BBGuy Essentials 064CE:
Granulocyte Transfusion with Ron Strauss
Released February 27, 2019

Ron: Hi, I'm Dr. Ron Strauss, and this is the Blood Bank Guy Essentials Podcast.

Joe: Hi, everyone! Welcome to Blood Bank Guy Essentials, the podcast designed to help you learn the essentials of Transfusion Medicine. This is Episode 064CE and my name is Joe Chaffin.

Today, I have an interview with someone who is legendary! His name is Dr. Ron Strauss, and Ron probably knows more about granulocyte concentrate and neutrophil function than just about anyone on the planet, and he and I are going to have a conversation about granulocyte concentrate, which I consider to be a really very widely misunderstood blood product. But before we get to that, since this is a continuing education episode, here is an important announcement:

The free continuing education credit is provided by TransfusionNews.com. Transfusion News is brought to you by Bio-Rad, which has no editorial input into the podcast. This podcast offers a continuing education activity where you can earn several different types of credit, including one *AMA PRA Category 1 Credit™*, one contact hour of ASCLS P.A.C.E ® program credit, or one American Board of Pathology Self-Assessment Module for Continuing Certification (which used to be called "Maintenance of Certification," or "MOC"). To receive credit for this activity, to review the accreditation information and related disclosures, please visit www.wileyhealthlearning.com/transfusionnews.

So, granulocytes have been available for transfusion for really decades, and they go all the way back to the time in the 1960s when what we now know as apheresis technology (which we use to collect platelets all the time) was used not only to collect platelets but also to collect granulocytes. And, in fact, in those early days, as Dr. Strauss will discuss later on, we collected granulocytes from donors with chronic myelogenous leukemia, which is pretty amazing to me. But unlike platelets, granulocytes have really kind of waxed and waned in popularity. They've come in and out of favor. Some say they work really well to treat infections in patients who don't have enough of their own granulocytes, some say they don't really help all that much. And the literature has really been mixed like that as well.

But my guest today, Dr. Ron Strauss, has been around through all of those debates, and, in fact, Ron's going to tell us a story that you'll love. He actually helped operate one of those very early apheresis machines, and wait till you hear how long it took to set those things up and tear them down! It's pretty crazy. Ron is a pediatrician with board certification in

Pediatric Hematology/Oncology, as well as Blood Banking/Transfusion Medicine. He spent decades doing amazing things at the University of Iowa and I did this interview with Ron actually just a few days before his official retirement last month in January 2019 from LifeSource (which is now Vitalant Illinois) in Chicago.

Ron has, I kid you not, more honors, awards, and publications than you could possibly believe, and I'm serious about that. The man has published over 300 peer-reviewed papers and over 120 book chapters. He is incredibly prolific over the years. But despite all that, if you meet Ron, as I've had the opportunity to do, you would just say that he is a kind and generous man who really just cares about people learning and patients getting taken care of in the best way possible. I am so grateful that he joined me for today's episode of the Blood Bank Guy Essentials Podcast.

So let's get right to it: Here's my interview with Dr. Ron Strauss on granulocyte transfusion.

****

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

Ron: Thank you. Glad to be here.

Joe: Ron, I think with granulocyte transfusions, it's really, really important for us, before we get into the specific details about the modern ways that we collect and transfuse granulocytes, I think for a lot of people that might be listening, whether they are clinicians or pathologists, or people that work in blood banks, the thing that I think we have to establish a little bit is the "why," is the background, is the where all this came from. And I think, again, there are very few people that could describe this better than you, and you've written extensively about this. I wonder if you would just start by taking us through a little bit of the history of how we got to the point of transfusing granulocytes.

Ron: Well, I think the best way to think of it and that will be most familiar to many people is to remember that this whole idea or concept is very closely entwined with the treatment of acute leukemia. Back in the early days, the 50s and 60s and all of that when leukemia first started being treated by chemotherapy, it became obvious within a short period of time that there were two tremendous blood banking problems that occurred, thrombocytopenia with bleeding and neutropenia with infection. And as chemotherapy became more intense and the benefits, of course, were more prolonged remissions and actually cure at the present time in many patients with acute leukemia, these problems of bleeding and infection persisted.

Development of platelet pheresis technology and the ability to give whole platelets from whole blood units, but mainly the apheresis technology,

allowed platelets to be collected in sufficient numbers to really be lifesaving and permit intense chemotherapy. And so it was logical for people to think, "Gee, if we could get some granulocytes, we would be golden." Well, it turned out not to be so easy at all. One of the interesting things that is always kind of fun to think back on is Emil Freirich at the National Cancer Institute and George Judson, who was an engineer at IBM, and the tradition is that Mr. Judson (perhaps "Dr. Judson," I don't know his full education) had a child with leukemia and so he had a vested interest, and the two of them worked together to develop a centrifugation type of leukapheresis machine to collect leukocytes.

They initially used patients with chronic myelogenous leukemia, who of course had very high numbers of neutrophils, to try to collect these cells. And back when I was a fellow at the Children's Hospital Research Foundation in Cincinnati, which is across the street from the Hoxworth Blood Center, we had one of these early machines, and it's kind of interesting to think about it because we actually collected some granulocytes and gave to patients. But think about the modern machines, now go back and compare this. This was a giant console like a huge TV set. It had 64 feet of plastic tubing, 50 nylon friction fittings, 10 three-way stopcocks, 11 intravenous solutions with all their sets and bottles all hung up, and it took hours to set up, to do the procedure, and to tear it down. And of course everything for the most part was reusable at that time, so it required a lot of sterilization and all of that. And the reason I'm mentioning this is that the success with collecting granulocytes at that time was not very good, despite using CML patients.

The numbers were small, but when you think about the way in which they collected it, it gives a lot of reason for why you must do certain things in a proper way now in order to get a satisfactory product. They ran a lot of blood through this machine, they anticoagulated the blood with ACD. The red cells and white cells, I think as most people know, have a similar density, so a centrifugal field sort of collects together, and it's very hard to separate them out without use of a red cell sedimenting agent, which we now have. And there was no donor stimulation at that time, and so the number of cells going into the machine from the donor blood was relatively small, resulted in a very inefficient collection. But they were hopeful that this would be improved in the future, and as we talk a little bit later about, "if you want to collect granulocytes, how should I go about doing that properly?," I can come back to some of these points.

Joe: You bet, absolutely. I'm wondering, Ron, in those early days, and I know that we kind of have an idea of this now, but back then was there kind of a risk tipping point for white cell counts that was recognized, and if so, what were the things that those patients were at risk for? What kind of infections were those patients most at risk for?

Ron: Sure, well that's a very logical and good question. Neutropenia, severe neutropenia, is always classically described as being less than 500 cells/mcL. But in actual fact, most of these leukemia patients during induction therapy/consolidation therapy have literally NO neutrophils that you can find in the blood. And when this is prolonged, and by that I mean over several days or a couple of weeks, then infections with bacteria are the primary ones we worry about, but also yeast and fungal infections start to appear. And part of the reason for that is not only the prolonged neutropenia but I think everyone realizes that the chemotherapy agents and, if a patient is a transplant patient, the conditioning therapy, the emergence of graft versus host disease, all of these things are very immunosuppressive and so there are abnormalities of cell-mediated immunity and all that that come into play, and [the] neutrophil is just one small part of the body defense as it tries to protect you and control infection.

So by general rule, people will consider a person "eligible" for granulocyte transfusions if they have a neutrophil count less than 500 and if they have a strong clinical evidence or (even more desirable) microbial proof of a bacterial or a fungal infection, in order to be eligible. Now the other factors that come into play, the risk is increased by the presence of any kind of portal of entry like a mucosal lesion, presence of an intravenous catheter, breakdown of the skin, and any other immunosuppression that might be going on, like the chemotherapy, presence of graft versus host disease, and the like. You can lower the risk of infection, and this is kind of important in modern day, by effective antimicrobial agents, either therapeutic or given prophylactically; by the use of peripheral blood progenitor cell infusions as opposed to marrow, because the period of neutropenia is shortened, engraftment is much quicker with that sort of a combination; and also with myeloid growth factors like G-CSF and the like. So infections that were very severe and frequent in the past many times are less frequent now because of these supportive care things.

And that leads to the quandary in many clinicians' eyes as to whether granulocytes are really helpful, or are they not?

Joe: And that's a huge thing to discuss, and I think, Ron, in some of the papers that you've written, you've taken a really extensive look at a whole bunch of early studies that were done and then some additional studies that were done after that set of early studies. I wonder if, we don't have time obviously to even come close to go through all of them, but I know that you've described 30-some early papers that had kind of mixed results and then seven more controlled studies in the 70s and 80s. I wonder if you'd just kind of summarize what we were able to glean from those before we get to the more modern looks at granulocyte transfusion.

Ron: Sure. The early studies were very interesting because they teach us some important lessons. And, as you mentioned, there were seven randomized

controlled trials that looked at the use of therapeutic granulocyte transfusion. There were quite a few, just off the cuff, that looked at prophylactic transfusions also to try to prevent infection. But we won't talk about those today, they'll spend too much time. But among the seven controlled trials, three of them actually showed benefit for granulocytes, which was kind of surprising because the doses were pretty small, donors were stimulated either not at all or with corticosteroids only, and in some studies some patients seemed to do better than others, for example bacterial infections are usually more treatable by granulocyte transfusions than fungal infections. But when you come right down to it, even in those early days there was some statistically proven and sort of clinically significant improvements with granulocytes.

I should mention one other factor and that is, studies that showed success, in general the granulocyte doses were higher than those that did not show success. And so people sort of lamented that, "W"e really can't give many granulocytes. When you think about granulocyte production and myelopoiesis in normal marrow in response to infection, under optimal conditions the most granulocytes, neutrophils that could be transfused would be only about 5% of what the marrow could make! So you can kind of see it was kind of wishful thinking that they would do very much. And so that led us, then, to forget about granulocytes. Some people were enthusiastic and still gave them, other people felt, "Hey, they're not worth it, we'll just go with their antibiotics," because improvements in antibiotic therapy, as you might imagine, really became marked, and it was really quite a wonderful pharmacologic victory, I guess, over infections.

But bacteria, being as they are, learned how to get around these antimicrobial agents. So infections still are a problem. But in the modern era, when G-CSF came about, granulocyte-colony stimulating factor, it was evident that neutrophil counts in donors could be greatly increased by stimulation, and commensurate with this increase in blood neutrophil counts, in these modern efficient cell separators, blood separators, could collect very large numbers of neutrophils, and the hope was raised, "Hey, maybe we can give enough now to make a difference."

Joe: I think we're at a really prime point to talk about the two main "modern" trials of granulocyte transfusion that have been done, both of which used at least granulocyte-colony stimulating factor to stimulate donors. So one was reported in 2008 in "Bone Marrow Transplant," and the other was reported (and you were a part of this one) the famous "RING Study" in "Blood" in 2015. So can you take us through those two papers, just starting with the one from 2008?

Ron: This was a European study, a multi-center study, and in my view just fell short in many ways, and it reflects not entirely the fault of the authors of this paper or the investigators, but sort of the way granulocyte transfusion trials go. And that is that the study was designed pretty nicely, the main

drawback being that they were pretty cavalier, I guess, or lax, in the way they were going to give granulocytes. Instead of the usual way we think they would be most effective, which is high-dose every day, they gave sort of moderate dose. They intended to give high-dose but it didn't really work out that way. And they only gave them every other day and if there were weekends, they did not give them. So it was sort of haphazard. As I know that I often mention, if you think about a patient who is bleeding and is thrombocytopenic and you wanted to give them platelet transfusions, if we gave platelet transfusions at low dose or variable dose and only gave them every once in a while, then of course we would think, "Gee, platelet transfusions don't really work."

Well, obviously they do work if they're given properly, and the same sort of thing falls back for neutrophils. So as a consequence, this study, which didn't really show an effect with neutrophils the way they were given, or granulocytes the way they were given, it's really not surprising. That study I just sort of put back as kind of interesting in a way to NOT do things if you're going to try to prove a point.

Joe: Okay. So no benefit was shown in that study to the granulocyte transfusions, is that correct?

Ron: That's correct, but it's not surprising that it didn't work.

Joe: What about the RING Study, Ron?

Ron: The RING Study...RING comes about from real words. The title was "Resolving Infection in Neutropenia with Granulocytes. And it was a multi-center trial funded by the National Heart, Lung, and Blood Institute, which involved multiple centers throughout the United States, and it was actually a very well-designed study, using granulocytes sort of the way that we would like to do that. The patients were eligible if they had severe neutropenia due to marrow failure, if they had a proven or a probable infection with known bacteria or a fungus or yeast, they were randomly allocated to either get standard antimicrobial therapy, and there were guidelines provided to the centers as to what optimal antibiotic random microbial therapy might be, realizing you can never get two Infectious Disease people to agree on the same thing, but there were guidelines provided. So they either got those or they got that therapy plus daily granulocytes from donors that were stimulated with G-CSF and dexamethasone.

The goal was to provide a minimum of 4 x 10^{10} granulocytes for transfusion, in hopes that the higher dose of 6-8 x 10^{10} could be given. And the transfusions were given until either the neutropenia got better because of marrow recovery, or until there was response to infection, or unless some serious adverse effect happened, which really didn't happen. And so, I should mention one other thing, that the endpoint was a

combined sort of thing where the patients had to survive to 40 days and then there was a judgment made as to whether they improved, whether their infection got better. And the other very important point is that this was a study that was, then the data were reviewed by an adjudication committee, experts in infectious disease and granulocytes and leukemia and marrow transplant who were not part of the study group at all but were sent the data, and then they could decide whether the patients were eligible, whether they really were treated correctly, whether data were accumulated and analyzed correctly, and blah, blah, blah, in order to give a third-party objective view.

Joe: So you've mentioned kind of how RING was set up, what you were trying to do with it, and the doses that you were looking for of granulocytes, so I think it's fair to say that when many people look at RING...let me put it this way, if you only read the first page of the RING study, if you only were one of those people that you look at the abstract and walk on, you might be tempted to say, "Not really a successful study, and what did we learn from that?" So I know you well enough to know that you don't necessarily feel that way, so can you tell about what the results were and what you've been able to learn since about those results?

Ron: Sure. Let me tell you about the results first and then come back with little caveats as to how one might think about these and analyze these results. The study was set up like all wonderful randomized trials to be analyzed by "intention to treat," which means that everybody who fit the criteria and were randomly allocated to one arm or the other, their outcome was included in the final analysis. So by intention to treat, it means you include everybody. So if somebody was involved and for some reason they never got a granulocyte transfusion at all, they still were analyzed in the granulocyte transfusion arm. And by the same token, if somebody in the antibiotic arm only got some granulocyte transfusions, nonetheless their results were included in the antibiotic arm only. So intention to treat, the statisticians all love that because it's sort of the "clean" way to try to look at things, and when it's done in that way, the successful outcome in the control arm was 43% as opposed to 42% in the granulocyte arm, so it was identical, it didn't really work.

Now the other way to analyze it, and clinicians, like all of most of us, want to say look, if the patient was treated the way they were supposed to be, they either got granulocyte and antimicrobial agents or they only got antimicrobial agents, you know antibiotics only, how did it turn out then? And in that instance, it still was statistically no different, but there was a little hint that the granulocyte people might have done better, if you really wanted to press a point, 41% of people who got antibiotics were improved versus 49% if they got granulocytes and antibiotics. Not statistically important but a little tempting to you to kind of overextend the data if you wanted to do that.

But let me come to the second point that I wanted to mention, that there were problems with this trial. One of the key problems was that the number of patients that were supposed to be allocated could never be reached in the period of time allotted for funding by the National Heart, Lung, and Blood Institute. Not their fault, it just was a very slow accrual of patients in entry, which is very typical for a lot of randomized controlled trials but especially for granulocytes. So there were still nearly 100 patients to be analyzed in a very large trial, but only about, if I remember correctly, about a third of the number that we really wanted to get in.

And then secondly, and this is, I think, a very important point we can talk about, when all of the patients were looked at and graphed out and all of that stuff, it appeared that there were actually two populations of people who got granulocytes. There were those that got the dose everybody wanted to get and everybody thought was being given, and another group who got very small numbers of granulocytes for reasons that we never really could figure out, for a whole bunch of reasons we don't have to go into now, but they just didn't get "high dose." And **when one looked at a comparison of people who got granulocytes the way they were supposed to get, high dose, as opposed to those that didn't, there was a pretty striking and statistically significant benefit in that success was seen in 59% of patients that got high dose as opposed to only 14% of low dose**.

Now this is a sort of after the fact analysis and all that kind of stuff, which means you have to take it with a grain of salt, but it was very suggestive that if you give granulocytes the right way, patients very well may benefit.

Joe: Let me summarize what I'm hearing, and if you tell me if I'm wrong. If you just look at the data, you would have to fairly say that there is no clearly proven benefit, but there is a pretty strong suggestion that if an appropriate amount of granulocytes are given in an appropriate interval, as in daily is what I think I'm hearing you saying, that there's at least a very strong suggestion that there could be significant benefit. Is that a fair way to put it, Ron?

Ron: Yes, that's very fair. All of us who are scientists, I guess, and want strong evidence-based medicine would then balk and say, "The only way to really answer is to redo a clinical trial where patients really do get the proper amount of granulocytes," but this is just never going to be done, in my view. And the reasons for that are what I wanted to come back to anyway and that why is there such a problem with doing these granulocyte trials. And one of them is, these are products that many blood centers are just not used to making. So in order to have a quality granulocyte or neutrophil concentrate becomes problematic, and we can come back to that in a minute, as to the best way to be able to do that.

But the other is that clinicians are all well intended and they want to do what's best for their patients. And at the time you're planning these trials, you get the oncologists and transplant physicians, to say, "Yes, equipoise is present." And equipoise means that we really don't know whether granulocytes are helpful or whether they're not, you can make equal arguments on both sides of the coin. And therefore, you need to study this. But when push comes to shove and patients are actually going to be enrolled, then their prejudice comes bouncing out again because they want to do what's best for their patients.

And even though they might profess to think the granulocytes are at equipoise, granulocyte therapy, in the back of their minds think, "If I have a patient who is really sick and of course I care a lot about him and by then I have a personal relationship with the patient and the family and on and on and on, I may not want to enroll patients that I think are really sick because I don't want them to miss out on the chance of maybe getting granulocytes." Accrual and entry becomes bogged down and you end up never getting the patients that you really want, or you get them skewed in some way because only certain patients get enrolled and others don't. And of course you can't force people to put their patients in the granulocytes. And all clinical trials are set up so that the patient can elect to drop out whenever they want. And this becomes a tremendous problem in centers like most of us are in, where you can get a granulocyte if you order it, granulocyte concentrate, so that whether you're in the study or not, you can get them.

It would be much better if the transfusion committee at the hospital would say, "You know, equipoise is really present, we don't know if these things work or not, and therefore the only way you can get granulocytes is if you're in the trial." Well, that's a very big step for many people and so there just is not going to be another granulocyte transfusion trial in my lifetime. And so, I think one has to apply the same kind of principles they do to any clinical problem where there is not definitive information. You have to review the data that's available and decide what you're doing at your center and whether you want to consider granulocytes or not. And if you want to consider them, then they have to be given in the correct way.

Joe: Well, and that is a perfect segue into the rest of the time that I want to spend with you, Ron, talking about what is, in your view, the optimal way to do this. So let's start from the beginning with the basic raw material. In 2019 when we're recording this, Ron, who is the optimal donor of granulocytes? When donor centers are looking for someone, they get a request to provide granulocytes, who do they look to, what donor pool do they look to to try and find a granulocyte donor?

Ron: Well, the ideal granulocyte donor, in my view, is someone who is an experienced apheresis donor. And so that means in your stable of platelet donors, plateletpheresis donors, it's where usually you can recruit the

most experienced and committed donors. Now some people prefer to use family members or they use regular donors that come in for the first time and agree to do apheresis. That, I think, is kind of fraught with problems and it's much better to, if you decide you want to set up a granulocyte transfusion program at your center, is to go to your platelet donors and inform them what's involved and say, do you want to be involved in this or not?

And so you give them a full picture of what it's like to get G-CSF, which usually means an injection 12 hours before the procedure is going to be given, sometimes that's problematic, who's going to give that shot, and the blood center might be closed. So some hospitals, they go to the marrow transplant unit, the oncology floor, and the nurses there give it. Some people work out with the local Walgreen's or whatever the other pharmacies are, I don't mean to be commercial, to give that. And then they also take a dose of dexamethasone orally.

The risks to those medications are really minimal. There is some sort of nuisance muscle soreness and joint soreness from G-CSF, and if you're a patient with sickle-cell anemia, you don't want to take G-CSF (or you're not likely to be a granulocyte donor anyway if that's the problem". So there are these nuisance problems, but in surveys that have actually been done, donors would say "I would still give granulocytes, that doesn't bother me. It's short term, I take an ibuprofen and I'm better."

Joe: So, Ron, one more thing on donors before we get into the stimulation. One of the things that I've heard people talk about is not only getting apheresis donors, but getting apheresis who have donated recently, as in the last 30 days. What's the importance of that? Why is it important for them to have donated recently?

Ron: Well, granulocytes have a short shelf life. When they're collected, you should transfuse them as soon as you can, certainly ideally within six or eight hours or so. They have a permitted shelf life of 24 hours, that's more for sterility and the like of that, but they begin to deteriorate in terms of their function, and so you like to do them as soon as you can. And depending on the speed with which you can turn around infectious disease testing for the normal donor, you may or may not be able to get that all done within a few hours, usually not. And so the idea is, if you have experienced donors who've been donating regularly and have negative infectious disease tests, you can draw your infectious disease testing, send it off to be done, but release the product ahead of time, with consent, of course, and transfuse it, and then the results of infectious disease testing come back later.

Joe: Let's talk about stimulation, and you've already mentioned the two main options, the use of corticosteroids, whether that's dexamethasone, which is, I think what you prefer, or prednisone, and then granulocyte-colony

stimulating factor, or G-CSF. One of the things you've provided, Ron, which I think is an excellent graph, showing the relative effect of these. And I wonder if you'd just talk to us about it? Is this an either/or situation? Is it better if it's additive, if they're done together, or how do you view that?

Ron: Well, if you look at this one image you were talking about, back in the olden days when there was no donor stimulation at all but you had a good blood separator and could process donor blood in the presence of hydroxyethyl starch and all the other good things I'll talk about in a minute to make collection optimal, with no stimulation you usually would get about 4-6 x 10^9 granulocytes per unit. Really pretty much what you get in a unit of fresh whole blood. And so the question is, is it really worthwhile doing that or not? And the quick answer is "No!", and I'll come back in more detail in a second.

If you use corticosteroids only, and there are a variety of ways of giving them, much of the time it's using prednisone 20 mg the night before, like at suppertime, 20 mg when you go to bed, 20 mg when you get up in the morning, with the idea that the collection will begin three to four hours after that final dose, then you can get 1-3 x 10^{10} granulocytes per unit, usually around 2, but it's quite variable. Sometimes it will be as though you never gave the steroid at all, or other times you'll get a nice higher dose of 3-4 x 10^{10}, so pretty inconsistent but nonetheless, as I mentioned before in the historical trials, it showed efficacy even in that large sort of yield.

With G-CSF given in an ideal way, which is one of the vials, usually they're 480 mcg of G-CSF subcutaneously, and then taking oral dexamethasone, which will increase the yield by about 25%, given the night before about 12 hours, you can get 5-8 x 10^{10}, aiming for the middle ground of 6 x 10^{10}, which is a very nice dose. You can pretty consistently get that, but occasionally like before with the steroids, it'll be like you didn't do anything at all. But that's sort of the dosage you're hoping on. And if you look a the one image that you have, you can see that the peak is usually around 12 hours after you give the G-CSF, ranging from 20-40,000, and then that peak will last for a few hours so that when you're collecting, most people will try to say, "Let's time the leukapheresis between 8 and 16 hours after giving the G-CSF."

So anyway, that's the story with the collections. Now the reason I'm mentioning that, maybe if you're not going to stimulate correctly, you shouldn't do it at all, is not only for efficacy of the yield, trying to help the patient, but we have a responsibility to the donors. We're going to put them through a fairly vigorous, it's not that big of a deal, but nonetheless it's a commitment of time and effort to give granulocytes, and if we're giving a product that we don't think is really going to be efficacious or help the patient, then I don't think we should be subjecting donors to that kind of a procedure.

Joe: I agree. So Ron, I wonder if we could put this in context a little bit? You had mentioned that with no stimulation, generally you can expect in the range of 4 x 10^9, which is, if I remember my numbers right, that's like four billion granulocytes or so, if you do corticosteroids only 1-3 x 10^{10}, or 10-30 billion, or with G-CSF and steroid it would be 5-8 x 10^{10}, so helps us understand, if you would, the context of that. What are the requirements, at least in the United States, from AABB for a unit to be considered an "acceptable" dose and how does that compare? You mentioned before the marrow's capacity. What is normal marrow capacity for daily granulocyte production?

Ron: Granulocyte units are still not licensed products by FDA and the like, so there's no sort of set in stone number that you have to have. On clinical experience, like I mentioned, people would like to give as a minimum 4 x 10^{10} to an adult patient. We're hoping for the 6-8 x 10^{10}. Studies in pediatric patients show that you can probably get efficacy from granulocytes if you can give 1 x 10^9 per Kg of body weight, so when you try to multiply that out for adults it becomes sometimes not always a nice linear relationship but those are the doses that one would like to give based on clinical experience within the context of what one can reasonably collect at the present time. So if you're setting up a program at a hospital, I think you say, “I want to always try to get 4 x 10^{10}, but if I can get more that would be great.”

So when you think about normal neutrophil production and how many cells are made per kilo, per day, and all of that in the marrow, even these modern granulocytes are giving maybe 10% of what the normal marrow might put out in a day, so it's very small. Under the stress of infection, you can increase the neutrophil production or output from the marrow close to 1 x 10^{10} in a 24-hour period. That's very generous, on the high side, some would quibble that it ought to be a little bit lower, but nonetheless even if it's a 7 or 8 or 9 x 10^{11} per day, you're still far ahead of the 6 or 8 x 10^{10}, if you're very lucky, that you can give with the granulocyte collection, so one might say you're still giving quite a bit below what you would love to have if you were a person trying to respond to a serious infection.

Joe: If I remember right, Ron, I think you're 100% right about there being no FDA licensing or requirements from FDA. I believe AABB says that 75% of the units you collect should have at least 1 x 10^{10}, so a really minimal dose in the grand scheme of things.

Ron: One comment about that, I think that 1 x 10^{10} in the standards for ABB is based on the fact that not everybody is willing to give G-CSF stimulation. Which I think is kind of shortsighted and not based on very much science. At the University of Iowa, where I spent a bunch of time, we've been using G-CSF for probably 20 years without a problem. Yet other centers, even today, are reluctant to use G-CSF and I think, just to make a very long story short, if one reviews the scientific literature on G-CSF and its

potential toxicity, and one interesting place to look at that is in progenitor cell collections, you know, where G-CSF is given daily for five days and then you collect your progenitor cells or so-called "peripheral blood stem cells," and look at the toxicity, almost everybody will have some aches and pains and all that sort of stuff with G-CSF, but it's all quite tolerable.

There have been long-term studies looking at these donors years later without problems. There were some theoretical problems related to what does G-CSF do to sort of the molecular genetics and all of that of stem cells and that in patients and finding that on long-term followup, nothing adverse happens. There are some short-term changes with chromosomal things but they're transient and they go away. So this concern about giving one dose of G-CSF to a granulocyte donor is just way overplayed in my view, and there are quite a lot of data that show that it's just not a problem.

Joe: One of the things that people will say is, "But hey, G-CSF isn't licensed, FDA-licensed, for donor stimulation," to which I say, "Well, physicians give patients medications that aren't licensed for indications all the time," and as long as you're appropriately consenting people, I'm right there with you, I don't see an issue. There are a couple things that I think are important to discuss, and the first is the use of the sedimenting agent that you mentioned earlier, hydroxyethyl starch, and the second is how long they need to stay on the machine or how much blood needs to be processed. Could you address those two things?

Ron: Yeah, sure, because they're very important points. One is you should have a continuous flow of blood separator, and almost all of these modern machines have been able to do that, and you have to use the program that the manufacturer recommends to collect granulocytes. You advise the donor in advance that they're probably going to be on there about three hours or so because most people as a minimum would like to process seven liters of donor blood through the machine, upper is probably ten liters, so somewhere in that range in order to get the full effect of trying to separate these cells out.

Secondly, during the infusion, the entire collection, there should be an infusion of hydroxyethyl starch. There are a couple of different preparations or formulations, so the one that's best used is the higher molecular weight, higher molar substitution substitution hetastarch as opposed to pentastarch, which is a lower molecular weight. Now the way that works, it promotes rouleaux formation among the red cells. In a centrifugal field, the red cells and white cells have a similar density, but if you can clump up the red cells then they are heavier, and so they separate better in the collection channels of the blood separator. And so it's very important to use that all the way through the procedure. Some people use one bottle and then stop. No, you have to use it all the way through.

Secondly, the starch is anticoagulated with a concentrated citrate. Remember back I was mentioning in the old days when Dr. Freirich and his colleague used that early machine, they used ACD-A, and that's not a very concentrated citrate solution, so it dilutes everything out. The starch gets diluted out and it doesn't work very well as a rouleaux-forming agent. So you must use this concentrated citrate in order to permit the hydroxyethyl starch to work optimally. And so you infuse that throughout the whole procedure, you collect as long as you can, and then you have your product available.

You must remember, that as you'll see when you collect those cells, the leukocytes, there is a lot of pink tinge and red cells in there, so the product has to be transfused with red cell compatibility ensured between the donor and the recipient, so you have to use the same blood. Now there are ways in which you can sediment out the bulk of the red cells. This occasionally is of practical importance because if you have a recipient who makes it impossible for you to find (or very difficult) red cell compatible donor, you can collect the granulocytes and then sediment the red cells out and still give the product safely. But that's another whole discussion, just to keep in mind that you can do that, so don't give up on finding a granulocyte donor if a patient really needs granulocytes.

Joe: So we've got that product. Before we give it to a patient, and you've already mentioned one thing, I want to just reemphasize it for a quick second, and this is something that you would probably take into account before you collected from this particular donor; if a recipient, for example, had one of the more common red cell alloantibodies, just let's say for example they had an anti-K or anti-D, what do we need to know about red cell compatibility for a unit of granulocytes before it's given to a patient?

Ron: Well, you would select a donor to not have those antigens, for the most part. Now, if you had a person that had a complex antibody, for example, some of the patients who get granulocytes have chronic granulomatous disease in childhood, which people who are blood bankers know that they can develop broadly reactive antibodies in the Kell blood system, and there are literally no compatible donors. And actually, I was involved in a marrow transplant for a patient with that who had antibodies and was successfully given granulocytes by just sedimenting the red cells out. There are some published techniques for enhanced sedimentation, but you can get rid of the vast majority of red calls and they can be given without risk.

Joe: Okay. So generally speaking, obviously avoid alloantibodies unless you've removed the vast, vast, vast majority of the red cells, they do need a cross-match just like a unit of red cells, correct?

Ron: Sure, exactly. That's exactly right. I should mention, maybe you're coming to this, once you get these cells collected, they often cannot be transfused

immediately, the patient is getting antibiotics or platelets or parenteral nutrition or something like that, and so they have to be stored, and the recommendations are to store them at room temperature, so you put them in a platelet storage unit, but you don't rotate them, just put them in there on the shelf.

And in my experience, and I don't know how many people will actually do this because it's not written down in Standards at all, you can let them sit around for hours. Let's say they're not going to be given for eight or ten hours, maybe twelve hours after they're collected, the neutrophils, the granulocytes, will tend to start to clump together and you'll start getting little strands of fibers, and I suppose is what it is, and other kinds of junk, and the cells just all clump up, and then when you transfuse them through a standard blood filter, of course they're going to be lost. So as a very practical point, which I don't think the FDA has written down anyplace and all that, but if you want to use common sense, about every two hours put the granulocyte unit which you have in your platelet rotator, on a rotating shelf and let it rotate for 15 or 20 minutes or some short period of time to sort of mix everything up again. And then take it off and put it on a non-rotating shelf in the platelet rotator or in a platelet storage unit.

Joe: I have to tell you, Ron, that's the first time I've heard anyone say that, and I've been around for a while, so that's a great tip. That's a great tip, really important. One last thing before I leave compatibility, Ron, when we transfuse granulocytes we are often transfusing them to patients that are transfusion-dependent on other products, for example, they may have platelet refractoriness, they may even be in scenarios where either they have developed either HLA antibodies or HNA antibodies (that would be obviously very difficult), but if you have someone in particular who is refractory to platelets and has established HLA antibody problems, I guess my question is, is it possible, or how difficult would it be, to find HLA-compatible granulocyte donors for these folks?

Ron: Well, the whole compatibility issue is complicated and there are no set in stone rules. The early papers, way back in the 60s and 70s, there actually was concerted effort to try to select donors that were leukocyte cross-match compatible or HLA-compatible (very difficult to find compatibility for granulocyte-specific antigens and antibodies), but there were efforts and the early papers suggested that was helpful. Now there also are a number of papers to show that if you have patients that are alloimmunized, generally they are picked up because they are refractory to platelets and then you do testing and you look and you find HLA antibodies or antibodies against neutrophil-specific antigens and if you transfuse cells, neutrophils, into these kind of patients and if you indium label the cells, the leukocytes, you're going to transfuse so you can track them and see where they go, there are a couple of very nicely done papers that show that in alloimmunized patients, depending on the kind of antibodies they have, these cells may go and get hung up in the lung, sometimes in the

liver or spleen, and they don't go to the areas where the infections are, as opposed to patients who don't have alloimmunization, where you can actually show the neutrophils going and because of the indium labeling you can image them and see that, "Hey, they're going right where we want them to go."

But despite those interesting papers, they're hard to interpret for clinical medicine because not all leukocyte antibodies act the same. Sometimes they don't interfere at all with the migration of the cells, other times they do depending on what type they are. The clinical experience has been...actually there is a paper as part of this RING study looking at patients that did develop leukocyte antibodies, showing that they just don't seem to have any clinically important endpoints, that the response to infection and all of that seems to be the same. And certainly there don't seem to be any transfusion reactions related to the presence or not of alloantibodies.

Joe: When I teach pathology residents, and often when I'm talking to clinicians about granulocyte transfusions, one of the things that tend to confuse them a little bit is when I mention that before this product is transfused, there is one other thing that we need to do it, which is to irradiate it, and they gasp and they say, "Well, why would we irradiate it? Won't that knock out the function of the neutrophils?" So can you talk to us about irradiation and granulocyte concentrate?

Ron: Yeah. Given the standard dose, neutrophils are really pretty resistant to radiation, so the usual dose given to our patients who are getting irradiated blood products will of course "do in" the lymphocytes and so diminish the risk of graft versus host disease but has little to no effect on the neutrophils, and so they can be given that way. And since many of these patients are getting irradiated products, they are especially mandatory for stem-cell transplant patients, then that's the thing to do.

Joe: Another thing that sometimes confuses learners is they are accustomed to discussions (though these discussions can be controversial) about the effect of leukocyte reduction on prevention of CMV transmission, and when I start frowning when they start talking about leukocyte-reducing granulocytes, they get a little confused. So can you talk to us about, I know this is an insulting question to ask someone as brilliant as you, but I want to make sure our learners know about leukocyte reduction and granulocytes.

Ron: No, obviously you can't leukocyte reduce, you don't want to remove what you want to transfuse.

Joe: That would be bad, I'm right there with you.

Ron: This is the one instance, actually the only instance in my mind, where you need to do CMV antibody testing. So you want to use a seronegative or antibody-negative donor when you collect granulocytes. When it comes to preventing CMV for other products, such as platelets and the like, then I think leukocyte reduction as done in the blood center, emphasize is done PROPERLY, is the optimal way to prevent CMV. There's no need for so-called "belt and suspenders" where you do select a donor who's antibody negative for CMV and then leukocyte reduce afterward, it's just not necessary in my view.

Joe: The patient is sitting there getting ready to receive this granulocyte product and the clinician calls you up and says, "Dr. Strauss, with your vast expertise, what should I be looking for in this patient? Is this patient at risk for any type of reaction from getting this product?" What would you tell them?

Ron: I would say, think of this as you would a platelet transfusion and try to transfuse it in a reasonably short period of time, probably not more than an hour, a little bit shorter, and just watch for the usual things. And actually there have been some studies comparing patient reactions following granulocytes versus platelets, and on average there's just no difference at all. Now, in the olden days, and people still have a great fear of pulmonary reactions, there were some patients that just had sort of "whiteout" of their lungs, they were tremendously serious reactions to granulocytes. And why this actually occurred was never worked out very well, but I think is maybe partially related to the technology by which granulocytes were collected in those days. This has turned out to be very rare and uncommon, but nonetheless something that someone can watch for, and if the patient becomes suddenly short of breath and all of that, then of course you would want to stop the granulocytes as soon as you could.

Ron: The other thing is that granulocytes are often given to patients that are extraordinarily ill and they're getting antibiotics and they're getting often parenteral nutrition and they're getting platelets and all kinds of stuff, and so I just think always being mindful of fluid overload is a very important thing to try to keep in mind when you give these cells.

Joe: Absolutely. One other question that has come up from time to time over the years, Ron, do we have any information either debunking or supporting the thought that oh, if you're giving granulocytes at the same time you're giving amphotericin B that leads to increased risk?

Ron: Yeah, I'm glad you brought that up. In the back of my mind, I knew there was another thing I should mention, and that is that amphotericin, these early papers that showed pulmonary problems showed that the cells were given along with amphotericin or shortly before or afterward, and in some nice in vitro studies you can show that amphotericin can interact with leukocytes and really cytokines and the like all of that. But in the body and

all, it just doesn't seem to make that much difference in a strictly scientific sense, but the common practice says to try to separate granulocyte infusions from amphotericin infusion by some arbitrary period of time. I think at our hospital, we try to separate them by six hours, other people say two, some say four, but to not infuse them at the same time.

Joe: Okay, Ron, so I have to ask one last thing before I let you go, because this is one of the things that people do ask me. My center infuses a few of these granulocytes. So let's just set up a scenario. Let's just imagine that we have a patient in one of our hospitals who's had chemotherapy for a cancer of some sort, he has a reasonable chance of recovery, but now he's got the bad combination that we've already talked about of a very low absolute neutrophil count, less than 500, and a fungal infection that just isn't responding to antifungals. So we go and we stimulate a donor appropriately, as we've talked about, we collect an apheresis granulocyte product that has a final count of let's say 6 x 10^{10}, and we give that product to this patient. Well the thing that people ask me, Ron, at this point, is now what? How do we measure whether this product is being effective? I mean, with red cells we check the hemoglobin to see if the patient has had a response. Do we do the same thing with post infusion ANC counts, and finally (I know it's a long question), but finally how do we know at what point we're done, whether this is working or not? How do you measure response, I guess is where I'm going?

Ron: Well, it's a little more complicated than with a red cell or a platelet transfusion where you can check a blood count afterward and part of the measure of success of the transfusion is the post transfusion increment, or what you get. With granulocyte or neutrophil transfusions, it's really more complicated than all of that. The endpoints of giving a course of granulocyte transfusions are almost always one of two things, either the infection responds and gets better or there's evidence of marrow recovery so that the patient has his or her own neutrophils. The other endpoint, which occasionally will come up, is if there's some toxicity from the granulocyte transfusions so you want to stop them. But most of the time it's a clinical endpoint, and the clinical endpoints are really not very well correlated at all with what the post transfusion increment or increase in leukocytes/neutrophils might be.

Now having said that, common practice for most of these patients is to get a blood count every day, partly because you're looking at what the platelet count is and hemoglobin and all of that, and so there will be always a total leukocyte count and sometimes there will be a differential, it kind of depends on how it's being done. When the counts are very low, the neutrophil counts, it's difficult to quantitate them, and so sometimes laboratories will not try to do a differential. But anyway, the common practice is to get a count and so what happens afterward to the leukocyte count? Well, in the olden days before these modern granulocyte transfusions, there was literally never any increment. Maybe you might

notice a couple hundred cells, a little blip or so, but most of the time it was so low you weren't sure if you were actually counting anything or not.

But with these modern ones, you actually very frequently get an increase in count between 1000 and 1500. And precisely why that happens is not totally clear, because when you think about normal neutrophils that are being made in the marrow and being released, they enter the circulation and they disappear in six hours or so, and they disappear in sort of a random fashion, it's not newer ones that are released get out quicker, they just sort of are released and then they disappear and they stay in the tissues a day or two to do their work. But these cells which are transfused, if you notice an increase, sometimes if you do a count the next day you can still see them floating around, so there's a whole different sort of kinetics going on that I don't understand very well, I'm not sure many people do, probably related to differences in the profile of those surface membranes that have to do with migration on membrane sites.

So anyway, the reason that I'm going through all this is that you can see an increase afterward and then let's just say after two or three days of neutrophils where you know the infection's not better yet, and so the question comes up, "Well, is the patient's marrow recovering? Can I stop these granulocytes or not?" And to me, that's more of a clinical answer than a laboratory one based on blood counts. I mean, once you have some idea as to what stage you are in your chemotherapy or in the recovery from a hematopoietic progenitor cell transplant and beyond the basis of previous marrows or just knowing what the course of neutropenia is following therapy that you're giving, and so if there's any question about it, one can do a marrow, which seems kind of drastic unless the patient is already scheduled to have one at that time, or you have to make a clinical judgment as to whether to keep going or not, and most of the time people will say, "If you get an increase in the count following a transfusion and you're wondering if marrow recovery is going on, let the count stay up for a day or two. Continue the granulocytes for another transfusion or two and see if there is a further increase in the count, up to 2000-3000 or so, and you might think there's marrow recovery and you maybe will do a marrow to confirm all of that."

Joe: Okay, so Ron, for those that are listening, I wonder if you'd give us one last practical tip? If you were looking at your practice in your particular hospital somewhere and you were trying to decide whether to incorporate granulocytes into the care of your neutropenic patients, how would you go about that?

Ron: For me, the message is, look at your neutropenic patients at your hospital. Are they having serious infections, primarily with bacteria, that are causing morbidity, mortality that's above some accepted standard at your hospital, and if they are, they need something else. Granulocytes are one of those things you should think about. Then if you're going to be giving them,

make sure that the product you have is optimally produced, giving you the dose you want. Otherwise the risks to the donor and the risks to the recipient are just not worth it if you're not going to give them good product.

And I should mention, just because I want to not forget this, is there is a paper in the January issue of "Transfusion" for this year from the UK and from the Netherlands that looked at leukemia patients in the presence of infection and found that still in modern day about a third of the patients had substantially or clinically significant infections with an 18% mortality rate. Mortality wasn't totally from infection, the endpoint was a little complicated, but nonetheless patients with infection did much worse than those patients who did not have an infection. And a second interesting point is that among all of the patients that were deemed "eligible" for granulocyte transfusions in the UK and in the Netherlands where these clinical centers were located, only about one quarter of them actually got granulocytes, about three quarters of them didn't for reasons that weren't clearly elucidated in the papers, but it points out there still is a potential need.

Joe: Well, Ron, this has been amazing for me. I have just enormous respect for you and your incredible accomplishments in our field and not only that, you're just such a nice man! It's just such an honor and a pleasure to talk to you! So thank you for everything you've done, not just for this podcast, but for your entire career of contributing to the health and safety of both blood donors and patients everywhere, so thank you so much, my friend.

Ron: Well, you're very welcome and thank you for the kind words. As I mentioned before, my mother would be happy and there's a lot to be said for good parenting.

****

Joe: Hi, it's Joe with just a couple of closing thoughts. I think really to me the take-home message is pretty straightforward, and I think it's this. If you're going to give granulocytes, the data really suggests that you should only do so if you're willing to go to the proper lengths to collect a high-dose product from a properly stimulated donor. In all transparency, that's what I do at my center in California, and I will admit that I actually stimulate donors with G-CSF only, and it's in large part because my staff does an amazing job at collecting granulocyte products with yields that average in the range of 6.0 x 10^{10} or more.

So, remember, you can go to www.wileyhealthlearning.com/transfusionnews and get your hour of totally free continuing education credit, and that's for both doctors and laboratorians. You can also go to the show page for this episode, which I really hope you do. The show page is at BBGuy.org/064. Ron has some images there that illustrate some of the things that we've talked about in this interview today, and you'll also find

the transcript for this episode and links to articles that Ron and others have done on granulocytes, including the link to the RING study article that we discussed so extensively in this episode.

The easiest way to make sure that you don't miss anything in terms of these podcasts is to subscribe, either on Apple podcasts or Google Play, or Spotify, or Stitcher Radio, or heck, you can even just "Ask Alexa" on Amazon devices, which is kind of fun. Anyway, while you're there, please take a moment and just give the podcast a rating and a review. It really does help more and more people see the podcasts, and more people learn, which is what I'm trying to do.

You're going to want to hear some of the episodes I have coming, I have no doubt about that. I have new interviews with blood bank superstars like Dr. Jed Gorlin, my man, Dr. Jed Gorlin, who's going to share his thoughts and experiences on caring for Jehovah's Witnesses patients and others like Witnesses who refuse transfusion. And coming soon is a wonderful interview with Dr. Jerry Sandler from Georgetown, who is going to help us understand anaphylactic transfusion reactions. I'm excited for you to hear all that.

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