DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

BLOOD PRODUCTS ADVISORY COMMITTEE

69TH MEETING

Friday, June 15, 2001

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C O N T E N T S

C O N T E N T S (Continued)

P R O C E E D I N G S

DR. SMALLWOOD: Welcome to the second day of the 69th meeting of the Blood Products Advisory Committee. I am Linda Smallwood, the Executive Secretary.

Yesterday, I read a conflict of interest statement that applies to today's session. If anyone is interested, that statement is available for your review.

At this time, if there are any additional declarations that need to be made by any of the participants, please do so at this time.

I would just like to remind you that according to the agenda, we have a packed agenda today, but in a much smaller time to take care of the business. We ask your participation and cooperation in trying to move this agenda along.

I have no additional announcements at this time, so I will turn the meeting over to the chairman, Dr. Kenrad Nelson.

DR. NELSON: Thank you, Dr. Smallwood. Hopefully, we can compress the discussion, so we can all have a weekend at home.

The first report is from Dr. Nightingale, a report on the TSE/BSE Action Plan.

Committee Updates

DHHS TSE/BSE Action Plan

Steven Nightingale, M.D.

DR. NIGHTINGALE: Thank you very much. My report will be very brief. What I can report is as follows.

On February 22nd, the Secretary of Health and Human Services asked the Public Health Service to prepare an action plan for the Department. That action plan went through 17--count them--17 revisions before it was presented to the Secretary.

We did have a meeting with the Secretary a week ago, and I anticipate that the Secretary will endorse the action plan in the very near future. I am waiting for my cell phone to go off to see whether or not he, in fact, endorsed it yesterday, but since the cell phone has not gone off, my presentation ends here.

DR. NELSON: If your cell phone goes off before 3 o'clock, you can tell us what they said.

The second is another report on the TSE/BSE Action Plan by Dr. Mary Elizabeth Jacobs.

OBRR TSE/BSE Action Plan

Mary Elizabeth Jacobs, Ph.D.

DR. JACOBS: Thank you, Mr. Chairman.

You probably are familiar in the BPAC audience with our use within the Office of Blood of action plans when we have a comprehensive effort. Our group has a core team which is meeting once a month, and I want to go through the major topic areas we are working on. We plan to finalize this after the Department effort is final.

It will be on our web site. If you go to CBER, you look under Manufacturer's Assistance. There is a heading for Action Plans, and you will be able to find it there. But I want to emphasize that we are already working on this.

[Slide.]

The first initiative we are working on is under FDA's overall action plan. I know we all think of blood as being Topic A, but actually, Topic A is covering efforts involving food that goes to animals in preventing BSE. So, the first one is on preventing potential exposure through transfusion and through tissue transplantation.

The first task is publishing revised final guidance on donor deferral to address travel/residence in BSE countries. You know that we have a TSE Advisory Committee meeting, which Dr. Asher is going to discuss at the end of the month.

We will be issuing a draft guidance after that and then final guidance.

I just want to remind you that when the first deferral was adopted, Dr. Satcher asked us to review those policies every six months. We do that internally, but most of them have also taken place at the TSE Advisory Committee which has met frequently.

The second thing in monitor developments in detection of TSEs.

The next one is monitor and reexamine scientific data on chronic wasting disease exposure of blood donors.

[Slide.]

The next one is monitoring data on potential exposure--this is of blood donors--through dietary supplements. That, we will be doing in conjunction with our Center for Food Safety.

The next one is monitor and protection of adequacy of the blood supply as part of our assessment of the effectiveness of deferrals, and looking at developments of detection of TSE infectivity in blood. We had a workshop on that about a year ago.

[Slide.]

The next one, Initiative C. This is potential exposure through other products.

We have listed here injectable blood products. Those are regulated under drug authorities, and that is why they are listed here rather than on the prior one.

I want to draw your attention as an aside to two things that are already on our web site that are important, looking at the question of animal source materials. Just go to the CBER web site and search under BSE and you will find these two things.

The first one is April 16th, 2001. It is a presentation by Mr. Mark Elengold, who is our Deputy Director for Operations of CBER. This summarizes all of our policies on BSEs. A number of them are on vaccines, but it is of interest because it lists all of our communications on sources.

The other one is a letter that is dated April 19th, 2000. It is signed by Dr. Zoon, who is our center director. This is a letter to all manufacturers of biological products regarding BSE sourcing. So, that is an important one.

I want to mention here to anticipate questions that you will see that we are distinguishing between injectable blood products and the IVDs which can be either HIV diagnostics or blood screening tests which don't involve direct patient contact.

The second item is that we are working within FDA addressing animal source materials. We are already within the office asking questions of manufacturers when INDs or other submissions come in about what the sources are of their materials.

We are monitoring developments in TSE safety of fractionated plasma products. A lot of you are aware that we also had a workshop on manufacturing and clearance issues.

[Slide.]

We have a separate initiative on education and outreach. That involves information on the web site. We already have quite a lot on the FDA web site on BSE efforts, and also separate ones on providing public information and examining our product labeling.

[Slide.]

We also include in the action our internal regulatory research agenda. We have internal

projects on TSE infectivity in blood and methods to reduce it; on PrPc physiology and TSE pathogenesis; on detection of TSEs, and also on blood donation and supply.

We expect to go final maybe in a month or so, so if you have any comments or suggestions, we would be happy to hear them.

DR. NELSON: Questions or comments?

Another issues is that, as everybody knows, there is a TSE Advisory Committee which is meeting, I think it's June 28th and 29th in case anybody wants to be there.

Next is Dr. David Asher. He is going to talk about this. Thanks.

Agenda Items for TSE Advisory Committee Meeting, June 28-29, 2001

David Asher, M.D.

DR. ASHER: Thanks for the segue. Thank you, Mr. Chairman, good morning.

I will review the agenda for the next TSE Advisory Committee taking place in two weeks, on Thursday and Friday, the 28th and 29th of June.

[Slide.]

The TSE Advisory Committee will consider three topics: The suitability of blood donors who

lived or traveled in BSE countries. This issue is an extension of discussions initiated at the last TSE Advisory Committee meeting of 18th and 19th of January, and is occasioned by FDA's concern about new information regarding BSE.

The second. The safety of plasma derivatives prepared in manufacturing lines previously used to fractionate plasma from donors not meeting FDA suitability criteria regarding BSE exposure. To my knowledge, this will be the first time that FDA has entertained a public discussion of cleanup and decontamination procedures for facilities and equipment potentially contaminated with TSE agents.

Finally, on the last morning of the meeting, the committee will consider new information regarding the safety of bovine gelatin from BSE countries, an issue that mainly concerns FDA Centers for Drugs and Food Safety.

[Slide.]

Once again, fulfilling our obligation to revisit the issue as frequently as needed, at least every six months, we will address the issue of blood donors who have been in BSE countries, whether to defer them and on what bases.

As you may recall unlike other forms of CJD, in variant CJD, the abnormal protease resistant prion protein accumulates to substantial levels in lymphoid tissues, a finding that early on raised concern that the relatively reassuring epidemiological evidence suggesting that blood was unlikely to be an important vector for classic form of CJD might not be predictive for variant CJD, a disease with which we have only limited experience.

[Slide.]

The uncertainty prompted CBER's current policy announced in November of 1999 recommending deferral of donors resident in the UK for any cumulative period of six months from the presumed start of the epidemic, the BSE epidemic, to full implementation there of a series of measures to prevent human exposures.

Those measures intended to protect the human food chain are a so-called age-based slaughter scheme requiring that all cattle used for meat products be slaughtered within 30 months of age, careful removal of specified risk materials from carcasses, and prohibition of mechanically recovered meat.

Taken together, those measures should have

substantially reduced potential contamination of products, at least with large amounts of BSE agent and reduced opportunities for human infection.

[Slide.]

The donors of main concern are those that were infected in the UK before 1996, as well as in other BSE countries where measures to protect the human food chain were introduced only recently, if at all.

Rates of new cases and deaths from variant CJD have continued to increase in the UK, fortunately not in France. Evidence suggests that in other European countries a substantial part of the supply or beef and beef products may have come from the UK, presumably causing the three cases of VCJD seen in France in people who had no history of travel to the UK.

Cases of BSE recently increased in France and several other European countries and new countries have recognized BSE in native animals, raising a concern that in addition to exposures to UK beef products, indigenous BSE may have become an important source of human infection.

[Slide.]

Last year's reported transmission of BSE

through blood of a sheep experimentally infected with BSE following an early report of infectivity in the blood of BSE-infected mice served to increase concern that infectivity in blood might be a general property of BSE, implying that the blood of persons incubating variant CJD might also serve as a source of infection.

Fortunately, the few studies addressing the infectivity of human blood in VCJD, both experimental inoculations of blood into animals and a lookback study of UK donors, 8 donors, 22 recipients traced, are all negative so far, but several are in early days, so that they provide only limited reassurance.

[Slide.]

This will be the third time that the TSE Committee will consider suitability of donors potentially exposed to the BSE agent. In their January meeting, the committee advised FDA to adopt no changes in the recommended UK deferral policies, that is, deferring donors resident in the UK for any cumulative six-month period from the start of 1980 to the end of '96.

The reduction in risk expected from deferring donors based on a three-month aggregate

exposure in the UK was not considered great enough to justify the additional donor losses that might result.

[Slide.]

A majority of members also advised deferring donors resident in France for 10 years or more from 1980 to the present by accepting the idea first put forward at the June 1999 meeting, that exposure of the French population to UK beef during the years of high BSE prevalence in the UK might have been about 5 percent of the exposure in the UK itself. The number of VCJD cases in France as of that time was about 5 percent of those in the UK, as we. Fortunately, the number of French cases has not increased since then.

[Slide.]

The TSE Advisory Committee overwhelmingly declined to endorse an FDA proposal to defer donors who had traveled or lived in any of the other BSE countries for an aggregate period of 10 years or more after 1980 until the present. Exposures to UK beef for smaller in the other BSE countries than in France, and except for Ireland, none of those countries has diagnosed a VCJD case, however, some members expressed a concern that in addition to

risk from imported UK beef, risks from indigenous BSE at some European cattle may now be significant.

[Slide.]

Apparently, because of that concern about possible human exposures to the BSE agent in other non-UK beef, a very closely divided committee went on to advise that the proposed deferral policy be recommended, but only for those two countries with the largest numbers of cattle diagnosed with BSE outside the UK, namely, Portugal and the Republic of Ireland.

[Slide.]

They also declined to advise deferring donors for a combined period of residence in more than one country because they worried that tallying combined exposure would be logistically difficult and fraught with errors.

[Slide.]

A majority of the committee also advised against treating donors potentially exposed as active duty U.S. military or dependents in Europe to substantial amounts of beef products from the UK, estimated on some bases at certain times to have been from 20 to more than 30 percent of total supply, that those donors be deferred as if they

had been in the UK for the same period of time. Members expressed concern that the impact of such a deferral would have both on the DOD blood program and the general U.S. blood supply might be excessive relative to the potential reduction in risk.

[Slide.]

They did advise the FDA to develop some less stringent policy for deferring U.S. military donors, presumably those exposed for longer periods of time than six months. The committee suggested first attempting to estimate effects that various deferral policies might have both on the military blood program and on civilian programs where military retirees and former dependents lived.

[Slide.]

The FDA acknowledges the committee's concerns expressed in January, agreeing that the risk of transmission of variant CJD is theoretical and that the potential loss of blood donors from increased deferrals is substantial. The FDA also understands the reluctance of the committee to lump all 30 countries that, in addition to the UK, are now on the USDA-BSE list together in one deferral policy. However, the FDA is not sure that singling

out donors resident in France, Ireland, and Portugal for deferral can be justified scientifically.

First note that the vast majority BSE cases, more than 180,000 have been diagnosed in the UK. As late as last year, Great Britain still reported 1,352 cases and 177 more were reported through the end of April of this year.

Although Ireland and Portugal indeed have the next highest numbers of cases, 613, 568, BSE cases in Switzerland were also substantial, 382, exceeding the number reported from France. Numbers of BSE cases in cattle born in Germany and Spain seem more modest, 75 and 46, but it is troubling that all those cases were reported just within the last year.

Furthermore, the recent finding of BSE in the Czech Republic last week, a country to which the European Commission's Scientific Steering Committee had previously assigned a low probability of BSE, casts some doubt on the ability of current risk assessments to provide reliable estimates of potential human exposure to BSE agent in various countries. By the way, the USDA, on which the FDA has relied to determine countries with a

significant risk of BSE place the Czech Republic with the rest of Europe west of the former Soviet Union on the its BSE list at the end of 1997. So, it is difficult to be confident that some bright BSE line can be drawn distinguishing exposure risk in France, Ireland, and Portugal from the risk in other European countries.

[Slide.]

For that reason, the FDA has again solicited advice from the TSE Advisory Committee asking them to review several options for revised FDA donor deferral recommendations in view of recent information concerning BSE in cattle and BCJD in humans.

We plan to present several possible options including the following: One, the policies previously advised by the TSE Advisory Committee; two, a more aggressive policy recently announced by the American Red Cross; and, three, other options.

The TSE review will be considered in a planned revision of the current FDA guidance, a revision that we anticipate will be issued for public comment within a reasonable time after the meeting.

[Slide.]

In considering the options, the committee will be presented with a summary of the European geographic BSE risk, a series of qualitative risk assessments attempting to estimate probable prevalence of BSE in cattle of various countries by Dr. Joachim Kreysa, who chaired the Scientific Steering Committee's Working Group that developed the assessments.

Then, the committee will hear a review of potential human BSE exposures in BSE countries, epidemiological models based on BSE risk, food chain protections, and other elements by Dr. Crystal Donnelly from the University of London.

Then, Tony Giulivi of the Canadian Blood Services will report on potential BSE exposure of Canadian travelers, blood and plasma donor deferral policies, and estimated effects on the Canadian blood supply and public health.

[Slide.]

Jean-Hugh Trouvin of the French blood authorities will present a risk assessment from a European perspective and describe European Union policies, and then CBER's own Dr. Alan Williams will estimate the effects of possible changes in FDA blood donor deferral policies, both the

potential reduction in risk of total donor exposures to BSE agent resulting from various options and their potential impact on regional and national blood supply in the USA.

Finally, we anticipate having a frank and spirited open public hearing.

[Slide.]

The afternoon session will begin with a review of the scientific bases for decontamination of the TSE agent and their potential application to the manufacturer of plasma derivatives by Bob Rohwer of the University of Maryland and VA Medical Center in Baltimore.

That will be followed by a summary of current and proposed cleaning procedures for facilities manufacturing plasma derivatives to be presented by an industry representative who will, we hope, give their views concerning risk assessments, practical approaches for cleaning and decontamination, and other approaches to the problem and consider their feasibility and possible effects on supply. That open public hearing should also be informative.

I look forward to seeing some of you at the meeting and thank you.

DR. NELSON: Thank you, Dr. Asher.

Comments or questions? Dr. Nightingale.

DR. NIGHTINGALE: I think this is the opportunity I needed to take to present the one piece of information that I would have liked to have presented yesterday, which is that the Department shares the public concern for the impact of the proposed donor deferral strategies, and shares the public concern that projections can be made that project what we really need are some hard facts.

I believe that most members of the audience are aware that the Department has for the last year and a half starting in October of 1999, had a contract with the National Blood Data Resource Center to collect data from a representative sample of 26 blood collection centers throughout the United States on the supply of blood to the United States.

We have recently--and this is with the full support and endorsement of the Secretary--initiated the process of getting what I will call for the record here a "quick and dirty" estimate of the demand for blood throughout the United States.

Our plan right now is to use largely existing resources perhaps with a supplemental agency tap to sample hospital transfusion services geographically distributed throughout the United States. We hope to have this program initiated on or about July 1, 2001.

We are in active discussions with transfusion services throughout the country and with a vendor to provide the logistical and collection support. We are aware, I would say simply for the record, of the necessity to absolutely protect the confidentiality of the data that we will be collecting.

One of the comments that was made at an advisory committee meeting by Dr. Weinstein and by others is the premature release of incomplete data can exacerbate a shortage, as well as lead to its correction. We are acutely aware of that as we proceed with our plans.

Our plans are to move as quickly as we can without falling into that, at least the other traps that we have anticipated, and to try to solicit the assistance of the public in avoiding those traps and moving as expeditiously as possible.

There will be a meeting of what I will

call right now the Advisory Committee on Blood Safety and Availability because I can call this meeting. It will hopefully serve as a workshop to obtain public input into the best way to monitor the blood supply in the United States.

We are looking for input onto the limits of how much data should be collected, how should it be stored, how should it be reported access to it, and so this will be August 23rd is a Thursday, August 24th is a Friday.

When I get yet another signature, there will be a notice of this in the Federal Register, and I recognize most of the people in the audience, if there is anyone in the audience who thinks that I might not recognize them and would like to be on the mailing list for this committee, please let me know.

My telephone number is 202-690-5558, and that is direct.

Thank you.

DR. NELSON: Thank you, Dr. Nightingale.

The next topic is by Dr. Martin Ruta, Final Rules on Requirements for Testing Human Blood Donors for Evidence of Infection Due to Communicable Disease Agents and General

Requirements for Blood, Blood Components, and Blood Derivatives; Donor Notification.

Final Rules

Martin Ruta, Ph.D.

DR. RUTA: Good morning, everyone. Thank you.

On Monday, the FDA published two final rules on donor testing and donor notification, so I am going to try and brief summarize what is in the rules. I can't do that in a few minutes, so I am going to hit the highlights. I would recommend to all of you to go and read the rules.

For those of you who sent us letters and comments on the proposed rules, in the preamble to the final rules, we summarize the comments and we address each comment, so if you want to see where your comment is and response to it, it is in the preamble and I apologize for turning my back on some of the committee members here.

What I am going to do is try and talk only for about 10 minutes. It really would take me much longer to got through the whole rules, so I am just going to try and hit the highlights here and again suggest that since this is something we are going to live with for a couple decades now, it is

probably time to go ahead and take a look at them.

The rules came about in part because of FDA internal efforts to review the regulations in part because of GAO recommendations and in part because of oversight hearings by the Government Reform Committee. In this rules, we address comments and recommendations by the GAO and the oversight committees, as well as our own thoughts and comments from the public.

[Slide.]

So, starting off with the beginning. If you collect human blood and you want to make it into a product, you have to test it. Now, this includes blood intended for transfusion, blood intended for use in preparing plasma derivatives, and blood intended for use as a component or to prepare a medical device.

[Slide.]

Now, what do you have to test it for? You have to test it for HIV-1, HIV-2, hepatitis B, hepatitis C, HLV-1, HTLV-2. There was some discussion about dropping syphilis. We did not drop the requirement for testing for syphilis, so you are so required to do syphilis testing. If the committee remembers, we brought this topic in

September to the committee for discussion. For technical reasons, it is not in this section of the reg, but it appears further down in the reg.

If you notice, what is a little different here compared to the current 601-40 and 45, is we did not specify the specific tests that you use, so it doesn't say antibody to HIV, it is technology-neutral.

One of the reasons we did this is because of evolving technologies in anticipation that new technologies would come along, and as they come along, the FDA will issue a guidance document to interpret the reg to say that we think at this time particularly new technology would be useful.

So, for example, when that gets approved, if the committee were to say, you know, we think NAT testing should be used in donor screening, then, we would issue a guidance document to say that NAT testing for HIV, hepatitis, et cetera, would then be required.

The preamble to the rule actually contains a chart, summarizes current guidance documents and gives our interpretation of what we think would be required right now. So, for example, for HIV-1, it would be the p24 test and the antibody to HIV-1

test, et cetera.

[Slide.]

Now, what this says is that you have to use one or more tests, and there are screening tests to test for evidence of infection. These are screening tests that have been approved by the FDA, and you must follow manufacturer's instructions. Again, the one or more is where we will issue a guidance document, for example, for HIV. As I said, we would currently think that p24 and antibody to HIV would be required.

[Slide.]

Now, there are some exceptions. One exception here is for what we call dedicated donations. So, this is a one-to-one donation where you know the donor, you have an identified donor, and that his blood or phoresis product is going to an identified recipient.

In that case, you only to test once at the beginning of a 30-day period. So, if you collect blood on day one from a known donor to a known recipient, you have to test the blood for all the agents. If you collect a second unit within the 30-day period, you don't have to test those units. There are specific labeling requirements added

here, but if you collect again on day 31, you have to go back and test.

[Slide.]

Again, there are labeling requirements here which would be intended recipient information label. This reg contains a number of labeling requirements, and I am not going to go through all of them, so again I am going to send you to the regs and ask you to look at those carefully.

[Slide.]

Another exception, source plasma. For source plasma, you are not required to test for HLV-1 and 2. In addition for medical devices, you don't have to test for HLV-1 and 2 unless the final product contains viable leukocytes.

[Slide.]

Autologous donations. We have a number of comments, probably most of the comments about autologous donations. This is written in the negative, but I am going to try and say it in the positive sense.

It turns out technically to be easier to write it in the negative sense, but what comes out is that if you have a crossover program, you have to test all of your autologous units. If you ship

autologous units, you have to test all of the units. So, for the small hospitals that we are collecting autologous units that didn't have crossover programs, and don't ship, they would not have to test.

[Slide.]

This basically spells it out. It says if you allow autologous donations, any autologous donations to be used for allogeneic transfusion, you have to test everything, or if you ship to another establishment, then, they also have to be tested.

[Slide.]

I am going to skip this one. There is a slight exception here, if you ship to an establishment that doesn't allow autologous transfusions to be used for allogeneic use. Again, there are labeling requirements here.

[Slide.]

I am just going to point things out. There are additional labeling requirements within the reg, so you are going to have to look at it in detail. So, for autologous donors, it would either be labeled as donor untested, that would be if there is no crossover, no shipping, it would be

tested negative, it tested reactive on the current collection or reactive in the last 30 days or tested negative in the last 30 days.

[Slide.]

Supplemental testing. There is now a requirement that supplemental tests be performed when there is an FDA-approved supplemental test, and these are for the HIV, the hepatitis B, the hepatitis C, and HTLV, which unfortunately we don't have a supplement to test yet. There are again some exceptions within here.

Then, there are additional sections of the reg that I am not going to go through right now, and they deal with release of units prior to testing, similar to the provisions that are in the current reg. They have to do with release if it is a medical emergency or if you have approval from FDA.

There are also sections of the reg that deal with restrictions on use, what you can do with a reactive unit in terms of shipping, and I am not going to go through those because they get rather long and I am trying to be brief this morning.

So, I want to turn to the next section which has to do with a requirement for donor

deferral.

[Slide.]

Now, there is a specific requirement that if one of the donors tested reactive by a screening test, HIV, hepatitis B, hepatitis C, HTLV, or syphilis, you have to defer the donor. There are some exceptions here. Again, one of the exceptions is, for example, for HTLV and for anticore, you defer the donor when they test reactive on the second donation.

I should maybe point out that we have gone to the term "reactive" here instead of "repeatedly reactive." We did that because it is technology evolution that some of the future tests that may be coming along, like NAT, the term "repeatedly reactive" doesn't quite apply, but you see the preamble explains that we actually mean for the serologic tests, "repeatedly reactive" is explain in the manufacturer's instructions.

So, now you have a donor and he tests reactive, you have to defer the donor. There are a couple of exceptions in there. HTLV is one of them.

[Slide.]

There is one exception here, but I

actually wanted to go to the B section, but one of the exceptions here is No. 5, is if you defer a donor and he is an autologous donor, that you can use the blood for autologous use. So, if someone comes in and donates for autologous use and tests reactive, their blood can still be used for autologous use.

The exception that I want to go to is B, which says that if you defer a donor, it is actually a provision that allows for reentry even though it is not called that. It says that if FDA has come up with some scheme that allows for a donor to be reentered, that this is a provision that would allow it to occur.

Okay. That summarizes seven years of work. I am going to turn to the next reg. My apologies to everyone. You have got to read the reg in detail because there are many things that I left out, and your comments are addressed in the preamble to the rule, so in order to keep things brief, I am going to move on to the notification rule.

[Slide.]

The donor notification rule. So, now that you have deferred your donor under the testing

rule, there is a requirement that you notify him. You have to make reasonable attempts to notify a donor including autologous donors who were deferred because they tested reactive for one of the tests listed in or were deferred under the previous section, or they were determined not to be suitable under the current reg 640.3, 640.63.

There is another provision here that says that you should try and get the supplemental test results before you notify the donor. If for some reason you don't get them before you can notify the donor, you notify them again with the results of the supplemental test.

[Slide.]

We will go through the specifics of what you tell the donor. First of all, you tell the donor that they are deferred or not found suitable. "Not found suitable" refers to 640.3 and .63, the suitable regulation.

So, you tell the donor they have been deferred and you tell them why they have been deferred or found not suitable, where appropriate, the types of donations that the donor should not donate in the future.

[Slide.]

Where applicable, you see there are a lot of modifying phrases in this section. So, if the donor was deferred because they tested positive for infectious disease tests, you can tell him the results of the tests including the supplemental test, and if it is appropriate, you tell him about information concerning the need for medical followup and the need for counseling.

[Slide.]

The time period for notification. Here, we said that you must make reasonable attempts to notify the donor within 8 weeks after determining the donor is deferred or determined not to be suitable.

For many of the suitability criteria, we expect that people will be notified right when they are sitting there. You must document that you have successfully notified the donor or if you are unsuccessful, that you made reasonable attempts to notify the donor.

[Slide.]

The previous requirements also included autologous donors, so you have to tell an autologous donor if they were deferred because they tested positive. We had comments on this and some

of the comments said notify the donor, or others said notify the physician. We put in requirements for both. So, in this case, because autologous donations are done under prescription, we say to also make reasonable attempts to notify the donor, to notify both the donor and physician. You would tell the physician why the donor has been deferred.

[Slide.]

Again, it parallels the previous section. Where appropriate, the type of donations that the autologous donor should not make in the future, the results of any tests including supplemental tests, and we give again, making reasonable attempts within an 8-week period.

I am going to stop there only because I am trying to keep things brief this morning and my apologies for turning back on the members.

DR. NELSON: Thank you. Questions? Blaine.

DR. HOLLINGER: Just one question. If I am an autologous donor and you have tested my blood in the past, and I am deferred for some other reason. I have donated before, and I have got some disease and I am deferred, and I want to be an autologous donor. I wasn't sure in there if it

said that my blood could be acceptable to myself or not.

I know you talked about syphilis, you talked about labs that don't ship, and so on, don't have to test it, but say it has been tested and I am found to be positive for anti-HBC or HBsAg, and so on, anti-HCV.

You probably say it somewhere in here, but does that mean that I can receive my own blood?

DR. RUTA: Right. We are not prohibiting autologous donations from donors who test reactive or found unsuitable as long as the medical director thinks it is okay to collect the person's blood for the suitability reasons, but there is no prohibition that someone who tested reactive for one of the tests for giving them back their own blood.

DR. HOLLINGER: And it says that somewhere in the regulations.

DR. SCHMIDT: Although you have changed "repeatedly reactive" to "reactive," I think in the table that you showed I saw the word "negative." So, are things consistent, are there some negatives and some non-reactives? Reactive is a difficult word for a donor, but is it all in that

terminology?

DR. RUTA: Going to the term "reactive," was not meant to change what is in the current manufacturer's instructions, so the current manufacturer's instructions will say "repeatedly reactive" for most of the screening tests, but not all.

Those would be the terminology that you could use now that would be suitable.

DR. SCHMIDT: I did see the word "negative" on your table there, it was the second line on an early table.

DR. RUTA: Where a donor tested negative?

DR. SCHMIDT: Yes. I am not sure, I am sorry, I can't identify it, but it was a table with four or five items on it, and I think the second line, I saw the word "negative."

Anyway, is it generally in there or is "negative" and "positive" gone?

DR. RUTA: We didn't change negative or positive in terms of the manufacturer's instructions, so the instructions from the tests say you tell the donor they are positive or you tell them they are negative, then, those are unchanged.

We went with the term "reactive" only because we didn't know how to deal with evolving technology like NAT, but you still do "repeatedly reactive," and if the insert says that you would consider the donor positive if they are repeatedly reactive, then, you can tell them they are positive, and if they are nonreactive, you could them they were negative, those would still apply.

DR. NELSON: Thanks very much.

The next topic this morning is Transfusion-Related Acute Lung Injury. The introduction and background will be given by Dr. Holness from FDA.

IV. Transfusion-Related Acute Lung Injury

Introduction and Background

Leslie Holness, M.D.

DR. HOLNESS: Good morning.

[Slide.]

The topic is Respiratory Distress Syndrome Associated with Transfusion, known as Transfusion-Related Acute Lung Injury or TRALI.

[Slide.]

The reason the topic is brought to the committee is because of the FDA fatality reports. TRALI has been implicated in 10 to 14 percent of

the fatalities in each of the last three years, actually, each of the last four years, also a rise in the reported reactions, three reactions in Fiscal Year '97, 12 reactions in Fiscal Year '98, 17 reactions in Fiscal Year '99.

Of the fatalities, 75 percent of the implicated donor products tested HLA/granulocyte antibody positive.

[Slide.]

Strategic options for reducing morbidity and mortality due to TRALI include deferral of donors implicated in a single unit or in more than one multiple unit TRALI case; the identification of donors with risk factors followed by donor deferral; screening of units for HLA granulocyte antibodies; diversion of plasma to non-injectables; and the establishment of improved physician educations about TRALI and improved surveillance mechanisms for donors implicated in non-fatal as well as fatal TRALI cases.

[Slide.]

Our presenters for this morning will be Dr. Mark Popovsky of the Haemonetics Corporation, Dr. Patricia Kopko, Sacramento Medical Foundation Blood Centers, and Dr. Lynn K. Boshkov from the

Oregon Health Sciences University.

They will be followed by Dr. John Finlayson, the Associate Director for Science, OBRR, FDA.

[Slide.]

The questions that the committee will be asked to consider would be:

1. Should FDA consider interventions at this time to identify donors and/or donations with an increased risk for producing TRALI in a recipient? If not, what data are needed to define appropriate measures?

[Slide.]

Question 2. If Yes, would it be appropriate to identify blood donors with a history of multiparity, defined as three or more pregnancies, one of more multiple allogeneic transfusions, implication in a single unit case or in more than one multiple unit TRALI case?

[Slide.]

2B. For donors with risk factors defined in Question 2A, would it be appropriate to limit collections for transfusion to plasma reduced products? For example, washed-resuspended red blood cells or apheresis platelets.

Divert the plasma collections to the manufacture of non-injectable products?

Screen for anti-HLA or granulocyte antibodies and permit negative donors to continue donating routinely?

Defer such donors?

DR. SIMON: Apheresis platelets are not plasma reduced.

DR. HOLNESS: At the moment, I don't believe so, no.

DR. SIMON: So, you are saying to change them to plasma reduced?

DR. HOLNESS: Yes, to plasma reduced apheresis platelets, yes.

DR. NELSON: Are there any other questions?

Our next speaker is Dr. Popovsky.

Mark Popovsky, M.D.

DR. POPOVSKY: Good morning. I want to thank the Food and Drug Administration for providing me this opportunity to present this morning, and thank you, Mr. Chairman, and Committee.

[Slide.]

Even today, in 2001, relatively few

transfusing clinicians are familiar with transfusion-related acute lung injury. There are several reasons for this, but one of which is a lack of awareness because there are actually a number of synonyms for TRALI, particularly in the early literature - by "early literature," before 1985 when the syndrome's name was first presented in the peer-review literature.

The publications prior to 1985 appeared in a number of disciplines with relatively few case reports. Some of those synonyms include leukoagglutinin reaction, pulmonary hypersensitivity reaction, noncardiogenic pulmonary edema, adult respiratory distress syndrome related to transfusion, and allergic pulmonary edema.

[Slide.]

First, a definition at least when it was first described by Dr. Brendon Moore and myself in 1985. In its fulminant and classic presentation, it includes the following: acute respiratory distress, severe hypoxemia, hypotension, generally moderate in degree, acute bilateral pulmonary edema, fever with temperature increase of 1 to 2 degree C. above baseline, and these manifestations occur within one to two hours of a transfusion of

plasma-containing blood products although there are a handful of cases in which a six-hour time frame has been reported.

The pulmonary edema may begin in the dependent portions of the lung, but it will involve the entire lung fields within a matter of hours.

Now, what are the predominant first presenting symptoms? In a retrospective study that I will quote several times, performed by Dr. Becky Haley and myself, using the American Red Cross database from its then 38 regional bleed centers, involving 46 cases, which to my knowledge, is the largest such report, three symptoms that caught the attention of the clinician that led to suspicion and ultimately diagnosis were: respiratory distress, hypotension and, interestingly enough, in 15 percent of cases, hypertension, that than proceeded to hypotension.

[Slide.]

This x-ray, which admittedly is not projecting particularly well, is very typical, that within a few hours one will see an interstitial infiltrate that involves the entire lung fields, that will progress over time in most cases, which we will talk about in a moment, will in fact clear

completely, typically within 96 hours.

[Slide.]

So, what is the differential diagnosis of respiratory distress in the setting of transfusion? It includes anaphylactic transfusion reaction, circulatory overload, bacterial contamination, and cardiac failure. Because of limitations of time, and the purpose today is to focus on TRALI, I won't discuss what distinguishes and needs to be distinguished for the clinician between these entities and the diagnosis of transfusion-related acute lung injury.

[Slide.]

But who is at risk? If you take 15 years of publications and summarize them, I would say today we don't know who is at risk. There is no common thread. There is an approximately 50-50 ratio between males and females for those who have the TRALI, and patients have ranged from 2 and 3 years of age to octo- and nonagenarians, and there is no common underlying diagnostic theme.

[Slide.]

The blood products that have been implicated include, from the very old literature, whole blood, fresh frozen plasma, red cells of all

types collected in all types of anticoagulant preservatives including additive solutions, granulocytes collected by apheresis, cryoprecipitate, platelet concentrates, apheresis platelets, and more recently, several rare reports of intravenous immune globulin.

The cryoprecipitate cases and platelet concentrate cases illustrate the fact that this is not dependent on a large volume of plasma.

[Slide.]

What do we know about the frequency? I think it is fair to say that we don't know. In the Mayo Clinic study from 1985, 1 in 5,000 plasma-containing transfusions were associated with transfusion-related acute lung injury. For those of you who aren't familiar with the setup at Mayo Clinic, the transfusion and I.V. team transfuses the great majority of transfusions at Mayo, in fact, all of the outside of the operating room, and these are trained staff who are very much aware of transfusion reactions and stay with the patient at certain time points both before and during and after looking for signs and symptoms.

So, this was the ideal environment in a medical center that was sensitized to the

condition, and therefore might be in the optimum situation to, in fact, make the diagnosis. So, this was 1 in 5,000.

[Slide.]

There is evidence to suggest that this is under-diagnosed. There are more than 240 cases that are in the medical literature, peer-reviewed literature, or who have been reported and described, but not published.

In a paper by Clarke, et al., from Canada, in 1994, 0.32 percent of severe respiratory reactions to random donor platelets were diagnosed. These were found to be more common, as one would suspect, in patients with hematologic disease and cardiac disease as recipients of these platelets, and the average age of these platelets was 4 1/2 days.

[Slide.]

In a paper dating back more than 30 years now, from the University of Pennsylvania, Cooperman and Price, looking at patients with pulmonary edema in the operative setting, looking for causes, found that 50 percent were associated with circulatory overload, but 50 percent also were for unknown causes, at least raising the specter that this may,

in fact, have been transfusion-related acute lung injury.

[Slide.]

Now, what is the clinical outcome? In the Mayo Clinic series of 36 patients, all of the patients with TRALI required oxygen support, nearly three-quarters required mechanical ventilation. The pulmonary infiltrates cleared in four-fifths of these individuals rapidly, in other words, within 96 hours. In 17 percent, there was slow resolution meaning it required more than 7 days, but eventually, these infiltrates cleared. However, two patients succumbed to TRALI and died, and there were no long-term sequelae.

[Slide.]

Now, Dr. Holness hinted at this fact in his introduction, that from the FDA's data on deaths from transfusion, if you look at the published cases from the period of 1990 to 1998, TRALI had moved to number 3 in terms of causes of fatal transfusion-related deaths.

[Slide.]

Then, from the UK's SHOT program, Serious Hazards of Transfusion, their most recent report, they described 18 cases of TRALI in which they said

12 of those were associated with major morbidity, 6 individuals died, and it was the second most common cause of death from transfusion in their hands.

[Slide.]

So, what is the pathogenesis? Again the precise mechanism is unknown, but we do know the following: That in 60 to 85 percent of cases, HLA antibodies in the donor or granulocyte-specific antibodies in the donor of various specificities have been associated with transfusion-related acute lung injury in a recipient of that donor's product or products.

In 50 percent of cases, the HLA specificity of the donor's antibody corresponds with an HLA epitope in the recipient. We know that these antibodies activate complement.

[Slide.]

And we know that receptors on neutrophils, C5a, promote neutrophil aggregation and sequestration in the microvasculature in the lung, and this is well described in an experimental literature of ARDS.

We know these neutrophils marginate in the pulmonary microvasculature, and we know that these activated neutrophils release, among other things,

proteases, super oxide radicals, which again in experimental models results in endothelial cell damage and pulmonary edema.

[Slide.]

Perhaps the most elegant paper on the subject from Seeger, et al., they used the next vivo rabbit model of lung injury in which they perfused rabbit lungs with various cocktails that included 5b-negative polymorphonuclear cells, anti-5b antibody, and a source of complement in various combinations, perfused those cocktails through the lungs, and then looked at changes in lung weight to reflect pulmonary edema in the animals.

[Slide.]

What this shows is that if, in fact, you have only antibody and the polymorphonuclear cell is negative for the antigen, and you look over time, you don't see any significant change in weight, or if you have only two of the three combination, you don't have any change in weight.

However, if you have both the source of complement, you have the antibody and you have the presence of the antigen on the PMN, that one sees in the time frame that is comparable to that seen

in humans, a significant increase in weight, suggesting that there is, in fact, a relationship between the presence of the complement source, the antibody, and the corresponding antigen.

[Slide.]

Now, if you look at implicated antibodies in various reports, you see that both donor and recipient antibodies have been implicated, but by far and away it is the donor that is the more common source of these antibodies, so in a paper that Dr. Haley and I published in 2000, we find that in 50 percent of cases, either HLA or granulocyte antibodies were associated with an implicated product with that of the recipient, in 11 percent total, but recipient HLA antibody or granulocyte antibody, pre-transfusion, of course, were associated with these cases.

[Slide.]

Now, what is the role of parity in these cases? We perhaps were helped by the recent report by Palfi, et al., in which they conducted the first prospective randomized study in the field in which they looked at 102 ICU patients receiving two or more units of FFP, and the two groups were controls versus multiparous donors, defined as three or more

pregnancies, and these patients received plasma.

What they found were five patients who had clinical reactions, one of which was clearly TRALI. That donor was a multip, but maybe more interesting, was in looking at a marker of injury, they looked at the ratio of PaO_2 to FiO_2 and found that there was significant depression of that ratio in the recipients of multip plasma versus that of control plasma.

[Slide.]

Now, I don't want to steal the next speaker's presentation, Dr. Kopko, but for the sake of completeness, what I have been describing up to now is the presence of HLA Class I antibodies. Dr. Kopko and colleagues have, in fact, shown that in some cases, HLA Class II antibodies may be involved, and they have now published a growing series of cases that were negative for granulocyte or Class I antibodies, for which they found Class II antibodies.

[Slide.]

Now, there is another hypothesis meaning another hypothesis that looks at another pathway to the lung injury, and this has been most strongly advocated by Dr. Silliman and colleagues in

Colorado. It may, in fact, combine the antibody model that I have described with a cytokine model of injury, in which the patient has an underlying disease, in which that disease for whatever reason results in the release of endogenous cytokines and now product, the blood product is infused that either contains HLA or granulocyte-specific antibodies or, in their hands, biologically active lipids that results on granulocyte activation and then the endothelial damage that I have described.

[Slide.]

So, what research is necessary today to move this field along? First, in the field of prevention, we need to better define who is at risk, and to do that, I believe we need prospective and multicenter studies to find out who, in fact, develops TRALI.

Today, in the United States, probably anywhere from 3 to 7 percent of donors have HLA-specific antibodies who are donating every day, and yet, we have only a handful of cases of TRALI that come to us as clinicians.

So, clearly, there are a lot of infusions of plasma containing these antibodies that does not result in TRALI. We don't know why.

The second issue is, is there a role for leukodepletion in preventing TRALI? Based on our knowledge to date, we would suspect not because most of this is donor antibody driven, and not leukocyte driven from the donor into a recipient, a donor's product into a recipient that has antibody against leukocytes.

[Slide.]

What recommendations might FDA consider in this setting? First, to quarantine untransfused components traced to implicated donors in cases that are under investigation.

Secondly, to defer donors who have been previously implicated in cases from future donations of products, such as plateletpheresis because of the volume of the plasma involved in most plateletpheresis collections.

Thirdly, to divert plasma-containing components from future donations of whole blood from implicated donors, so that their products would be made into either a red cell or washed red cell product.

[Slide.]

So, in conclusion, TRALI is an under-diagnosed serious problem in the transfusion

world, which represents a spectrum of lung injury that ranges from noncardiogenic pulmonary injury to pulmonary edema, to full-blown adult respiratory distress syndrome.

Thank you.

DR. NELSON: Thank you, Dr. Popovsky.

Questions? Toby.

DR. SIMON: Even though it has been described with all the different components you showed including cryoprecipitate and red cells and additives, would you say that the vast majority of cases are from units that contained a substantial amount of plasma?

DR. POPOVSKY: Yes, by "substantial" meaning more than 60 to 100 ml, yes.

DR. SIMON: So with red cells made with--

DR. POPOVSKY: Additive solution. But there are still cases that are coming in, Toby, so, you know, we described the syndrome in '85, and most cases you see are still associated with FFP or plateletpheresis, but the majority of cases would encompass those products that have more than 60 ml, but not entirely.

DR. McGEE: Can I ask you a couple questions about the numbers? What period was your

46 cases? I mean how long a period?

DR. POPOVSKY: That was I believe 1991 to 1998. Becky, is that right? Yes.

DR. McGEE: On the Mayo, there were 36 cases?

DR. POPOVSKY: That was 36 over two years.

DR. McGEE: That was two years. Then, your slide where you had the implicated antibodies, that is the same 46 cases?

DR. POPOVSKY: Yes.

DR. STRONCEK: In my past life, I directed a laboratory, one of the few laboratories that tested for neutrophil antibodies around the country, so I would get the results of these tests in a lot of these cases. We have to remember about HLA antibody tests, the neutrophil antibody tests, most of the time it is not one unit that is implicated. Usually, you get tests on up to 2 to 3 to 4 to 5 to 6 units, and then you start testing these units for antibodies. If you start thinking about the numbers, if you took any six blood donors, you know, half of them are women, so you probably have a couple of them that are multiparous, so in any of these cases, by definition, you probably just by chance are going

to have a multiparous woman involved, and then a lot of those are alloimmunized.

So, what really Mark is presenting, which is the best data, is 46 anecdotes. It is not controlled. Nobody has ever said, well, let's look at TRALI cases, then, let's look at case controls, other people that has similar medical conditions with similar numbers of transfusions, and how many of those people got transfused with units that contained HLA antibodies.

So, I think there is a lot to be said about the pathophysiology. The other thing about the disease is that I think it is very difficult to make statements like most of these are involved in plasma-containing products, because there is plenty that don't have a lot of plasma.

You know, if we are speaking about anecdotes, there are case reports where people don't have granulocytes and get transfused, and they do have TRALI reaction, so you have neutropenic patients transfused that have TRALI.

There is also anecdotes, which I published one, where you will find a donor that is involved in TRALI, and you go back and look, and yes they have that neutrophil antibody, but, you know, they

have given 20 other transfusions, and none of those other units were associated with TRALI. So, what is going on? Why do you have TRALI in one case with this unit, and not in the other 20 donations? So, I think that even though Mark gave a very eloquent presentation, as you will hear from other presenters, what is going on with this syndrome is not really known at this time.

DR. NELSON: Are there data on the repeat occurrence of TRALI from the same donor?

DR. POPOVSKY: It is very limited. There is one published report of a patient who, in fact, had two consecutive cases of TRALI, so the data are sparse.

DR. MACIK: I have a lot of questions about the information because I was not familiar necessarily with this syndrome. In particular, based on what I have seen, is this really a new syndrome or a newly recognize syndrome, do we really know that it is due to blood or to something that is used in transfusing the blood or even a medication given simultaneously with blood in some of there patients, and then also getting back to the point, what information do we have that this same donor's blood is causing this in multiple

people, in other words, by deferring these donors because their blood has been linked to somebody with this disorder, do we really know it was their blood, or is there something in that patient that they would be reactive to multiple bloods?

I think what you have presented is nice, initial information, but I still have a lot of questions about what the link is, and some of the things that you recommend, such as deferring of donors, I don't know that there is enough information there to really defer people at this point.

DR. POPOVSKY: Those are all good questions. First, to speak to your first point, I concur with what Dr. Stroncek said, is that except for the prospective study from Palfi, all the other data are retrospective and they are not controlled, so we agree.

However, is it a new syndrome? No, it was probably first recognized in the late 1940s, but it was called by these other terms. The term was first coined in the early 1980s because of the clinical impression that this was strongly related to, in fact, transfusion. Why these patients develop it, I think is unknown.

Let's go to your next point, which is why a recommendation that you would defer a donor who is implicated. Because at Mayo, we had a single donor who was implicated in six cases of transfusion-related acute lung injury. She had a history of 14 pregnancies, and she, through a period of time before we realized what we were dealing with, her blood, and because of the unique system there, they collect blood and they could follow it through in a controlled way, as it were, her blood was implicated in multiple cases of transfusion-related acute lung injury, and these recipients had nothing in common in terms of the types of conditions for which the blood was being transfused.

DR. HALEY: A short comment. In the American Red Cross, we do defer the donors from giving other plasma products as soon as we find a confirmed case, so we can't answer the question would they repeatedly.

We do know that most of these donors have given many, many times before, and not been implicated in the case, but we cut it off at that point.

DR. HOLLINGER: Do you find it unusual

that IVIG and so on has only been associated with rare cases of TRALI?

DR. POPOVSKY: Well, there are some thoughts about that, one of which is that these antibodies are in the process of fractionation procedure being diluted. I suspect, in fact, there are more cases than have been reported, but at least it is a clinical impression that it is an infrequent association. Why? We are not clear.

DR. HOLLINGER: If you examine them for HLA and granulocyte antibodies, almost all of them are positive for IVIG?

DR. POPOVSKY: Yes.

DR. HOLNESS: Mark, I would just like to mention that we have had one case at the FDA where the same donor had 11 TRALI reactions and 2 fatalities from the same donor FFP.

DR. POPOVSKY: Thank you.

DR. NELSON: The next presentation is by Dr. Patricia Kopko from Sacramento Blood center.

Patricia Kopko, M.D.

DR. KOPKO: Good morning. I would like to thank the committee and Dr. Holness for inviting me to speak today. I will admit four years ago I was what I call a TRALI skeptic, I didn't really

believe that this reaction really existed or we really knew anything about it, because when you get one of these cases, they can be very difficult to work up, you are often dealing with multiple donors, you have to get the donor back to work them up, and even when you get the donors back, you often do not find an antibody.

Well, I would like to say that my opinion has changed for two reasons. The first reason is that I now work at a blood center that keeps a sample from each donor for at least nine months, so every time they come in and donate, we have a tube available to go back and test up to nine months later.

The other reason is the following case.

[Slide.]

This case was of a 60-year-old female who had been hospitalized with a two-week history of vaginal bleeding and a recent near syncope. She presented to the emergency room and when she presented her hemoglobin, as you can see, was actually pretty good for somebody who had been bleeding for two weeks. It is 13.1. That is high enough to donate blood.

Her platelet count was normal at 156,000,

her I&R was 4, but it had been 5.8 two days prior, so they were reversing a coumadin effect. She was on coumadin because she had had a prior hospitalization for syncope secondary to an arrhythmia, which was probably why she had the syncope this time.

But the resident on that night decided she had had a syncope from blood loss, decided she needed to have her coumadin effect reversed emergently, and he wrote an order for 200 milliliters of FFP to reverse the coumadin effect.

[Slide.]

Shortly into, about 45 minutes into that transfusion, the nurse walks into the patient's room, the patient is in respiratory distress, the nurse calls the code, the intubate the patient, send her to the ICU.

These are her first few hours of vitals in the ICU. You can see when she gets here this red line is her heart rate. She is already tachycardic. Her blood pressure, this is her systolic blood pressure. Her systolic blood pressure is below 100 when she gets there. Her respiratory rate is at 40, and the resident, deciding that this has to be a fluid overload from

that 200 ml transfusion, decides to give Lasix.

When he gives Lasix, she gets more tachycardic, up to over 140, over 150, her blood pressure goes down, her systolic less than 60. He then decides he has to start dopamine because she is hypotensive. He starts the dopamine and you can see, after the starts the dopamine down here, he gets to the point where he gets her blood pressure back up, so he gives her more Lasix. Again, she gets up hypotensive, and yes, I know, I am a pathologist, but it would just seem to me that they would have figured out that if you are giving both Lasix and dopamine, maybe you shouldn't be giving both.

Anyway, the resident didn't realize what this was. He thought it was a fluid overload, it wasn't until the pulmonary ICU attending came in the next morning that he realized that the attending said you him you do realize this is a TRALI, don't you. The resident did not know what TRALI was.

This woman spends two weeks in the hospital, 12 days of that two weeks she is in the ICU.

[Slide.]

I need to remind you that the problem with treating this like a fluid overload, which is what it is often attributed to, is that this person has just taken a large amount of fluid from the intervascular space and dumped it into their lungs, so they are not fluid overloaded, their intervascular space is actually fluid depleted. You give them Lasix, you can cause severe problems to them.

So, we did the standard workup, which at the time for us was an AHG-CDC for HLA Class I antibodies. That was negative. A granulocyte antibody test, that was negative. We have a rare opportunity here. Not only did we have access to this donor's plasma, but it was the only donor's plasma. There was no other transfusion involved, and it was clear-cut TRALI, so it had to be from this unit.

The PMN priming agent that Dr. Popovsky talked about that is Chris Silliman's theory, has never been shown to be an FFP. So, we knew it couldn't be that. We knew it wasn't HLA Class I, we knew it wasn't granulocytes, what else could it be.

Somebody got the bright idea has anyone

ever looked at HLA Class II antibodies, and that is what we decided to do in this donor.

[Slide.]

Using flow cytometry, we were able to show that this donor's serum contained HLA Class II antibodies to DR53 and DR51. The recipient's HLA DR phenotype was DR53. So, we had a direct match there.

[Slide.]

Based on that, we decided to change the way we did our TRALI workups, so we decided to add HLA Class II antibodies to, if at all possible, both the donor and the recipient. We have, as Dr. Popovsky mentioned, a growing number of cases with HLA Class II. We have submitted a paper to Transfusion that has been accepted for publication, and what I am going to show you is not that data entirely. I have done something a little different for today, because as I mentioned earlier, you often have a number of donors, and it is very difficult when you have a number of donors, to know which donor did it.

As Dr. Stroncek mentioned, you get six donors, there is a good chance one is just going to have an antibody by chance.

[Slide.]

So, what I decided to show you is the cases that we have where we either have just a single unit transfused or the timing of the units that were transfused was such that it had to be one unit.

What we have here is 17 cases in the last two years.

[Slide.]

We have found HLA Class I and II antibodies in five cases, in the donor, of course. We found HLA Class I and II antibodies in one recipient, not in the donor, in that donor/recipient pair.

We found HLA II antibodies in one donor and HLA Class I and II antibodies in the recipient in one case. We found no antibodies whatsoever in two cases, although I have other reasons to be believe there is an unidentified antibody in at least one of these cases.

[Slide.]

We found granulocyte antibodies in two cases in the donor, granulocyte antibodies in one case in the recipient. HLA I antibodies alone in one donor, which I might add we could not pick up

on AHG-CDC, we only were able to pick it up on flow cytometry. And HLA Class II antibodies alone in four cases in the donor.

[Slide.]

So if we go on to how would we prevent this, I would first like to say something about how we screen for this, how we test this, why this wouldn't work, screening would not be practical on a large scale donor basis.

The AHG-CDC test is a transplantation test, and whereas it works well for transplantation labs, to think we would have to screen 6 million donations a year, the capacity isn't there.

Additionally, as Dr. Stroncek mentioned, there are so few granulocytes lab in the country that you could not get routine granulocyte testing on every donor.

Then, the flow PRA that we are using to detect HLA Class I and II antibodies using flow cytometry, I would like to emphasize it is a research test with a Capital R. It is in very initial phases of being used. It would not be applicable to large-scale donor screening.

So, even if you just said we did granulocyte and HLA antibody testing on donors or

even on female donors or multiparous donors, I would like to show you why that probably wouldn't work.

[Slide.]

We would not have been able to detect 10 of these 17 cases with that strategy. In two cases, we detected no antibody. In four cases, we only found HLA Class II antibody. In one case, there was granulocyte antibody in the recipient; one case, the HLA antibody in the recipient; one case, we were only able to detect the HLA Class I antibody by flow cytometry, and this was antibody on the donor.

One more case. The donor had HLA Class II antibodies, which would not have been detected using that testing scheme, but the recipient had HLA Class I and II antibodies.

[Slide.]

Also, I broke down what components were implicated in those 17 cases. You can see it is almost a third, a third, a third; a third FFP, a third red cells, and a third platelets, and I would just like to say for the record that the vast majority of our red cells are AS-1 red cells. We do produce some AS-3 red cells, but to my

knowledge, none of these were from AS-3 units.

We usually only provide CPDA units for neonates and for sickle cell transfusions, and things of that nature. I would doubt that any of these were CPDA units.

[Slide.]

So, the next question is so they have antibodies, does that mean it causes the reaction? Just because the antibodies are there, does that mean we know those antibodies cause it? I think the answer would be no, we don't.

What we do know is that the presence of antibodies is way too common to be coincidence, but that doesn't mean that is what is causing the reaction. For all we know, all these people could have an endothelial cell antibody and that is what is causing the reaction. I don't think we have proven that the antibodies are causing it.

So, the next question you would have to ask is can you find that the antibodies in the donor correspond to the recipient's antigen? So, we have been working on that.

[Slide.]

Eleven cases now we have antigen typing on the recipient's white cells. Four of those cases

we have been able to prove that the antigen and the antibody are identical. Eleven of these cases were somewhat inhibited by the fact that some of these donors have--actually, most of these donors have multiple antibodies. You get the PRA reports back, and they react to 80 to 100 percent of the panel. So, there are so many antibodies, it is hard to distinguish which antibodies are there. We are working on that part.

[Slide.]

I would like to show you the four that we have found. We have an HLA 24 pair, a granulocyte 5b pair, that original DR51 pair, and then we have one case where we are pretty certain there is a B62 there. It's a multiple antibody case, but we are pretty certain there is a B62 there, and we think that there is a DQ3 there.

[Slide.]

What I would like to tell you about next might partially answer some of the questions the committee was asking of Dr. Popovsky. How do we know how many reactions these people are causing or are they causing reactions?

Well, we had a case recently, I believe it is the case that Dr. Holness was referring to, that

illustrates a number of points. The case was from our local hospital where they had a fatality, and I never would have even known of it, that the fatality had occurred except the resident called me and asked me if the donor had ever taken penicillin.

My question was why do you want to know. Well, it turns out that about a half-hour 45 minute into a transfusion, the patient went into respiratory distress, coded, and expired within four hours, and they were thinking that because the patient was allergic was penicillin, maybe if the donor had taken penicillin, that this was the cause of the fatality.

I did my best to try to convince these people that this was, in fact, TRALI. I met with a considerable amount of resistance on the part of the physicians. I was told, and this is my favorite one, "Everyone knows FFP does not cause TRALI."

So, after some education, and I might add a little bit of arm twisting, I did finally get them to report the reaction to the FDA. This patient was a man in his 50s. He was going in for knee surgery, they were reversing his coumadin,

which I think might be a bad thing these days, anyway, they were reversing his coumadin, they gave him the one unit of plasma, he had the reaction, he expired.

Our FDA inspector showed up for the usual post-fatality workup, and he asked us to do a lookback. Now, I am sure that lookback is familiar to everyone on this committee with the recent hepatitis C lookback.

We also do lookback for HIV. If a donor comes back and is newly seroconverted for HIV from the last donation, we go and we look back at the recipients of those units to see if they have contracted HIV. So, we did what we call a TRALI lookback.

[Slide.]

The donor was a 55-year-old and by the definition used here today multiparous female. She had been pregnant three times, had two sons in their 30s, and had spontaneous abortion over 30 years ago. She had never been transfused. We found a strong 5b antibody in the donor.

This was the very first time this donor had ever been implicated in TRALI, despite a 15-plus year history of frequent donation. When I

am talking about frequent donation, I am sure most of you are thinking red cells six times a year. We run a frequent plasma program where under the proper medical supervision we allow our frequent plasma donors to donate jumbo plasma up to 60 times a year, and we use that all for transfusion.

So, this woman had been donating not just frequently, but very frequently.

[Slide.]

So, our FDA inspector asked us if we could back a year and look at all these donations because there were so many donations, and we were happy to oblige.

[Slide.]

And I decided that instead of just asking them to see if a reaction had been reported to the transfusion service, I wanted to know if the patient really had a reaction. So, I asked the transfusion service medical directors to go back and actually open the chart and look at the chart, see if there was a reaction at the time of transfusion.

When you preface odd requests like this with, "The FDA wants us to," it is amazing how cooperative these people can become.

[Slide.]

The donor had made 28 plasma donations that had been transfused in the previous 12 months. Additionally, we still have about 3 liters of plasma quarantined in the freezer, and a couple broke in the water bath, so the donor had been really donating quite frequently.

The recipient's clinical condition precluded evaluation of the transfusion in four cases. What that means is if you are already giving this unit to a patient that has ARDS or pulmonary edema, how do you know if they have TRALI.

[Slide.]

Nine of the 24 transfusions or a full 37.5 were associated with a transfusion reaction, and I need to remind you that 5b is probably present on greater than 90 percent of people. We classified the reactions as mild to moderate or severe.

Mild to moderate we decided was fever, chills, dyspnea or oxygen desaturation. There were four of these reactions of 16.7 percent of transfused units. Severe reactions meaning new onset of pulmonary edema or the need for mechanical ventilation were found in five cases, which is 20.8

percent of transfusions.

I would like to add at this point that the FDA has asked us to go back two more years, and we are still working on that, but in those two years I can tell you we found two more mild to moderate reactions and two more severe reactions.

[Slide.]

This is the interesting part. Only four of these nine reactions were ever even reported to the transfusion service, and the very most interesting thing about this is it wasn't the severe reactions that we reported, and the one severe reaction that was reported, the fatality, they refused to believe it was a TRALI.

I have a theory for this. My theory is everyone knows, every clinician knows you can get a febrile reaction from transfusion. I think this is further proof that very few clinicians know or appreciate or even believe that you can get this type of a pulmonary edema reaction from transfusion, which is why I think most of these reactions were not reported to the transfusion service.

Of those, only two of the nine were reported to the blood supplier, which was us. Of

those two, one was the fatality and one was a mild reaction that happened at a very close time to the fatality and was only reported because of the fatality.

[Slide.]

So, where are we? I think that HLA Class II antibodies are associated with TRALI, as well as Class I and granulocyte antibodies. I don't think that screening blood donors will work. It is too complex, it is too complicated. The technology isn't there for wide-scale screening of blood donors.

As important as the clinical implications, as important as the screening implications of blood donation, I think also what we really need to get at is how frequent is this, how many of these reactions are never being reported, not only to the blood supplier, who then cannot work up the reaction, but to the transfusion services.

That concludes my remarks.

DR. NELSON: Thank you. That is very interesting data.

Any questions? Mary.

DR. CHAMBERLAND: Have you or do you have plans to do these antibody evaluations in a

controlled series of patients and recipients?

DR. KOPKO: At this point, we are working on where we want to go next with this. A controlled series would be nice.

DR. STUVER: I think it would be very important because I mean that is really the problem. You have looked up this one case. I mean if you were to go to another donor who donated as frequently as this woman, and looked back, would you be able to find the same frequency of these unreported reactions? I mean I think that is a huge question.

DR. KOPKO: I think it is a very good point, and we certainly have the data now at least to do a retrospective study like that, because I have got a number of donors now that have been implicated and probably well over 100 transfusions, and to take that and do a parallel series seeing if just transfusion reactions are under-reported would be a wonderful thing to do.

DR. NELSON: Is there any dose response suggestion like the level of antibodies in the donor or when the recipient has antibodies and receives one unit or a small, is the clinical reaction milder?

DR. KOPKO: I don't seem to see one at least with this series, because this woman was consistently giving those 600 milliliter jumbo plasma and everyone was getting them, and you would have to assume at least 90 percent of people were positive for that antigen, only 37 1/2 percent reacted, and there was a spectrum of reactions from mild to death.

DR. SIMON: I also think this data is very interesting and hope that you will pursue the controlled studies. I do have an alternate theory. Back in the days when I used to interact more frequently with transfusing physicians, surgeons would tell me all the time that they see many more pulmonary problems in their patients who are transfused than their patients who are non-transfused, and I think many physicians accept the potential for pulmonary problems as a complication of transfusion. They may confuse it or it may be pulmonary edema in some cases due to volume overload, but it may be that there is a certain tolerance of this that also interferes with reporting.

DR. KOERPER: I think that this is a very interesting report. I am just reflecting on my own

practice. I take care of kids, oncology and BMT patients who are so sick anyway that frequently, these kids have fever, hypotension, and respiratory distress, and it is ascribed to sepsis, and they are transferred to the ICU and taken care of for several days and they get better. Of course, they are put on antibiotics, so the improvement is ascribed to the antibiotics.

I take it there is no test that we can order as we are transferring the patient to the ICU that says this is TRALI, because I suspect that we have cases like this that we have never recognized because of the clinical setting in which they occur.

DR. KOPKO: One of the things we are working on, and I did not bring the data because it is too preliminary, we have a study going where we are taking the white cells from the recipient, reacting them with that specific plasma, and seeing what happens to the white cells.

Additionally, we are pulling just a random plasma off the shelf and reacting the recipient white cells to a random type-specific plasma and seeing if we can find a difference, and it is too early to represent the data.

DR. KOERPER: The other problem, of course, is that a lot of these kids don't have any neutrophils because of chemo or because they are transplants, so there is a piece missing in this theory of how this occurs.

DR. KOPKO: You are right, they don't have neutrophils, and what we are actually looking at more than the neutrophils are the monocytes, and looking for monocyte activation, because HLA Class II antibodies are not on granulocytes, they are not your run-of-the-mill lymphocyte, they are on monocytes.

Then, the other question is how do we know it is not through endothelial cells, and I think that might be a possibility, too, that it is actually reacting through the endothelial cells, and not the white cells, because there is certainly HLA antigens on endothelial cells.

DR. NELSON: Some time ago, Victor McKusick made the comment that he thought that the entire population should know their HLA type, and that we should type the whole population. Maybe that would be some interesting background data if his recommendation was ever carried out.

DR. STRONCEK: The clinical situations

that speak to, it is more than just granulocyte, activated granulocytes. Hemodialysis membranes used to activate complement, you know, there have been several studies that showed C5A was activated, granulocytes were activated, people became neutropenic for a couple hours while on dialysis, and those granulocytes actually ended up in the lungs, and they could show desaturation, but those people never went on to develop TRALI. That is when dialysis was stopped, their neutrophil counts came up and they felt fine.

The other situation is that with granulocyte transfusions, sometimes people get alloimmunized and while they are alloimmunized we may transfuse them with a granulocyte concentrate, not knowing they are alloimmunized, and they will get pulmonary reactions, but those reactions are more transient and seem to be more consistent with just the trapping of neutrophils in the lungs, and as their neutrophils clear over a matter of a few hours, the patients get better.

DR. NELSON: Thank you very much.

The next presentation, Dr. Lynn Boshkov from Oregon Health Sciences University.

Lynn Boshkov, M.D.

DR. BOSHKOV: Good morning. I would like to thank the committee and Dr. Holness for inviting me to speak here.

I have been working for the past few years on TRALI with Chris Silliman in Denver, so I am going to present a slightly different view of TRALI, and our view is basically that this is a two-hit mechanism. This is like ARDS. You have a patient who is susceptible and often you infuse a product that contains granulocyte activating factors.

[Slide.]

I am going to give you an overview of the model because it is a bit different from the model that we have been dealing with earlier this morning. I will tell you how I have been defining TRALI. The background to my interest, which was basically an epidemic of TRALI that came to me as medical director of the transfusion service at University Hospital in Edmonton.

I will show you a number of lines of evidence supporting the two-hit model and a major role for neutrophil priming activity generated during the storage of platelets, specifically whole blood platelets, and red cells.

I will talk about some candidate biological response modifiers and interventions that do decrease this neutrophil priming activity.

[Slide.]

Basically, the two-hit model says that TRALI is a syndrome of abnormal neutrophil activation whereby the bactericidal arsenal of neutrophils becomes focused on the pulmonary vascular endothelium rather than on the bacterial target.

The first hit is neutrophil priming by physiological substances, such as endotoxin LPS, complement, platelet activating factor, which is released in ischemia reperfusion, et cetera, et cetera, and that as a result of this first hit, neutrophils adhere to the pulmonary vasculature.

The second hit then that precipitates clinical TRALI is the sequential administration of a priming agent, which augments the respiratory burst of the neutrophils and causes a release of their granule contents with the resultant damage to the endothelium, capillary leak, and a picture of non-cardiogenic pulmonary edema.

[Slide.]

This is normal neutrophil function where

you have an infection releasing mediators that cause the neutrophils to adhere to the endothelium and then diapedese through it and attack the invading organisms.

[Slide.]

In TRALI, on the other hand, the neutrophils adhere as a result of that first hit, but then with the second hit, they release their granule contents on the endothelium, causing damage and capillary leak.

[Slide.]

As I mentioned, I got interested in this. I am a clinical hematologist by training. I was work actually as a clinical hematologist with a primary interest in coagulation in Edmonton with an appointment at the Red Cross, and then they invited me to head the transfusion service at University Hospital there.

That was in 1993, but we had had an epidemic of TRALI starting around the summer of 1991, and I am going to define TRALI here as respiratory compromise being the predominant syndrome. These patients were critically ill with severe hypoxemia. They had at least peripheral cyanosis, often central cyanosis. Their PO_2s went

down, their O_2 saturations went down into the 80s, sometimes even lower. They all required supplemental oxygen.

We have several intubations. We had one death. So, the reactions were serious to life-threatening, intervention was necessary, and respiratory compromise was the predominant symptom. Almost invariably there were fever and chills associated with it, and the reaction was temporally associated with transfusion of a blood product.

Interestingly enough, the onset here was virtually immediate, within minutes of receiving the offending product, and cleared rather quickly. I think we have a variety of mechanisms that culminate in a clinical picture of TRALI. I think this is a multifactorial disease.

I think this is the neutrophil adhesion, the granulation type of situation. In the cases where chest x-rays were done, there was no evidence of volume overload, the radiological picture was classic for TRALI, non-cardiogenic pulmonary edema, and we excluded cases where other cause was evident. The patient had a pre-existing pneumonia or ARDS or whatever.

We tracked between October '91 and January

of '98, 121 TRALIs.

[Slide.]

The reason we began to think something besides antibody was going on was a number of reasons. The first was that we started to see a upswing in these reactions that accompanied the use of a new blood bag by our blood supplier, so they had gone from one manufacturer to another, they had had to modify collection procedures and centrifugation speeds, and so on, and so forth, to keep up platelet yields.

We saw almost no reaction to the plasma. Six patients, interestingly, had recurrent reactions to products from different donors, and when we first looked, we found little convincing evidence for major involvement of anti-granulocyte or anti-lymphocyte antibodies, so in our initial reactions, we looked at 21 donor plasmas, could implicate an anti-HLA Class I in one of those. None of the first eight recipients we looked at had evidence of anti-granulocyte or anti-lymphocyte antibodies.

[Slide.]

I started to freeze down plasmas from implicated products. I started to freeze down

plasmas from non-implicated products, and patient pre- and post-transfusion plasmas.

We have subsequently been able to test 87 implicated donor plasmas, and have found evidence for anti-granulocyte antibodies in five of those. None of them have evidence of anti-lymphocyte antibodies, and most recently we have tested for anti-DR antibodies, not by flow cytometry, but by a fluorescent bead.

So, although there is some evidence that anti-granulocyte and anti-lymphocyte antibodies were involved in these reactions, I don't think that they were the major radiology in this series of TRALIs.

I didn't know what we were dealing with. We looked at complement generation, implicated blood bags. We looked at anti-plasticizer antibodies in recipients, and none of these avenues indicated there was anything going on there.

[Slide.]

The implicated products--and this is the sort of the timeline here--as I mentioned, over this period of 1991 to January of 1998, we tracked 121 TRALIs. The overwhelming majority of them were to whole blood platelets, and you can see the

incidence here, up to 0.5 percent of whole blood platelets were associated with TRALI type reactions.

Apheresis platelets, which were about 20 percent of the platelets we used at that point in our patients, were associated with very few TRALIs, and one of these was in a patient that had had a previous TRALI to the random donor platelets.

Red cells, much lower incidence, still significant numbers, and plasma, University Hospital in Edmonton, probably we transfused about 10,000 red cells a year and about 4,000 plasmas. Only one TRALI to plasma during that time.

Very interestingly, after we had a fatality due to TRALI, multiple intubations, and we kept on reporting these reactions to the Red Cross. The Canadian Red Cross was our blood supplier. They requested a special inquiry. That report came out in 1994, and they said basically we don't know what is causing these reactions, but, boy, your leukocyte contamination and your platelet products is real high in Edmonton, so why don't you reduce that leukocyte contamination and issue fresher products.

They did that right here. The incidence

of TRALI declined after that, but it had already declined a bit before because actually, we had recognized the population at risk and had started to prioritize our single donor platelets to that population at risk.

[Slide.]

The other thing we did was a nested case controlled study. This has only been published in abstract form by Gwen Clarke, who was my resident at the time. We took 46 confirmed cases of TRALI due to random donor platelets prepared from whole blood and we looked at 225 randomly selected controls who had received random donor platelets during that time and who we verified by chart review and blood bank records had not had reactions.

We looked for patient and product factors that were associated with TRALI by logistic regression analysis.

[Slide.]

We found no difference between cases and controls with regard to age, sex, recipient and donor blood groups, previous transfusions, history of previous transfusion reactions, et cetera.

We looked also at platelet increments at

the next platelet count. We didn't consistently do one-hour platelet counts, but most platelet patients had a reasonable rise in their platelet counts following these very severe reactions.

Interestingly enough, the diagnosis was very important in that the cases were much more likely than the controls to have hematological malignancy or cardiac disease. So, this just wasn't the fact that, you know, if you have hematological malignancy, you are going to get platelets. It is that there is something in having that that predisposed you to having a TRALI.

We have subsequently undertaken a chart review, a detailed chart review. We have over 60 cases now, and they are growing. This is a work in progress. We are pulling them off microfilm and doing detailed chart reviews, but I can tell you that at first go-around, it looks like these reactions are particularly frequent during chemotherapy, particularly induction chemotherapy, and one of the things that has struck me is that a lot of these patients have not received a lot of blood products before they had these reactions, and a lot of the patients have had cardiopulmonary bypass recently.

I have seen a couple of these reactions myself. You know, when we started to see them, if you care for patients with malignancies, you know, they are always having reactions, they rigor, they have febrile reactions frequently to random donor platelet products. They are febrile because they are septic, because they are getting their amphotericin, but these reactions were other.

The nurses were terrified of them. The physicians were terrified of them. Family members used to tell me, you know, she looked like she was going to die. I saw a lady rigoring off the bed. She was just blue, and she said to me I thought I was going to die. So, these are very, very serious reactions.

Post-op status, GI bleeds. I think although I am just reviewing, this is not a nested case control. I think these are probably risk factors. These things do release platelet-activating factor. Ischemia reperfusions does, and I think TRALI is a multi-hit phenomenon.

So, the end result of this nested case control, I think we can say that certain patients seemed to be predisposed to these reactions although the nature of that predisposition is

unclear.

[Slide.]

Interestingly enough, there was a weaker effect of platelet age, which was evident by continuous analysis in that the cases tended to have older units than the controls, and this was consistent with some sort of metaboloid or mediator that is generated during product storage.

What this metaboloid or mediator was, I had no idea. I had been freezing stuff down, and the penny dropped actually when I went to a talk by Dan Ambruso at the ASH in 1992, and he produced evidence that packed cells, whole blood, and platelets contained the priming agent, which enhanced the response of neutrophils and response to a test stimulus fMLP.

Chris Silliman and Dan Ambruso have subsequently gone on to show that exposure to this priming agent does cause shape change in neutrophils, increased expression of CD11 and CD18, which are the adhesion molecules for them to adhere.

This PMN priming activity is seen by two days of storage for platelets, by 14 days for whole blood and red cells. There is no priming activity

in FFP, and that whole blood platelets have a lot more priming activity than apheresis platelets.

The priming agent has also been shown to damage pulmonary endothelial cells in a cell culture model and activate pulmonary endothelium. I don't have time to show you that. It is published in abstract form, and there is an animal model, which I will show you some data from, which Chris published in Journal of Clinical Investigation a couple of years ago, of isolated perfused rat lungs, which has basically confirmed the fact that this sort of two-hit injury with the second hit being PMN priming can cause pulmonary damage.

[Slide.]

This is the priming assay. Neutrophils are isolated. You give plasma with the second hit. You activate with the fMLP, and you measure the super oxide anion production.

[Slide.]

This is stored red cells. You can see that the priming activity in response to fMLP at day zero is a little over 2, and that by 42 days, it has risen several fold.

This priming activity seems to be in the

lysophosphatidylcholine moiety that can be isolated by HPLC from the day 42 plasma from the red cell units.

[Slide.]

When we sent Chris and Dan our products from Edmonton, the results were interesting. These are our platelets from Edmonton, and clearly, the priming activity increases with the age of the platelet. Interestingly enough, these dark bars here are priming activity in the presence of WEB 2170, and one of the points that you will see come out in this is that some of this priming activity is chloroform extractable. It does to be a lyso PC, but some of it is not.

So, WEB 2170 is a selective blocker of the PAF receptor that the lyso PC priming activity works through.

We also were able to show that the units associated with TRALI had higher priming activity than five-day-old controlled platelets.

[Slide.]

Again, that the priming activity was, in significant part, in the lyso PC section of the platelet supernatants.

[Slide.]

When we looked at patient post-transfusion plasmas, and there were 28 paired samples that I gave to them, you can see that the priming activity rose in patient post-transfusion samples as opposed to pre-transfusion samples, but it was higher than normal, and buffer and FFP run together pretty well here in standard plasma, but it was high to begin with in the patients.

So, these are patients that have high neutrophil priming activity, and you bump it even higher. Again, this is the patient plasma, and there is a significant rise in the lyso PC portion of it.

[Slide.]

We were looking for that other part of that priming activity that wasn't lyso PC, however, the part that wasn't WEB inhibitable, that wasn't chloroform extractable, and so we tried to round up the usual suspects, IL-6, IL-8 have been shown to achieve high levels in platelets, and certainly in our platelet concentrates that were implicated, we saw high levels of IL-6 and IL-8 both.

When we looked at the IL-6 and IL-8 levels from the patients, this is the IL-8 and this is the IL-6, and there were rises in both, but the rise

was only significant for the IL-6 although this may be due to small numbers.

[Slide.]

We don't think this increase in priming activity is an epiphenomenon of any transfusion reaction. This is other work published by Chris where he basically looked at patients with simple allergic and febrile reactions, and showed that in these patients, the priming activity did not go up in the pre- and post. These were an additional 8 TRALI patients. Again, he could see this increase in priming activity.

[Slide.]

There is an animal model, and that model is a two-hit model. This is a rat model, so you inject intraperitoneal LPS as your first hit, isolate and perfuse rat lungs, and then enter the perfusate, you add the second hit, which is plasma from the implicated products, and you measure lung weight and pulmonary artery pressure.

[Slide.]

You can see that this is the pulmonary artery pressure here, and you can see that with a double prime of LPS and day 42 red cells, the

pulmonary artery pressures rise markedly. This is not seen with fresh product, day zero, and again there is marked change in the lung weight.

Some of this seems to be WEB inhibitable particularly with regard to the rise in pulmonary artery pressure. Some of it, however, is not particularly with regard to the pulmonary edema.

[Slide.]

We were also struck, and we are doing a detailed review of the Edmonton cases, by the fact that in some cases, the recipients don't seem to have granulocytes, so how can you say that there is neutrophil priming activity that is causing these reactions.

I had a very interesting pair of cases which we published in abstract form, and was presented as a poster at ASH last year. Basically, this was 5-day-old apheresis product, which was a double donation, so it was divided and right before out date, the first half went to a CML patient on STLI-571, which we use a lot of in Portland, and that patient had a classic TRALI, whited out his lungs, just about required intubation, and had a radiological picture of non-cardiogenic pulmonary edema.

The second recipient, however, and I did not know this was a double donation, went to a 10-year-old girl with Burkitt's lymphoma, and an absolute neutrophil count of zero. She also had a TRALI, a milder one. She did desaturate. They said, boy, you know, we thought it was epinephrine ICU time for her, but her picture was not quite as marked as the first patient's.

Interestingly enough, we weren't able to see any evidence of anti-granulocyte or anti-leukocyte antibodies in either the donor or either of the recipients, however, the priming activity in the product was quite high, and interestingly enough when we measured a vascular endothelial growth factor, or VEGF, which is released by platelets during aggregation and accumulates in platelet concentrates during storage, the levels were ski high.

We have been looking at VEGF levels, and it is a bit too preliminary to give you any data on this, but some products do have extremely high levels, and this was one of them, and indeed in the neutrophil priming activity in the post-plasma on the first recipient, who had the neutrophils, went up, in the child it did not.