VOLUME II

ADVISORY COMMITTEE ON BLOOD SAFETY AND AVAILABILITY

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Thirty-fourth Meeting

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The above-mentioned meeting of the Advisory Committee on Blood Safety and Availability was continued on Friday, May 30, 2008, commencing at 8:35 a.m., at The Hilton Rockville Hotel, 1750 Rockville Pike, Rockville, Maryland 20852, before Robert A. Shocket, a Notary Public.

REPORTED BY: Robert A. Shocket

APPEARANCES:

PARTICIPANTS/MEMBERS:

ARTHUR W. BRACEY, M.D., Chair
JERRY A. HOLMBERG, Ph.D., Executive Secretary
RICHARD J. BENJAMIN, MBChB, Ph.D.
ANN MARIE BENZINGER
JAMES S. BOWMAN, III, M.D.
WILLIAM DUFFELL, JR., Ph.D.
JAY S. EPSTEIN, M.D.
ANNE MARIE FINLEY
HARVEY KLEIN, M.D.
LIEUTENANT COMMANDER LOPATKA
ILEANA LOPEZ-PLAZA, M.D.
KLAUS NETHER
GREGORY J. POMPER, M.D.
GLENN RAMSEY, M.D.
LINDA THOMAS-WADE
DARRELL J. TRIULZI, M.D.

(Appearances Continued on the Next Page)

APPEARANCES CONTINUED:

GUEST SPEAKERS/PRESENTERS:

H. FRANKLIN BUNN, M.D.
JEFF CARSON, M.D.
LARRY DUMONT, M.B.A., Ph.D.
MARK GLADWIN, M.D.
SIMONE GLYNN, M.D., MSc, MPH
COLLEEN GORMAN KOCH, M.D., M.S.
TIMOTHY McMAHON, M.D.
MARIA STEINER, M.D., M.S.
DARRELL J. TRIULZI, M.D. (Written Statement)

PUBLIC PARTICIPANTS:

LISA CARBO
BASIL GOLDING
WILLIAM G. MURPHY, M.D.
TERESA WIGMAN

P-R-O-C-E-E-D-I-N-G-S

DR. BRACEY: Good morning and welcome to the second day of the 34th meeting of the Advisory Committee on Blood Safety and Availability. We heard a great amount of data yesterday regarding platelet safety issues. Today we have a number of distinguished presenters who will share information with us regarding red blood cell physiology and outcomes associated with red blood cell transfusion. Mr. Secretary, would you like to take the roll call?

DR. HOLMBERG: Sure. Thank you. Dr. Benjamin?

DR. BENJAMIN: Present.

DR. HOLMBERG: Ms. Benzinger?

MS. BENZINGER: Here.

DR. HOLMBERG: Ms. Birkofer is absent. Dr. Bloch is absent. Dr. Bracey?

DR. BRACEY: Present.

DR. HOLMBERG: Dr. Duffell?

DR. DUFFELL: Present.

DR. HOLMBERG: Ms. Finley?

MS. FINLEY: Present.

DR. HOLMBERG: Oh. Dr. Haley is absent. Dr. Ison had to leave. Dr. Pierce is absent. Dr. Lopez?

DR. LOPEZ: Present.

DR. HOLMBERG: Dr. Matyas, Mr. Matyas? Juan Pierce is absent. Dr. Ramsey?

DR. RAMSEY: Good morning. Present.

DR. HOLMBERG: Dr. Pomper?

DR. POMPER: Present.

DR. HOLMBERG: Ms. Thomas-Wade?

MS. THOMAS-WADE: Present.

DR. HOLMBERG: Dr. Triulzi?

DR. TRIULZI: Here.

DR. HOLMBERG: And then on the government side, Dr. Epstein?

DR. EPSTEIN: Here.

DR. HOLMBERG: Dr. Klein?

DR. KLEIN: Here.

DR. HOLMBERG: Dr. Bowman?

DR. BOWMAN: Here.

DR. HOLMBERG: And Dr. Kuehnert is absent. And Lieutenant Commander Lopatka?

L.C. LOPATKA: Here.

DR. HOLMBERG: We have a quorum, Mr. Chairman.

DR. BRACEY: All right. What I would like to do is yesterday in our discussions regarding the various reports on adverse events, we did hear about some potential gaps and we discussed earlier three specific recommendations that we thought would help improve the ability to monitor and assess adverse events. So what I would like to do initially is to simply to flash up on the screen -- the file is labeled ACBSA Recommendations for 5/30 and Transplantation. And, I don't want to really finalize this now but I've got a working group who, that's volunteered, Dr. Epstein and others, but specifically, I'll just read it through.

"Whereas the HHS Advisory Committee on Blood Safety and Availability is charged with advising the Assistant Secretary on public health issues related to safety of tissue and organ transplantation, the Committee recognizes the need for the following measures: One, acquisition of data on tissue use to allow current surveillance activity done by HHS to better determine the frequency of adverse events reporting; two, capture of appropriate data regarding etiologic agents of infections reported following organ transplantation to allow for better assessment of infectious risk related to transplantation, and, three, support for improvement of infectious disease assays to meet the need for accurate tests with rapid turnaround time to allow for efficient organ procurement to enhance organ availability. Current systems approved for diagnostic testing should be evaluated for screening potential organ donors."

So the broad issues are stated and I would like basically to have the working group, that this come back to the Committee, so later in the afternoon we can digest it. Are there any major grasp seen of what we have thus far? Okay. That said, that will be the plan.

Moving on to today's business, this morning's business, then, Mr. Secretary, could you flash up the questions from the Assistant Secretary? Today the information that the Assistant Secretary seeks from us is the following and I would ask you to keep this in mind as you hear the presentations. One, do current data support a change in medical practice from transfusing red cells stored for as long as 42 days to transfusing red cells that are stored for much shorter periods of time? If so, what impact would the shift in practice have on blood availability in the U.S.?

Two, is there a need for additional research to evaluate red cells stored for longer periods of time, are as safe and clinically effective as red cells stored for shorter periods of time? And also to understand the nature of the red cell storage lesion.

Third question is what impact would a change in transfusion medicine practice have on blood availability? And four, should the blood banking industry strive to produce improved red blood cell products? So as we hear the information today, I would ask you to keep those questions in mind because our task is to advise on those specific issues.

To begin, I would like to introduce our first speaker this morning. We're very privileged to have Dr. H Franklin Bunn present on clinically significant biochemical physiologic changes in red cells during storage. Dr. Bunn is research director of the hematology division of Brigham and Women's Hospital. He's done major work in the field of red cell physiology, including work on leukoreceptors and many aspects of red cell physiology. Dr. Bunn is past present of the American Society of Hematology and a fellow of the American Academy of Arts and Sciences. Thank you, Dr. Bunn.

DR. BUNN: Thank you, Dr. Bracey. It's a pleasure to be here. What I wanted to do this morning is to present an overview of the nature of the so-called storage lesion and how it impacts on viability and function of transfused red blood cells.

As we noted, when the blood is taken from, fresh from the body, red cells appear as really uniform appearing biconcave discs.

When they're stored in a standard medium, for transfusion purposes, the red cells undergo a multitude of changes and that then impacts metabolism of the red cell function, the hemoglobin, complex membrane structure function changes. These impact on the flow of blood through the microcirculation, the rheology of the transfused red cells and also impact on the viability, the survival of the transfused red cell in vivo and you end up with a cell that has lost some hemoglobin so it has higher hemoglobin concentration. It has less conformability and it has a shape change. I'll get into these in a little bit more detail as we go further.

So, in order to first address the issue of metabolism, this is an outline of the primary metabolic pathway in the red cell, the Endon-Meyerhoff (phonetic) pathway, lipolytic pathway from glucose to lactate. Now, also included on the slide is the conversion of pyruvate into the Krebs cycle, the TCA cycle. And that is -- oh, here we go, yeah, the TCA cycle, here, that's present in all cells having mitochondria; however with the red blood cells conditioned in the bone marrow, it loses both its nucleus and its organelles including mitochondria and therefore it loses its Krebs cycle.

So its metabolism then is anaerobic, not dependent on oxygen. And you see then that the energy accumulation through ATP is greatly limited by this process. Instead of making 36 mols of ATP per mole of glucose oxidized, one has only two ATP molecules per mole glucose. So the red cell is very limited in this regard.

Now, the red cell is special compared to any other cell in the body and having a very prominent shunt from 1-3 diphosphoglycerate to 2,3-DPG through the enzyme, DPG mutase. And that DPG then can cycle back into three phosphoglycerate through aphosphatase. Turns out that these two enzyme functions are actually on the same polypeptide but that's not important information for our discussion today.

So, 2,3-DPG is present only in micromolar concentrations in most cells of the body whereas in the red blood cell it's very high concentrations, 5 millimolar. Indeed, hemoglobin tetramer and DPG functions to bind the hemoglobin tetramer to mediate a marked and physiologic reduction in oxygen affinity to the red blood cell. And this is very important in the events that accompany blood storage.

So, and basically the red blood cell has modest metabolic obligations and they include, important ones are shown here, the maintenance of cationic pumps, maintenance of 2,3-DPG, reduction of met-hemoglobin and maintenance of membrane integrity.

Now, the normal red blood cell contains five millimols DPG, as I mentioned, whereas there's a marked fall during blood storage in 2,3-DPG. This is shown from earlier studies that I did when I was in the Army years ago, with a marked fall in 2,3-DPG levels over time, and ACD even more marked fall, in ACD adenine. The decay in 2,3-DPG during storage can be delayed by the addition of inosine. The more recent data from Bennett-Guerrero, et al., Dr. McMahon's group, is shown here, and below, and allowing for a difference in the time scale on the X axis, the data are really very similar for CP2D, very rapid decay in 2,3-DPG.

Now, that's accompanied by a very rapid decay in the P50 red cells during blood storage. P50 is an index of oxygen affinity. Normal P50 is about 26 millimeters of Mercury, and, during blood storage there's a rapid decay during the first week to a P50 of around 15. So this signifies an increase in oxygen affinity.

So the two phenomenon, falling DPG and increasing oxygen affinity of course are tightly linked because DPG is the main allosteric modifier of hemoglobin function in the red cell. So, that here we have two oxygen binding curves, fresh blood with P50 of 26. That's, the 50 percent saturation would be about 26 millimeters of Mercury and then the marked shift to the left with increase in oxygen affinity with blood that's stored over a week or ten days.

Now, the importance of this is at the degree to which oxygen can be unloaded from fresh blood versus stored blood. Fresh blood again is five millimolar DPG and with a marked decay with storage. So the unloading with fresh blood is shown here going from an arterial PO2 to a mixed venous PO2 of 40. And you can see that about 15 percent on the average, of the oxygen is unloaded to the tissues in contrast with the left-shifted oxygen binding curve, with stored blood, very much less oxygen is unloaded, maybe a third as much.

Now, these, of course, are a highly hemotized diagram and the amount of oxygen unloaded in different tissues, different organs is highly variable but the overall picture is depicted reasonably well by this simple diagram. And you can see here the correlation between the P50 and the DPG level during blood storage.

Now, importantly, as red blood cells from the bank blood are infused back into patients, there is generally a rapid regeneration of 2,3-DPG over time.

You can see here this is recent data from Heaton, et al., 1989. It confirms previous studies showing that over time and specifically in about six hours that half of the DPG has been recouped in the stored blood, as shown in this lower diagram. These studies were done by an Ashby technique to recover the transfused red cells by antibody panning.

So, that the problem with increased oxygen affinity of stored blood is a transient phenomenon; however, there's an important caveat here and that is that these studies, all the studies I mentioned are done in reasonably healthy recipients. In very sick patients it's not at all clear that the time for recovering, recruitment of 2,3-DPG is as short as half time of six hours. So that's something that's worth pursuit and further investigation.

Now, ATP is an equally important player in determining the viability and function of stored red blood cells. The fall in ATP, as I will show you, is less dramatic. Normally there's about one millimolar ATP in fresh blood cells, varies with the age of the red blood defeasor (phonetic) -- 21 day life span of the red cell but with storage there is a dropoff in ATP levels.

And with the decay in ATP, there is consequences. There's leaking of potassium and as a result water from the red cells of the hemoglobin concentration, the red cell goes up somewhat. This alone makes the stored red cell more rigid, less deformable. In addition, there's loss of membrane through microvesicles.

This is a very important research topic. It's not one that has gained a lot of support but it's one that has attracted the interest of a number of investigators and in concert with microvesicles from other cells including platelets is a topic that deserves a lot of scrutiny because micro red cell vesicles can be, can have pathophysiologic consequences. I don't have time to go into detail on this but it's something that bears concern with the knowledge that during red cell storage there is shedding of microvesicles. And then there is some hemoglobin loss, as I will show you.

Now, I think even more important than loss of these materials is the fact that the perturbations within the red cell membrane during storage. There is oxidation of proteins. There's an impairment of the assembly of spectrum of band 4.1. This can contribute to the rigidity of the red cell. There appears to be loss of sialic acid residues which decreases the negative charge on the red cell which allows the red cells to agglutinate or aggregate each other more than they normally would.

There's loss of phospholipida. Asymmetry, which may have pathophysiological consequences and then morphologically one sees echinocytosis, which I tried to diagram as a scalloped border but what it really looks like is -- I'm sorry you can't really see it, it just doesn't show up well enough but anyway these are spiny, spiculated red cells. Not all red cells during storage develop this appearance. It's logical to think of the ones that do are the more damaged and have more perturbation than red cell membrane structure and function.

So, there are important pathophysiologic consequences of red cell storage which I'll talk about individually, decreased deformability, impaired blood flow, impaired oxygen delivery, hemolysis and disordered nitric oxide homeostasis. We're going to hear a lot more about NO later but I did want to touch on it in this introductory talk.

First decreased deformability, these are data from D'Almeida, where a micropipette is used to suck a small portion of the red cell membrane through negative pressure into the narrow bore of the micropipette. The pressure, the negative pressure required for pulling a bleb of a membrane into the micropipette is a very accurate and reliable measurement of red cell membrane stiffness and overall red cell deformability.

And you can see here that during blood storage there's kind of a shift to the left, if you will, of these nomogram, the bar graph here. So this is fresh blood and as during storage there is an increasing shift to the left here, which indicates progressing, a progressive decrease in red cell deformability. And then these, of course, the cells vary considerably and heterogeneity is an important theme that I'm going to come back to during red cell storage, that it may be that a few bad actors, a minority of the red cells that undergo storage could have the most important deleterious effects when transfused into certain patients.

Now, the key question is does this decrease in red cell deformability have in vivo consequences, does it impact on blood flow in vivo and oxygen delivery in vivo? And there are a number of studies that have been done to address this but I think the one that perhaps is to me is among the most convincing is a paper published by Tsai, et al., from the group, University of California, San Diego.

And what they did was to take a hamster model on which they did an isovolemic exchange, transfusions, with the idea of first challenging the animal by removing the bulk of the red cells from the animal and then once the animal has maintained the normal bloodline but with a marked reduction in red blood cells, asked the question as to whether it matters whether that animal has been transfused with fresh red blood cells or stored red blood cells.

And so that the idea is that there's this progressive removal of red blood cells going from a hematocrit of 47 to 28 and then a level two, to 19 and the colloid that's replaced is Dextran 70 and then in the in level three, the endogenous red cells of the hamster are replaced either with fractionated or replaced with either fresh red blood cells or stored red blood cells. And you can see here that both arteriolar and venular blood flow is compromised somewhat when the replacement is with fresh red cells but, markedly so, marked decrease in blood flow when there's replacement of stored red blood cells.

And, the impairment of oxygenation follows suit, that the middle bar shows tissue oxygen tension. These experiments are done in a capillary window that's engineered into the skin of the abdomen of the hamster.

And you can see here that, of course, there's a drop from arteriole to venule, venular oxygen tension but importantly at the tissue level the middle bar in the lower panel, there's a much lower oxygen tension when these animals have circulated stored red blood cells as compared to circulating red blood cells.

Now, in addition to impairment of blood flow and impairment of oxygen transport there's also a concern about hemolysis. The AABB requirements now for transfused blood is that within 24 hours 75 percent of the circulating red cells be viable and remain in circulation at that time period. That means that 25 percent of the transfused red cells are destroyed, can be, up to 25 percent can be destroyed in blood units that are issued to patients for transfusion. This can be a huge amount particularly in patients who are receiving multiple units of blood. So, this is an issue of considerable concern and we need to delve into the consequences of this hemolytic load on the patient who is receiving this blood.

So just to put this into context, the study from Dr. McMahon's group looked at the accumulation of hemoglobin in the plasma during storage and what you see here is a significant although rather modest rise in hemoglobin in the plasma during storage of blood. It goes to about O.02 millimolar during, after a couple weeks of storage. Now, this in itself is, you know, reflects, as I mentioned before, a leak of a number of materials from the red blood cell during storage but even more concerning is the fact that once this blood is transfused, if you consider that up to 25 percent of the red cells can be destroyed within a day and the actual data show that with these bad red cells that are destroyed are actually destroyed even sooner than a day, is a rapid collapse and survival of the nonviable red cells during storage, that following infusion, I made this back-of-the-envelope calculation that the relative amount of hemoglobin released into the plasma goes from .02 to a 50-fold increase of one millimolar. So there's a vastly higher amount of hemoglobin that is the least following infusion.

Now, one can argue with this to some degree because there's a lot of assumptions that are made in this calculation. It has to do with how short the survival is of the bad red cells that were destroyed. Extravascular hemolysis would be higher than intravascular but it's important to realize that even with extravascular hemolysis there's still hemoglobin leak into the plasma. The T-1 half of nonviable transfused red cells is roughly of the order of five hours. The T-1 half of hemoglobin from lysed red cells is roughly the order of one hour.

So putting all this together, I think one can, this figure may be off by a factor of five but I don't think it's off by more than a factor of five and can go in either direction. So that one can end up in any case with facing, particularly in patients receiving multiple units, that you can have a very large load of plasma hemoglobin in patients who get conventional blood transfusion.

Now, what are the consequences of this? One would, and, hemoglobin is a subject that was recently covered in an FDA meeting that I had the pleasure of attending and the therapeutic use of HBOCs, hemoglobin-based oxygen carriers, so that there's a ying and yang of hemoglobin. There's a very strong reason to think that hemoglobin products can be developed in a salutary, positive way for therapy particularly in acute medical situations but the yang is that legal hemoglobin can be toxic.

And there are many reasons why hemoglobin can be toxic including release of oxygen free radicals, release of heme and other issues which were not germane to today's discussion but I would like to consider briefly disordered nitric oxide homeostasis with the idea of course that in Dr. Gladwin's and Dr. McMahon's talks there's going to be much more consideration of nitric oxide.

So first of all we have the issue of in vivo hemolysis. When you think about the large load, as I mentioned, of hemoglobins going to be unloaded into the plasma in rapid red cell destruction, transfused blood, this hemoglobin is much more, has a much higher potential for an NO scavenging than does the red blood cell. I think there's about three orders of magnitude's difference in the ability of circulating red blood cells to establish NO owing to the fact the parameter of blood flow as red cells circulate through the microcirculation and a huge perfusion barrier for red blood cells to capture the nitric oxide that's made in epithelial cells. In contrast with free hemoglobin circulating in the plasma in immediately juxtaposition to the endothelium, so it's right there where the NO that made in the endothelial cell can leak out and be captured.

So this is an important consideration. Now, it would be a transient phenomenon but it still could be clinically important, particularly in patients, in critically ill patients who are receiving a large amount of blood. The other issue that's been raised is the decay of SNO hemoglobin during storage. This is a topic that Dr. McMahon is going to be presenting in detail. I just had a couple of comments on it.

This is a diagram that was published by

Isbell, et al., from the Alabama Group that I understand has just come out in Nature Medicine. And it shows in this diagram, there are three independent mechanisms that have been proposed to mediate a physiologically crucial phenomenon and that's hypoxically mediated vasodilation. This is an adaptation whereby hypoxic tissue can undergo appropriate vasodilation to enhance blood flow to address the need of that tissue for oxygen.

Now, I won't go into ATP at all although just to mention that ATP can be released in stored blood, may be important in that way and so that may play a role. Mark Gladwin's going to be talking about the importance of nitrite and on the right-hand side is the, a depiction of a very interesting and heuristic proposal from Jonathan Stamler's group that postulates a critical roll of the Beta 93 cysteine and the hemoglobin molecule for reversible binding of nitric oxide through as a nitrosal file.

And this is presented, this is proposed to be an allosteric process. It's well known that the hemoglobin when it's in the oxygenated state has a -- reactor, Beta 93 file can take up a number of reactants and adducts but that when hemoglobin changes to the D-Oxy structure that the Beta 93 is much less reactive. And so Stemler and his group proposed that this could allow for a very elegant process whereas red blood cells which have taken up SNO through, from nitric oxide released from endothelial cells in microcirculation, once they encounter hypoxic tissue that NO is released and can serve as a vasodilator.

Now, this has been an area of great interest and also considerable controversy. This paper in Nature Medicine actually presents data on a knocked-in mouse model where the mouse has circulating human and hemoglobin A and all the red cells, except that the Beta 93 has been replaced by, cysteine has been replaced by an allomine. In that study they do not find any disorder or disturbance in vasoregulation. However, I think it's very important to point out this is a valuable animal model -- perhaps the wrong challenges -- it's very important for this animal model to be studied by many labs and the proper test done on the mice to really test the important issue of whether or not Beta 93 cysteine is physiologically important for NO regulation.

Now in addition to, one question that arises is two recent papers in PNAS which both show both from Duke University, that show a decline in SNO hemoglobin over time. And you can see that the decline is extremely rapid. And in the case of Dr. McMahon's group it's three hours of decline and in the studies from the Stemler group there's a marked decline by day one and it falls even further.

Now, the question is -- this may well be physiologically significant and we'll hear more about that from Dr. McMahon's talk but one question is does it address a critical, a widely read and quoted recent paper from the March issue of New England Journal of meds by Cochlar, et al., which showed that there was a deleterious effect in terms of complications, clinically significant complications including mortality when patients receive blood that's prolonged storage of blood that would be greater than 14 days versus blood that was of short duration storage which would be less than that.

I think that their findings if their findings are corroborated cannot be explained by this NO phenomenon. This SNO decay, this SNO decay is occurring so rapidly that it would not distinguish between the short, shortish preservation time versus the longer preservation time, which was the crux of the New England Journal paper.

So, in conclusion I hope I made a case that the storage lesion is a misnomer. In fact, that it's extraordinarily complex, biochemically, structurally, functionally and it's clearly multifactorial. I have the prejudice that the impact of storage on hemoglobin oxygenation and NO homeostasis may be important but probably only important in critically ill patients where a timeframe of hours during transfusion therapy is critical for that patient's morbidity and mortality whereas in other settings and in fact in general, I think that more emphasis should be placed on studies that further investigate the red cell membrane because perturbations of the red cell membrane are, certainly are likely to affect blood flow adversely in the microcirculation; therefore, impact on tissue oxygenation and these changes would affect not only the acute situations but even patients who are surviving beyond an acute period of time and in which in the case of the red blood cells recoup this DPG, has recouped its NO functions, the NO scavenging phenomenon has taken its course. But yet these membrane changes are probably not reversible.

And therefore I would like to leave you with an earnest plea that those who are in charge of convincing or influencing the spending of research dollars for blood research should put a very high premium on studies that will investigate in detail the nature of the molecular mechanisms involved in deleterious effects of blood storage on the red cell membrane because I think that there's a good chance that practical measures can be adapted on the basis of better knowledge and more research that could address this defect in blood storage and have a salutary effect on transfusion therapy. Thank you.

DR. BRACEY: Thank you, Dr. Bunn. Questions or comments from the committee to Dr. Bunn? I did have one question that did come to mind, and that is in terms of the hemolytic load, you mentioned that the microparticles can have other pathophysiologic effects. One of the effects that I began to think about is the role of the RE system and is there an up-regulation a down-regulation, you know, what exactly, what are your thought in terms of that?

DR. BUNN: Exactly. I think that the microparticles have been shown in experimental systems to impact on the RE system. There's concern about their functioning in a prothrombotic way that could be particularly, again in, inflamed or critically ill patients could exacerbate microthrombi and impair blood flow through the microcirculation. And this is an area where, as I mentioned, there are a few investigators who have a major interests in responsibility but it's an underly-developed area of research.

DR. BRACEY: Thank you. Question from the audience? Could you use the microphone please?

DR. GLADWIN: Frank, that was a lovely presentation. I just want to make a comment about these microparticles. There's a lot of data coming out that's unpublished from Western and other groups now, there's a Danish group I saw them present their data showing very striking relationships between microparticles from the red cells, like a foreign -- microparticles on activation of the hemostatic cascade. So at one point to TAT a variety of pathways and these microparticles express PS, phosphatidylserine, which activates the system.

And the other comment about micro particles is they, the ability of the effective nitric oxide scavenging is proportional to the relative surface are of hemoglobin so as you decrease red cell size to the microparticle there will be dramatic increases in NO scavenging, so you also knock out the other pathway which could affect platelet activation. And I was just in Seattle, and there's a young investigator there named Tim Watkins, who has analyzed the aired-Ness, the randomized trial transfusion of leukoreduced and nonleukoreduced red cells and in that trial leukoreduction, which we do across the United States, did not modulate the clinical outcome. It wasn't leukoreduction that was responsible. And what he sees is the more older the blood, the more lung injury, and that there was strong correlations with the number of measured microparticles. So I absolutely agree that this is a very important area for a number of reasons.

DR. BRACEY: Dr. Holmberg?

DR. HOLMBERG: Thank you for your presentation. You commented about the hemolysis between the storage and the transfused five-fold increase and also the membrane changes. Are you aware of any studies out there that have looked at this in regards to mechanical damage, for instance, like through pump action on the red cells?

DR. BUNN: That's a very interesting issue. In fact, in our practice at our hospital, the great bulk of our consults where the issue of transfused blood safety arises, we happen to be a cardiology hospital and a lot of patients who have, in, post or during cardiac bypass surgery, and there is no question that mechanical trauma can, you know, exacerbate the storage lesion. It hasn't been properly investigated to my knowledge and it's another example of the interface between the patients who need a fair amount of blood and the fact that in the setting of a mechanical trauma that blood can have more adverse consequences than it normally would. So it is a very key question.

DR. BRACEY: I think we have one last question from Ms. Thomas.

MS. THOMAS: Excellent presentation, and as a patient advocate, I would really like to see that we recommend and implement what has been discussed today. I just want to thank you for that presentation.

DR. BRACEY: Okay. Well, thank you, Dr. Bunn, and then we will move on to our next speaker. The next speaker is Dr. Mark Gladwin. Dr. Gladwin is the chief of pulmonary vascular medicine at the NIH

NHLDI, and he will present on new insights into healthy red blood cells, the red blood cells regulate blood flow and inflammation.

DR. GLADWIN: Thank you, everyone. That was a wonderful presentation. I think everything that I will present will really emphasize and perhaps go into slight more detail, many of the pathways that Dr. Bunn presented so I think this will be easy to follow and it really amplifies these messages.

The major point I want to make today is show you three examples of how the red cell is not just a bag of hemoglobin, as I've stated here, that it's a living, breathing cell with impressive functionality and despite growing appreciation of this we really have left the research of this important cell backwards in time, you know, where 25 years ago this was an area of, investigation, and we really dropped all research in this important area.

And I'll make a point at the end that this is one of the most used human therapeutic agents, that's never been approved by the FDA, and it's one of the most understudied and under-understood human therapeutic agents. So I think it's vital that we study this in the interest of great science but also public health. And I think if everybody can just relax and look at this presentation, and I just want to show you sort of a canvas of the stars and say, wow, you know, this red cell is really interesting and complicated and just make a point that there's a lot of work that has to be done.

So the old view of the red cell is that it's a bag of hemoglobin and as you can see it's one of the most tightly packed collection of protein of virtually any cell. And you can see the 64 angstrom hemoglobin molecule here and how -- let's see if I can use my pointer -- yeah, how it's just packed within the red cell. 99 percent of the protein is hemoglobin, which is your oxygen-carrying molecule. One percent of the cell contains many, many enzyme systems, as Dr. Bunn highlighted as well as structural proteins that give the red cells characteristic, important shape.

So what else does it do? Well, Frank

mentioned these very important nitric oxide retardant properties. The red cell is designed to block the entry of nitric oxide and I'll review that. It has very important antioxidant and energy-generating properties that limit its oxidative damage and limit its hemolysis. And I agree with Frank that this is probably an important lesion in stored blood. It's got very important blood buffering properties. It's very important when you transfuse eight units in critically ill patients during resuscitation, and probably most importantly -- and I think we agree with this -- it has a rich collection of enzymes and structural proteins organized beneath the membrane forming energy and vasodilatory metabolomes.

Phil Lowe has elegantly shown that there's a glycolitic apparatus that assembles and deassembles underneath the membrane of oxygenation and deoxygenation. There's the data that Frank talked about with ATP generation and release. That's an enzymatic system. It's G protein coupled. There's a nitrite reduction pathway, which I will briefly review.

There's the ability to release an NO file, NO equivalent which will be reviewed by McMahon, and there's very provocative new data and I'm going to show this to you just to make you appreciate how rich this field probably is as we study it more that maybe the red cell action can make NO by NO synthase enzymes.

Finally, I want to point out it has other properties not related nitric oxide that may be of more importance than nitric oxide. It has interesting antiinflammatory properties. There is very nice work from a young investigator, Janet Lee. She's shown that the Duffy blood group potently scavenges cytokines, and with the aging of blood in storage there's oxidative damage in Duffy and it stops finding cytokines. Our red cells are cruising around our bodies sucking up inflammatory cytokines and we infuse old blood, that we may giving blood that can't scavenge these inflammatory cytokines.

I'm going to talk about nitric oxide, however. And this is not nitrous oxide which the anesthesiologist would you give you as an anaesthetic.

This is a little more boring. It's a diatomic gas molecule like oxygen or nitrogen but it has an unpaired electron so it's a free radical and it turns out to be a critical stabling molecule that maintains our blood vessel flow and vascular health.

We now know that the endothelium, the cells that line blood vessels make nitric oxide. They have these enzymes called nitric oxide synthases, right here. They convert the amino acid arginine to citrulline and make nitric oxide. This nitric oxide diffuses into smooth muscle where it activates guanylyl cyclase to make cyclic GMP, which is a downstream signaling molecule that opens up and relaxes the smooth muscle so it increases blood flow.

And this is why I say it's a vital molecule for maintaining our vascular health. Nitric oxide regulates our blood flow. It increases our basal blood flow by 25 percent. So, if you block your nitric oxide in your body your blood flow drops 25 percent, big effect on resting blood flow. It blocks clotting by inhibiting platelet aggravation and attachment. It blocks the production of very important adhesion molecules which stick our cells within our blood vessels, such as VCAM, ICAM and E-selectin. It inhibits the release of a vasoconstictor and growth factor, endophilin-1, and it inactivates superoxides, which is really a diffusion-limited oxidant. If you were to destroy nitric oxide, as happens in cardiovascular disease, sickle cell disease, other conditions, all of these pathways are then impaired, so having a big, creating a big problem for normal blood flow and perfusion of our vital organs.

So one of the paradoxes in vascular biology is that nitric oxide is made by our endothelium, by these cells that line our blood vessels; yet, within our blood vessel there's a massive quantity of hemoglobin, the oxygen-carrying molecule. The reason this creates a paradox is that hemoglobin, as Frank mentioned, destroys nitric oxide. It reacts with the heme group here to bind or sequester the nitric oxide and it reacts with the oxygenated heme group to oxidize the nitric oxide to nitrate, which is an irreversible oxidation. And these reactions are very fast when we look at the chemical reaction rates. So if you calculate how much hemoglobin we have inside our blood vessels and how little nitric oxide we make, you would calculate just based on chemical kinetic calculations that all of the nitric oxide would be destroyed, that it couldn't function; yet, we know that it does function.

So how is this possible? And how this works becomes vitally important to potential storage lesions in red cells. And this slide outlines in cartoon form how this pathway works. And, here you have the normal red blood cell, the normal functioning red blood cell and around it in yellow we have these nitric oxide diffusional barriers.

So, nitric oxide is made by the endothelium, by the nitric oxide synthase. The NO can get to the smooth muscle to do its, to exert its functionality because the hemoglobin is safely compartmentalized in this normal formed red cell. And there's an unstirred layer, shown in yellow here, and there's a cell-free zone in laminar-flowing blood. And by the way, the glycocalix also helps create this distance between the red cells and the source of that production. And this reduces the reaction rate with the nitric oxide with the hemoglobin by a thousand-fold. So it prevents this scavenging or consumption reaction.

So what happens when we infuse cell-free hemoglobins as therapeutics or we give aged blood that forms microparticles or hemolyzes, as this free hemoglobin now gets between the glycocalix, it gets between the endothelium and smooth muscle and it destroys the nitric oxide very quickly, a thousand times faster than what is in the red cell.

The other area, which is a very rich area of scientific discussion -- and you will hear about the work from McMahon's group and Stamler's group later in the day -- is the idea that the red cell is not only destroying but it has functionality to generate NO. And I'll share with you some of that data as well. This can form as s file via the small anine salt nitrite. It turns out the red cell has a nitrite reductase ability, ability to convert nitrite back to nitric oxide.

So, my best guess and that of my colleagues is that the NO pathway is disrupted in the transfusion of aged blood for three major reasons. One is nitric oxide scavenging by the hemolyzing aged blood, just as Dr, Bunn suggested. We think there's an important nitrite reductase or an ability of red cells to convert nitrite to nitric oxide and that this would require functional submembrane nitrite reductase metabolomes that may become disruptive with aging.

And finally there's provocative new data emerging that the red cell may have enzymatic nitric oxide-generating ability, that it may have enzymes, the nitric oxide synthase, which we've always thought as in the endothelium but maybe the red cells possess these enzymes as well. And all of these pathways really should be studied in terms of the effect of these pathways during storage.

So, just to talk about hemolysis, Frank

presented the data very elegantly that aged blood cells have a much shorter survival time after infusion. So even though you might not be infusing that much free hemoglobin in the plasma of your packed red cell, these red cells rapidly hemolyze both intravascularly and extravascularly. These cells are also energetically more prone to oxygen stress and stress-induced hemolysis in vivo, and again patients that are critically ill are going to have more oxygen stress, driving more of this. Aged red cells form extensive microparticles which we discussed. And NO is destroyed by microparticles and by hemoglobin by attacking the NO, as I showed you. This is now known to be one of the major side effects of the artificial blood substitutes. We haven't been able to get around red cell therapeutics because of the problem with these cell-free hemoglobin destroying nitric oxide and we found that this hemolysis is a major problem in human hemolytic diseases like sickle cell disease and like malaria, for example.

So, the concept is that normally your

endothelium is making nitric oxide and tonically regulating our blood flow but then when you get microparticles or hemolysis you'll disrupt this nitric oxide signaling by scavenging reactions.

And this just shows the case in sickle cell disease and it illustrates how little hemoglobin you need to knock out the nitric oxide signaling. So in this case these are 27 patients with sickle cell anemia in orange, and normal African-American volunteers in green, and with plasma heme concentrations of only zero to 25 micromolar we have a linear scavenging of the nitric oxide. And we looked at these patients and we actually put catheters into their four-arm blood vessels and infused a nitric oxide donor medicine called sodium nitroprusside. Sodium nitroprusside releases nitric oxide and dilates, increases blood flow about 200 percent.

In these patients we infuse the nitroprusside and we measure the plasma heme levels. And you'll notice that even with plasma heme levels of only 6 micromolar in heme -- this is about 5 milligrams per deciliter plasma hemoglobin -- we saw an 80 percent drop in the responsiveness of their blood vessels to nitric oxide. And this was seen at high, intermediate and low doses of nitroprusside. These levels would easily be obtained after infusing more than ten units of aged red cells.

So, the concept here is that during blood flow we have these healthy red cells and they retard the scavenging of nitric oxide but if we infused aged or damaged red cells, or during hemolytic conditions, these cells release microparticles and hemoglobin that oxidizes the nitric oxide to nitrate and creates vasoconstriction. So, in an effort to look at how we can prevent hemolysis or target the endopathway, for example, with inhaled nitric oxide may allow a restoration of this nitric oxide kind of flow.

The second thing I would like to briefly talk to you about is the possibility that hemoglobin has enzymatic properties in nitrite reductase and understanding this pathway may help us understand the normal function, that normal function of red cells. We found back in 2003 that nitrite at very low doses, in this case only 2.5 micromolar, vasodilated the human circulation. And, it did it by generating nitric oxide within the red cell. So, as we look at the dropping hemoglobin oxygen saturation as hemoglobin releases its oxygen it forms more nitric oxide. These are all studies in normal volunteers. And, we discovered a chemical pathway that was known for many decades, since 1937, that nitrite reacts with deoxygenating hemoglobin and a proton to make nitric oxide. So this is an enzymatic nitrite reductase pathway, very similar to bacterial nitrite reductase pathways that generate NO under hypoxia.

So, according to this hypothesis the deoxygenation within the blood vessel, nitrite in a deoxygenating normal functioning red cell can lead to hypoxic dilation. Obviously we want, under low oxygen, we want to dilate to bring in more red cells and more oxygen to that region. So, this pathway allows a linkage of hypoxia and the formation of the vasodilatory equivalent.

Now, what I would like to talk to you about is the possibility, or share with you some data coming from another group, in this case, this is coming from Warren Zabel's group at the Massachusetts General Hospital, illustrating the importance of intact red cells and nitrite reductase pathways in regulating blood pressure. And what they did is they did studies in mice, and here they have a mouse that has a normal nitric oxide production and here they have a mouse with a genetically knocked out nitric oxide synthase enzyme. So, these mice can't make nitric oxide.

Now, down here in yellow they're infusing whole blood, so these are freshly isolated red cells that are intact. And you'll see that these red cells don't vasoconstrict. Blood pressure does not rise. But if they infuse the same amount of hemoglobin without a red cell, in this case tetrameric hemoglobin, or a hemoglobin-based oxygen carrier, they get dramatic increases in blood pressure in vasoconstriction.

And we would hypothesize that aged blood that hemolyzes, as Dr. Bunn suggested, would behave more like this tetrameric hemoglobin or HBOC and that could link the old blood infusions to cardiovascular risk that we're seeing in epidemiologic studies, especially when you give massive transfusions to at-risk patients. And this shows that it is a nitric oxide problem because here they infused all three solutions into a mouse that doesn't make nitric oxide and while the mouse has higher blood pressure at rest because it doesn't have nitric oxide, there's no additional effect now of these infusions. This provides evidence that it is a nitric oxide scavenging mechanism that leads to this.

Now, related to nitrite, another very provocative result is they could pretreat these animals with nitrite and then they could infuse in red this tetrameric free hemoglobin and now the free hemoglobin behaves like an intact red cell because this nitrite reductase activity maintains vasodilation. So both of these pathways could be quite important in terms of the normal function of our red cells and hypothetically the function of aged cells.

The last part of the data I want to share with you is data that I personally did not believe, I still struggle to understand, and I just want to share it with you as sort of a "wow" result to show you how rich the science of these red cells are and to suggest how much work is needed to be done. I explained to you that the endothelium is where we make our nitric oxide. That is the absolute state the art, the absolute scientific dogma that our endothelium makes nitric oxide and that our red cells destroy nitric oxide. However, data has been presented from a German group that maybe the red cell makes its own nitric oxide, that it has its own nitric oxide synthase enzymes. And there have been three studies.

Two of them were very limited in data but one of them was a little more complicated, published in Blood, showing that red cells have a functional endothelial nitric oxide synthase. This is a very good laboratory group in Germany but I'll tell you that nobody, that they show that these red cell nitric oxide synthase inhibited platelet activation so it inhibited clotting, it made the red cells more deformable, so they flowed better through the microcirculation and that it made nitrite, which as we've shown you can regenerate NO. But I will also tell you that not a single scientist believes this paper could be possible. So, we wanted to look at this and I'll say I didn't believe this was possible.

So we wanted to say, do the red cells have a functional endothelial nitric oxide synthase that's active in nitrite homeostasis in blood pressure regulation? And we did this in one of the most rigorous ways we can think of and that's using cross-transplantation in knock-outs. So what we do is we can get bone marrow from mice. We can lethally irradiate a recipient mouse so it doesn't make cells any more, it doesn't make bone narrow, and we can give it the bone marrow from another mouse so that we can give it bone marrow from a wild-type or an eNOS knock-out mouse and then we could measure things like nitrite and blood pressure.

So to explain how this answers our

question, we can take the bone marrow from a donor that's wild-type, meaning it has the enzyme, nitric oxide synthase; it can make nitric oxide. But, we give it to a recipient, this is a control, that also can make nitric oxide synthase. So that in this mouse it has nitric oxide synthase in the circulating blood cells and the vessel wall and it's a positive control. It's a normal mouse except for being transplanted. But then we can take a mouse that can make nitric oxide in its cells but we transplant it into a mouse that doesn't have any nitric oxide synthase capability in its aorta or its blood vessels. This mouse has circulating cell eNOS but no eNOS in the vessel wall of the blood vessels. And we call this a plus-minus mouse. And this mouse tests whether the circulating blood cells make nitric oxide.

We can do it the other way. We can get a mouse that doesn't make nitric oxide in its cells but makes it in the vessel wall, and then we have another control which doesn't make it in either compartment. So if the German group's right, then the mouse that makes nitric oxide only in the blood vessels will be able to control its blood pressure but the mouse where you knock it out in the blood vessels will be hypertensive.

So, we did a lot of experiments using differential surface markers just to prove that we effectively had gotten the right mixtures when we did these transplantations. And we did Western blots of the aorta. This is the aorta looking at the eNOS and you can see the mouse that's control that has eNOS in both its cells and its aorta, does have eNOS in its aorta and the double, the animal, importantly, that any animal that has eNOS in its aorta has eNOS in the aorta by Western but any animal that does not have eNOS in the recipient has no eNOS.

So the experiment worked. And then we measured a bunch of parameters in blood, and just to show you the data, this is the measurement of nitrite which we think again is a storage reservoir for NO, and this is just looking at the wild type and knock-out and it confirms that if you don't have eNOS or nitric oxide synthase in any of your body, both your plasma and your red cell nitrite levels drop.

So then we looked at the four groups. So this is the nitrite in the plasma. Okay? And, if you have eNOS in our your blood cells and your endothelium, you have a normal plasma nitrite level and if you knock out eNOS in the blood cells and in the endothelium, you have low levels. So what happens in these mixed animals? Surprisingly if you just have eNOS in the cells but not in the blood vessels, you have a higher plasma nitrite level, and the opposite, if it's just in the blood vessels, and it looks like your plasma nitrite comes from both compartments.

Well, then we looked at the red cell, and it's only -- and this kind of makes sense if nitrite in the red cell is coming from a functional eNOS, the levels are lower in the animals that have no blood cell eNOS but have endothelia eNOS, showing that the red cell nitrite is coming from the blood compartment.

What about blood pressure? And this is the result that really shocked me. We saw more hypertension, high blood pressure in the animals where we knocked it out from blood but they still had it in their blood vessels. We also saw, of course, higher blood pressure if you knocked it out in both but notice the blood seemed to be more important in blood pressure regulation. We were so surprised by this result that we got hold of the Harvard eNOS knock-outs. These are the Chapel Hill knock-outs. We got a whole other strain, repeated all our experiments in the Harvard knock-out. And, it's really incredible. Notice that you have a 40 millimeter mercury increase in blood pressure if you knock the eNOS out of the blood cells but not from the blood vessels. And you can see it's equivalent to what we see if you knock it out of both compartments.

And very provocatively the most important correlate with blood pressure was our measurement of red cell nitrite, that is, the red cell nitrite dropped, blood pressure rose. And the fact that this blood cell eNOS was functional we confirmed by treating them for five days with LMNA, an inhibitor of nitric oxide synthase, and both the plasma and the red cell nitrite levels drop when you give them a nitric oxide synthase inhibitor even if the eNOS is only in the blood cells. And we're now looking at which cell is responsible. So far we've knocked the platelets out and we still have a hypertensive effect and we're now knocking out white cells. It's possible it's not a red cell enzyme but all of our data so far is suggesting indeed it is indeed a red cell eNOS.

So now you could imagine that your endothelium generates NO from eNOS but also your red cells generate NO from eNOS. And in preliminary data we're now seeing that as these cells age they lose this eNOS functionality because this enzyme becomes oxidized and unfunctional. So you can imagine that you give old blood and this enzyme property, we know enzymes degrade over time, that there could be a storage lesion in the ability of these cells to actually make nitric oxide.

So, in conclusion, I just wanted to present a rich canvas of functionality of cells as they relate to nitric oxide and just say we absolutely need more research in this area. Again these are complicated living, breathing cells, as you can see here, and to just emphasize again, it's one of the most-used human therapeutic agents. I'm a critical care physician and I think I prescribe more blood than any other single drug in my whole life; yet, it's the most under-understood and incompletely understood, understudied, incompletely understood.

I'm leaving NIH so I think I can say without risk of going to jail, that we have to fund this and I think one critical plea I'd make is that we can't just ask the NIH to fund this and shift dollars around, that the whole NIH infrastructure is under extreme duress. Clinical research is really starting to collapse, the intramural program. We need to shift additional new friends in NIH research.

As I travel through Europe I see a rising tide in the commitment of Europe to fund basic science. In the same way we lost the automobile industry, we're going to lose one of our crown jewels, biomedical research, if we don't get our act together and fund it.

This is bipartisan. Companies love the technology that we're developing. It's good for America. So I think we have to fund -- and this is a great area for research where right now all the lead scientists with the exception of this German group are in the U.S. So I think we should continue to fund this. It's very important. Thank you.

DR. BRACEY: Thank you Dr. Gladwin. Questions or comments from the committee? Dr. Triulzi.

DR. TRIULZI: Mark, great talk. I was surprised that the double knock-outs still have substantial nitrites so why would that be, you know.

DR. GLADWIN: What we've found and other groups have found is that about half of the nitrite comes from diet so it turns out that nitrate, which is very abundant in leafy green vegetables in the Mediterranean diet, the nitrate is converted by bacteria in the mouth to nitrite and taken up. So, if we take all nitrate out of the diet of rodents, we drop the nitrite level in blood in half. And, so it looks very clearly like from eNOS knockouts experiments, that half of the blood nitrite comes from diet and half of blood nitrite comes from eNOS. And that's something that multiple lab groups have seen. So I think what we're dealing with here is the blood authentic formation rates, which, of course, become very important when you don't eat healthy foods like salads because then you don't get that dietary source.

DR. BRACEY: Question or comment from Dr. Klein?

MR. KLEIN: Mark, again, thank you very much. And I guess to paraphrase the former CEO of General Motors, perhaps what's good for NIH is good for America. But I was wondering from all these slides it looked as if you're doubling eNOS knockout mouse had a lower blood pressure than the one that had red cell eNOS and knocked out endothelium eNOS. Was that a statistically significant difference and, if so, why didn't you have higher blood pressure in your double knock-out?

DR. GLADWIN: Yeah, it wasn't statistically significant but we see that pattern over and over. And what it is, is we get more hypertension if you knock out blood alone than if you knock out both. That's what you're talking about, right? You see the inverse with nitrite. And we see that less in the Harvard knockout. We see that more in the UNC knockout. But it's always the case that the blood knockout's a little more hypertensive.

And what we think it is, just to be complicated, it's almost like a conditional eNOS knock-out, that in the background mouse that's eNOS knock-out for its whole development, it appears to be up-regulated Cox 2, to make prostacyclin compete, compensate, excuse me, for the loss and when you do the minus into the plus, you create almost a conditional knock-out. It's only knocked out for six weeks. And right now we're actually doing Cox 2 inhibitor experiments just to confirm that. But it's been well described both in sickle cell disease with a lot of hemolysis that there's a compensatory up-regulation of Cox 2 and the eNOS knock-out, there's a compensatory up-regulation of Cox 2. That's what we think it is.

DR. BRACEY: Question or comment from Dr. Benjamin?

DR. BENJAMIN: Again thank you for a wonderful talk. So, a very simplistic question, hypothesizing that free hemoglobin and all these microparticles may be critical versus intrinsic membrane defects, simplistic experiments clearly to look at washed red cells versus unwashed red cells, do you know if anyone has done that in the animal models yet? Because clearly it hasn't been done in humans yet.

DR. GLADWIN: I'm not aware if that comparison been done. Frank, do you know? But one thing I would point out is exactly what Frank pointed out and that is that I don't think the problem is in the stored product right before infusion. The blood bankers, including the members of this room, have done an incredible job. We actually did a study very similar to Dr. McMahon's, how much hemolysate was in these units and it's pretty well-controlled with modern preservation and storage and oftentimes these cells are washed, which clears as well.

But as soon as those cells go in the chromiolabel studies have shown as Dr. Bunn suggested 25 percent will hemolyze in situ within three days, even as short as one day. And just to give you a comparison, that's equivalent to the hemolytic rate of a patient with sickle cell disease. So you're going to turn a critically ill patient into a patient with an in vivo, hemolytic anemia by infusing a large quantity of these old blood, these aged blood units.

DR. BENJAMIN: Do we really know that, in that the 25 percent, lasting 24 hours, have we actually looked whether that's introversed or extroversed, whether there's actually a drop in the nitric oxide?

DR. GLADWIN: No. What we know is that there is a strong correlation between microparticle numbers and activation of thrombosis and association with acute lung injury. We know that this turnover rate does happen with chromiolabel studies and then we can extrapolate from other diseases but we don't. We need to study this.

DR. BENJAMIN: So what I'm hearing is that we really are slightly overstating the case because, that lasted 25 percent, no one's really shown yet these correlations; these are extrapolations at this point in time?

DR. GLADWIN: Yes. Although I will say that they have shown that that rate of turnover occurs, just not what the biological effect of that turnover is.

DR. BRACEY: Comments from the floor?

MS. CARBO: I just had a question related to that. How do we know that the old red cells aren't sticking to the endothelium in the microvasculature rather than hemolyzing? Because I think that that is more likely since we don't really see tons of increase in hemoglobin or bilirubin or a decrease in heptaglobin but we do see multiorgan failure with microvascular --

DR. BRACEY: Could you introduce yourself for the record.

MS. CARBO: My name is Lisa Carbo. I'm from WRAIR.

DR. BRACEY: Thank you. Dr. Bunn?

DR. BUNN: I agree, that there have been studies on adhesion of packed red cells with the endothelium and that is a phenomenon. Also, potentially of considerable importance I think that with massive transfusions of blood it's rather common to see an increase in, in direct bilirubin and drop in haptoglobin. Like I say, it's obviously dose-dependent but certainly in a clinical setting that can be observed. So, I think both things are going on.

DR. GLADWIN: I would echo that, that you do have sufficient hemolysis to see clinical parameters change in these massively transfused patients. And it does seem like in clinical trials, again the patients at risk are these very severely injured patients, for example, patients that receive more than eight units and then see relationships with acute lung injury later. And I think as you expand from a 300 patient study to thousands of patients you will start to see, as this epidemiologic study indicated from a few weeks ago, that you will start to see a more subtle toxicity.

The other point to make there is what's remarkable is how little hemoglobin it takes that's extracellular to knock out the endopathway. And just to give you a relationship there, the normal, if I convert things to the micromolar concentration but it's somewhat analogous to milligrams per deciliter, with a normal plasma hemoglobin it's less than two micromolar.

It's a highly regulated, our bodies work very hard to capture and sequester that, and then in sickle cell disease, it will go up to 20 micromolar steady state and up to 40 micromolar with crisis. But on cardiopulmonary bypass for two hours, you'll go up to 150 micromolar and in malaria you'll see levels up to 200 and 400 micromolar.

And remember that each unit of aged blood has 200 micromolar just in that plasma, with the packed red cells. So if you were to do a massive transfusion of eight units, you would be in the range of the intravascular hemolytic rate of malaria just by giving that plasma, again, not only something we'd only see in massive transfusion. So what we would argue is as you increase your population size that you're studying, you're going to start to see cardiovascular toxicity of lower and lower levels of hemoglobin.

DR. BRACEY: Discussion is good. We have three more questions and then we'll stop for sure. Dr. Bianco?

DR. BIANCO: Celso Bianco. Now, the question is, with the classical studies of infusion of red cells with antibodies and all that, a substantial proportion of this 25 percent is taking up by macrophages, in the spleen and in the liver and we have at least the lower volumes, very little free hemoglobin that happens. They're just cleaned up. So is that, are we passing, exceeding that capacity?

DR. GLADWIN: Absolutely. So there's a very highly evolved hemoscavenging system. We maintain about 16 micromolar. Again, that's, think of the range of hemoglobin I described, 16 micromolar haptoglobin and haptoglobin is a hemoglobin scavenger protein. It binds the hemoglobin dimer with one of the highest protein-to-protein affinities known. And it binds the hemoglobin dimer and then exposes this neoepitope called C-163, which is the hemoglobin scavenger protein and it takes it into the -- system for uptake. And by the way when it does take it up, it also activates the downstream cascade of signaling. Activates IL-10, heme oxygenase 1 -- reductase and all those enzymes are in catalytic antioxidants. They prevent vessel injury.

So, what happens when you have higher than 16 micromolar release is haptoglobin has to be resynthesized so you swap that system. The haptoglobin goes to very low levels and now you start developing free circulating hemoglobin. The other problem is these microvesicles outside of PS clearance by the spleen, the microvesicles, they're not scavenged via that system. So it's, what we would argue is that you have to saturate the systems. So, as Frank said -- I agree with him -- this probably isn't going to be a problem for a healthy person getting two units of blood at all. I don't think we should, you know, scare the general population about the risk of a very safe product but the problem is when you get critically ill patients and you get a lot of blood, it's very clear that you develop very apparent toxicity.

DR. BRACEY: I think there was a comment from the floor or question. Could you introduce yourself?

DR. McMAHON: Tim McMahon. Mark, very nice presentation. As you know, one of the questions about nitrite and red blood cell hemoglobin is what is the final product and how does it get out of the red cell. You show data shown in showing the correlation between mean arterial pressure and nitrite levels but how exactly are these linked? I mean, it's been also shown before that nitrite is a marker for eNOS activity. That may be, you know, anywhere from a marker of the eNOS activity in the experiments to -- do you have any mechanistic data on the nitrite effect there, functional data with those red cells producing eNOS equivalents?

DR. GLADWIN: Not from those particular experiments. You know, one of the clear issues, if you looked at that data that I presented from Zabel's group, if you give the same dose of a nitric oxide, an authentic nitric oxide donor, so you give 10 micromolar systemic levels, donor, and then you give two grams per deciliter hemoglobin, as you know you will see no dilating effect. So nitrite appears to behave uniquely in its ability to interact with the hemoglobin to promote a vasodilating signal. Mechanistically we published a paper this January in Nature and Chemical Biology, where we explore the ways that a signal could get out.

Our favorite theory is that we form a nitrite met-hemoglobin intermediate that develops a radical character and develops a nitrogen dioxide RD-2 (phonetic) radical character. And we show that with density function theory calculations with EPR with rapid reductive neutro-insulation. We also have five lines of evidence to support that. And then when you form them with a nitrite, you get a radical, radical reaction that forms N2O3. We think our best guess is that N2O3 is our primary export species. That of course forms SNO as well. They're very fast with file in vivo. We don't see evidence for small file export like in SNO but that would be a possibility.

DR. McMAHON: Thank you.

DR. GLADWIN: But I think it's obviously a very important, rich area to study more because all of these pathways can become potential lesions.

DR. BRACEY: Last question. Dr. Benjamin? That's been covered? Thank you very much. Our next speaker, continuing in the theme, is Dr. Jeff Carson. Dr. Carson is the Chief of the Division of General Internal Medicine at the Robert Hood Johnson Medical School. His topic today will be a review of the clinical significance of red cell age and contributing factors to outcome. Dr. Carson is well known to many of us in the field of transfusion medicine and has made many contributions. Thank you.

DR. CARSON: All right. Good morning. Thank you so much for having me present today. It's a pleasure to be here. My task is to look at this at a clinical level. And so what I plan to do in my twenty minutes is to present some background considerations, what the basis of the hypothesis is, and I'm going to delete this stuff from my talk that describes some of the material that was previously discussed, and just talk about clinical stuff. I'll then show you what little we have on clinical trial data. Most of the evidence we have clinically is observational studies and we'll discuss those and then I'll give you my take on this evidence.

So a potential conflict is that I attended a meeting for the ABLE Investigators. This is a group that's designing a clinical trial, so, just so you know, done that, although I have no current active role although we're discussing getting involved with it.

So here are some background considerations. As this group certainly knows, that blood can be stored up to 42 days and that the time of storage is not based on clinical observations but on laboratory parameters and to say the least if we reduce the time of storage this might create some challenges. And my personal bias based on that simple fact is that you need clinical trials and even if the observational data was consistent in demonstrating improved outcome with shorter storage times, I would not recommend changing any of our regulations, that we need definitive trial evidence and I would prefer to see two, not just one clinical trial because this would be so, so disruptive to our blood supply. So I think we need a very high level of evidence if we're going to change anything that we do here in this country.

So the basis of the hypothesis at the laboratory level has been described but let me tell you about where it began at a clinical level, and it starts with the results of the TRICC trial. T R I CC. This is a trial that I'm sure this group know well. This is the only large randomized clinical trial that has ever been done looking at the efficacy of red cell transfusion. It was done in Canadian intensive care units in which they randomized euvolemic patients who had hemoglobins less than 9 to a restrictive transfusion strategy, which was a 7 gram threshold or a liberal transfusion strategy, which is a 10 gram threshold.

Now, what was striking about these results was that if you look at the, there's about 800 patients in this trial that the overall mortality was 18.7 percent in a group that got less blood, and higher, 23 percent, in those who got more blood. Now, while these results are not significant and we usually just look at this as a negative study, one wonders why it's trending in that direction. And, in fact, if you look at two subgroups, those who are less than 55 years of age or those who are less ill, as defined by an APACHE score less than 20, there were statistically significant reduction in mortality in those who got less blood. There also were less MIs, less patients who went into just pulmonary edema and there was a trend toward less ARDS in those who got less blood.

So this raises the question, are these extra red cells that the patient in the liberal group received, are they toxic, are they harmful? That's where one of the clinical hypotheses lie. Now, there's an awful a lot of observational data -- that's the only clinical trial that you can comment on because everything else is too small but there's a lot of observational data out there as well and with few exceptions the observational studies also show that blood's bad for you. It increases the risk of infection and death.

But I urge you to be cautious, that these I think are biased and unreliable observations in cohort studies. Physicians decide on who to give blood to and the clinical characteristics of patients getting blood and those not receiving blood often differ. And typically physicians are going to look at a case and one patient looks okay and the other patient looks sick and it's that sick patient who gets the blood and so I think that sicker patients receive more blood transfusions and sicker patients of course develop more infections and die. So I think this association is almost surely a biased association and it cannot be adjusted in analyses.

Now, this is relevant and the reason I'm taking the time to emphasize it in this talk is because one needs to think about, as we look at the observational data that looks at age of blood, are these same sort of potential biases in those analyses, so if age of blood is related to the frequency of transfusion, that is, if patients who get more blood have on average longer storage times, that maybe all that is a marker for who is sick and maybe it's the same bias in this analysis. I raise that as a consideration.

Now, I'm going to skip this part of it, other than, these are, this is from a trial that we're doing but I just want you to look at the pretty red cells and how nice-looking they are and how these are kind of ugly. And this is all the stuff that our other speakers were discussing and I'm just going to skip all that.

So to summarize the background considerations, there's some evidence that blood could be harmful but it's based on only one clinical trial. And true, the cause is unclear. We've talked in prior talks about morphology in 2,3-DPG. And I think that ultimately what we need to make good decisions here, I is reproducible highest quality evidence since we'll have such a profound impact on our blood supply.

So, what clinical trial evidence, that's our highest evidence, so that's what we want, well, in fact there are no clinical trials examining clinical events. They have not been done. They're certainly under, they're in the planning stage and I think you're going to hear about those this afternoon. But there is one small study. I don't know that this is all that really but there was done by Dick Weiskopf, published in the Anesthesia Letter, includes only 9 subjects, and they're young folks, 23 years of age, and this was looking at the impact of fresh and whole blood on cognitive function.

Basically what they did was they did isovolemic hemodilution down to 75 grams. They got a baseline neurocognitive test. Then when those patients were anemic and you determined that they were not functioning normally, then they gave one group randomized back fresh blood three and a half hours old and another group older blood, which had an average storage time of 23 days, and then they repeated the memory test to see how these patients did. So they used this test called a digit symbol substitution test. Here in BL is baseline in both groups here and shorter time means better function. And then they led these people down, down to 5 grams per deciliter and their function declined. And then they gave them back blood here up to a hemoglobin of 7.

And basically the differences between these groups are not significant, those who got fresh blood or older blood, there did not appear to be any significant differences. Small study using surrogate outcomes, the relevance certainly could be questioned but it certainly does not support the hypothesis that older blood is incapable of delivering oxygen.

Now, what about observational studies? This is really what I was asked to spend my time on and so I'm going to go through these relatively quickly, and just a few selective ones but keep in mind the following comments related to the design considerations. The first is what outcomes do we care about? Well, I think we care about mortality, morbidity but not things like length of stay, which are included in some of these analyses so I'm not going it to review those studies. Keep in mind that the challenges in analyzing these kinds of studies is that patients receive more than one unit of blood and so the age of one unit may differ from the other. And how do you deal with that? Do you look at mean age? Do you look at youngest age? Do you look at oldest age? There's all different ways that have been in these studies and I don't know what the right way to do it is.

Keep in mind that the likelihood that patients randomly receive blood stored for different lengths of time, that is, the basic premise of these kind of analysis is that when a patient is given a unit of blood, it's the most, it's the oldest available unit that matches for that particular case. So in principle it should be a random process and therefore you would expect that those who get younger blood and those who get older blood would look about the same, when you look at the classic table one of any kind of study where you line up the two groups. If that's true, we should look for that and see if it's really there or it's not.

Now, to come back to the bias question because I think it's really a critical issue here, if age of blood is related to the frequency of transfusion, then storage duration is just another indirect marker for who is the sicker patient, and the sicker patient is going to have poor outcomes. And you can't adjust this for in the analysis so I'm going to show you a number of studies in fact have this issue.

All right. So, this is a table that comes from a nice systematic review that was published in Transfusion, in 2006. This is data up to June of 2006. Here are the first authors. You can see that most of these studies were done in either in cardiac surgery or trauma. They're all cohort studies so none of these are randomized trials. Some of these studies are reasonable sizes. Some of them are quite small, you can see here, and some of these studies looked at things like length of stay and I've told you I'm not particularly interested in that, in trying to assess this question.

So let me, now -- I'm going to step through quickly -- I got twenty minutes so I'm talking fast -- some of these studies to give you a sense for what's out there. Okay? So the first paper, which is the oldest on the table that I just showed you, was from Vamvakas, published in Transfusion, in 1999. There are 261 patients undergoing coronary bypass surgery, received at least one unit of blood. They looked for, their outcomes were pneumonia, wound infection, bacteremia, sepsis. They used CDC criteria. They evaluated the mean length of storage examined after adjusting for confounders.

Curiously, they found that the use of CBDA, one, was not associated with any of their outcomes and so they just eliminated that that's from their paper and they only looked at the 192 patients receiving red cells preserved with adsol. Here's a table that comes from that paper in which they look at pneumonia or wound infection as the outcome. The mean storage was 15.2 days of those who had that disease versus 12.2 days. For pneumonia only is 15 versus 12 days of storage. Wound infection was not significant; that was 14.4 times 12.7. They do an adjusted analysis and the pneumonia and wounded P value was .02; pneumonia only was .04.

So what are some of the limitations, conclusions? Examined multiple outcomes and multiple preservatives. You consider many comparisons that they've done. This would not be even statistically significant. And they actually did another study published the following year looking at length of stay that they found no association. So, I don't mind these results particularly convincing.

Next paper, age of blood in cardiac surgery. This is 897 patients. This was published by Nobel in Anesthesia, 2004. You can't quite see that on the slide. There are a lot of patients here. Storage was defined as a mean of all units and the oldest unit. This was done with buffy coated depleted stored red cells in saline, adenine, glucose, mannitol and citrate anticoagulant. It had multiple outcomes that they looked at including length of ICU stay, mechanical ventilation, MI and post-op infections including pneumonia, mediastinitis and sepsis. It turned out that pneumonia was the only finding that was positive that was associated with the age of blood, for each day of storage was associated with an increased risk of pneumonia by 6 percent. All the other outcomes were not associated with age of blood.

The next paper comes from Basran published in Anesthesia and Analgesia. This is also a reasonable-sized study of 300 patients and this is a higher risk group. These are reoperations of, who underwent coronary bypass or valve surgery, so this is a group that's going to typically require more blood and is not going to do as well. These patients, the blood was stored in AS-type preservatives as described in the paper. In-hospital mortality and up to eight years after surgery was evaluated and was adjusted for using Cox models, duration of storage evaluated by mean duration of all units and the oldest unit transfused. Patients receiving more units of blood were more likely to receive units of longer duration.

So this comes to that theme that I started out with, is they clearly demonstrate that there was a correlation between the number of units transfused and the maximum duration of storage as well as the mean duration of storage. So this confirmed this potential bias in this study. Here are the basic results. This is a multiple regression, these are regression models. The hazards ratio is significant. Now, this doesn't look like much but this is per day of storage. This is maximum red cells duration of storage, once again per day of storage. And if you were to classify this and create categories between 1 and 19 days, 20, 26 and so forth, you can see that the longer the storage, the higher the mortality that they see in this analysis.

The next study I want to show you -- there's just a couple more. This was performed by van de Watering, published in Transfusion 2006, about 2700 patients, so this is a bigger study. They looked at

Buffy coated depleted stored in saline, adenine, glucose, mannitol solution. This is, once again it's coronary bypass surgery. They analyzed the effect of storage time by mean, youngest, oldest, and above and below the mean storage time. So they looked at many different ways of defining the duration of storage. This is table one. This is too complicated to read quickly but if you look over here, these are the groups less than 18 days, greater than 18 days and if you sort of scan the results, the groups look pretty similar. Everything, pretty, lines up nicely although they don't describe all that many clinical variables.

They did show once again that the age of blood rises with the numbers of units transfused so this is storage time in days by numbers of red cell units transfused. And you can see here the average age of blood goes up the more units of blood you receive.

These are the basic results from a table that I copied. If we just go down to this section down here where they looked at all the different ways they define storage time, none of these results are statistically significant and so this is a negative study. And so I've shown you a few positive studies; this is a negative study. Even with that bias that I described, they did not find that association.

And the last study to show you is one that you're going to hear from the primary author, that's already been referred to a number of times here, which was published in New England Journal by Dr. Koch out of the Cleveland Clinic. This was a study in which they looked at patients undergoing cardiac surgery who received blood. This is much bigger than any of the other analyses, 6,000 patients, and they compared the complication rates of those who exclusively received blood stored for less than 14 days or exclusively received blood stored for more than 14 days. This is a very nice part of the design that they included in this project.

Blood bank, they stated the policy at the Cleveland Clinic was blood bank was released, oldest matched unit of blood. Storage time was defined as the longest time of any unit of blood received and their primarily outcome was a composite outcome which included death and 16 events. That's a real composite outcome.

Now, this is a table from table one and all I have done is circled some of the parameters that were different between those who received newer blood or those who received older blood, and there are some differences between these groups. Here's some more. I think had is probable more important. The cardiac function was somewhat different between some of these groups. Leukodepletion I think was different in these groups as well. So, that when you line up as to how random this process is, it looks like there's differences although this is a big study and therefore you're going to detect small differences between those two groups.

Now, they display for us the number of red cell units transfused in relationship to the duration of storage. And these basically in yellow are the newer patients who got newer blood and blue are the people who got older blood, and these seem to pretty much be right on top of each other. It would be nice if they matched on this but they didn't. And then I illustrate just to come out to the section which is probably where the action is. This is probably where more of the events are because these are the people that got a lot of blood, and that there are small differences. Are they meaningful differences? I don't know. You know, the average number of units was 3.1, in those who got newer blood, 3.4, in those who got older blood, in excess of about 2,000 units. Does that matter here? I'm not sure. I just raise, I raise the question.

So, what were the basic results? If we go down to the bottom here, the primary outcome, composite outcome occurred in 22.4 percent in those who got new blood and 25.9 percent in those how who got older blood. This was highly statistically significant and if you just look at mortality, the differences are a highly significantly difference but the absolute difference in mortality is actually quite small, at 1.7 versus 2.8, I think that is. So, we're looking at an absolute difference in mortality that's quite small despite the small P value.

So what are some of the limitations, conclusions? It's a large and carefully performed analysis. There were some differences between the patients receiving newer and older bloods. How important this is I'm not sure. More, slightly more blood was used in the older group as well. I think without question this is the best evidence supporting the possible adverse effect of older blood.

So, the overall conclusions I draw is that most of the evidence -- and now this is sort of my bottom line here -- is most of the evidence supporting the hypothesis that storage time is associated with poor clinical outcomes is inconclusive or weak. The age of blood is related to frequency of red cell transfusion in a number of studies and there have been no clinical trials that have evaluated clinical outcomes for this particular question.

I have this sort of goofy slide here which, "Does your spouse beat the children?" But isn't this really the question that we're worried about, that, you know, this is a question you really want to know the answer to but you don't want to ask and isn't that really the case here? Because, gee, if we really are left with a situation where we demonstrate that the storage of blood is too long, we create lots and lots of problems for our blood community, don't we?

So, in summary there's inadequate evidence to support reducing storage time for red cells. I think, however, there's enough evidence to warrant performing clinical trials. I would urge that if we do consider changing our regulations here that we require at least two well done clinical trials demonstrating improved outcome with younger blood. Thank you very much and I'll be happy to entertain questions.

DR. BRACEY: Thank you, Dr. Carson. Questions or comments from the Committee? Dr. Klein, do you have any?

DR. KLEIN: Yes. Thank you, Jeff, that was a nice review and I wanted to do ask whether all of the retrospective studies you analyzed were single-institution or were some of them multiple-institution, since those of us who issue blood know that an outlying hospital may have a lot older blood than a center-of-the-city hospital which turns it over rapidly and certainly the practices may differ between the different hospitals.

DR. CARSON: Harvey, I think they were all single-center studies. I think they're all single-center studies.

DR. BRACEY: Dr. Epstein?

DR. EPSTEIN: Jeff, first of all, thank you for taking on the difficult literature review in a very coherent way. My question harks back to a point that Jerry Holmberg made earlier about the vulnerability of stored blood to mechanical damage -- and it raises the question of whether these findings that are perhaps valid are setting-specific, in other words, the same age of blood may be a risk in some settings but not in other settings. And just what's your perspective on that?

DR. CARSON: Well, again, most of the

studies were done cardiac surgery, so, and that's obviously a setting in which people go in bypass and there could be the mechanical issues that you're alluding to. I think unless you directly compare it, you know, I don't know. I think that the proposed trials that are being discussed, one would be in ICUs and one would be in cardiac surgery and we have a chance to look at that.

DR. EPSTEIN: Yeah.

DR. CARSON: But, see, if that's true, that would argue against when I made the case that you need two trials because if it turns out you've got an ICU trial that doesn't show anything and the cardiac surgery does, maybe that would support the hypothesis that you just raised, is that it's this mechanical problem.

DR. EPSTEIN: Yeah, but it has tremendous implication for the scope of studies. You know, you may have to do studies in GI bleeders completely apart from, you know, cardiac surgery; in other words, if it's setting-specific and then we have to ask setting-specific questions, it's many, many more studies.

DR. CARSON: Yeah. Nothing easy about this field.

DR. BRACEY: Question from Dr. Triulzi.

DR. TRIULZI: Jeff, really well done. You know, we're building a case here for equipoise, meaning that there is sufficient evidence on either side of the question to justify clinical trial versus making a change in practice immediately and I think you make a good case for that. What I did want to mention is one other study, which is a small randomized trial, which as you had mentioned, the ABLE study, and Paul Hebert, and I have the same disclosure that I was involved -- Paul's not here, is he? And they published a pilot study of --

DR. CARSON: Yeah, I should have reviewed that.

DR. TRIULZI: -- 66 patients that followed the protocol that they planned to use for their 2000-plus patient study. So it was really a feasibility study. It wasn't meant to be an outcome study. But, quite interestingly, at 66 patients you can imagine there weren't enough to ensure that randomization created equal groups. So the groups weren't entirely equal but the composite endpoint of morbidity, mortality, was twice as high in the fresh group, the 33 patients who received fresh blood as it was in the group that got older blood, and that was a less than 7 versus 21 or more age. So, the event rate was 26 percent in the fresh group and 13 percent in the older blood group.

DR. CARSON: Yeah.

DR. TRIULZI: And I bring that out not to say that that ignores that definitive trial, wasn't meant to be, that it's not statistically significant but I think it adds to the status of equipoise around the question that would justify being able to randomize patients to older units and the need for the randomized control trials.

DR. BRACEY: Thank you.

DR. CARSON: You know, I didn't -- I should have presented that. As you mentioned that, I said, "Yeah." Well, I just would, you made the proper cautions, which is you're looking at small numbers. This is not stable data. I wouldn't -- I definitely wouldn't look at this and say, oh, gosh, this fresh blood's bad stuff. I mean, I think that's really the wrong conclusion. And if you look at lots of clinical trials, you know, that if you look, for example, at the early thrombolytic trials that were done for acute MI, the first couple hundred patients actually showed a statistically significant increased mortality in patients who have thrombolytics and it was only after you had several thousand patients that the real results show. So, be very, very cautious in small datasets.

DR. BRACEY: Question from the floor?

MS. CARBO: Back to setting, I think it might make a difference if you look at patients who are massively transfused versus patients who receive one or two units, so maybe trauma is a good place to look at that.

DR. CARSON: I think it's clearly the

other -- I mean, I think there are sort of three obvious settings. One is coronary bypass, two is ICU patients and three is trauma. So, I completely agree with that.

DR. BRACEY: Dr. Bianco, you have the last question.

DR. BIANCO: Yes, Celso Bianco. The clinical trials are things that are difficult and I won't take years to mention. I think there are simpler experimental things that can be done that could give a more immediate answer, like Dr. Epstein mentioned, in terms of the damage done by a cardiac bypass machine, that is, to young or older red cells, like microparticles, hemolysis, and all that and I think that they should also be encouraged.

DR; CARSON: Sure.

DR. BRACEY: Okay. We will take a 15-minute break, so, reconvene in 15 minutes, "quarter of."

(There was a break in the proceedings.)

DR. BRACEY: Okay. We are ready to

reconvene. Our next speaker is Dr. Colleen Koch, and she is from the Cleveland Clinic. Dr. Koch is the director of education, the vice chair for education and director of research in the cardiothoracic anesthesia department of the Cleveland Clinic. Dr. Koch will present on the age of red cells in cardiac surgery.

DR. KOCH: Thank you. It's actually "Cook" but that's okay.

DR. BRACEY: Okay. "Cook," all right.

DR. KOCH: Because -- I answer to everything.

DR. BRACEY: All right.

DR. KOCH: So, as mentioned, I'm going to talk on blood storage duration and patient outcome, cardiac surgery. Before I get started I want to talk about four studies that our group had in print that dealt with outcomes in transfusion, in particular in the cardiac surgical patient population. I have no disclosures or any financial conflicts of interest.

Now, without question the notion that red cell transfusion as we all know has been considered beneficial both to replace lost blood volume and to increase oxygen-carrying capacity. This was really unchallenged for years and as advertised blood saves lives. Now, the concept of risk really changed with that and infectious transmission and the association between morbidity and red blood cell transfusion really became more of a focus of research.

I'm going to talk about risk and risk in relation to morbidity, so these are serious adverse events related to major morbid organ system failure and mortality and survival. Now, controversy was generated for a number of years, in recent years with publications that report an increase in risk with red cell transfusion and patients as compared to those who did not receive it.

And as noted by our prior speaker, these investigations were cohort investigations; however, I think they really shed light and provide a starting point for future trial investigation. These studies have shown an increase infection. In cardiac surgery this would be an increase in deep and superficial sternal wound infections as well as pneumonia. There has been an increased risk of multisystem organ failure of death, and that's both in-hospital death and survival after discharge. There's an increase in lung injury that's been associated with transfusion, and this is primarily manifest at least in our cardiac surgical population as prolonged postoperative ventilatory support beyond 72 hours. And there has been association of renal injury, that is, renal failure that necessitates hemodialysis.

Now, the first of the four studies I want to talk about before we get to storage duration, this is an investigation we looked at, red cell transfusion, and patient outcomes in isolated CABG. This is a prospective cohort investigation. We have two very large registries that prospectively collect data and then follow patients through the hospital course. We looked at transfusion in 11,939 patients. Isolated CABG refers to coronary artery bypass grafting and isolated means that there was not an additional valve or aortic type procedure done. So, this is a relatively homogeneous patient population.

We looked at seven morbid outcomes. And You can see these listed on the Y axis here. We have overall mortality, renal, intubation, there's infection -- it's hard to read -- cardiac neurologic injury such as stroke and an overall composite outcome. On your X axis are your odds ratios. Number one is here. We know an odds ratio greater than one is associated with increase risk. The column to the right represents the odds ratios and the confidence limits associated with each morbid outcome. The closed square boxes represent adjusted and the open unadjusted odds ratio for each of the morbid outcomes. Our study reported that transfusion of red cells in comparison to those not receiving red cells was associated with an increased risk of virtually all the outcomes that we examined.

This is a very well-studied patient population. The Society of Thoracic Surgeons have risk models developed for isolated CABG patients and included the risk factors that we know of to be associated with adverse outcome, and we toll for those in our statistical modeling. From the same study, I want you to look at this frequency histogram for red blood cell units transfused. On your Y axis you have your frequency counts in thousands and on the X axis packed red blood cell units.

Now, the arrows pointed to one thing I want to highlight. Almost half of our patients receive red cell transfusion but most commonly they receive one to two units. And a one-to-two unit transfusion in my clinical practice is not an amount that's associated with massive blood loss.

The next thing we wanted to look at, this is a separate investigation. There was some recent evidence around the time of this investigation that the development of atrial fibrillation was associated with inflammation. And we know that red cell transfusion results in a direct increase in inflammatory markers and it also augments and modulates the inflammatory response to cardiac surgery, mediasternotomy and cardiopulmonary bypass. So, we wanted to test the hypothesis that infusion of red cells would increase our risk of atrial fibrillation.

This dataset included almost 6,000 patients. We looked at patients on pump and we also looked at patients off pump. On the Y axis we have a probability of developing postoperative atrial fibrillation and on the X axis number of red cell units transfused.

Now, in our patient population a lot of these patients are on statins to lower their lipids. Statins have pleiotropic effects and that has some antiinflammatory effects so we also want to make sure we also took -- note of that. Results of our study demonstrated that increasing units of red cells transfused increased probability of developing new onset, postoperative, atrial fibrillation.

We looked at initially our postoperative morbidity and mortality and we wanted to look at whether or not there might be persistent effect of red cell transfusion on survival in our cardiosurgical patient population. In this investigation we looked at red cell transfusion and long-term survival. This is over 10,000 patients in open heart surgery. On the Y axis we have survival and on the X axis years after cardiac surgery. Each of the colored lines represent different transfusion status.

The black line represents patients who did not receive a red cell transfusion; the green, one; the yellow, two units; the blue line, those patients who received between three and five; and six and greater is represented by our line that is in red. Patients who received red cell transfusion, it was associated with a dose-dependent, decrease in survival throughout the follow-up period. We used again the risk-adjusted models in cardiac surgery that is well-studied, everything that we collected and we know that is out there that's associated with survival. We adjusted for it in our statistical modelling.

And finally, the last thing we wanted to look at was quality of life. How do the patients feel about what's going on with their disease process with their surgical procedures? So what we did is we had a dataset of patients that had information on Duke's activity status index or the DASI score. This is in 7,321 patients. They were asked about the functional health-related quality of life before surgery and then three to six months of follow-up.

There are certain risk factors for poor quality of life after open heart surgery, namely, poor baseline quality of life influences follow-up quality of life. There are a number of other risk factors including postoperative morbid events that we included in our statistical modeling. What we're looking at here on your Y axis is your predicted probability of reaching the highest DASI score, at a 58.2. Your in tip-top functional shape, is what that's reflective of. On your X axis you have age in years and then we have transfusion status.

As you can see from here, increasing age decreased your probability of being in the highest quality of life category as did red cell transfusion, which lowered your quality of life in the follow-up period.

I will talk a little bit about storage

duration. The panel knows that red cell storage duration simply refers to the time a unit is donated until the time it's given to a patient. And there have been a number of the prior speakers who have discussed a number of both biochemical and structural changes that occur in the red cell unit, some reversible, some irreversible, but these functional and structural changes may actually decrease microvascular tissue flow and decrease oxygen delivered to the periphery. And some of these changes may contribute to some of the complications we've been observing. It's important to know this area is not well understood and necessitates more research.

You saw one of these slides a little bit earlier. This represents an electron micrograph. The slide or panel to your left represents blood and population that's five days old. And then to your right is a 42-day old storage duration, red cell population. As you can see on the left of the day five blood there's a lot of smooth biconcave dysosites (phonetic) that predominate amongst the red cell population. And you look to the right and there's progressive morphologic change, structural change in the red cells. You get spicule formation which can break off form little vesicles. You get iconocytes (phonetic) as well as irreversible steroiconocytes (phonetic). So there are structural changes that do occur in the red cell product with increasing storage duration.

In terms of aggregation, this was already mentioned but with routine storage there's an increase in red cell aggregation as well as an increase in adherence, particularly a strong adherence to the endothelium at the microvascular level. We have deformability. Deformability just refers to the ability of a red cell to be able to remain flexible in shape through the microvasculature, or pressure applied to it. The red blood cells you can imagine is about eight microns in diameter, about two microns in thickness. The microvasculature is about three to eight microns so as you can see here, the red blood cells need to be able to remain flexible to be able to get through the microvascular for oxygen delivery. An increasing storage duration has been associated with a decrease in the deformability index and this may be due to the increase in microvascular tissue flow.

And, finally, a number of people have already discussed -- and I'll go through this quickly as well -- biochemical changes that occur in the product with increasing storage duration. We have a decrease in a number of compounds, which was discussed earlier, to ATP and to 2,3-DPG, a decrease in you pH and your nitric oxide.

Again, some of these changes are reversible; however they may impact the immediate delivery of oxygen to the periphery when you give that red cell transfusion. There's an increase in bioactive compounds. Some of these are probably flammatory, some of them amino-modulatory. There's increases in free hemoglobin as well as soluble lipids with increase in storage duration. So the combination of these structural and biochemical changes offers some biological plausibility to some of the adverse events that we may be seeing in our cardiac surgical patient population.

So we wanted to investigate this effect, the effect of increasing storage duration on complications in our cardiac surgical population. Our patient population consisted of over 6,000 patients, who were adult cardiac surgical patients, and over 19,584 red cell units were transfused among the groups. The red cells were delineated into groups by median storage duration of 15 days. Those who received newer blood, was less than 14 days. This constituted 2,872 patients. Patients in the older blood group were those who had blood transfused greater than 14 days and constituted 3,130 patients.

Our outcomes again included morbid outcomes reflective of serious adverse events to the organ system, similar to the outcomes that we looked at in our prior work and similar to the outcomes that the Society of Thoracic Surgery accumulates and documents for patients undergoing open heart surgery. We looked at in-hospital mortality and we also looked at survival in the follow-up period. We used modern statistical techniques.

Again, this was not a randomized control trial. We used multivariable logistic progression -- as to the outcome. We used propensity methodology which is a standard when your are analyzing observational datasets, greater propensity score, and forced it into our multivariable progression model to control for additional confounding. We used a parametric, hazard decomposition model -- that's kind of a full sentence there of words -- but what that refers to is that survival after cardiac surgery is not proportional so you couldn't use one -- past (phonetic) model to look at survival after surgery.

So you've got an early risk that usually goes out to about six months and then a later risk that follows out for as long as you do a follow-up period. There are risk factors in cardiac surgery that impact early survival and there are risk factors that affect late survival. And some of these are not similar so you need to model the data in a very particular manner to be able to account for the time-bearing hazard for data as well as the time-bearing hazard for many of these risk factors associated with the hazard of death.

You've seen this figure before. The panel to the left represents the number of units transfused and percent within each group, the percent within each group is on your Y axis on your X axis are your red blood cell units per patient. From our prior work we knew that increasing red cell units was associated with increased morbidity so we wanted to make sure that we had an even division between the distribution of red cells. We wanted to make sure that the patients who got older blood just simply didn't get more blood. So as you can see here to the left there was no statistically significant difference between the old and the new blood in terms of red cell units per patient.

Now, to the right you have mean days of storage, on your Y axis, in red blood cell units per patient, on your X axis. The lower and upper margins of the box represent the 25th and your 75th percentile and the heavy center line represents the mean days of storage.

Now, this is an unadjusted figure here. This represents, we wanted to get an idea of the dose response relationship between the maximal days of storage and the probability of composite outcome. These were two separate models that join at the center there. What you have here is your probability of composite outcome on your Y axis and on your X axis maximum days of storage. There's an increasing linear trend toward the increased probability of composite adverse outcome with increasing storage duration. This is a composite table a little bit shortened up from one that was shown earlier.

What we're looking at are complications. This is unadjusted results for storage duration and outcome in this patient population. The first column represents your complications, the next younger, blood, followed by older blood and the far right column represents statistical significance. This is our unadjusted comparison. There was an increased risk in complications in patients who received older blood. There was an increased risk of in-hospital death, prolonged ventilation, renal failure, sepsis, and a composite outcome. Multisystem organ failure was also increased in this patient population for those who received older blood.

Now, let's take a look at survival and our hazard curves. What you're looking at here is a survival curve. Survival is represented on your Y axis and your years follow-up is on your X axis. The blue line represents older blood; the yellow represents newer blood. The numbers above and below the lines represent patients at risk during that time interval. Next at the top right-hand corner of the figure represents the hazard function. We have the rate of death in percent, the Y axis, years follow-up on the X axis. Old blood is similarly represented by blue and yellow is represented for the newer blood. Patients who received older blood had reduced survival during the follow-up period and an increased risk of death. Superimposed on this figure, the open circles represent the Kaplan-Myer survival and the solid lines represent our decomposition model results.

From the dataset we're able to form a model to look at varying age of red blood cell storage duration and outcome. This figure represents a predicted survival and maximum age with our hazard decomposition model that I mentioned. Survival is on the Y axis and years after surgery on the X axis, following these patients out to seven years. The different colors represent different days of storage duration. Day one is represented by orange. Red is represented by 15 days storage duration, blue, 30, and the black line represents storage duration of 42 days. So you can see from these results in this risk adjusted model there was a decrease in survival associated with increase in storage duration.

I just want to make four points of my gestalt from this research. Number one, we really, we're not asking from our results here to dump blood that's younger than 42 days old but what the results of our finding really tell us and the message that we want to put out is that, you know, one, this is a cohort investigation, it's not a randomized control trial, and two, we really need research in this area. The studies are consistent. Many are consistent in terms of adverse outcome in transfusion but as well as some of the storage duration in our patient population. Although this was not a randomized control trial, our blood bank during this study period simply allocated the blood the night before. A technician in our blood bank would allocate between two and four units per patient in heart surgery. So when we would need additional blood, if we did, we knew if we used less than 40 units it didn't necessitate a call to the blood bank. So in a sense they were somewhat blinded to the patient as far as the patient morbidity and illness, how severely sick they were; nevertheless, it was not randomized.

There were some differences that were noted in the table. I didn't put that up. Between our two groups, actually 60 percent of the patients in the younger blood group, if you believe leukoreduction reduces risk, 60 percent of these patients did not have leukoreduced blood. So, it was biased against the newer blood. In terms of LD function, the patients receiving newer blood had higher -- heart functional class, meaning they were little big sicker; however, if you look at abnormal versus normal, LD function, on the slide, those who received older blood had more abnormal LD function but they could have an ejection fraction of 45 percent; you probably should have more clearly delineated LD function there.

But nevertheless it really moves us to increase the research funding in this area looking at the impact of storage duration, not only the basic science level. We really don't understand the functional consequences of increasing storage duration but we also need increased research in the clinical arena. We have a trial on going at the Cleveland Clinic that took a considerable amount of time on age of red cell transfusion and now we're trying to get it to multicenter sites. We're trying to get additional funding so we can go to multicenter sites. If you can imagine the complexity of working with the blood bank and trying to make sure there are enough units to allocate once a patient enters the trial to be able to go through the trial in cardiac surgery because some bleeding is unpredictable so we have to make sure there is enough inventory onboard. So we're close to about 100 patients in that trial right now.

Conservation I want to bring up. We simply need to use less blood. And I know that sounds trite and it's easier said than done; however, we really need to, you know, institute more blood conservation measures. If you look at that one histogram I presented, most patients only received one or two units of blood. These are patients who aren't bleeding to death. You know, we transfuse blood that actually saves lives of patients in cardiac surgery but the one-to-two unit transfusion I think is really an area that has considerable variability amongst our own surgeons, amongst different centers nationally and internationally.

There's just considerable variability of transfusion. And I think that this reflects the lack of randomized control trials in particular in cardiac surgery. Typically cardiac surgery is the largest consumer of blood products in-hospital. And if surgeon doesn't know, okay, you can go to a hematocrit, be more conservative and go to a crit of 24 or 22 and it's safe in this patient population. Irregardless of the ICU studies that had been done, cardiac surgeons want to see, and anesthesiologists, is it safe to go that low. We really need randomized control trials looking at hematocrit thresholds in this patient population to really be able to get a handle on, among the variability in transfusion practices, decrease that variability but decrease usage.

Distribution seems to be a little bit of a contentious issue. Actually I'm in business school right now and I just had a class in inventory management and thought a lot about inventory management with blood banks. And I'm not a blood banker, I'm a clinical and cardiovascular anesthesiologist but there peculiar things that really differentiate blood banking inventory from regular business models, dynamic optimization, things like that, that are practiced in the business world, you know, with your donor constraints, with your shelf life that has a limited expiration, limited shelf life and then your donor, which is a little bit more predictable.

Again, our blood bank, and a lot nationally, we use FIFO inventory management strategy, that is the first in and first out. So when I'm in the operating room and I need blood, they'll give me the oldest unit first and that's what gets transfused. I know. I don't know if there's an opportunity for research funding to explore really and mathematically model some of the different inventory management strategies. There have been a few studies recently but most were done, you know, 20, 30 years ago with the thought of cost minimization and minimization of wastage. But certainly as these new studies come out you wonder about modelling different inventory management strategies to blood banking and see what our results show.

The other comment on distribution, I'm part of the Cleveland Clinic, which is a consortium of a lot of other neighboring hospitals, and you wonder whether or not more regionalization of blood banking services wouldn't be a little bit more efficient. It's true that the blood bankers will tell me that in the community they don't reorder blood unless that 41, 42 day old blood has been used and then they'll reorder. But I wonder if you wouldn't have more of a centralization of blood services at least within the consortium of hospitals to more effectively manage inventory and move inventory around, that might be a little more efficient.

And finally in terms of rejuvenation, there's a lot of neat research going on in adding solutions to the storage median to perhaps the research they mentioned on nitric oxide, things to add back or prevent the storage lesion from occurring, I think are very intriguing and we probably need more research in that area of storage media to make a better product. Thank you.

DR. BRACEY: Thank you, Dr. Koch. We will take questions or comments from the Committee. Dr. Ramsey?

DR. RAMSEY: Thank you very much for your presentation and, by the way, thank everybody for their great presentations today. I have two questions. One is just a kind of background question about the study. There are almost 3,000 patients in each group but I was wondering given your vast experience at the Cleveland Clinic, how many patients would have been in the middle group that got a mixture of both ages of blood that would have been excluded from the study during that time period?

DR. KOCH: Well, actually, there were close to 2,800 and I think 72 patients that received an admixture of blood and these patients were every different. They received more blood than the other two groups. And we really wanted to try to get a handle on what, you know, having looked at the --

DR. RAMSEY: So that about two-thirds of the surgeries were included in your study and about one-third were not included, is that what you're saying?

DR. KOCH: Yes. Yes.

DR. RAMSEY: Thank you. Okay. And then the other question, would it, have you ever considered doing, we heard today about concerns about massive transfusion particularly in patients getting lots of units and I was wondering whether it might be feasible in yours or other studies or maybe it's been done in some of the other studies looking at subgroup analysis of patients who got lots of blood. I mean, from a day-to-day standpoint, a particular patient, I note in your study patients who got lots of blood, over nine units, tend to be, get a little more than -- than younger, younger group, I believe, is that --

DR. KOCH: Yeah, there wasn't a statistical difference in the distribution there.

DR. RAMSEY: Okay.

DR. KOCH: The problem you have is with statistical modelling, when you start looking at the subgroup analysis. For example, when we have a dataset originally we thought how do you define age and try to eliminate as many confounders of admixture. So if you just took a patient who received one unit of blood, that would be very clean and as you can see from that probability curve the number of patients I think it was that group, that was much higher than the patients who received massive transfusion. And even that patient number wasn't enough to support statistical modelling adequately to be able to look at an outcome. So, down in that group that received a lot more, you know, you can do subgroup analysis but the statistics don't hold up.

DR. RAMSEY: Say on a day-to-day basis on a particular patient, one patient who uses lots and lots of blood in an individual hospital, of course, the tendency is, okay, this patient is using lots of blood but you have to call the supplier and get more blood in and that tends to, that might tend to be younger blood in terms of the reinforcements that are being brought in. So it would be very hard, for me very hard to look back but it's very interesting. Thank you.

DR. KOCH: Thank you.

DR. BRACEY: Dr. Benjamin?

DR. BENJAMIN: That was a great, great presentation. I appreciate you coming here today. A couple of questions. The first one as a blood banker, kind of confused by the fact the leukoreduction would be not equally distributed between the two groups, given that the study was done over the time period when universal leukoreduction was being introduced by many blood centers. Two explanations come to mind. One might be that the two groups were performed at different time periods or that there were different surgeons actually asking for leukoreduced or nonleukoreduced blood between the two groups. Do you have any explanation why there should be a mismatch with leukoreduction status?

DR. KOCH: Well, first our surgeons can't ask for leukoreduced. They'll get what they get from the blood bank. As big as our cardiac surgeons are, they can't unless it's a cardiac transplant patient, you would see leukoreduced products coming up to the OR before 2002. They can't, they don't ask for that. The second question -- and I think I answered it in my reply letter to the Journal from you -- that the time -- thank you very much -- and I wanted to meet you. I read your letter. I got to meet this guy. He's giving me such a hard time. In a USA Today interview I think said -- well, Dr. Benjamin said -- and I went, oh, my goodness. I got to meet this guy.

Anyway, but on that time point the data surgeon is a variable, what we always include in the statistical modelling so it's part of the variable selection procedure so should practice change over time, that would be captured in a time variable. So that was considered. I don't know why, and again it wasn't a randomized trial so I can't tell you why there were these differences in leukoreduction.

DR. BENJAMIN: I do find it strange, because I was running a major blood bank in a major hospital during this timeframe myself, and our surgeons did have the option of asking for blood during this time period. But I'll take your word for that. The second question I have for you, though, you make a lot of points about the statistical modelling you do and the risk adjustments you do but you don't show risk adjustment in any of your tables or figures. And I was wondering, having previous studies that show similar data -- but when you do adequate risk adjustment it disappears so I was wondering why you didn't show risk adjustment in your tables and in your mortality survival analysis.

DR. KOCH: Well, actually in the survival -- if you read, and actually I replied as well to that point -- in the survival curve if you read the figure legend, the parametric estimators are superimposed on top of the Kaplan-Myer for your survival. In the text the results from the multivariable logistic progression are written in. The table is just simply the univariant outcome table. So, the results are all in the paper, both from the multivariant model as well as the survival model including the appendix that includes the parametric model with the figure. Some of them are just more cleanly presented in that manner with the, it's very clearly stated, unadjusted for the dose response with maximum days.

DR. BENJAMIN: I guess my concern is the discussion was based on the unadjusted figures and strong recommendations were being made for changes in transfusion practice based on unadjusted numbers. And many of us are still trying to work out whether this is, you know, we agree that you have identified a major issue that requires further research. We're not yet at the point of saying that we should change the way we practice medicine in this country based on the data presented in the study, if the discussions spend a lot of time suggesting things we might do.

DR. KOCH: That's always a good starting point but if you follow the statistical methods section you'll look at, it goes from univariant comparison to multivariable and then to the survival so it was pretty clearly delineated there. In terms of recommendations there are recommendations we can look at and we did recommend this is something we need to think about. You know, cohort, prospective cohort investigations are really where, is your starting for randomized clinical trials. When you design a trial -- we've got a trial going on in the clinic on age of red cell -- how do you design composite outcome to know that you don't do a trial when you have three limitations in each arm.

We need close to 3,000 patients to be able to detect a difference in our patient population, at Cleveland Clinic. So these studies are very important and again they form a starting point even if they, you know, raise a few hairs on the backs of some people's necks, it's something that really needs to be looked at. As far the other suggestions of exploring changes in inventory management, I think that's something that should be looked at. I think it's something that can be done mathematically modelled rather than no one is saying dump the blood that's old but they're saying, hey, let's take a look at this, this is important and why don't we take a look at managing inventory a little differently. And that involves no patient care. That just takes some people who have the wherewithal to do mathematic remodeling and perhaps put some cost measures in of patient morbidity.

So let's say see if some of the trials do find some more effective or some more adverse outcomes of -- blood, and when they start looking at inventory they need to pick that up and possibly change it because you're going to have to model the cost of a patient on a ventilator for three days in an ICU because they've just received older blood and none of these things were considerations in prior molding.

DR. BRACEY: In the interest of time we probably need to move on.

DR. BENJAMIN: One last point. I strongly agree with you that we need more basic research to prove whether this effect is real or not.

DR. BRACEY: Dr. Klein, the last question.

DR. KLEIN: Thank you, again. That was a very nice presentation. But I wanted to get back to the first four studies you presented because again the issue of lots of blood and toxicity is one that is of particular concern. And I know you did multivariant analysis and I think I understand that but I'm still not sure how one can draw a causative relationship when clearly sicker patients who receive more blood and probably just as importantly frequently sicker patients receive blood inappropriately, and if that just looks as if the more blood is related to mortality and morbidity, how do you, really control the bad without prospective randomization?

DR. KOCH: Well, number one, I don't want to mention causality, so, this is an association because it's a cohort investigation so it's a strong association. And again, yeah, sicker patients tend to do more poorly. We know risk factors that make patients sicker in cardiac surgery. Certainly there could be some unknown risk factor. One unit of blood increased risk in these patients for infection and for a lot of other adverse outcomes. I'm not talking about buckets of blood, you know, eight to ten units certainly increase the risk as well but a one-to-two unit transfusion increased risk. You got to remember there could be something going on.

Back in the old days of kidney transplants, surgeons would give patients red blood cell transfusion because it would immunomodulate them. And the renal allografts would last a heck of a lot longer, in those who didn't receive the red blood cell transfusion. Now, we don't do that anymore but there's something about a blood transfusion that does have some persistent effects that does have immunomodulatory effects and we do have some basic science presented here today in the literature that gives some biological plausibility to the findings. It's pretty persistent across the cardiac surgical literature as far as findings of adverse outcomes. Again no one is making it causative but there is an association.

DR. BRACEY: Dr. Gladwin?

DR. GLADWIN: I just want to make one point to link the basics of the clinical research. There's a lot of the data about whether, for instance, you may, looking at this cohort of patients but I just want to point out there are clinical trials that have been performed where a drug was given blocked the inner pathway. So LMNA was given for sepsis and the trial was stopped, the P value were harmed .003; it was one of the most lethal trials ever conducted in the human research experience. And then the dioxaphospholane hemoglobin Baxter trial was equally lethal and -- drug with nitric oxide.

Even with the diffusion barriers that I mentioned -- if there's any tip towards more hemolysis in vitro we have a dramatic effect. So I do think we have a rich experience of translational basic science suggesting that these pathways when really pushed can be quite harmful. So I think there's a very strong basic science, basis behind this clinical observation so we just have to, I think we should keep connecting the dots there. That's my comment.

DR. BRACEY: That's a nice segue to our next presenter.

DR. KOCH: Thank you.

DR. BRACEY: Dr. Timothy McMahon, Dr. McMahon is the medical director of the medical ICU at the Durham VA Medical Center. He is associate professor of medicine at Duke University. He will present on evolution of adverse functional changes in stored red blood cells. He's done much work in the field of red cell physiology and we learned today that SNO not only affects traffic in D.C. but perhaps traffic within the blood stream.

DR. McMAHON: Thank you, Dr. Bracey, for the opportunity to speak today. The inspiration for the study that I'll describe today comes from questions that have been raced by good studies in the clinical literature, some of which we talked about today. And there are two questions, two bottom-line questions -- let me back up and say that as a clinician I note even with these trials in day-to-day practice I find it difficult to know when to transfuse and when not to transfusion. I'm also a critical care physician. I certainly know, have a good sense from the TRICC trial, from Paul Hebert and colleagues that transfusing to 10 is not a good idea relative to transfusing to a hemoglobin of 7 in comparable patients.

Aside from that there's not a lot for us to hang our hat on in decision-making for transfusing critically ill patients. And among other questions are whether a marker can be developed that will help us decide in a given patient whether the potential harm from a transfusion for them will outweigh its benefit. But two questions that come up today that motivated and framed our study, are one, is a transfusion better than no transfusion for our patient in a given setting? Transfusion versus none or is more transfusion worse than none, and why? The study that I will present is a basic science study looking at red blood cell mediators that may go back to storage and the functional consequences of those changes.

And the second question is, are fresher red cells better than older red cells? And that's been very well framed by Colleen in terms of the clinical data there, her study I think being the most informative and compelling in that arena. And of course these two questions may be interrelated. It may be that there's a continuum and many of the same lesions that we see initially when blood is stored worsen further over time.

And, the function of the red blood cell that I will focus on today is one that Mark described and gave a good background for, and that is a relatively recently appreciated function for the red cell in oxygen delivery and that is its ability to regulate blood flow. And I think when we transfuse or not we should be thinking about dysfunction of the red blood cell, which is key for its classic function of oxygen delivery.

We know a few things about this. We know that this turns out to be a red blood cell function. Hypoxic vasodilation is a response where there is vasodilation in tissues with low PO2, getting more blood flow as a result. This is a red blood cell dependent activity and this is nicely demonstrated here in work from Saltine and co-workers, where the blood flow in a leg of a normal human subject was inversely proportional to hemoglobin oxygen saturation. So this is a function not only of oxygen per se but of hemoglobin oxygen saturation so it appears to be governed by hemoglobin. In contrast, in the second panel below, when you look at this as a relationship between PO2 and vascular conductants there's really no relationship.

This is the way that saturation was manipulated here, was to use CO, use carbon monoxide to keep hemoglobin in R-state but with lower oxygen binding. So when it's in saturated a R-state, that inhibits blood flow. When it desaturates, that promotes blood flow. And ongoing work and with it all, review, addresses some of the molecular mechanisms behind that.

In similar experiments we know that that phenomenon is NOS independent. In exercising subjects increases the blood flow with hypoxia or blocked by a NOS inhibitor -- I'm sorry, are not blocked by a NOS inhibitor. It's NOS independent.

And so summarizing these data blood flow is dynamically regulated by changes in tissue oxygen concentrations but the transducer appears to be hemoglobin saturation rather than PO2. The sensor is blood-borne and in the red blood cell hemoglobin is a good candidate. It's also a NOS independent phenomenon. And we and others can model this in isolated blood vessel rings.

So shown here are experiments where we preconstricted isolated blood vessel rings from a rabbit and then exposed them to red blood cells after he equilibrating them out to varying PO2s. What you see is that there's a graded change in the vasomotor response to red blood cells as a function of the starting oxygen tension. When you're at a PO2 of 63 that you might see in a peripheral artery, you get largely constriction when the red cells are added. But the lower the PO2 goes, the less constriction. In fact, you can you convert to a vasorelaxant response of PO2s of 3 or 7, seen in respiring tissues. And this is in contrast to responses to another NO donor, in this case -- where responses are largely PO2 independent. There is hypoxic potentiation of vasodilators and especially nitro-vasodilators but that alone does not account for this phenomenon. And one way that hemoglobin can carry out this dual oxygen sensing and vasodilator dispensing function is through -- hemoglobin. It's well established that -- binds to the hemes of hemoglobin. That's the basis for some of the NO scavenging effect of free hemoglobin that's been talked about.

In addition, NO combined at reactive file sulfur groups, the S representing sulfur in hemoglobin. These are highly conserved residues and the binding at this reactive file group is reversible. When SNO hemoglobin so formed it gets back to the T-state, it will release the NO from those reactive file groups. When hemoglobin is alone, typically the released NO equivalent will go back to the hemes in hemoglobin but if there are other NO or SNO receptors it may go to other molecules.

And so moving on to this in the context of the red blood cell itself, you have hemoglobin frequently alternating between the deoxygenated T-structure and the oxygenated R-structure. Along with this transition there's a change in the ability to sustain SNO bound to hemoglobin, a change in its stability. We believe that the primary source for NO bindings of hemoglobin to form this SNO hemoglobin is NO from NOS. It may be endothelial NOS or it may be NOS within the red blood cell. It's also possible for hemoglobin to take nitrite and convert it into SNO in a SNO synthase function of hemoglobin.

And of course in addition to hypoxia the other major physiologically relevant trigger for transition from the R to the T-state is increasing acid level or decreasing pH, where again the red cell wants to increase its flow to meet metabolic demands.

So, filling out the scheme here, and some of these are known knowns in the system and some are more speculative. As hemoglobin releases its SNO, it traverses red cell membrane and the membrane protein -- exchange of 1 is a key relay point for SNO. The identity of SNO outside the red cell is unknown but as -- is a candidate molecule, this is formed by -- isolation of glutathione. And then there are questions about how this SNO would get into cells of any kind, endothelial cells or vascular -- muscle cells, for example. An enzyme called GGT or -- that has an established role including -- stasis, is capable of cleaving this GSNO to a smaller molecule that can get into cells and that's CYSGLY NO. In some cases this needs to be converted further and then an L type immunotransport may carry in, for example, NO cysteine, a single immunoassay, NO bound. We're still learning about the cell-specific requirements for those various processing and transport enzymes. On the other hand, the GSNO in the extracellular space or within cells is degraded and tightly regulated by an enzyme called GSNOR -- that's GSNO reductase -- creating the inactive products GSSG and ammonia.

So here are results from experiments where we said, well, if GGT is important in transducing the SNO-related red blood cell vasorelaxant response, we should be able to inhibit with an inhibitor of that enzyme, GGT. So GGT inhibits this conversion of GSSNO to CYSGLY NO. And in fact you're able to nearly abolish the response in the presence of acitisine, suggesting that these responses go through GGT signaling.

We're also interested in another red blood cell derived vasodilator, and that's ATP. ATP is released from red cells in response to a variety of stimuli, including hypoxia and deformation. That release has been shown to be abnormal in some disease states including pulmonary hypertension. It may play a role in the perinatal translation or transition, rather, of the pulmonary circulation, the transition from fetal oxygenation to lung air breathing in the neonate.

We don't know much about the mechanisms of ATP release and we don't know much about the relative roles of ATP with SNO from the red blood cell. Studying this in detail, these are preliminary data where we have worked with Eduardo Lazarowski to develop a technique, to measure -- under the conditions of our assistance, ATP and its metabolizer precursor so here we get good recovery of spiked ATP, concentrations and we also see release of ATP from red cells in hypoxia, but in addition we see ADP and adenosine monophosphate that accumulate outside these cells -- hypoxic. So I think experiments like this will require analysis together of these interchangeable mediators to put them into context as well as the use of receptor antagonism and knock-out applies to learned functional data from these.

So what happens to this vasoregulatory activity of red cells when they're stored? Until recently we've known very little. We do know that ATP is depressed. As was pointed out earlier it's a relatively slow decline, slower than the decline in 2,3-DPG, for example. We didn't know much about SNO lost during storage until recently and we consider the loss of both of these to be relevant to the blood flow control by the RBC that's relevant in terms of transfusion medicine. So we hypothesized that storage would lead to depletion of ATP and SNO hemoglobin and that in turn the ability of red cells to regulate blood flow would be compromised.

We enrolled 15 healthy volunteers. They gave consent. We used standard AABB and American Red

Cross techniques. The red cell units were leukofiltered and stored in CP2D and AS3 solutions. We did a blinded analysis of multiple functions and multiple molecules. Unless otherwise stated the results that you will see relevant, the results you will see in red cells themselves are in washed red blood cells. So to answer some of the questions, someone had a question about washed versus unwashed red cells and it affects a wear of the biology of free hemoglobin. We took that out of the picture in these studies. We assessed red cell vasoactivity using isolated vessel ring assays. We measured SNO hemoglobin by photolysis chemiluminescence. Since this area is controversial, we also used a second method. This is a chemical reduction method -- use of copper and cysteine and carbon monoxide. And this study -- disclosure here -- this study was funded by a company called Nitrox.

And, we paid close attention to several key allosteric effectors, allosteric effectors of hemoglobin function, that is. And these have been it all studied before but we wanted to use them to benchmark a study, and to closely correlate changes across different parameters as well described pH falls a lot earlier with exposure to this lesion and continues to fall over time.

PO2 starts out with a low venous level and it comes up slowly, getting in through the gas permeable PBC bag, and finally getting to the 200 level. Of course, this is of course -- not -- cold storage, the conventional 40 degrees storage. Hemoglobin oxygen saturation rises reflecting a complex effect from the change in PO2, change in pH, the loss of DPG and others. There's also CO2 loss across the bag. And these, some of these changes are potentially relevant not only to hemoglobin stability and function itself but also to the chemistry of -- stored red blood cell unit. We looked at -- and related -- and apparently we looked at its functional correlate, red blood cell bioactivity.

And in this part of the study we wanted to address we wanted to address, we wanted to dissect out the effects of processing and time. And so we acquired blood and processed it in the way that I've described. From the very fresh blood in a separate set of donors, we studied these parameters immediately and then three hours later, with no processing, just a three-hour hold, whereas in the processed samples it was impossible to get the datapoint before three hours. We did that and then at eight hours one day, four, seven, two, three, four and then six weeks typically.

And what we found is that the total -- bound hemoglobin fell significantly by that three hour time point and again irrespective of processing for exposure to the additive solutions. That level was comparable to the seen in the first post-processed samples, and this did not change at least not significantly over the remainder of the studied duration. Bioactive SNO hemoglobin fell to a similar degree.

Again the first post process day before was similar, no significant changes, a trend here but there is not significant. We had a resurgence in SNO around a week. We also looked at SNO in the red blood cell membrane. As I mentioned, membrane protein AE1 as one example binds SNO in accepting it in transfer from hemoglobin as part of the process for SNO getting out of the red cell. And that pool of SNO was profoundly depressed at the first time-point measure, which was eight hours. This was a process sample.

In addition to the SNO hemoglobin measure, photolysis chemiluminescence, we also made similar measurements using the 3C technique that I mentioned, another SNO measurement technique that measures the total SNO in the red cells and that was depressed to a couple degree. And, the data here are a median between 25 to 75 percentiles probably. Red cell bioactivity was significantly depressed in the first time-point, that is, at about a three-hour point without any processing or solution exposure. And some moving around -- no significant change from here on out for the remainder of the studied duration.

As Colleen mentioned, the red cell is often asked to get through capillaries and other microvessels that are narrower than a cell and to do so it needs to be able to deform. And this is a sign of a healthy red cell and when impaired can impair regional blood flow. We looked at red cell deformability as a function of storage, choosing two clinically significantly sheer stress levels, 3 and 30 Pascals.

This was done in a so-called NORKA device or ECTA cytometer in which the red cells are in between two cylinders, one of which is rotated and you optically measure the elongation effects, that is, how much the red cells elongate as a function of that sheer stress and the more they elongate, the more deformable they are.

The decline in deformability has a much different time course as you with see decline in vasoactivity. And, the values, the P values here are for the entire curve. There really is a change that takes place over a matter of weeks. These are raw datapoints from our ATP assays in the study and ATP fell to a comparable degree compared to previous studies that have looked at this. Again this is a slower decline compared to the early declines in NO.

We looked at several other potential players, interleukians, six and eight, TFNL -- really very little -- no significant change over time. This is in the supinates. Again this is in the supinate whereas this red cell data, this red cell and SNO data are from washed samples from the units.

We also looked at the availability of these stored red cells to adhere to stimulated endothelial cells and there was none, essentially consistent with other studies in leukoreduced red blood cell units. There was no significant increase in phosphatidylserine, PS exposure over time. I mentioned the lack of change in cytokines, that there was no bacterial contamination of the units.

Free hemoglobin toxicity is another lesion with red cell storage. But, I have listed this here to remind us that in some cases we separated out the supinate.

Summarizing the main new findings, red cell vasoactivity and its mediator, SNO hemoglobin are depressed early during red cell storage and independent of exposures. The timing differs for at least two different significant functional changes in the stored red blood cell, one being very early and one taking place here.

Further study is needed to determine our ability to improve clinical outcomes with red blood cell transfusion. And I think directions suggested by there kind of research are studies to see whether we can prevent some of these lesions in the first place, to test whether we can correct the loss ex vivo, that is in the blood bank, or in vivo, that is in the patient once we've given a transfusion or while we're transfusing and I think you get a high -- need for markers. Some of these parameters can be used for markers that will to help us decide when to transfuse, what we should be looking for in the first place to decide if our patient really needs a transfusion.

Future directions for our group, we're interested in defining the relative contributions in vivo, these different functional changes that we see in vitro, specifically the deformability changes, the vasoactivity, lesions, and the adhesion changes preventing it. The mediators of interest are ATP and SNO hemoglobin. They play into the vascular dysregulation as a function of storage and to help in ways to see whether we're depleting ATP, for example, with regudisol (phonetic) or RBC SNO, which can be done in a few different ways or preventing their loss may correct the RBC storage lesion in the big sense in vitro and in vivo.

So, specifically, and coming back to this schematic, that outlines how the system works, as we understand it, you know, there are a number of forces during storage that act to grade SNO in hemoglobin. For example, one, you're keeping the red cell unit at a relatively low PO2 and that's a normal, we call that a normal PS PO2 but it's not normal for blood not to be cycling back and forth between T and R structures. I think it's also worthwhile to investigate optimal pH for preservation of these mediators and of function, to investigate whether the extracellular files, for example, the plasma that's there present for those first few hours while a red cell unit is being acquired and processed, whether that is beneficial or not and so on. I think I'll stop there.

DR. BRACEY: Thank you. Given the rapid fall-off and I guess the clinical observation that transfusion is worse in some instances than none, this, you know, one sort of the simplistic way of thinking about this is that this underscores just the risk of transfusion, period, rather than necessarily the storage related, long storage related risk because of fall-off, so, so rapid.

DR. McMAHON: I think that's fair to say.

DR. BRACEY: Could you comment on that?

DR. McMAHON: In other words, at least the new findings from the study, at least directly speaking, say more about why a transfusion may be worse than no transfusion or more units may be worse than fewer units rather than what's worse about 28-day old versus 7-day old blood, right. And I think, you know, I think it will be important to address these things in concert and, you know, if there are lesions that can be corrected or ameliorated early on, they may have downstream consequences. For example, you know, early correction of NO in the red cell may improve the ability of the red cell to maintain its deformability of storage.

There's a link between those two, that is, we will get into, and also, and, you know, similarly we know from Stemler's -- that it's possible to get NO back into the stored red cells and get the function back on lipid. What we don't know and we started to work on is whether that's a good thing for deformability or a bad thing, we know with sepsis there's leukoreduction and deformability of red cells gets worse. So it might be the red cell -- worse. This preliminary series of experiments, we seem to get just a little bit better with -- but these kinds of coordinated approaches to the multiple questions I think are needed.

DR. BRACEY: Additional questions or comments? Dr. Epstein?

DR. EPSTEIN: Thank you for helping us to understand a complicated subject. I guess my question is, it's been presented that there's rapid restoration of SNO hemoglobin after transfusion and the question in my mind is, if you look at long-stored blood versus short-stored blood, is there a difference in greater constitution in vivo of SNO hemoglobin?

DR. McMAHON: You mean there's demonstration of DPG getting restored?

DR. EPSTEIN: Well, I guess I'm asking about SNO hemoglobin. Is it not --

DR. McMAHON: It's not all the same.

DR. EPSTEIN: Yeah, is it not rapidly reconstituted and does that differ with younger versus older stored blood?

DR. McMAHON: That's a good question, that we don't have the answer to that, haven't done that. But it won't necessarily be the case. It may be the case that you get restoration of SNO after transfusion if your patient is okay and can make in NO properly and so on. But the opportunity may be, may have been lost to get NO to the right targets. For example, you know, we know there's this link between red blood cell NO and its deformity but we don't know a lot about the molecular link between the two, is it spectrum, is spectrum getting isolated, and it might be that oxidative changes that have taken place during storage are such that NO given later is too late, as an example.

DR. BRACEY: Any other questions? Doctor, thank you very much.

DR. McMAHON: Thank you.

DR. BRACEY: We are at a point for Committee discussion. Oh, yeah, Dr. Pomper?

DR. POMPER: I just had some comments.

DR. BRACEY: Okay.

DR. POMPER: I have been taking some notes. I just want to comment that from all the information we've seen from this morning that there is very little evidence presented that has shown on the benefits of old blood. So, it's I think for me reasonable to think that, I can hardly think of any benefit to older blood;

rather, there's a lot of detriment, seemed to be gained in the information and the research. The only benefit I can come up with so far is that it is there on the shelf, so, it's better to have the older blood there than none.

And, so, what's missing for me from this morning is an estimation of, well, how would keeping fresher blood on the shelf impact availability? I think Dr. Koch had referenced this by suggesting that it would be very, it would be a good idea to have mathematical modelling of inventory management. On a more simplistic level, that some of this information is in the journal paper, that there may be differences in the age of blood on the shelf based on blood type and there are also, I think there's quite a variable amount of blood that's issuing practices from hospital to hospital.

So, we really don't know whether a hospital, what age the blood is when a hospital gives out the blood. In fact, it's very difficult for me to tell the age of blood of a unit when we issue it. In fact, I have to go through a little spreadsheet and really can't just look at the unit and tell. I know when it will expire but I don't know how old it is. And so I think that's difficult for a lot of blood banks to actually ascertain, how old is the blood. So, my first comment was that it would be nice to have more information on this. And finally as these restrictions get layered on, we would all like to have fresh blood that's very, very specialized and it's very, essentially the best we can provide and so as these, as any new restriction becomes added to a blood transfusion order it's for me difficult right now to gauge how this would affect what blood is available.

DR. BRACEY: Well, actually that's one of the considerations that Dr. Holmberg had in preparation. You want to make a comment on that?

DR. HOLMBERG: Yes. Thank you, Dr. Pomper. That's one of the questions we really struggled with in preparing for this meeting and numerous people within the government were asking the same question. You know, we have anecdotal stories as far as what is the average red cell age. For instance, I heard somebody talk in Chicago that, you know, you know, at his hospital -- he's a surgeon, cardiovascular surgeon -- the average age of red cells, 29 days. You know, you go somewhere else, it may be older than that, may be younger than that.

And, I think that it depends a lot on institution. Whether the institution is a large user, many times the distributor may send the blood that may be older to that facility so that it can be used. And that's an assumption, too. I don't know that for a fact. But what we are trying to do is within our blood safety or blood availability safety information system, basis, is that we're contemplating going out with a question in that daily inventory to ask what is the average or actually I should say what is the median red cell on your shelf and at a specific time ask for that information. But there's other questions that we can possibly ask in that survey.

So, I'm open to suggestions on trying to get to that information. I think any information, you know, whether the results, the final results in clinical studies are one way or the other, I think that there's some basic information that we need to have as far as the availability of blood products.

DR. POMPER: Depending on the system setup at a particular institution that could be an easy or a difficult question. In fact, I would love to know how that's handled at Cleveland Clinic, how they measure this. For us the way to determine the age of a unit of blood would be to look at a computer system and find out what storage solution it's in, then we would take the expiration date and based on the storage solution subtract the appropriate storage time available to calculate some estimated date of collection. Then you would have to compare that to the date of issue to find out how old the blood is. It's not something that you can do fairly quickly.

And, so, it's a, you literally have to go back unit-by-unit and pull this out. It's a hard number to come by and our computer system will not generate that for us readily. So it's more difficult to estimate the age of blood on a day-to-day basis but yet it's critical for a lot of these issues. We have a high-volume transfusion service and we looked at a tiny little element of this a while back and the age of blood was vastly different from what the, in other words, what you had suggested, so.

DR. BRACEY: One of the internal conflicts that we have as part of our strategic plan addressing availability, we sought to have so many days of supply and the notion of having the fresher component is really, it's a contradistinction to that issue. So again these are challenges that we face and I think that, you know, one of the things that we have heard from the investigators is that the data are definitely suggestive and we need to proceed with analysis to study but maybe right now is not the time to make a significant change though we should know what the impact would be in terms of, if we make a change in terms of inventory availability. Dr. Epstein, you had a comment.

DR. EPSTEIN: Well, my comment is just that it's hard to disassociate safety of blood from the question of when is blood needed. Because safety is not an absolute thing; it's relative to the intended use, in other words the need setting. And I think that part of the problem here is that it may well be that older blood is not as good as younger blood. After all, you know, you're talking about living cells and they're perishable. You know, we know from recovery studies that recovery climbs as bloods age in storage. So these are perishable goods. We have the same problem going on with platelets. The question is, how bad can they get and still be of clinical benefit in the setting where there's a need? And this is where it ties into the issue of availability.

In other words, if older blood is not as good as younger blood but older blood is still better than not getting blood, we die without it, then it becomes an availability question, or, in other words at what point can you no longer manage an inventory and provide blood? So I see it as a good, better, best type situation and that there's really, it will be very, very hard for us to figure out at what point aged blood is no longer acceptable if we can't figure it out in the context of using blood where blood is needed. And that's my point.

DR. BRACEY: Ms. Finley?

MS. FINLEY: I actually echo what Dr. Epstein said but as we move forward in looking at the schedule this afternoon we don't have a lot of time for discussing but I wanted to just alert my fellow Committee members to the fact we're looking at broad issues of policy here and not, I just want to make the point that we can't get mired in the concept of whether, you know, a certain number of days too long versus others. We don't have enough information in that regard and we don't have frankly the authority to do that. That's strictly, it's a regulatory issue. So, I just wanted to before we get too deeply involved in all of this, make those points so you think about them in the back of your mind as we move forward this afternoon. There are a lot of interesting questions. There are utilization issues that were raised in the hallway that I think we should include in some of our recommendations but I just wanted to make sure that we understand that we do policy and BPAC does more scientific evaluation.

DR. BRACEY: Well, that's understood but I think that what we have heard from many of the investigators today in terms of policy, support of research, not redirection of dollars but accumulation of more dollars driving policy to support answers to the questions is important.

MS. FINLEY: Agree.

DR. BRACEY: Dr. Klein and then Dr. Lopez.

DR. KLEIN: First of all, I wouldn't entirely agree with that because I think if there is an issue -- we've heard a lot of the data but if there is an issue where there are a significant number of patients who are dying or suffering morbidity either because of too much blood or aged blood, we need to find out why and what to do about it. And that's a research investment issue and I think that is a broad policy issue. I also wanted to point out to my colleague that while I'm not advocating for older blood although I have more respect for age as the years go by, that both cell-associated viruses and graft versus host disease we're associating with younger blood so it depends again on where you cut it. There could be other disadvantages as well.

DR. BRACEY: Dr. Lopez?

DR. LOPEZ: I just wanted to make one comment. We have talked about this component from the point of view of age of red cells, having a number of red cells available but we have not really addressed another very important component, that is efficient practices. Are we really, when we talk about the negative effect of blood are we questioning that transfusion was needed at all? And I think we really need to look more at transfusion practices and review our standard guidelines.

DR. BRACEY: Well, yeah, and tied into that would be perhaps education because I would venture to say that clinicians that use blood products -- and we have heard today that they're one of the most commonly used therapeutics -- in no way do they think at the level of what we've discussed today and I think that if there is clearly a need, that is to educate people about what exactly what it does. Dr. Triulzi?

DR. TRIULZI: A couple points. One, Dr. Steiner, who this afternoon is going to present some pilot data that we needed to collect for the study design on age of blood in which University of Minnesota, University of Pittsburgh have about a hundred units worth of blood issued to cardiac surgery patients, so there's a frequency distribution to see at least in two high volume centers what that looks like. So that will give us a picture. I was going to mention the same thing Harvey did on older blood. There's some things that may have an advantage. I'll just add microchimerism, which is something that we're learning about, which does seem to be a property of younger blood as opposed to older blood, the clinical significance of which remains to be seen and is not abrogated by leukoreduction.

So, there are reasons to not reject older blood out of hand, other than that. And then I think Ileana raised a good point, that perioperative blood management has become a real banner for transfusion medicine anesthesia and surgery and hospitals that have embraced that, truly, like Richard Spence and Englewood have about 90 percent of cardiac surgery patients don't get any allogeneic blood. And so we have a long way to go to look at some of these studies. These patients are getting on average three, four units of blood that we could probably eliminate much of the risk just by improved practice. And that's not just transfusion trigger but optimizing hemoglobin preoperatively, optimizing platelet function and coagulation status and use of salvaged blood during surgery. So, I think that there's probably as much to be gained in that as there is with the actual blood that is required.

DR. BRACEY: So other thoughts of the Committee? Let me just then go back to the basic questions again. And we will hear this afternoon about trials that are planned and we will also hear from Dr. Dumont regarding the best collaborative practice in terms of looking at the use of older blood. Dr. Hebert will not be with us. He's had reasons for why, an emergency came up so he won't be with us. But back to the basic question, one, and I think the big question is, based on the information that we have at hand, should we recommend a change in medical practice, in terms of what we do on a day-to-day basis?

And, at this point, even though there is concern, and obviously one recognizes that the changes occur with storage, I think I heard from a number of the experts that while we're not quite there, what is the consensus of the Committee? Does the Committee concur with maintaining? Again, but one of the issues -- and this actually, this sort of crosses because this really does become sort of a regulatory, yeah, this kind of gets into the regulatory area, and perhaps this sort of a question may not really be germane to our deliberations but nevertheless I don't hear a strong, I don't sense a strong consensus that we should suggest that the regulators actively revisit, you know, the storage shelf life. Ms. Finley?

MS. FINLEY: I think you could, it would be a fair policy statement to say that, you know, the Committee has taken testimony on issues, that we are concerned about the impact of longer shelf life but we do not have or we do not believe that all of the scientific data is available. We can express concern in that regard and just say that we think other studies are needed or acknowledge that we believe that there are studies planned and encourage the department to hearing this out; that's, that's appropriate, which I think gets to the heart of concern without, I think, overstepping our bounds relative to the information that we have.

DR. BRACEY: Okay. Dr. Klein?

DR. KLEIN: The major piece of information that we don't have, that we didn't get this morning -- I don't know whether we'll have that this afternoon either -- is what impact would it have, shortened by a day or a week or three weeks; what would the impact be in the United States? And I think even if blood were extremely toxic, you need to know what the impact would be before you can say, well, this is what we need to do. And I don't think we have a clue right now.

MS. FINLEY: I agree. One other piece of information that's missing that's important is -- this goes back to Dr. Lopez-Plaza's conversation with me last night -- which is if, you know, as blood becomes more expensive and/or less available as in other countries, you know, the utilization will decline as a direct result. So, if we have that information about what our utilization would be, if there were certain other conditions including, you know, the requirement that we use less aged blood, then I think that would be an important factor to consider.

DR. BRACEY: So perhaps as the evolves so would the analysis of the inventory impact; in other words, the modelling of what with would happen if, you know, we would only have blood for 21 days, 14 days, five days, three days.

MS. FINLEY: And I didn't express it well. My concern here is that we don't say that if we were to reduce the storage time for red cells, therefore we would reduce availability and we would never, we just look at the two issues as a see-saw rather than looking at the rest of it. It's a much bigger question here. So, in other words, it's not an excuse to either not use, to use less, less old blood just because we might have some availability issues, is what I'm saying.

DR. BRACEY: Well, looking at ways to ramp up --

MS. FINLEY: Exactly.

DR. BRACEY: -- to be able to address it. We've got one comment from the floor. Dr. McCurdy?

DR. McCURDY: Paul McCurdy. I was a director of a reasonable-size blood center, close to 200,000 units a year, during the time when we went from 21 day storage to 28 day storage to 35 and ultimately to 42 day storage. And it is my recollection, I collected fairly careful data that we almost never had what I considered an adequate supply of blood. But managing inventory supply in the region, going from 21 to 28 to 35 to 42 days, the principal effects were considerable increase in the inventory of A and AB red cells, which we were not short of to start with. The effect on old availability was not very large, if any effect at all. It went out about as quickly as it came in. So I think there are some differences there. And it's my opinion that with adequate inventory management going down to 28 days would not have a serious effect on availability; going below that might. And it might conceivably help in having seasonal shortages but perhaps we can overcome this.

DR. BRACEY: Dr. Holmberg.

DR. HOLMBERG: Yeah, I just want to add that, you know, in the, I should say the data that we receive on weekly basis supplied to us from the blood collecting agencies, we get days of supply. And, such things as O negatives run anywhere from a 1.8 days to maybe as plush as 2.5 but usually never more than 2.5 days of supply for especially O negatives.

On the hospital level, there's a general rule of thumb that there's probably about an eight day supply of red cells sitting on the hospital shelf. And so I just, that's as much as I have as far as being able to tease that out. Now, that eight day supply, what percentage of that is older blood, what percentage is newer blood? I think that I would have to agree with Dr. McCurdy and my experience also is that, you know, the O positive -- are usually ones that are fresher and that the ABs are definitely the ones that go much a longer period of time. For instance, the ABs usually run about 17 or 18 days of supply in the hospital.

DR. BRACEY: Was there a comment on that? Dr. Pomper? No? So in terms of again a general sense, am I -- let me see if I can extrapolate. Is there anyone among the committee members who feels strongly regarding a need to change practice?

DR. LOPEZ: Regarding age?

DR. BRACEY: Regarding age. So I think the answer to that question, again, in relatively straightforward ways, come out with certain provisos that follow, and the provisos being that there's not an adequate evidence yet to make that move, though there are a number of suggestive studies and this should be studied more. We need to do more investigation. Dr. Pomper?

DR. POMPER: Just hopefully, I mean, maybe we could have a comment that it would be helpful to encourage or recommend active surveillance or monitoring of safety, the age of blood, including hospital demographic data so we can characterize if they're rural versus urban, high, if they're trauma center, not trauma center, large hospital, small hospital, et cetera, to get, to try to get a better perspective on how blood is managed at various blood centers.

DR. BRACEY: Actually that's a good point one of the things that Dr. Koch mentioned is that perhaps we should look at our distribution model and it could be that certain categories of institutions have blood that tends to be older than other categories. I'm trying to avoid anecdotes we actually contacted Dr. Ben Guerrero at our hospital and we collect blood in our hospital and therefore the turnover is quite rapid and our age was relatively fresh. So we assumed that our older units were the units that in fact had been distributed by the blood centers so they would get rid of, you know, shorter outdated blood to the larger volume units and in fact that was not the case. So it was rather surprising. So I think it really would behoove us to look at what the models are and maybe even to engage the providers to see, well, how do you distribute the blood in the community, because, you know -- Ms. Wigman?

MS. WIGMAN: Teresa Wigman from AABB. Just some background on that issue. In the national blood collection and utilization survey, that's done every other year, we asked that question in hospitals in the past in terms of what's the average age of a unit transfused in your facility for red blood cells and what have you, and, I believe, I don't have the figure right in front of me but for red blood cells in the last survey, from 2004, they, I think the average age was about 15 days. But we have done a follow-up question in this more, most recent survey to figure out whether hospitals are basing that on calculating the averages, average age or just doing estimates and our preliminary findings are that the vast majority of hospitals are just giving an estimate. And so, I would say, suggest that when you're, if we do collect data on that, it would be look at it carefully because the value of the data may not be as strong as we would want. I think only 3 percent of the hospitals were actually calculating average ages and I think that reflects the difficulty that the hospital has in supplying any information like that because they don't have it in their systems.

DR. BRACEY: That's an excellent point because when we got the information that we had on our age, it took a little bit of arm twisting. Dr. Klein?

DR. KLEIN: I would just caution also on how we use those data because an average or a mean might be a nice number but if you don't have the ranges, I think the issues with supply in the City of Washington are quite a bit different than the issues with supply -- where I think blood on the shelf might be quite a bit older for a variety of reasons and it might be safe at Johns Hopkins Hospital. The other comment I wanted to make on it for me is that, if I may, is I would like to take a page perhaps from Jay's book from yesterday and say that we have heard some data that raises some concerns about these issues. And so a frank no, I think, is maybe a little bit rigid because I think clearly we don't have answers and there's a potential issue here of very broad medical significance to the country and we need to investigate that -- and --

DR. BRACEY: Thank you. Dr. Epstein?

DR. EPSTEIN: Yeah, it troubles me if we would move to, you know, a yes or no answer to such a question. I think it's more valuable to the Department for the Committee to make a finding that the available information has raised concerns which ought to provoke suitable research.

DR. BRACEY: Okay. Additional comments or questions on those? Oh, yes. Sorry.

MS. BENZINGER: Yes. I would just like to reinforce what Dr. Epstein just said and also recognizing -- and I'm partial to lung patients -- there seemed to be a variance in there that's more impairment on them on the oldest sounds as to what I gather that, so, we want to take it as presented, on the data that was presented --

DR. BRACEY: Could you say that again?

MS. BENZINGER: I'm sorry. I was reinforcing what Dr. Epstein said.

DR. BRACEY: Oh, okay. Under the question of should there be more research on, I think that we would have general agreement that we need more research both in terms availability and the ways to understand the complex storage lesion and obviously the blood bank would strive to have improved products. I mean, that's what we do.

DR. LOPEZ: I have one more comment.

DR. BRACEY: Yes.

DR, LOPEZ: I think number two, we should specifically address that. We need to be looking at the clinical guidelines that are in use right now because it's a very big component of availability and then also maybe we should really be looking at not only hemoglobins or platelet counts but other levels, of clinical assays or evaluations that would help determine the need for transfusion and also the outcome of transfusion. I think we need to look at more data.

DR. BRACEY: Dr. Bianco, you have a comment?

DR. BIANCO: Yes, Celso Bianco. I would like to extend some of the research to the -- set of the transfusion. I think that we are treating in clinical data that we have, pipette -- patients as a generic. If the problem was specific floor population, this may be a couple percent of the blood that is distributed and the impact would be much smaller than a general change in age of blood. So I think it would be very important to look at different status --

DR. BRACEY: Thank you. Dr. Benjamin?

DR. BENJAMIN: Can I just agree wholeheartedly with Dr. Bianco on this one? Because I think the papers that have been presented raise serious concerns especially in -- surgery, on patient group, and, this really does get to the confidence that the patients under surgery might have around the blood supply and safety of blood supply. So I do think we need to have some comment around that issue, that there really is, I think, an urgent need to understand the biology and clinical relevance of red cell agent given this patient group, especially.

DR. BRACEY: Dr. Ramsey?

DR. RAMSEY: Yeah, just, I agree with what's being said. One other aspect comes to mind would be that I guess there have been efforts to try to extend red cell storage using various added solutions beyond what might be possible now. So I guess one suggestion that comes to mind would be that, that when in terms of efforts, interventions that would be made on a red cell, in a red cell storage system for other reasons such as extending the shelf life, obviously that would have the impact on many of these biochemical markers that we heard about.

Another aspect would be pathogen reduction technology, I don't know that there's any connection between pathogen reduction technology and the red cell biochemistry we're hearing about but it would be something to keep in mind, I guess, for those who know a lot more about it than I do in terms of how these two things might interact.

DR. BRACEY: Right.

DR. GOLDING: In listening to this morning's session, and discussion, it seems to me there's a logistical issue that we discussed and that is what is very clear that the data raises concern -- policy statement right now but the data that's missing is to do prospective studies. The only question there I would ask is how many they're going to take, three years, five years, before you get the data. And, meanwhile there is this concern that we haven't done anything about. My question is from a logistics point of view one of the missing things is, what is the impact?

The reason why we don't want to take an action because there may be a very negative impact on blood supply but maybe, maybe the answer to that question could be more quickly answered by, I would think I'm not sure, but it maybe you could find out in a month or a few months or a year what would be the impact of changing the storage time from 42 days to 28 days to 14 days, and if that is known, impact is small, depending on the change, isn't that a way to go forward and to say, well, then see what the impact is, then do a risk-benefit analysis and make a decision so we don't wait five years or longer to find out if we really have a major issue here that a lot of people have been adversely affected.

DR. BRACEY: Thank you. If there are no more comments, I think we have had a good discussion of the issues at hand and we're now ready for a lunch break and we'll rejoin in an hour.

(There was a break in the proceedings.)

DR. BRACEY: Good afternoon and welcome back for the afternoon session. As I mentioned before, unfortunately Dr. Hebert will not be able to join us. Our next speaker is Dr. Simone Glynn. Dr. Glynn is in the Transfusion Medicine and Cellular Therapeutics

Branch of the Division of Blood Diseases and Resources from the NHLBI. Dr. Glynn will tell us about the plans for future red blood cell studies that we're all so looking forward to. Thank you.

DR. GLYNN: All right. Well, thank you and good afternoon. And I wanted to thank you for giving me the opportunity to present to you our plans for the red blood cells transfusion studies at the National Heart, Lung and Blood Institute. So just a reminder, I am in the transfusion medicine cellular therapeutics branch, which is in the division of blood diseases and resources so this is an extramural division. That means that our major role there is to fund and support and manage a large portfolio of grants and contracts in research areas that we do specialize in. So, just to mention also that you can have investigator-initiated grants or you can have also institute initiated programs.

And, just to remind the Committee that we have two programs that they may be particular interested in today. One is the transfusion medicine and hemostasis clinical trial network program, which includes 13, I'm sorry, 17 clinical centers and one coordinating center, nearing, and as the name indicates, this network is charged with conducting clinical trials in the areas of transfusion medicine and hemostasis.

The other program that was also initiated and is of interest if today's discussion is the Retrovirus Immunology Donor Study Program or RIDS. RIDS is in -- phase, it consists of six blood centers and one coordinating center, Westat, and it is charged with conducting our lab survey and AE, epidemiological studies related to blood donation safety and availability. That's just a reminder of what we do.

Okay. So, I also wanted to inform you that the institute recently released a strategic plan to serve as a guide for its research and training programs for the next five to ten years. And, the process initially involved a series of thematic strategic planning meetings involving members of both extramural and intramural research communities.

And one such group concentrated on issues related to global blood safety and availability. So, what this group recommended, the group met in May of 2006 and came up with a series of recommendations. And I just listed the first two major research needs that were identified and these were to define the immunobiology and the immune consequences of transfusion and to define the biology and the clinical indications for red cell transfusion.

So, I took a quote from the minutes of the workshop that you have here below and the group said that the impact of component factors, including storage age -- so that's what we're here to discuss today -- on the function of transfused red cells and physiology at clinical levels are largely unexplored so essentially requiring more research.

So what we usually do when we have a workshop is we follow that up with kind of specific working groups that are able to flesh out the details of exactly what kind of research is needed. And we did have a such group. It was convened, it was in May of last year, and this group essentially came up with very similar recommendations as a workshop, which was good, and also came up essentially with the idea that there is a strong need for studies on transfusable red cell units as a function of preparation and storage.

So, why is there a need for research in this area? And I think we heard about this quite a bit this morning. We've heard that there is a growing volume of literature that reports that there is an association, and again I'm not using the term causal association, it's just an association between transfusion, specifically the number of transfusions, and an increase in length of hospitalization, postoperative infection, lung injury, tissue, hypoxia, bleeding, thrombosis and multiorgan failure.

We also have another body of literature that's emerging and again with some studies, as we heard this morning, that do show an association between the transfusion blood that has been stored for longer period of time and some poor clinical outcomes. We also heard this morning that we do have some studies that do not show association and that these reports are often very difficult to interpret and potentially confounded by severity of the illness. Although we tried to adjust our models for various confounders, this is all, you know, within, we're always making some assumptions in statistical models and it's really difficult to really adjust for differences at baseline and approach. I don't think you can, actually.

The potential mechanisms that have been suggested, essentially two major hypotheses, one would be the storage lesion defects cause some immune and inflammatory complications in the transfusion recipients, as we heard this morning, and then another hypothesis is that there may be susceptibility factors which predispose certain patient populations to the potential adverse effects of red cell transfusion. So essentially this probably, if they do exist, coexist. We know there a storage lesion defect but I think the research question is whether this has clinical consequences.

So, essentially we are now faced with a

research question of importance, which is again whether the storage of red cells somehow predispose you to have poor clinical outcome if you are transfused with a red blood cell unit that has been stored for longer periods of time.

So how are we going to be addressing this question? And, I guess -- I'm going to talk about clinical trials in a minute -- but usually when we address research questions we try to or at least I try to think about it in what are the research tools that I should be using to address this research question, and I kind of categorized the research tools into three broad categories. One has to do with the epidemiology observational studies, research tools that we have. The second one has to do with the phase one to four clinical trials, the clinical trial research tools, and then the last one are of course our basic animal model and early human physiological research we can do in the lab.

So, first thinking about this new research question, so one thought was, is there a need to do any additional epidemiological associational studies? And we decided that there would be a need if we could find a database that would provide a lot more information on many more patients than -- available so far. So, we're trying to establish a collaboration for our RIDS program and more specifically our UCSF Center for Drs. Murphy, Busch and Custer, with investigators in Sweden and Denmark, who have established a very large donation and recipient information database which is called the ScanDat database.

And essentially it's very comprehensive and it includes information on both the donations and on the clinical outcomes and on mortality, of course, on the recipients. And we think that we would be able to do an observational study that will include about 400,000 recipients, which, of course, is much larger than what you have been able to see so far. And the nice thing about that is then we would be able to evaluate some of the subpatient populations that are of interest, much better because then the numbers would be bigger. So, that's what we're going to be trying to do in the epidemiological observational arena.

Going on to the clinical trial category -- -- relate that there is a need for phase three clinical trials, and we also believe that it should be done probably with different patient populations because I think, as was discussed this morning, what you find in one patient population is not really reflective of what you may find in another patient population.

So, it's unfortunate that Dr. Hebert will not be able to join us to discuss the ABLE clinical trial. I can tell you a little bit about what I know but I may, I don't know a lot of details about it. What I know is that it's going to be a phase three randomized clinical trial, in intensive care unit patients, and they are going to be comparing, if I'm not mistaken, less than 7 day old red blood cell storage versus standard of care. And I do not know what the standard of care is in Canada, so unfortunately I can't answer that for you. What I know also is that it's been funded and it's going to get, you know, it's going to go and get started pretty soon, I think in the next couple of months, I think. So that's really exciting.

And then the other clinical trial that we're trying to give a lot, and that's within the transfusion medicine and hemostasis clinical trial network, is called RECESS and Marie Steiner is going to tell you about our plans there, but essentially this patient population is the cardiovascular surgery patients.

And going on to basic research, we also feel strongly that there is a need for basic research to better characterize storage lesion elements and to also, maybe foremost, understand the interaction between the storage lesion elements and the host, so the vessel wall, host cells such as pulmonary endothelial hematopoietic cells and of course the role of the storage lesion elements on microoxygenation. And pretty much regardless of what we find, we review the clinical trials or, you know, over observational studies. I think it's always important to try to improve our red cell therapies and to be able to do that we need to understand what's in those bags; otherwise, we can't change them.

So, thinking about basic research and how to support that, the first thing we usually do is we look across NIH and we try to find out, well, how many grants are being supported in this area so we did do that search. As you can see, there are not many -- kind of listed them here -- there are not that many grants that are being supported throughout NIH so that's 27 institutes and centers and none of them have anything to do with blood products.

So, there was no doubt in our mind, in regards that there was a need for NIH initiative to stimulate research in this area. And we did that and Dr. Nabel approved -- Dr. Nabel is our director, and this request for application was released in March of 2008. So, it was just released. And it's an initiative in blood banking and transfusion medicine that proposes to support basic and translational research including basic human physiological research, and it's aimed at again characterizing storage lesion elements and then again trying to understand the interaction between the elements and the host.

NHLBI intends to commit up to three and a half million in fiscal year '09 to support an estimated, we hope, five to eight meritorious projects. Support will be provided for four years as long as our sponsor is successful and the scientific review of the applications will be managed by NHLBI so that means we will convene a special review with particular expertise, in that particular -- so this is different from the usual grants that are reviewed by the Center for Scientific Review.

And I've just put some dates. If you missed last week's application due date, then please consider applying for the January 1st one, and we hope to be able to fund -- by September 30 of '09. And then finally I just added this as a reminder to everyone -- that of course we always encourage investigators to submit applications, R-1s and R-21s -- to -- RFA or PAR or something, we have. So that's, all I have.

DR. BRACEY: Thank you. We're certainly

happy to heat that there are plans afoot to support this important area. Questions and/or comments from the Committee regarding the presentation? Yes, Dr. Holmberg?

DR. HOLMBERG: Dr. Glynn, you commented that 3.5 million will be available in fiscal year 2009 and this potentially could go for four years. What would be, is it anticipated how much would be a sustained amount for fiscal year ten, eleven and twelve?

DR. GLYNN: The anticipated amount again, depending on the, you know, what we find appropriate, the same amount for every year for four years.

DR. BRACEY: I have a question. And on the relative scale, perhaps if we used Canada as a mark, what is our degree of investment contrasted to other nations for these sorts of efforts?

DR. GLYNN: Boy, that's a good question and I don't know the answer. I think definitely we know certainly in the clinical trial -- agents have first -- some of the -- major clinical trials, TRICC trial, --

are definitely ahead in their thinking in terms of this question about the number of transfusions and clinical outcomes. So, I will say they are, they have been ahead in the clinical arena. In terms of the basic research, I don't think that actually that much has been done.

DR. BRACEY: Yeah, because I know one of the things that we heard from the investigators earlier today is that, you know, we have a number of leaders in the field and those leaders need, you know, funds to continue. I was interested in the comparative data. Dr. Epstein?

MR. EPSTEIN: I had a similar thought in mind but along the lines of international collaborative study opportunities because clearly the issue of establishing scientific evidence based for transfusion practices is a global concern. There has been lots of discussion about the various -- in Europe, you know, Council of Europe, World Health Organization bodies, and I just wondered whether there are opportunities to leverage the U.S. effort such as, you know, international sites and collaborative arrangements.

DR. GLYNN: I'm certainly very hopeful to consider, you know, such collaboration, so, and again as soon as we, whenever we can we try do that. So, for example, just Canada as an example of what we're trying to do there, so, and certainly we should certainly think about how we could collaborate on some of these clinical trials, you know, that are hopefully going to go forward. So, that's certainly something to consider. Of course, it's always very difficult to enroll all those patients. That's the most difficult thing in the clinical trials.

DR. BRACEY: Thank you.

DR. GLYNN: You're welcome.

DR. BRACEY: Our next speaker is Dr. Maria Steiner. Dr. Steiner is at the University of Minnesota. She's in the department of pediatrics in the Sections of pediatric critical care and hematology, oncology, and bone marrow transplantation. She will present on the NHLBI Transfusion and Hemostasis Clinical Trials Network proposed studies.

DR. STEINER: Thank you very much, Committee Chair, members of the Committee, ladies and gentlemen. And I am very honored to be here today. I am also honored to be the one representing this very distinguished group of investigators who have been working in a very dedicated fashion. We've had teleconferences once a week, almost once a week for most of the past two years in order to develop this protocol. And, some of the names I'm sure you recognize as to those you know very well.

So, at any rate, our proposed study which is developed through the NHLBI's transfusion medicine and hemostasis clinical trials network is a red cell storage age study, The Pediatrician, it came out, it's called RECESS. Not that one, not that one -- manager environment, right? That one. There it goes. Okay. See, my kids put together my PowerPoints, I'll be honest. My conflicts of interest you see listed there. None of them are impacting today's presentation.

I think we've spent the greater part of today discussing the fact that there still is equipoise about the effects of the age of the red cell products we transfuse to our patients. We've talked about the fact that there are some retrospective studies which do show poor outcomes in patients transfused with longer storage age red cell units but then we also have discussed a little bit about the fact that there are some studies which actually show no deleterious effects if longer age products are given to our patients. And you see that some of those are small studies and some of them are larger studies which folks brought up earlier today and then last but not least is the pilot randomized control trial which is the backbone of the ABLE study that Dr. Hebert is putting forward in Canada, in which ICU patients were given blood either less than eight days old or their standard of care, which I believe is around 19 days of age, 27 percent in the less than 8 day group had life threatening infections as compared to 13 percent in the -- group, and although this is not statistically significant, it was just a pilot to put here to assignments -- ages it provides proof of to carry this forward.

. I think it's a true statement that we can say that there has been no large randomized control trial which has evaluated the effect of transfusion of red blood cell units stored for different periods, on any one of these outcomes that we could choose to look at, whether a clinical outcome, on immediate oxygen delivery enhancement, on microvascular -- changes or on even standard hemodynamic variables and end organ function measures. We give red cells -- oxygen delivery and make our patients better but we really haven't demonstrated that that is the case.

So our proposed phase three clinical trial has the primary hypothesis that there will be no significant difference in clinical outcome and mortality between recipients transfused with shorter storage age red cells and recipients transfused with longer storage age red cells. We're being politically correct in saying shorter longer also.

The study design is patient population -- am I too loud?

STENOGRAPHER: Actually, if you could speak up a little bit.

DR. STEINER: Speak up? All right. Do understand, I used to be a cheerleader and when you tell me speak up, you don't know what you're asking for. All right. Patients who are over 12 years of age and 40 kilograms in size or undergoing complex cardiac surgery which we define as multiple procedures, re-dos, something that is worth the effort to enroll them and not a straightforward first single vessel coronary bypass patient, that doesn't seem like the right patient population which to look at this issue. We want patients who are likely to be transfused either intraoperatively or within 96 hours postoperatively and we have identified a tool by which we think we can successfully choose these patients in the preoperative arena and have been transfused afterwards.

They will be randomized to transfusions less than eight to ten days at the time of release or stored greater than 21 days at the time of transfusion. It will also, leukoreduced AS red cells of assigned age and the age will be the age assigned for all transfusions given intra and post-operatively through day 28. So they will get blood cells of this age right from the get-go, through hospitalization, which is something that hasn't been done two years ago.

Our primary endpoint is a clinical outcome which we're going to assess using a change in multiple organ dysfunction score, which I will refer to as Delta MODS, from the preoperative baseline to the highest composite, MODS through day ten or death or discharge for those who come first. So the highest multiple organ dysfunction score compared to the preoperative multiple organ dysfunction score and the highest composite because different end organs will misbehave at different points in time in the postoperative course.

Our secondary end points will be the change in MODS, discharge death, or postoperatively day 28 which we will call end of study, the actually 28 day mortality rate and then measures of end organ function and oxygenation. Globally speaking lactate levels or individual end organ dysfunction markers such as troponin, creatinine -- liver function tests.

The scheme looks like this. Patients will be consented preoperatively. They will be randomized preoperatively to either shorter storage age or longer storage age size. They will receive those cells in assigned age through their surgery into their ICU course and then through day ten which is our primary endpoint continuing on through our secondary endpoint which is day 28 after discharge. There is an optional physiologic substudy which was -- now, who gets in? First of all why are we studying cardiac surgery patients? Well, these folks commonly require multiple red blood cell transfusion and so if there are effects that we can ascribe to the age of red cells it ought to be in this population.

This is also a very large group of patients with very significant red cell usage. We were talking about the impact potentially on restricting age of our products and going back and doing some math with pen and pencil because I don't have a calculator with me. Dr. Goodnow published some data suggesting that there are around 14 million units of red cells transfused annually in the United States. There are other references that say between 10 and 20 percent of those units are given to cardiac surgery patients, so that's around two to two and a half million units of red cell also a year. And so that's a fair number of red cells being transfused in the population.

We've already talked about the fact that there's conflicting data for retrospective studies and some small prospective studies have evaluated association of red cell storage time in cardiac surgery outcomes. I particularly as an intensivist like the fact they undergo invasive cardiorespiratory monitoring and so there's data available on oxygen consumption and delivery and other physiologic parameters that will be readily available to correlate to the red cell transfusion, because, after all, this is what we're supposedly getting a red cell transfusion to positively impact.

And lastly, it complements the ABLE study in the ICU patients. These are different patient populations. We have talked about the fact that -- quarter of the bypass run -- that the hemolytic effect of the age of the product may have some impact and so it's complementary; it's not the same.

Now, I talked briefly for just a second about the fact that we're going to do something called a TRUST score to include patients in this study. The TRUST score was a scoring tool put together in Canada about five, six years ago originally whereby they looked at well over 10,000 patients and tried to decide how best to prevent whether or not someone coming into an operation was going to need a transfusion. There are multiple scores which look at this but this was a way that we could very easily look at somebody in the preoperative setting and predict whether or not we thought they would likely need a transfusion.

The risk features predict the need for transfusion and per these parameters over here, the age, the gender, the hemoglobin, the weight, baseline creatinine, whether or not the surgery is elective or not, whether or not they're a re-operation and had a previous cardiac surgery and then whether or not the tasks are an isolated procedure or multiple procedures necessary. Each of these is given either a zero or one. A maximum score is eight. And the predictive probability of a red cell, receiving a red cell transfusion either intraoperatively or postoperatively is dependent on the total score, zero less than twenty and greater than eight to the four, 80 to 100 percent likelihood that you will receive a transfusion either intra or postoperatively, at least one, maybe more.

In order to assess our feasibility and being able to do this study at our centers and in order to see if we could actually screen our patients this way for the TMH centers potentially interested in participating in this study, I screened a year's worth of our cardiac surgery patients to see if we could generate the score and then to correlate that with other -- that mirrored our own transfusion practice. And at our four centers we found an 88 percent probability of receiving a transfusion with a score greater than eight to the four so we were pleased.

Now, why those pediatric inclusion criteria? Well, we're talking about using a multiple organ dysfunction score for our endpoint and there really has been no organ dysfunction score validated for both adults and children. It's too bad but it's the truth. Specifically there are none for pedia-cardiac surgery patients. Although the RAC score is being developed, it's nowhere close to where we could use it yet. There are multiple organ dysfunction scores and pediatric modifications of scores. There's a P-MOD score developed by the folks at UT Southwestern and there's a score the -- use -- in Tri-PICU study, the PLOP (phonetic) score but they used different data in a different scoring range and so aren't interchangeable. Because they're not interchangeable we can't analyze our patients together and so we need an even larger study in order to use two different systems for two different populations.

So we chose pediatric subjects, who were greater than -- 12 years of age and greater than or over 40 kilograms. The "and" is important because some of the children with congenital heart disease don't grow well and they certainly don't mimic adults. But, fulfilling both the age and weight criteria, they should be physiologically similar enough to adults to justify using the adult scoring system.

Lastly, regardless of what I think and what I would do anyway, the trump card is the fact this the surgeons will not randomize our younger patients to older blood. In fact, we just went through an exercise with my new surgeon who will insist on the freshest, youngest product available for his neonatal and his toddler cardiac surgery patients recognizing we can't give him fresh whole blood, which makes most blood bank people's hair start on fire when you start talking about fresh whole blood. Turns out that many of the major pediatric cardiothoracic surgery centers in the country, they do want the freshest, youngest product available. This information is, this bias, I want to say is based on the scans and old data and is not able to be delivered at most pediatric surgery centers. So, the bottom line is even if we wanted to include younger patients, at this juncture we couldn't.

Now, the transfusion arms -- and this gets at one of the questions which we were talking about a little bit earlier a today, why we chose less than or greater than 10 day versus greater than or -- 21 day old red cells. The greater than or less than ten day old product is something that we can meet demand for in doing the study. That means something harvested on a Friday is good until a week from the following Monday. Weekends being what they are, people -- go out free on weekends or try not to, anyway.

And the less than ten day old product is comparable to the younger product in other studies such as Hebert's study. We actually have modified the upper age range quite a bit. This is actually the third iteration of that upper age limit. And we chose greater than -- the 21 day -- didn't as the longer storage age product because it is comparable to current practice at many of our transfusion medicine hemostasis medical network centers.

We did a quick-and-dirty survey at PITS, MGH Minnesota. You see that in this diagram here. Each of these lines represents ten units of the product given to cardiac surgery patients. We just pulled records for a week and what we've been given through the course of a week. The lighter purple is less than 28 day and the darker purple is over 28 day old product. And, the range of product that was given in that given week was 20 percent over 28 days up to 48 percent over 28 days.

So, quite a varied practice in just a snapshot, and that's all it is, is a snapshot. But my own surgeons will say, well, of course we'll participate in the study because you give us all this crap you've got anyway. So, quite a variability in practice, very, longer storage age, shorter storage age.

Then Dr. Triulzi's institution and our institution added up all the units we gave in that particular week and bar-graphed by age, the purple area right here is 21 days. So you can see that all these to that side of the arrow are products given out that week that were 21 days or older and everything in this side of the arrow is less than 21 days. 21 days for the upper storage age limits is also comparable to that used in other studies, Dr. Hebert's study, the van de Watering study -- Basran study and Dr. Koch's study. So less than or equal to ten days versus greater than or equal to 21-day.

Now, the endpoint. What is the multiorgan dysfunction scoring system? Well, it's a scoring system that John Marshall developed back in the nineties after reviewing the literature on what multiple organ failure is defined as including in the critic care literature to that point, comparing that to 300 and some odd patients that he felt had multiple organ failure and then validating it against another 300 and some patients.

They ranked each of the five organ symptoms, sorry, six organ systems by degree of dysfunction, with zero being no dysfunction and four being very dysfunctional. The scoring system automatically gives a maximum score of 24, so four times six for anybody who dies. So, that includes both dysfunction and death in the scoring system itself. It uses very common, commonly acquired patient information. The respiratory index renal function, liver function is indicated by a bilirubin, something called a pressure adjusted heart rate, which takes into account filling pressures as well as hemodynamic status. Hematology is based on the platelet count and Glasgow Coma score.

This is, it's hard to get a reproduction of this because this is available only -- not as a PDF file, that's how old it is -- but the ICU mortality is along here and the multiorgan dysfunction is divided into categories here, one through four, five through eight. You can see it on your handout. Hospital mortality here, dysfunction score here, ICU length of stay here, organ dysfunction score there. So you can see that the organ dysfunction score, the light bars are the original data derived from the literature and from their first cohort of patients; the dark bars are the validation score in this ICU mortality percentage.

So you can see as the dysfunction score goes up, mortality goes up, hospital mortality goes up, ICU stay goes up.

So why should we use that as our endpoint? Well, like I said, it's easily calculated from readily available data. It correlates well with mortality. It incorporates mortality in that you can assign a maximum score to those folks who die of 24. In contrast to other scores like CASSIUS and the SOFA and PILAT, it's not based on management or interventions so you don't have to take into account whether or not someone manages pressors like you do, whether or not someone manages a ventilator like you do, and adjust your score on that basis.

It is widely used and well validated in the critical care literature and has previously been used as an outcome and an endpoint in transfusion studies, most notably the TRICC and the TRICC cardiovascular cycle.

Now, in terms of how we set the study up, we chose to do equivalence study. And why did we choose to do something that was harder than it might have to be? Well, because many people do believe that the storage duration of red cell product makes a clinically important difference in the patients to whom you transfuse it. And so and equivalence study is a more rigorous study. It starts out with a null hypothesis, there is an important difference, and then tries to rule out that important difference. The result that's generated is more compelling and we figured we have one chance to do this.

So the null hypothesis is that there is a clinically significant difference between less than 10 day versus greater than 21 day old product given to these cardiac surgery patients in terms of how we calculate the sample sizes. We generated a two-sided confidence interval. If the entire confidence interval then lies totally within the prespecified region of equivalence, the null hypothesis is rejected and you can conclude that there is no clinically significant difference in the changes or the development of multiple organ dysfunction between the two treatment groups.

So what's significant? Well, in the cardiovascular TRICC subset, the treatment arm difference in the clinical outcome patients, the Delta MODS was one point with a standard deviation, 7. In the TRICC study overall there was a one point difference in the Delta MODS, and that was felt to be not clinically significant. Paul Hebert went on to say in the ABLE pilot, the absolute difference in major outcomes such as mortality, organ failure, and infections less than three to four percent -- red blood cell ages may not be worth pursuing. And one point in the Delta MODS correlates to around 4 percent mortality.

So, therefore it seems the differences between treatment groups in their Delta MODS from their preop to the worst post-op compounds is for and less than one point wouldn't justify changing practice. Therefore our trial uses the next teeniest little increment over that, plus or minus 1.2 points as the smallest clinically important treatment difference in the Delta MODS between the two ages of red cell product.

To maybe explain it a little bit more easily in terms of a diagram, here's equivalence, over here is minus 1.2, over here is plus 1.2. This is the average change with the confidence interval here. This is a different simulation with the average change confidence interval here and because this confidence interval crosses minus 1.2, this is not an equivalent trial; you could not reject the null hypothesis because that 90 percent confidence interval includes the equivalence limit. So these would be trials where we would reject the null hypothesis and this is a trial where we wouldn't reject the null hypothesis.

So based on those statistical considerations, recognizing this is a two-sided equivalence test with type one of -- percent power -- the exercise we just went through, we'll need about 800 transfused patients per arm. Now, can we do it? Well, the blood bank underwent an inventory assessment survey and of the eight centers who answered -- mine didn't bother to answer which embarrassed me -- eight of eight centers could meet the needs for participation in this trial with one to two days notice, meaning that they could sequester between six and ten units of the appropriate aged red cell and maintain that inventory through that patient's hospitalization.

We then looked at patient accrual assumptions, which even though you need 800 patients per arm that doesn't mean that's all you need to find out there in the world. Based on other transfusion trials, we adopted a very conservative estimate, that 25 percent of those patients who were approached would consent to participate, based on our data from our own centers, 80-odd percent of patients with a TRUST score greater than or equal to four would be transfused in the intraoperative or postoperative period. Based on our, again our surveys at our own hospitals, 27 and a half percent of all of our cardiac surgery patients did have TRUST scores of greater than or equal to four.

And so working backwards if you need 800 per arm, you have to consent 1800 to make up for those who actually don't get transfused. That means you have to screen and find 7200 who have a TRUSS score greater than or equal to four, which means you need to prescreen or at least approach thinking about 2600 different patients. So you screen 2600, 7200 have a TRUST score of four, 1800 consent, 1600 wind up transfused. That's a lot of patients.

However, having said that, we have twelve of our fourteen centers, who have agreed to participate based upon their annual cardiovascular surgery population. That group has 15,000 patients a year, from which we start screening. So, this study could be done just within the centers that we have in the network in approximately two and a half to three years.

Now, the part of the study which is a little bit more labor intensive and a little bit more costly but which I maintain is just every bit as important as the other study is the in-depth physiology substudy. Basically we have our patients who are consented to receive other shorter or longer storage age red cells who go to the ICU, who within that first

96 hours of getting to the ICU, if they meet certain criteria are going to get what we call an index or an INDA study transfusion.

The primary hypothesis to doing this physiology substudy is that the shorter storage age blood does not differ from the longer storage age blood in its impact on physiologic parameters of oxygen delivery and consumption, specifically tissue oxygenation microvascular flow and other measures of end organ function measured before and after index transfusion. This is probably highly related to whether or not nitric oxide can be on or off-loaded and whether or not the red cell is as deformatable when it ages in the bag as opposed to aging in the body.

Our secondary objectives are to determine whether or not the physiologic parameters of oxygen delivery and consumption are associated with clinical outcomes. Remember, a lot of what we're doing is transfusing because someone hits a triggered hemoglobin, define what that trigger is indicating. Is it a number or is it indicating a physiologic process going on in the patient? As an example, if I'm trying to rehab after cardiac surgery, is the hemoglobin sufficient for me to being working on a treadmill or working on a Stairmaster and is my physiology the same as someone who is laying in bed pharmacologically comatose and immobilized because of increased intracranial pressure. Does that safe hemoglobin abate? Not enough.

The second objective is to try and determine whether the storage lesion, biochemical and biophysical changes are associated with the physiologic response to transfusion. Are those little cells that are not as deformable able to deform as they circulate? Are those little cells that don't -- nitric oxide as well able to regain that -- or nitric oxide or to generate nitric oxide? Does it happen immediately? Does it happen as they circulate? Does it not happen at all? Nobody knows.

So to be included in the physiology substudy you have to be enrolled in RECESS. The index transfusion needs to be ordered according to standard practice at your institution within the first 96 hours of ICU admission and you need to be clinically stable in the two hours prior to the index transfusion. This red cell product cannot be given because you're being resuscitated. It is the typical scenario that's just to make them feel a little better -- transfusion. Well, we well want people who have had no inotropic changes, no respiratory support changes, no fever, no changes of blood pressure, no -- no ongoing blood loss in order to have a stable physiologic baseline before this red cell transfusion is given to see what we do to impact physiology with just the red cell product and that alone.

Exclusion criteria are related to this phenomena of having a circuit that messes things up. So that if you are on renal replacement therapy because of renal dysfunction, if you have an LVAD, IABP or ECMO support, so you've got a circuit in line, if you are a cyanotic cardiac heart patient with a PO2 less than 60, you're going to have different physiology than someone who is normally pink like they should be. And because we're worried about central nervous system impact cells, deep hypothermic deep -- rest during surgery, excludes for this in-depth study.

The primary endpoint to the physiology, study is the maximum change and something called the thenar eminence tissue oxygenation parameter from the preindex red blood cell transfusion to the worst value through day three after the transfusion. What the STO2 is, is the difference between oxygenated and deoxygenated hemoglobin in the capillaries, in the muscle. So it's an index of how well your tissues are oxygenated, and we'll measure those indices right after transfusion completion, one hour, four hours, and one days, two days after the transfusion to see if they change.

The secondary endpoints are the maximum change in something called functional capillary density in the sublingual microcirculation using Sidestream, Darkfield illumination, SDF. This basically looks at red cells rolling around in capillaries under your tongue from the preadmixed transfusion to the worst value, day three, look at correlations between Delta MODS and both the thenar synapse and the SDF, tissue ischemia markers and red cell biochemical changes. We'll look at comparisons of the NIRS and SDF, end organ measures, some things like cardiac output, things like lactate.

We'll see if those are able to predict development or progression of multiple organ failure and/or death. They're being used in the Shock Trauma literature in order to do just that in terms of even using them in the resuscitation in the emergency room. And then we'll also look at changes in red cell characteristics as a function of storage age and how they impact all these parameters as well.

This is the Spectrophotomer technology, it's not invasive, used as an adjunct to invasive monitoring. It's just a little censor that goes over your thumb. The censor shoots light in. Light comes back out the other side. And through the software you get a capillary oxygen saturation reading.

The Sidestream Darkfield imaging using

technology called MicroScan looks like a little, it looks like something my mother used to take my temperature with. But that's actually where it goes, is this little probe which is about the size of your little finger, goes under your tongue and light goes in, light comes back out. And this is the kind of picture that is generated and this kind of picture can be used through software analysis to generate an idea of how quickly red cells are flowing through the individual capillaries and what that capillary density is in that region.

So, in other words, if you're giving red cells in order to enhance oxygen delivery and oxygen consumption, if you transfuse someone you do actually open up capillaries to feed tissues that have previously been hypoxic. The Shock literature would suggest that that's what happens with volume resuscitation. We'd like to know that's what happens when you give a red cell product. And is it impacted by the age of the red cell? Can the red cells be able to reopen small capillaries that are closed, and if they can get them open, can they flow through them and if they can flow through them how fast do they flow through them?

To show you we're not kidding when we talk about in-depth, these are the study measures here. These the times over here. And, we use the thenar saturation monitor over the sublingual probe, hemodynamic measures, cardiac indices. Remember, most intensivists they think that the cardiac output and the cardiac index is the gold standard to whether or not oxygen delivery and consumption are optimized. And we don't know that for a fact and we don't know what happens when we give a red cell product to that cardiac output. It should go up. Maybe it goes down.

There is some data that I'm not supposed to identify completely but there are some folks who are doing data looking at oxygen delivery and oxygen consumption using the thumb saturation monitor looking at different ages of red cell product and actually in their preliminary data, which hasn't yet been published, the oxygen saturation in the thenar saturation monitor drops with products that are over 14 days of age and goes up with products that are younger. And the drop is not insignificant. The drop is 7 percent if the product is old; it goes up 5 percent if the product is new. But, again, preliminary data.

We're also looking at blood gases and other measures of end organ function, troponin and lactates and we also want to actually look at the storage lesion, take out an alloquat of the product -- lesions, guess you should say -- take alloquats from the product and then take alloquats from the patient after they have been transfused an hour, day one and day three to see if there's any change in recovery, any impact of these red cell transfusions when they're given at various ages.

We also are even more committed after discussion today to create a repository so that folks who are interested in looking at the impact of varying ages of red cell storage of different phenomena can have access to these samples. In terms of a sample size for the substudy, the normal thenar saturation is

87 plus or minus 5 percent. It's been validated in a number of different series and is impacted only if you're in Miami Beach on a very hot day. Then the number is actually a little higher. A difference of plus or minus two percent so a change in the STO2 is probably not clinically relevant. And so incorporating that into standard statistical considerations, we only need a 120 physiology substudy in each of our two storage ages in order to be able to look at whether or not there is a change in the thenar saturation that's significant.

So, in conclusion we've talked about our proposed prospective randomized controlled trial which will evaluate the impact of red cell storage and development of organ failure in transfused cardiac surgery patients, why we chose those patients, why we designed the study the way we did. We've also talked about a proposed substudy of the impact of red cell age on oxygen delivery and oxygen consumption, which I maintain is the Holy Grail in why there are transfusion patients in the first place. And we use both traditional and nontraditional, novel assessment measures in order to try to get at those ideas. So the bottom line is stay tuned. We're hopeful that this will move forward fairly quickly. Questions?

DR. BRACEY: Thank you for that extensive review of the well-designed study. We got time for one or two questions but we have to move on so that we have enough time for discussion. Dr. Epstein?

DR. STEINER: Yes, sir.

DR. EPSTEIN: Well, first of all, thank you for that very comprehensive overview and much credit to yourself and NHLBI. The thing that troubles me is that in the end there's a critical parameter that Delta MODS of 1.2 decides all and the question is how broadly clinically is that endorsed; in other words, were there consultations and so forth, because, you know, if you get a result of a boundary of confidence of 1.1 or 1.3 you're going to have people that say, well, that was arbitrary and, you know, the answer could go the other way.

DR. STEINER: Right. That's why we felt

fairly reassured that that was the same parameter used by Paul Hebert in a restrictive transfusion strategy versus standard of care. And that that study has been fairly widely disseminated and actually in terms of changing practice in the recent survey that the Canadian Board of Anesthesia did, their finding and practice is indeed changing on the basis of that trial. A Delta MODS of 1 corresponds to only a few percent change in mortality. The question is whether or not you would actually change blood banking practice for anything less than that. The answer is probably no. Because if you actually then use some of the information that we need by doing our survey, if you recognize that, okay, there probably are two to three million units a year used in the States for cardiac surgery, if you want to cut out those that are 21 days or older because they impact outcome in terms of your Delta MODS, which is death or organ dysfunction development, what you're asking people to do in terms of changing practice is to take out, recognizing that 20 to 40 percent of the products are over 20 days of age, you're asking people to basically not use 600, 800, a thousand units of red cells annually. Does that cause a shortage? Some centers, probably; other centers, maybe not. But that seemed like a very reasonable parameter for us to use because that would provide incentive to tell us yep, things are changing, something is changing but we don't actually have to increase mortality to show that there is a difference.

DR. BRACEY: Is the study fully funded solely funded by NHLBI; what part of the 3.5 million that we heard about earlier today does this represent?

DR. STEINER: Completely different pot of money. This would be funded completely through the transfusion medicine and hemostasis clinical trials network budget which has been already allocated for five years. Conceivably the important part of developing a repository is to have those samples available to someone who is going to go in through that other mechanism to use some of that other funding money to be able to access the samples that are saved in these patients that are being given either shorter age or longer storage age blood and then follow them serially to see how the lesion changes, does not change, conceivably things we don't even think of right now, you know, transfusion related immmunomodulation. Maybe somebody will think of something that they want to look at. And, we just don't know a lot about it right now. So that would be why a repository would be important to be established as well.

DR. BRACEY: Dr. Glynn?

DR. GLYNN: If I could just add that the samples could be stored in the -- concentrate that we have in -- number of resources that --

DR. BRACEY: Dr. Carson?

DR. CARSON: Hi, Marie. What you said was that the change in MODS that you're looking at was equivalent to a 3 to 4 percent difference in mortality.

DR. STEINER: Yes. Yes.

DR. CARSON: What mortality are you estimating is going to occur in this population and what's the baseline; what's the mortality in this population?

DR. STEINER: That's why we can't do a straight-out mortality study. The mortality in this population of complicated or -- cardiac surgery patients looking back at other studies, looking back at Eliott Bennett-Guerrero's most recent data is probably only on the order of 8 percent. If you were going to look for a change in mortality that was statistically significantly different from 8 percent, we would have to have over 10,000 patients enrolled and therefore we would have to have upwards of 60 to 70,000 patients screened.

DR. CARSON: But also what you said was the MODS that you're looking at is one, is equivalent to 4 percent mortality.

DR. STEINER: Yep. Yep. Yep.

DR. CARSON: So why aren't you actually looking at the same thing?

DR. STEINER: Well, because those are actually, there's brackets and the brackets are a few point in each brackets and going from one bracket to the next bracket are Delta MODS of 1, I don't know exactly where in the bracket an individuals is going to fall. In the CB subset in the TRICC trial, the folks were sitting in the 7 to 8 point range and the difference in their Delta MODS was on the order of three to four points in either direction; yet their overall mortality wasn't any different.

So, we wanted to pick an endpoint which would translate into organ dysfunction development which may not occur simultaneously as you go through the perioperative period, which would include mortality but didn't want to use mortality as the primary endpoint because that would be a huge study, a long study, an expensive study and potentially expose patients to risks that they don't have to take. If a Delta MODS is on the order of -- if the difference between the average MODS changes are on the order of 2 percent, I'm sorry, two points, then you're actually crossing brackets and mortality will start going up.

DR. CARSON: And what about a composite outcome?

DR. STEINER: Well, it is, it is --

DR. CARSON: Well, that is but it's one that's hard to understand clinically. I mean, I don't understand what MODS is but I know what death and MI and infections are.

DR. STEINER: Sure. I mean, it is essentially a composite outcome. It's a score that standardizes respiratory difficulty, renal failure, liver failure, DIC, neurologic failure in a standardized scoring system and it ascribes the highest possible score to death. So you can look at the continuum from one organ not working to multiple organs not working to death without exposing all those patients to a mortality line --

DR. BRACEY: Sorry but we do need to generate a product later today. So can we move on so that we'll have success in our round-up? So, our next presenter is Dr. Larry Dumont. Dr. Dumont actually spoke to us yesterday. He also spent a lot of time more recently at the Dartmouth-Hitchcock Medical Center and he's done extensive work on blood storage studies and he will present to us on older red blood cells, Biochemical Excellence of Safer Transfusion under the BEST collaborative view of the evidence.

DR. DUMONT: Mr. Chairman, members of the Committee, thank you for another invitation to speak with you. And we'll try to get out of here early today, I hope. I want to give you a background on who in the world BEST is. It's actually an international research organization that's intended to improve transfusion related services through standardization of analytic techniques, development of new procedures, and execution of clinical trials in hemotherapy. And there's a Website that you can look at.

This is the Executive Committee of the collaborative and actually as I was looking at this it seems more like a Committee of Englanders and New Englanders with a few friends but I think you'll recognize a lot of these names. We're organized into four teams that look at specialized areas, areas of cellular therapy conventional components such as platelets and red cells, transfusion safety and clinical studies.

The people highlighted in gold are actually those that contributed most to what I'm going to speak on today. We have several scientific members, names are shown here, and associate scientific members. We meet twice a year together to talk about studies and work on those throughout the year. The collaborative is actually sponsored, the money comes from this group of companies and the companies also have a membership and they actively participate in design and execution of the trials along with the scientific members.

So we have heard a lot today about biochemical and biomechanical changes in red cells that happen during storage. And actually it's been a great day. I think it's been very stimulating and I've really enjoyed it.

The main question, though, is you know, which of these are clinically significant and which ones are important in what patient groups? I think we don't know that. Furthermore, I want to just once again remind us that any change in red cell inventory dating will have a dramatic affect on the availability of red cells and require a major undertaking to address in the United States. And this is just a snapshot that I took from the Website of the Americas Blood Centers and this is inventory availability of old red cells and this is the percentage of centers that are members of ABC that state that they have on their she was one day or less inventory, two days of inventory, three days or more. So, for old red cells that gives some indication of inventory and also something that is followed -- but we don't have enough information on that.

The main points, the best collaborative one to make today, one is that the current evidence does not support a change in transfusion policy. We further feel that observational studies are limited in determining causal relationships and they may not be generalizable and they need to be interpreted with a great deal of caution. We encourage adequate funding of prospective randomized controlled trials to test the hypothesis that have been generated that have been discussed today and we encourage funding of basic and translational research to examine the pathophysiology of the effects of transfused red cells.

And finally, as has been mentioned a couple times, we also encourage some funding for operations research. What do we do about this inventory? How do we understand it? Can we model it? What would be the effects if we cut back the storage age.

I want to touch on a few points in the limitations of observational studies that we have seen today. And I'm going to use an example the study that was published in the Boston Globe and Los Angeles Times and also slowed up in the New England Journal of Medicine that we have heard of today.

And I know you can't read this slide, I can't either, but this is table one out of a paper that shows the characteristics of the two groups, the new blood group and the old blood group. And if we look at this, there are several characteristics between the two groups that are significantly different. And, in fact, some of these are quite important. They're important to blood bankers, blood groups and we've got cardiac risk factors and those types of things. So there's really a heterogeneity between the two groups. So, that's an important thing to consider about observational studies.

Another thing that we can see from this table, that actually -- did a very good job of pointing out was the difference in the blood usage. And, blood is not issued randomly. I mean, blood bankers know that. I have seen other cohort studies where they make the assumption that blood is issued randomly and that's not the case. In fact, when I was reading this paper for the first time my wife, who is a blood banker, was cooking dinner and I said, I got to this table and I said, "Hey, Deb. Guess what." I said, "Here's briefly what this study is they give a table of blood utilization for the older and the newer. I said, "What do you think the table says?" She filled that table out while she was cooking dinner. So blood bankers understand how blood is issued.

The other thing that's very important about in this area that hasn't been considered is there are differences between not only just a phenotype of the red cells but other factors in these patients, some coagulation factors, for example, and there could be other factors that are different in these patient groups.

So why do we worry about that? Well, so that we can get a prognostic balance between the two groups that we're trying to ask the question about. And when we consider prognostic balance for this study, this was not achieved by the retrospective study design. That's not a terrible surprise and in fact the authors sought to adjust for this imbalance through appropriate statistical analysis. And there's a couple of points that I want to make regarding that.

First of all, when one adjusts for known risk factors, that still doesn't guarantee that we'll achieve prognostic balance in the analysis because in fact we don't know what we don't know about a clinical situation. That's why we do randomized trials to begin with. And, also especially there are so many factors and when you try to adjust for those in analysis you're generally not taking into account what could be important interactions in these factors so there's a real limitation in prognostic balance.

Well, let's look at the abstract from the study. And like most of us you read a paper, you read the abstract and you look at pictures. So, what does this abstract tell us? Well, it sites several important outcomes in hospital mortality, intubation beyond 72 hours, sepsis, composite complications. The numbers that are quoted here are all the proportions were unadjusted in the abstract. They did note that the adjusted risk or risk adjusted rate of a composite score that did carry over in significance but you have to look in the text to find that. And that's shown here with adjusted odds ration with a confidence interval.

And we saw this picture earlier. Again this is an unadjusted relationship. And, the legend for this figure didn't say that it was unadjusted. I had to look in the text to find that. And then this figure that we've all seen a lot of, I've made those numbers a little larger so we can see them. As we know, that this was an unadjusted comparison as shown. This is noted in the legend. And our suggestion would be for reporting clarity that uncorrected -- actually present a misleading picture of the true facts. And looking at this study we would suggest there might be some other ways to present the data that would help others and the reader to understand the effects. For example, survival that would be stratified on some key risk factors such as number of transfusions or the blood group or cardiovascular risk factors would be very helpful in trying to understand what the data is telling us.

And then finally we would appreciate to have some explanation of plausible biological mechanism where divergence occurs after the clearance of red cells, of the transfused red cells. That would be helpful for us.

So then we have to ask questions about generalizibility. And taken from this paper, there were a number of patients that received a mixture of older and newer blood products. And it said that they received substantially more blood than either study group. And we know that the more blood you receive the risk of mortality goes up. So, what about their outcomes? We don't know anything about those. And maybe the results from this study aren't generalizable within this group. We don't know that. We weren't given the data.

Were there differences over the eight year study period? You know, we heard earlier from Dr. Koch that they had this factored in their adjusted analysis but at least I wasn't able to pick that out of the paper. Were there differences in practice, not only in blood products but in surgical practice or other things that happened over that period of time? And what was the effect of intraoperative blood salvage? I mean, this can be a huge mechanical insult to the blood. How much was used? How much was transfused? We don't know that. That would be very helpful.

And then to generalize, the other patient populations, we have no idea what the effect is, if this effect would carry over to others. For example, you heard about Dr. Hebert's preliminary study in that ICU group where they were randomized, the young and old group. Even though it wasn't statistically significant, the group receiving fresh red cells had a higher mortality. And we'll find out when the ABLE study is completed if this holds up or not but it's kind of curious.

I would like to suggest that in papers like there this that you actually need to understand all of the factors and their effect sizes because we want to go after the things that have the largest leverage force to correct any problems that we can find. And I would like to see these effect sizes reported of all the independent variables, coefficients, and standard errors or odds ratios.

An example of this was actually from this group which was published earlier and my understanding is that the group of patients in this study was also included in the New England Journal paper. And so they published adjusted odds ratios, with these factors, transfusions, the number of units of red cells, FFP, preoperative risk factors, et cetera. And we find this very helpful in understanding the relative order of importance for these different factors. For example, mortality, that's a 77 percent increase in mortality per unit with red cells transfused. And you can see that, that curve here from the paper. Of course, you go out to more and more transfusions, the mortality goes up quite dramatically.

It's also instructive to see that FFP use, in fact FFP use had a protective effect, and this is consistent with data that the Army has published in the use of FFP. That would be very helpful to see in the total picture because maybe if there is an effect here or maybe there's an interaction where instead of making red cells younger we could just, you know, add some FFP to it and maybe mitigate some of those effects. And if we compare that -- and I don't know if it's fair to compare that odds ratio to that odds ratio, because they weren't analyzed together, but, you know, roughly speaking, you know, that number is smaller than that number. So, it would be good to understand that so we could direct our resources appropriately.

So just to hit it once again, we don't believe that the current evidence supports a change in transfusion policy. Observational studies are limited. We really encourage funding for prospective randomized controlled trials, basic and translational research and for some operations research in these areas. With that I want to thank you on behalf of BEST. I'll take any questions.

DR. BRACEY: Thank you. Questions or comments from the Committee for Dr. Dumont? If not, thank you. We are then at the point for public comment. We did have a statement from the AABB.

DR. TRIULZI: Yeah, I was asked to make this statement on behalf AABB, ARC and ABC and in the interest of time and to avoid being redundant, rather than read this statement I'm going to ask that be entered into the minutes of the Committee and just briefly summarize that the organizations would agree that the data are insufficient to change practice at this time and all three organizations support the performance of randomized controlled trials to address the issue.

DR. BRACEY: That sounds fine to me. Okay. So accepted. Why don't we take a 15-minute break -- how about a 10-minute break so then we'll meet somewhere around 12 after the hour.

(There was a break in the proceedings.)

DR. BRACEY: Okay. If the members could come to the table, we're ready to start our final task. So the task that we have at hand, A, is to reply to the questions from the Assistant Secretary regarding the issue of the day, and that is the issue related to the storage lesion of red blood cells. Over -- yeah, I can see it. Can everyone see this fairly clearly?

MS. FINLEY: Yeah.

DR. BRACEY: Okay. Great.

MS. FINLEY: It's big.

DR. BRACEY: So the first question that we have to respond to is, number one, do current data support a change, medical practice for transfusing red cells stored for as long as 42 days to transfusing cells that are stored for much shorter periods of time? If so what impact would the shift in practice have on blood availability. There was a working group that prepared a draft over lunch and this is the draft. Based on the availability scientific data -- I guess we need a comma there -- the Committee is concerned about the potential toxicity associated with progressive storage of red cells.

I guess we should say the progressive storage lesion, or, anyway, progressive storage of red cells particularly in certain clinical settings, e.g., cardiac surgery, ICU, trauma. However, absent the availability of definitive safety data from adequate, well-controlled prospective randomized trials, and in the absence of any analysis of the impact of shortened red cell dating on blood availability, the Committee believes that a change in practice is premature. The Committee recommends efforts to optimize blood management including blood transfusion practices in these settings through research and promulgation of clinical practice guidelines based on scientific evidence of safety and efficacy.

So it says a lot but in essence it says that we feel right now that it's premature to make a change, that we feel that more randomized trials -- no, not more -- randomized trials are necessary and that given the current state of the knowledge that we should emphasize the appropriate use of blood. So I open up the floor for comments.

DR. HOLMBERG: Mr. Chairman, during the break someone from the audience commented to me about the donor recruitment aspect and I was wondering if the Committee would like to consider putting something in that last sentence where it talks about to optimize blood management we consider putting donor recruitment and blood management or should that be a separate fragmentation?

DR. BRACEY: Well, you know, actually the donor recruitment aspect I think is an important consideration but I think we would like to leave this separate right now because it really gets to the point needing to make sure that we focus on fostering appropriate use of our resources. So I wouldn't want to dilute that. I don't know, how does the rest of the Committee feel?

MS. FINLEY: Great. We've recommended donor management, donor improvement many, many times before with much more detailed recommendation. Those are still our recommendations. So I think we've got that covered.

DR. BRACEY: Okay.

DR. KLEIN: I think we ought to focus on the question as you had it.

DR. BRACEY: Okay. Does anyone think that we need, given the statement at hand, is there something that's glaring or even not glaring that's missing an important element to answer this question or respond? Yeah, Dr. Murphy.

DR. MURPHY: Thank you. Could I just ask a question? Has anybody tried to get through an institutional review board yet? Do people not think that some efforts of the Committee's will have concerns about randomizing people to older blood, given the state we're in -- tend to do is randomize people to standard, which is blood -- compared to younger products and if it's going to be a problem in recruiting patients, recruiting examiners in trials in the future, maybe somebody should have a consideration on this.

DR. BRACEY: Yeah, I think I heard Dr. Triulzi make a statement earlier today about equipoise and as the trial that, the RECESS trial was designed, it was designed with the notion that the standard of care would be the control arm, and the treatment arm would be the better, so, you want to comment on that, Dr. Triulzi?

DR. TRIULZI: Yeah, I think that Dr. Murphy's question is valid. And the problem with standard of care is you get a mixture and you get into that issue of some of the patients in the, quote, control arm are going to get a mixture of fresher blood and older blood. And if we really want to answer this question most definitively it would be ideal to have the older blood group get only blood, we originally had it at 28 days older, the New England Journal paper came out and there was such a fervor, we said, you know, this may play into the mind of either patients and/or surgeons or IRBs and we moved it back to 21. And that came from approximate median age of what's currently being transfused is somewhere between 17 and 21 days.

So it's not truly standard of care but it approximates the median age of blood that's currently being used now. In the equipoise for IRB would say today a patient who goes for cardiac surgery could get exclusively blood that's over 40 days old. And so we're currently using that blood now. And so the control arm of 21-day or older would be trying to approximate the median age blood or older for that group. And I would be, when we discuss this specifically we would cite that there's data showing that older blood is no different than younger blood and that's why it's ethical to randomize patients to either arm of that study. And, as Dr. Klein mentioned, there are things that are lower risk with older blood, CMB transmission, graft versus host disease and microchimer. So, I think all those things would go into the IRB discussion.

(There was a loud echo from the microphone)

DR. BRACEY: Sorry. That was a "powerful" statement. Dr. Klein?

DR. KLEIN: Dividing those as you have over there, very valid, one is the IRB issue and the other is the recruitment of centers issue. I think one could further argue that the IRB, very justifiably, I certainly feel comfortable with the preliminary study from Canada suggesting that perhaps the younger blood is less beneficial. And while I don't really believe that's the case, what I do believe is that we don't flow.

And, I think if you don't know, and there's no obvious toxicity that in terms of equipoise, in terms of risk to the recipient that one ought to do this study. I do think it might be a harder sell for individual centers because no matter what you say, older is worse, so, they say, and I think it may be quite a trick to convince people that in fact we don't know the answer to this question, which is in fact why we're doing this study.

DR. BRACEY: Ms. Finley?

MS. FINLEY: If I can make a suggestion. Whether the trials are being randomized or not is a level of detail that I don't think we need in the recommendation for it to be effective. Those decisions will be made by the NIH or by funders or whatever, by FDA at some point in the future. And I understand that there is a very complicated issue, ethically as well as scientifically, and maybe we should just take out the word "randomized" and be done with it.

DR. BRACEY: Any other comments from the Committee members? Now, one of the things that we have heard from the multiple presenters is in fact that we should have randomized trials . So, Committee, what do the other members of the Committee think on this topic, on this subject? Dr. Benjamin?

DR. BENJAMIN: I would think it's a critical point here. You could take out more controls and take out prospective but the randomized is the key issue here that we need to have to get rid of all these confounding issues.

DR. BRACEY: Dr. Epstein, comment?

DR. EPSTEIN: I agree with Dr. Benjamin. I think if you do not have prospective randomization you will never resolve the situation.

DR. BRACEY: Okay. So the consensus is we'll keep -- okay. To move on then, I would like to move on to the second -- well, okay. I tell you what. Let's do it piece by piece. Motion for approval of this statement?

DR. RAMSEY: Can we see an overview of what's coming, I guess?

DR. BRACEY: No. Yes, you sure may. The next question is, is there a need for additional research to evaluate if red cells stored for longer periods of time are as safe and clinically effective as cells stored for shorter periods of time, and then also to understand the nature of the storage lesion.

And so, here the draft statement is, the Committee finds that the available scientific data from observational and limited prospective clinical studies are insufficient to resolve concerns regarding the safety of progressive stored red cells. Therefore prospective adequately controlled clinical research is needed to correlate basic science findings on the adverse effects of progressive red cell storage with clinical outcomes. In parallel, studies are needed to establish the efficacy of transfusion therapies in various clinical settings. Committee recommends new and sustained investment in basic and clinical research in this area -- yeah, that's good, I think that's a great point. So comments from the Committee? Dr. Epstein?

DR. EPSTEIN: Well, just the grammar again. If we add the word "randomized" there I think you need to move the "adequately," adequate prospective randomized control.

DR. BRACEY: Ah, yes. Right.

DR. TRIULZI: I thought the point that we're trying to get at there is that the basic science findings have yet to make any clinical correlation and logically you're going to go through some phase one, phase two studies that won't be randomized before you decide what to invest large sums of money in phase three. For instance, we don't know which of the nitric oxide compounds are most important or which of the, whether it's the membrane lesion or the content. You know, we've heard both sides of that equation so that not all the clinical trials need be randomized additionally. And, so, I wondered about maybe just taking that word out because that particular issue, which is to explore the clinical relevance of the basic science findings does not necessarily need to be randomized as we build the database for that. In fact, it probably won't be.

DR. BRACEY: Actually, that's a good point.

MR. LOPEZ: Perhaps it can be moved down to where you have in parallel studies are needed, maybe that's where, because that would be more clinical trials, so maybe that's where that order wording is needed.

DR. BRACEY: Down to --

DR. TRIULZI: It's really just a grammatical issue.

DR. BRACEY: Yeah, we just get rid of "randomized" because that opens it up. Dr. Epstein?

DR. EPSTEIN: Well, I guess this comes back to Ann Marie's point. If we simply say adequately controlled, it really does cover the waterfront. We don't have to define it right now. Just adequately controlled clinical research, clinical study.

DR. BRACEY: Yeah, so adequately controlled.

DR. EPSTEIN: I mean, I think most of us believe that unless it's ultimately done in a prospective randomized fashion, we're not going to have a definitive answer. We don't have to dictate that in this recommendation.

DR. BRACEY: Okay. Comments from the Committee? Does everyone feel comfortable with the statement? Then let's move on to the next one. Now, here we reached a point of actually not putting anything in. It says, what impact would a change in transfusion medicine practice have on blood availability? And I think what we heard is that, that the Committee is concerned about the impact on, of a change in storage life on red cell availability and we would like to see modelling to be able to assess the impact. Would that be a --

DR. BENJAMIN: Well, since we don't know what change we're advocating maybe we should be saying that any change should be adequately modelled and explored before implementation.

DR. BRACEY: Okay.

MS. FINLEY: I would question whether, do we even need to answer that in number three? We are calling for a change. We're specifically saying we don't have enough information to recommend a change.

DR. BRACEY: Correct. I think one of the things that we talked about, though, and I think a point was brought up is that rather than to wait until the time of the change is upon us that we should consider developing models in advance so that, you know, in a "what if" scenario if all of a sudden the data suggested that we need to be using cells that are 21 days old, we wouldn't have to do the modelling at that point. Why not assess it now?

MS. FINLEY: I don't have any, any actual objection to that. I just think it's overly prescriptive and sometimes when you're trying to send something up, up the chain, in HHS, you don't want to put anything more, more prescriptive than it needs to be.

DR. BRACEY: Right.

MS. FINLEY: I think, you know, just stating it has to be adequately modelled before we make a change is just, you know, water under the bridge.

DR. BRACEY: Okay. Dr. Epstein?

DR. EPSTEIN: I think we've already answered all the questions and that we ought to instead of parsing our answers, you know, one through four, here are questions one through four and here are our collective or aggregated answers.

DR. BRACEY: Yeah. Well, you know, actually when we were at the point that we had the break at lunch, that we pretty much, yeah, right, we pretty much were at that point but I just wanted to make sure that everyone feels comfortable with leaving numbers three and four as being addressed referred to the other question, the other answer as well.

MS. FINLEY: I think our answers are very comprehensive.

DR. BRACEY: Dr. Duffell, comment?

DR. DUFFELL: I was just going to say, number four, I mean, it's kind of a bad question. I mean how can you nerve say --

DR. BRACEY: Yeah, yeah, right.

DR. DUFFELL: I mean, the answer has to be yes, right? So, I'm not sure the answer, though, to number four is implicit in 2.2, though.

DR. BRACEY: Dr. Epstein?

DR. EPSTEIN: Well, I think calling for clinical research to establish the efficacy of transfusion practices is really the answer to number four.

DR. BRACEY: Okay. Then what I hear is

that given the response that we had to items one and two or questions one and two, that the Committee feels comfortable with the statements as made as drafted. Comments from the floor? Dr. Dumont?

DR. DUMONT: Just, do the answers in one and two, do they actually address some initiatives to evaluate operations research? We're talking about a lot of science research, but operation research, because that's really what item three is getting to. I think there needs to be some more and specific resources directed to that point.

DR. BRACEY: Okay. That's a good point. So let's see. The question then is -- oh, Dr. Klein?

DR. KLEIN: I would just like to make one comment on that and on question four. As I read question four I was a little taken aback because I don't think that the responsibility for improving red cell products in this country should be specifically the responsibility of the so-called blood banking industry. At the very least it ought to be a joint responsibility.

In many countries, of course, the blood banking industry is the government so the enemy is us but in this country I don't think that we have a mandate and certainly the Secretary has no mandate to tell the blood banking industry what they ought to do. So I this think the answer to that, which we don't have to do specifically, it's contained up above where it really suggests new investment is necessary but not that it comes specifically from the blood banking industry. In terms of operational research, I think that's something that the Committee might want to think about, whether that ought to be something that is investment from the federal government, whether that perhaps is the responsibility of the blood banking industry.

DR. BRACEY: Okay. What are the other -- how do any of the other Committee members feel about our making a statement specifically on operational aspects?

DR. LOPEZ: I have another comment. Do we need to even address number three and number four or did everybody address just one and two?

DR. BRACEY: Well, actually what we're saying is if we could cover the numbers three and four with the broader statements made under one and two but I think that the question that we are sorting right now is adding a piece with respect to operational analysis, operational studies, how to manage inventories. And, so, does the Committee feel that that's something that we should specifically insert? Dr. Triulzi?

DR. TRIULZI: Yeah, you know, unless we specifically ask, I'm not sure an outcome of those statements would be the blood centers going back and looking at what would be the impact of shortening the red cell out-date to 35 or 28 days, and we would be back at our next meeting and still not know what the potential impact of that might be.

DR. BRACEY: So one of the things that -- sorry. On of the things I was just looking at is right where the cursor is there, the Committee recommends efforts to optimize blood management. I was trying to insert blood and inventory management but that doesn't --

MS. FINLEY: No. I think that gets to the heart of it right there.

DR. LOPEZ: That's says it --

DR. BRACEY: Just, just blood management?

MS. FINLEY: Blood and inventory management.

DR. BRACEY: Blood and inventory management.

MS. FINLEY: I think it's an important part of this.

DR. BRACEY: Okay. So we'll put, so, optimize blood and inventory management.

MS. BENZINGER: Blood is the inventory.

DR. LOPEZ: Blood is inventory.

DR. POMPER: Yeah.

DR. BRACEY: Well, we're thinking of blood management in terms of hemotherapy.

DR. TRIULZI: Most of that management I think is in the blood center as opposed to in the hospital.

DR. LOPEZ: I mean, one of the concerns I have is that we cannot give all the responsibility to the blood center.

DR. TRIULZI: Yes.

DR. LOPEZ: I mean, when you talk about blood management, it's inventory, utilization. It's everything. I mean, you can't put all the weight on the blood center because, you know, on the hospital side we are responsible for the blood center to make blood available.

DR. BRACEY: Right.

DR. LOPEZ: And that's not the number of units you collect, used up blood.

DR. BENJAMIN: When you talk about blood management there are you talking about how you manage a patient to transfuse appropriately and to minimize blood usage? It's really -- so, I think there is a difference between inventory management and blood management.

DR. TRIULZI: It is the intent.

DR. BRACEY: So, if we -- yes, Dr. Pomper.

DR. POMPER: Just to, I agree with Dr. Dumont's comments that there is also probably a difference between just efforts to manage the inventory as opposed to, say, operations research and sort of trying to understand all the components that go into this. It's not just the blood center. It's not just the hospital. In fact, there's a lot of other variables that may affect the overall inventory. So I think operations research was a reasonable concept.

DR. BRACEY: Dr. Epstein?

DR. EPSTEIN: My suggestion would be that we remove the phrase blood management from the statement optimize blood management and blood transfusion practice. Let that statement just be optimize blood transfusion practices.

DR. BRACEY: And it covers both.

DR. EPSTEIN: And the "as" statement, as needed to be supportive of operational research to optimize, you know, blood inventories. So here we're now saying government should just do it but, you know, if there's an unmet need to get the job done, so as needed to be supportive of industry efforts or, or industry -- well, let's just go back -- of operational research on optimization of blood inventories.

DR. BRACEY: Just leave it as a separate statement.

DR. EPSTEIN: Right.

DR. BRACEY: Yeah, that parses it.

DR. EPSTEIN: Right.

DR. BRACEY: So, "as needed."

DR. HOLMBERG: As needed supportive of operational research --

DR. BRACEY: As needed.

MS. FINLEY: In management of blood inventories.

DR. TRIULZI: On management --

MR. EPSTEIN: On management of blood inventories.

DR. BRACEY: Okay. So then I guess we just need a -- operations, yeah, and maybe a comma.

DR. HOLMBERG: Where?

DR. BRACEY: After "needed."

DR. EPSTEIN: We need to turn it into a real sentence but as needed HHS should or the Committee recommends that the Secretary be supportive, something like that.

DR. BRACEY: Okay. All right. So I we've added another element. I think it's a good element. I would propose that we have you read through it. Dr. Epstein?

DR. EPSTEIN: Coming back to the issue about randomization, I think we hit on some good language when we were on the next question. We should now reflect it backwards up to the first paragraph.

DR. BRACEY: So to go "adequately control" again?

DR. EPSTEIN: Just "adequately control clinical trials."

DR. BRACEY: It's right after, adequately controlled, yeah.

DR. HOLMBERG: Right here?

DR. BRACEY: Yeah. That would be clinical trials, just trials. Great. Yeah. All right. Are we satisfied? Is there a motion?

DR. RAMSEY: So moved.

DR. BRACEY: Okay, motion by Dr. Ramsey.

MS. FINLEY: Second.

DR. BRACEY: Seconded by Ms. Finley. Any more discussion? No? In that case all in favor?

DR. HOLMBERG: We're just barely at a quorum.

DR. BRACEY: Okay. You have to catch a --

DR. RAMSEY: Chicago.

DR. BRACEY: Any opposed? Any abstentions? All right. So it passes. Now, we are not yet done because we've answered the responses, we've answers the questions of the Secretary, but yesterday when we heard the adverse event reporting, we also identified some areas for improvement in terms of transplantation activity.

And, so, the statement that we have prepared, we discussed a bit earlier today, first thing this morning and then we revised that statement so it reads, "Whereas the HHS Advisory Committee on Blood

Safety and Availability is charged with advising the Assistant Secretary on public health issues related to the safety of tissue and organ transplantation, after review of the current status of safety and availability reporting for organs and tissues, the Committee recommends, one, enhanced acquisition of data on tissue distribution and utilization to allow current surveillance activity to better determine the frequency of adverse events, i.e., we need a denominator; two, capture of appropriate data regarding etiologic agents of infections reported following organ transplantation to allow for better assessment of infectious risk related to transplantation, i.e., we need to know what the specifics are rather than just the total aggregate number of patients infected; three, support the acceleration of rapid -- we need to scratch -- rapid infectious disease assays for use in for use in the organ transplant setting as a strategy to improve both safety and availability of organs; four, -- and this is added -- enhance utilization of CMS data bases to improve monitoring of organ transplantation practices and related outcomes through cooperative arrangements with other agencies; and then five, the Committee recognizes that there is a gap in organ availability which needs further study."

So, the two pieces that were added, were added, Dr. Solomon made a recommendation that we add a piece on utilizing the CMS databases so that we bring all the information that we have at hand together. And then we did have some discussion yesterday in terms of the gap and understanding more about why that gap exists and clearly it's within our realm to consider availability. Dr. Benjamin?

DR. BENJAMIN: I think if you're going to mention a gap you should tell us where the other part of that gap is. It's between demand and availability.

DR. BRACEY: Okay. Recognizes that there's a gap between demand and --

DR. BENJAMIN: Organ.

DR. BRACEY: -- and organ -- yeah. Dr. Duffell.

DR. DUFFELL: Yeah, I'm not sure I

understand the last one, the way it's stated because, I mean, maybe I'm naive in my thinking but the gap is because of lack of donors, isn't it? I mean, why do we need to study this? The gap exists because there's not enough donors, or maybe I'm missing something.

DR. BRACEY: Well, but I think that the reason to study the gap is in much the same way that we want to understand what are the factors that prohibit people from, you know, signing on an organ donation card. I mean, we recognize that there is a gap and we just want to stimulate an assessment of how we can improve it.

DR. DUFFELL: Yeah, I guess what I'm saying is to be more direct, I mean, it's not just to study it, it's what do we need to do to get people to die, I mean --

DR. BRACEY: Okay.

DR. DUFFELL: Maybe I'm being --

DR. BRACEY: I understand what you're saying, yeah.

DR. DUFFELL: When you say a study, I mean, a study of what?

DR. BRACEY: It's further study to do what?

DR. DUFFELL: Study the demographics of those who do donate?

DR. BRACEY: Yeah.

DR. DUFFELL: You know, study the conditions under which they donate?

DR. BRACEY: Right.

DR. DUFFELL: I mean, I'm just saying the gap is there's not enough donors.

DR. BRACEY: Right.

DR. DUFFELL: So that's what we need to look at to say what do we got to do to improve donations.

DR. BRACEY: Right. Right. Exactly. I understand. Dr. Bowman?

DR. BOWMAN: Yes, I don't want to speak out of turn for Dr. Burdick from the Division of Transplantation, over at HRSA, but number five seems almost like an afterthought, after a progressive four-point progression of logic with -- three and four.

And even further the point is actually Dr. Burdick's division of HRSA is, that main focus of that decision is oversight of -- and organ donation efforts in this country. And, extensive studies have already been done and are ongoing and the Secretary already has an organ donor collaborative team -- about five, or six years now, some fairly, it's a breakthrough collaborative, a lot of important gains in exact factors that you raised about what keeps people from signing donor cards or consenting to the organ donation -- things like that. So I'm not sure if it really fits in with the intent of the rest of the points in the set of recommendations.

DR. HOLMBERG: I would also agree with Dr. Bowman. There has been one study report issued and I believe the funding for another study report through HRSA was just let so I think that there's adequate research. It's just the implementation of that.

DR. BRACEY: Okay. Dr. Epstein?

DR. EPSTEIN: Well, what happened here is that we saw three graphs, they were, kidney, heart, liver, and, you know, there were these diverging curves and it kind of raised eyebrows. We recognize that we did not have presentations or discussion of data on the gap between demand and availability of organ, we just were uncomfortable, those of us drafting this straw-man recommendation, leaving it at that. So perhaps, perhaps we should just strike it on the concept that it wasn't really presented or discussed.

DR. BRACEY: Yeah, I think so because again, as you say, it was the shock value of the curves that generated the concern and it sounds like initiatives have taken place and so let's strike it. All right. Oh, Dr. Bowman?

DR. BOWMAN: Yeah, and I have one other comment on number four, regarding the use of CMS databases, the CMS databases are there for researchers to use. There are some administrative -- and some requirements for confidentially and incription and all these other things that are required to use those databases. But, you know, again I wasn't here yesterday but more to the point I think is that the CMS databases do have fairly good information at least on diagnostic information on the kidney transplant recipients but the vast majority of actually liver transplants and pancreas transplants and heart transplants are actually not Medicare beneficiaries and are actually looking at commercial private health insurance systems in this country. So if you're trying to encourage a more comprehensive acquisition of clinical data to correlate with outcomes and things like that, with other federal agencies it will be a limited set of liver and heart transplants and it will be primarily for kidney transplant recipients.

DR. BRACEY: Would it be possible, would it be fair to say, enhance utilization of the CMS and other databases? I don't know what access we would have to those databases.

DR. BOWMAN: I just wanted to clarify for members of the Committee that it would be a limited set of transplant recipients that this data will be available for and it will include the commercial private insurers but CMS will not have difficulty in responding to whatever the Secretary asks the agency, for number four, that would be not a problem.

DR. BRACEY: All right. Ms. Benzinger?

MS. BENZINGER: Yes. Well, the OPTN data on a national level is available on Website, on patients waiting on transplant lists. And to say that there isn't, you know, a reason for us to be involved in it I think is just, you know, putting your head in the sand. At this point I have a very positive look at it, daily, in the last ten months there is an increase of over 3,000 patients waiting on the list. You're looking at 99,000 people waiting for a transplant. I don't think that it's unreasonable or unrealistic for us to be putting, in here, and we're trying, you got after, post cardiac gap which has not been approved everywhere for organ transplantation and that is a place we can improve donor availability as well as -- out, presumed consent. So I think that we can make those recommendations if we agree to do that. So I think there are options available for us to take.

DR. BRACEY: So you would, when you're speaking of donor availability, so you're speaking back to the bullet number five?

MS. BENZINGER: Well, it seemed like there was a negative that we didn't have a place in that, saying that there was a need to reduce that gap between the want-a-transplant and the get-a-transplants.

DR. BRACEY: Right. Right. Dr. Bowman?

DR. BOWMAN: No, I didn't mean to convey that impression at all. Actually, the gap is huge. There's a huge problem and the Secretary is very much aware of that and so was the previous Secretary, going back to I think 2001, and he was actually the one who initiated the donor collaborative program currently managed by HRSA. So I think my point was that there was at least one other advisory committee which is the Advisory Committee on Transplantation, which is similar to this Committee but has had a focus on, probably, primarily organ donation and to some extent recipient issues. And that advisory committee is under auspices of HRSA and I didn't want to -- I was a little reluctant to see maybe two ships cross in the night here passing each other and, not, making it look like one didn't know what the other is doing, is my only point about that. It's a very, very huge problem.

DR. BRACEY: Dr. Ramsey?

DR. RAMSEY: Would there be value in rewording that statement to say that the Committee supports ongoing efforts to improve organ availability; would that be useful?

DR. BRACEY: I mean, it would be useful in the generic sense but I guess the thought was that much of that activity is actually happening and there's a relatively intense focus on it. And I think that's the point that Dr. Bowman was making. I mean, it's good, it's a great statement, and, so perhaps the thing to do is for us, you run to this issue where there's, as you say, they're two ships and maybe the thing to do is first to have some presentation on that so that we can better understand what the current efforts are and how we relate to those efforts.

DR. BOWMAN: I'm sure Dr. Burdick would love to come here at some point present an overview on what that division has done and the advisory committee on organ transplantation, which is analogous to this advisory committee for blood safety availability, for over the last six, seven years.

DR. BRACEY: Dr. Triulzi?

DR. TRIULZI: Yeah I was along the same lines going to suggest that we not have that statement and ask the Assistant Secretary if he would like this Committee to address that issue, and, if so, then we should set you up the appropriate people to come and present the data that we would need to make some meaningful recommendations or he may feel comfortable that the Transplant Committee is adequately addressing it and this Committee doesn't need to address it.

DR. BRACEY: Okay. So we can make that -- okay. And I'll put that in the letter as part of the text that accompanies this. Dr. Lopes-Plaza?

DR. LOPEZ: Going to question number four, how are you going to address the non CMS database, are you going to put private healthcare or data or how are you going to address that?

DR. BRACEY: Well, that's actually, so, do we want to say CMS and other databases or just CMS? CMS and other databases. Oh, yes. Dr. Bowman?

DR. BOWMAN: I think it's appropriate to say other available databases.

DR. BRACEY: Okay. Other available.

DR. BOWMAN: Because actually HRSA does have oversight over OPTN and also has oversight over collection of recipient, transplant recipients, even those who were not transplant for CMS Medicare purposes.

DR. BRACEY: Okay.

DR. BOWMAN: So the scientific registry of recipients is maintained by HRSA.

DR. HOLMBERG: Let me also comment. I commented to a few people yesterday concerning this. ARC is in the process of finalizing the patient safety organization ruling and part of that is actually the various data elements that need to be collected for organs, tissues, blood, for all the safety measures. So I think that, you know, just adding other available databases is sufficient.

DR. BRACEY: Okay. So we have now four elements and maybe we could --

DR. LOPEZ: Number four you cut out the word "and."

DR. BRACEY: Oh.

DR. LOPEZ: Medicare service and other available.

DR. POMPER: CMS and --

DR. BRACEY: Oh, oh, oh, CMS "and," "and," right, right, right, sorry. "And," all right. So then the statement.

DR. RAMSEY: I was going to suggest another "and" actually in the first whereas, transplantation, comma, and after review, just to be help that along --

DR. BRACEY: You're saying and after review?

DR. RAMSEY: Yeah. Yeah.

DR. BRACEY: Dr. Bowman?

DR. BOWMAN: And in that same sentence this is a minor clarification but it says after review of the current status for safety and availability reporting. I think the reporting of availability organs and tissues is fairly extensive. I think it's a safety piece that may be insufficient. That's a good thing.

DR. BRACEY: So you would say strike availability from --

DR. BOWMAN: Right.

DR. BRACEY: Yeah, because availability is actually what got us to bullet five.

DR. BOWMAN: Right.

DR. BRACEY: Yeah. Right. Okay. Good.

DR. TRIULZI: What we heard was that we don't know how often the tissues are being used. Organizations that distribute know they distribute to a hospital X number of tissues but they really don't know how many actually make it to a patient versus outdate, destroyed or whatever. And so while we may have good data for organs, that doesn't exist for tissue.

DR. BOW: So maybe the term is utilization, not availability. The concern is the use of those tissues in terms of tracking, monitoring.

DR. BRACEY: Yeah, that's a good point. So safety and utilization. Okay. Are we near the point for a motion?

DR. TRIULZI: So moved.

DR. BRACEY: We have a motion and a second?

MS. FINLEY: Second.

DR. BRACEY: Okay. Motion by Dr. Triulzi and second by Ms. Finley. More discussion? Hearing none, all in favor? All right. All opposed? Abstentions? Thank you very much. We have a successful product. All right. Dr. Holmberg, there has some discussion about the change in the schedule. Our next meeting, there was some discussion about change in schedule.

DR. HOLMBERG: Our next meeting is scheduled to be the end of October; however, there have been some conflicts on that date and so we're still in a stage of flux in establishing that date for sure. We will get back to you hopefully in the next two weeks on the accurate date. The other thing that I should mention to you is that we are also looking at dropping back to maybe two advisory committees a year in the interest of trying to serve conserve funding. So, you know, if there are hot issues we can always have a third one but probably drop back to two advisory committees.

DR. BRACEY: Okay. Motion for adjournment? All right. So moved. Thank you.

(Proceedings adjourned at 3:57 p.m.)

State of Maryland.

Baltimore County, to wit:

I, ROBERT A. SHOCKET, a Notary Public of the State of Maryland, County of Baltimore, do hereby certify that the within-named proceedings personally took place before me at the time and place herein set out.

I further certify that the proceedings were recorded stenographically by me and this transcript is a true record of the proceedings.

I further certify that I am not of counsel to any of the parties, nor in any way interested in the outcome of this action.

As witness my hand and notarial seal this 18th day of June, 2008.

Robert A. Shocket

Notary Public

My Commission Expires:

November 1, 2010