there some drugs that do that?

**Pat:** Yes. That is true and cefotetan is a good example. You can make drug-treated cells and it will have a very high titer, like up in the 16, 200,000 range titer. It’s huge, it’s amazing. But they will also react if you just mix together serum, cells, and drug, but not nearly to that same high titer. Piperacillin will react by drug-treated cells and in the presence of the drug. So, yes, there are some drugs that will react by both mechanisms.

**Joe:** And piperacillin was one those big three that you were saying you were seeing.

**Pat:** Yes.

**Joe:** So again, we’ll get to that more, shortly. So, we’ve got the drug-dependent, again, we’re talking about the mechanisms of drug-induced immune hemolytic anemia and we’ve covered the drug-dependent antibody mechanism, which has the 2 subtypes. We’ve discussed the fact that sometimes these antibodies react by more than one method. So let us move on and talk about the second type...you know what? Actually before I have you do that, I did want to mention something and tell everyone, there will be a few slides on the website for people to look at. One of them, I’m hoping to put on there...I haven’t asked your permission for this. You have a slide that I’ve seen, Pat, that shows the “unifying theory,” that I think can help people. It’s widely available, obviously, it’s something that’s been published. You can find it in the Technical Manual and all over the place, but can you just take us through that image real quickly? It’s kind of illustrating what you were saying in terms of the different mechanisms, right?

**Pat:** As far as a drug-dependent antibodies? Yes, it does. It may explain something that’s going on with cefotetan. So you have the drug attached to the red cell membrane and the drug has a red cell interaction has many epitopes. So the drug antibody could be reacting just with the drug itself and that’s on the upper left hand part of that diagram. Or the antibody could be reacting with the combination of the drug and the membrane, together. And that’s what is illustrated on the lower right hand part of that diagram. Or, just to take that to an extreme, the drug antibody could be reacting mostly with the membrane and just a little bit of the drug. And this kind of is a possible explanation for what we see with patients with cefotetan antibodies because they have a drug-dependent antibody that will only react when the drug is present and now that drug antibody is very strong, but they also have a little bit of what we call a “drug-independent antibody.” It’s got a little of a bit of an autoantibody there that will react with red cells without the drug being present, and we think maybe that’s that lower left hand component that’s mostly reacting with the membrane and a little bit of the drug. And if

the drug is not there, it will still react really weakly. By weakly, I mean a titer of maybe 2, as compared to a titer of 2,000 that the drug antibody has.

**Joe:** I see. Well, I think that image really helps kind of pull things together, to an extent. I know it's not perfect, but I think that it helps pull things together, to an extent.

**Pat:** In my mind, it helps, yeah.

**Joe:** Yes, I agree. You've kind of given us a preview, then, to the **second mechanism of drug-induced immune hemolytic anemia**. Why don't you take us through that?

**Pat:** Well I have, but I haven't, because what I'm going to talk about, the drug-independent type is not what I'm talking about with cefotetan. Cefotetan antibody reacts with a red cell that's not drug-treated and there's no drug present. That is different than what is happening with these drug-independent antibodies. There's 2 different types of drug-independent antibodies, if you want to get really confusing...

**Joe:** Bring it on! (laughs)

**Pat:** Okay. So there's the drug-independent antibody like cefotetan where a component of the drug antibody will react without the drug being present. But then there's drug-independent antibodies that are not reacting against the drug at all, and that is typified by a drug like methyldopa, which isn't used so much anymore, or fludarabine, which is used more, now. In these patients, somehow the drug seems to trigger their immune system to make just a regular, old, idiopathic autoantibody. Not really sure how, maybe they're affecting the suppressor cells, that's not really clear, but, it seems likely that something like that is happening. This drug is affecting the immune system so our normally suppressed autoantibodies are now getting produced, and it looks like any other autoantibody, reacts with all red cells in the serum, you get a positive DAT, you get a positive eluate with all red cells; you cannot distinguish any other idiopathic warm autoantibody.

**Joe:** So, how do you prove it? How do you know that it really is that, or do you know?

**Pat:** Well, the only way to know is clinically; it's to take the patient *off* the drug, and you see if the patient is hemolyzing, that the hemolysis goes away, that the drug antibody goes away, and then, you have to put the patient *back on* the drug! And this is what they did in the '60s with methyldopa, they did this. Today, it's probably a little more difficult to do that, and people are a little more hesitant to do that, for good reason.

**Joe:** You might have a hard time getting that past an IRB!

**Pat:** Yeah. So that's a little bit harder to prove it these days.

**Joe:** OK, so the second mechanism of drug-induced immune hemolytic anemia is the drug-independent antibodies, and the classic one, as you said, that we think about is with what used to be called "Aldomet," or methyldopa years and years ago. But

fludarabine is a drug that is used quite often now, I believe it's used for chronic lymphocytic leukemia as I recall, so not an uncommon drug to be out there. But again, obviously, we are talking about a small proportion of patients that are on it, but I actually looked up the package insert for fludarabine, and it is one of the things that's mentioned in there: Severe immune hemolysis that could occur.

So, beyond those two mechanisms, there is another mechanism that, well, I don't know if it's quite as exciting as the other two, but it's something that...the third category or the third mechanism. Why don't you describe it for us, Pat, what is that third category?

**Pat:** It's called "**nonimmunologic protein adsorption**," and it happens just with a few drugs. There's a short list of drugs that will bind to the red cell membrane covalently, and when they do that, they somehow change the membrane to make it sort of "sticky," so that all your plasma proteins will start sticking to these red cells nonspecifically. So that means IgG, complement, IgM, IgA, fibrinogen, albumin, fibronectin, on and on and on. And if you have IgG coating the red cell, and you test it with anti-IgG, it doesn't matter that it's not a red cell-directed antibody, it just happens to be some IgG that was floating by and stuck to the sticky red cell; you'll get a positive *antiglobulin* test, and that can be confusing sometimes. It can cause positive DATs in patients who are taking those drugs, it can cause positive indirect antiglobulin tests if you're doing a drug antibody test, and if you don't run a control to know that that's going on, you can get the wrong interpretation with your drug antibody test.

**Joe:** I see. So I have to say, that's actually more interesting than I thought it was! I mean, the concept of a drug making the surface *sticky*, and all kinds of stuff binding to it; that's kind of fun!

**Pat:** Yeah, it is kind of interesting. You just have to be careful. Whenever we test drug-treated cells, we always run a normal serum control to make sure that that's not happening, or if it, that we know about it so we can do something about it. And it's interesting that we took some of those drug-coated cells that had been incubated with plasma and now had all these proteins on them including IgG and complement, and we incubated them with monocytes, in an assay called the "monocyte monolayer assay," and those monocytes gobbled them up, they were interested in those nonspecific IgG-coated cells because they don't care how it got there; IgG was on them!

**Joe:** Fascinating! So the name of the mechanism, nonimmunologic protein adsorption, would make it *sound like* it's not something associated with hemolysis, but that doesn't sound like I'm right there, does it?

**Pat:** Well, that's what we thought for many years. We thought it wasn't, we thought that it was just sort of an in-vitro problem, that wasn't going to cause the patient any problems. Turns out that there is some evidence that there may be some patients, especially patients with hypergammaglobulinemia, who may have some hemolysis due to this mechanism.

**Joe:** That's really interesting. OK, so we've got hemolysis everywhere, for crying out loud!

**Pat:** Yeah, that's the problem with this!

**Joe:** That is the problem. If you don't mind, Pat, let's talk a little bit about, in the time that we have left, let's talk about how we test for, and I should say how *you* test for, because you obviously are involved in what I would characterize as *the laboratory* with the most expertise in working these up...How do you guys work these up, and is there a difference in how much effort you put into, or is it even possible to do workups for all three of these mechanisms?

**Pat:** Right, we really only test for the drug-dependent antibodies, because the drug-independent antibodies like fludarabine, there's no way for us to tell the difference between an autoantibody that's due to a drug vs. an autoantibody that's not due to a drug. And, as far as the nonimmunologic protein adsorption, we can show that that's happening, but we don't have any way to prove that that's what is causing hemolysis in the patient. So, both with the drug-independent autoantibodies and the nonimmunologic protein adsorption, that's really a clinical thing that you have to take the patient off the drug, show that it goes away, then put them back on the drug and show that it comes back. So in the lab, all we can do is test for the drug-dependent antibodies by making those drug-treated cells and/or by testing in the presence of a solution of the drug.

**Joe:** I know that this has been published before, and I know that you've been involved in this, so I think that it's really important for people to understand that investigation of drug-induced immune hemolytic anemia is time-consuming, it expends a lot of resources. And so I know that Dr. Garratty and Dr. Petz, I believe you've told me before, at one point anyway, set up some criteria for what are questions that should be asked before you decide to go into working up one of these drug-dependent antibody mechanisms of drug-induced immune hemolytic anemia. So, would you mind taking us through those?

**Pat:** Sure! Happy to! When we get a phone call asking about a drug workup, we like to make sure that we are using our resources wisely. So, **we want to make sure that the patient is hemolyzing**, so we ask for indicators of hemolysis. Obviously, the hemoglobin, is it dropping? Are the retics [Note: Reticulocytes] going up? Is the indirect bilirubin going up? Is the haptoglobin going down? The LDH going up? All these things that usually point towards hemolytic anemia. So, we also, if we are looking for intravascular hemolysis, we also want to ask if there is any hemoglobinuria. If there's hemoglobinuria, there should be hemoglobin present in the plasma; it has to be present in the plasma before it can appear in the urine. And hemoglobinuria is different than hematuria! So if there's red cells in the urine that are giving it a red color, that's not the same as hemoglobin in the urine, so we try to differentiate those. That's kind of important.

**Joe:** A classic standardized exam trick question!

**Pat:** Yes!

**Joe:** Go ahead, Pat, I'm sorry.

**Pat:** So, once we realize that the patient is having acquired immune hemolytic anemia, **we ask about the direct antiglobulin test.** Because for a drug antibody to be causing hemolysis, there really needs to be IgG or complement or both on the red cells. We really like to get a sample at around the time of the hemolytic anemia. If we get a sample six months later, obviously, the antibody could have gone away, the DAT's not going to be positive anymore, you're not sure what you're going to get that point. So at the time of the hemolytic anemia, the DAT really should be positive. We like to know, **did they make an eluate?** With a drug antibody, you would predict that the eluate, if it wasn't tested in the presence of the drug, which it wouldn't be at the hospital, would be negative. But there are other reasons for patients having a positive DAT with a negative eluate: Maybe just hypergammaglobulinemia, or maybe they are a group A and they had a group O platelet transfusion, with anti-A in it. If you don't test A and B cells when you test your eluate, you wouldn't know. So there are other things to think about besides drugs. Surprisingly, what we're finding sometimes, though, even though we would expect a negative eluate from a patient with a drug antibody, sometimes the eluate is positive, and that really surprised us, but it seems to be when the patient is still taking the drug, and the drug is still in their circulation that sometimes we are finding a positive eluate without having drug in our test system.

**Joe:** Just to quickly interject, if anyone is sitting there saying, "eluate, eluate...I know that word..." can you give me the thumbnail? I know the vast majority of people know this, but if you don't mind, the thumbnail on what an eluate is?

**Pat:** OK, an eluate is...you take the red cells and you remove whatever is from the red cells as far as antibody. So you wash the red cells really well to make sure that you're not going to get any plasma contamination, and then you treat them with heat or a chemical, or some means of separating the antibody off the red cell, and you have your antibody in a solution. And that's your eluate.

**Joe:** Got it. Thank you for that. I did want to ask you, and I know that there aren't super hard and fast rules about this, but when you're evaluating the DAT, and again, we are generally working up the drug-dependent antibodies, obviously primarily those two subtypes, where the drug binds tightly to the red cell and the other one, where it's in solution with the drug, the red cell, and the serum. In terms of distinguishing those two, are there DAT patterns that can help you with distinguishing those two, or is it nonspecific?

**Pat:** Ceftriaxone very clearly always has complement on their cells, patients with ceftriaxone antibodies always have complement on their cells. Patients with cefotetan pretty much always have IgG on their cells; I mean they could have complement as well, and the ceftriaxones could have IgG as well. But other than that, I don't see any patterns.

**Joe:** OK, that was actually asked out of pure ignorance, I was not sure, so that's helpful, thank you! No trick question there, I promise, Pat!

**Pat:** Good!

**Joe:** OK, so we've talked about the first two questions that your lab asks, and really, beyond that, just to be clear with everyone, I think that *hospital labs* should be asking the questions before they're calling you guys, in terms of whether or not to work these up. So, we talked about: Does the patient have an acquired hemolytic anemia? Second, is the direct antiglobulin test positive, and in what pattern? And then, what's number three?

**Pat:** Number three is we want to know what drugs the patient is taking, but not only *now* at the time of the hemolysis, but also maybe *ten days ago* in surgery? There's many examples where the patient's taking a drug now that sort of the red herring. The true culprit was cefotetan that they got in surgery a week and a half ago, and they only got one dose of that, and it's hidden away in the anesthesiologist notes! So, sometimes you really have to do a lot of detective work to find out which drug might be the cause of the hemolytic anemia and the positive DAT. We do have a list of drugs; the most recent one was in "Immunohematology" 2014, if you have a chance to look at that.

**Joe:** And I will actually have that reference for everyone on the show page. I have that article, and it's a little exhausting, it's a big list, but it's incredibly useful in terms of day to day stuff. And I will admit, Pat, I have been guilty, as a hospital blood bank medical director (I've worn that hat in addition to my blood center medical director hat that I wear now), I've been guilty of having one of my techs bring me a long list of drugs, and just kind of glancing at them and going, "hmmm, hmmm, hmmm. Yeah, whatever!" It really does require a little bit of critical thought, doesn't it?

**Pat:** Yes, it does, but that leads me to the fourth question, **what is the temporal relationship?** Sometimes when we dig into this history, we find out the patient was hemolyzing before they got the drug that they are wanting us to test, which really doesn't make any sense at all! So you have to kind of look at the whole picture. The perfect picture would be: The patient's not hemolyzing, they start the drug, they start hemolyzing, they take them off the drug, they stop hemolyzing. That would be the perfect scenario, and we try to find what pattern fits them the best. We've had patients that had five drugs that they were taking and they were all on that list of possible drugs, so you have to start looking at the temporal relationship. But even then you can be fooled! Another reference that you might want to put on your web site is one by Dr. Petz about cimetidine and the fallacy of clinical association. There were two patients that were on this drug called "cimetidine." They started hemolyzing, they had a positive DAT, they took them off, they got better. At that time, they were able to put them back on a year or so later, and no problems at all, so obviously that wasn't it, but the temporal relationship looked so good, so sometimes even a temporal relationship can lead you down the wrong path.

**Joe:** Just when you think you have it figured out, and maybe it's just coincidence!

**Pat:** Right.

**Joe:** Well, we've walked through a whole bunch of things! Wow, the time has gone quickly! We've walked through some of the history of the different types of hemolytic anemias, we've talked about the different categories, specifically of drug-induced hemolytic anemia, including:

1. Drug-dependent antibodies
2. Drug-independent antibodies
3. Nonimmunologic protein adsorption

We've talked through how to do a lab workup, the questions you should be asking prior to doing a lab workup, and categorizing those drug-dependent antibodies specifically, as well as talking about the lack of great usefulness of lab workups for drug-independent antibodies and nonimmunologic protein adsorption. So, Pat, have I missed anything? Is there anything else that you would like for students and people learning about this to really be aware of in terms of this...it's your favorite topic! I'm sure you must have something! What else should people know? What have we not talked about?

**Pat:** Well, it's interesting because the estimate is that it's about one in a million, that it's a rare event, and of course it does depend on the denominator as we talked about. But I still think that some of these cases are getting overlooked, so I think people should just kind of keep it in the back of their minds, you know, if their patient is hemolyzing and they have a positive DAT, and the eluate's negative, and they're on piperacillin. You might want to start looking at, "when did they get the piperacillin?" and "when did they start hemolyzing?", and think about it. Interesting, some antibodies like anti-piperacillin can look like they have specificity, like little e specificity.

**Joe:** Whoa, now you are blowing my mind! Really?

**Pat:** yeah really! The patient looks like they have an auto-anti-e (depends on if they are e-positive, which most people are), looks like they have an auto-anti-e, or maybe an allo-anti-e if they are e-negative. And it's really the drug antibody. And that goes back to that picture, that unified hypothesis, where maybe that drug and the membrane are both the antigen and you need the e antigen there for part of that to happen.

**Joe:** Now, I admit that you just blew my mind with that, because that was one I was not aware of, and I'm thinking back to the times in the not-too-distant past when patients with what appears to be auto-anti-e have been sent for Rh genotyping to see which partial e they have, when perhaps a drug history might have been a little bit of a better use of everyone's time!

**Pat:** Or maybe both. I mean, it's easy to get a drug history, and there certainly are plenty of those partial e's out there, too, so you kind of have to put the whole picture together.

**Joe:** OK, that's really good, is there anything else that you'd like to leave us with on this?



**Pat:** I think it's important, first of all if you suspect a drug antibody, you should get serological studies to prove it, and if serological studies show that a patient has an antibody to a drug, that patient should be warned to not receive that drug again. Because it could be disastrous if they do. It's interesting that we've had people ask us about cross-reactivity of different cephalosporins, and we've done a little bit of in-vitro work, but we really don't know how that relates to in-vivo, so there's more to be learned out there!

**Joe:** That is scary, and obviously, people carry around drug alerts and allergy alerts and the like, and this is one that they definitely should be aware of, right?

**Pat:** Yes, definitely.

**Joe:** For sure. I think, Pat, you have really helped us kind of work through the categorization of these things, and work through really an intelligent approach, which involves some detective work, for sure, but I think you've given us some good guidelines and some good framework to hang our hats on. And I'm going to go back and look at those auto-anti-e's, by the way!

**Pat:** Great! Thank you.

**Joe:** Pat, thank you so very much for taking the time to be with us here on the Blood Bank Guy Essentials Podcast! I really, really appreciate it!

**Pat:** Thank you for inviting me! I appreciate it too.