The Effect of Hemodilution on Cold Agglutinins

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Abstract

***(J. Extra-Corpor. Technol.* 19[3] p. 290–296 Fall 1987, 14 ref.) The effect of hypothermia in patients with cold agglutinin disease was studied in a patient with a strongly positive agglutination at 4°C (1:16,000 titer, a thermal amplitude of 30°C or less, and a specific I antibody). The study investigates a method of hemodilution by which patients with cold agglutinin disease may safely undergo hypothermia during cardiopulmonary bypass. By diluting the blood with an isotonic solution, the tendency toward agglutination of red blood cells is reduced, along with the risks associated with hypothermia in the patient with cold agglutinin disease.**

It was concluded that as the degree of hemodilution of the blood is increased, hemodilution reduced the tendency toward aggregation of red blood cells during hypothermia when cold agglutinins are present in the blood. As a result, hemodilution may enable a patient with cold agglutinin disease to safely undergo cold cardioplegia and cardiac arrest during hypothermic conditions.

Introduction

The routine use during cardiac surgery of systemic hypothermia and cold cardioplegic protection of the ischemic myocardium have brought about two aspects of cardiopulmonary bypass protocol: 1) maintenance of increasingly lower core blood temperatures during bypass and 2) delivery of sanguinous cardioplegic solutions at temperatures as low as 4°C. The increased efficiency of heat exchanging devices for the extracorporeal circuit has made this possible.[7]

The potential problem of the cold agglutinin antibody has received increased attention in patients undergoing hypothermic cardiopulmonary bypass. Cold agglutinins are usually IgM antibodies that exhibit reversible, thermal-dependent equilibrium associations with erythrocytes, with the binding of these antibodies being favored at lower temperatures. Antigen-antibody reactions may take place in the blood of cardiopulmonary bypass patients during surgical hypothermia and may thus predispose the patient to several potential dangers. The potential hazard that is present with strongly reactive cold agglutinins against red blood cells is that agglutinates are formed during systemic hypothermia and with the use of cold sanguinous cardioplegia. These agglutinates increase the risk of cold-mediated problems during cardiac surgery which include perioperative myocardial infarction, hemolytic anemia with cardiac failure, immune complex nephritis with renal failure, hemagglutination, and microvascular thrombosis. Furthermore, patients with cold agglutinin disease may develop significant hemolysis postoperatively due to intraoperative hypothermia.[2,5,6]

Cardiac surgery on patients with cold agglutinin disease presents two problems; 1) the risk of a hemolytic or agglutination crisis if hypothermia is employed or 2) the risk of tissue injury secondary to inadequate myocardial protection during normothermic perfusion.[11]

Although several successful methods for myocardial and renal preservation have been proposed for cardiac surgery in patients with cold agglutinins, there is the need to determine the importance of the cold agglutinin antibody in patients during hypothermic cardiopulmonary bypass. Knowledge of cold-reactive

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hematologic disorders will allow the surgical team to choose the best management technique for these patients and possibly may permit the use of hypothermia to the patient's benefit where it might otherwise have been avoided.[2]

This study was undertaken to investigate the effects of hemodilution on the blood of a patient with cold agglutinin disease at temperatures commonly employed during hypothermic cardiopulmonary bypass.

Materials and Methods

Undiluted plasma containing a cold agglutinin antibody was obtained from the Department of Hematology. This cold agglutinin was of the I type with a 1:16,000 titer at 4°C. The undiluted plasma was then diluted with 0.9% sodium chloride to the following six concentrations:

1) No dilution–5cc plasma 2) 3:4 dilution–0.8cc NaCL : 2.5cc plasma 3) 2:3 dilution–1.3cc NaCL : 2.5cc plasma 4) 1:2 dilution–2.5cc NaCL : 2.5cc plasma 5) 1:3 dilution–4.0cc NaCL : 2.0cc plasma 6) 1:4 dilution–6.0cc NaCl : 2.0cc plasma.

These standard dilutions were then incubated at 37°C in a Precision Scientific Company water bath. A second water bath from the same manufacturer was used to vary the temperature of testing and to represent the heat exchanger used in the extracorporeal circuit.

Beginning at 37°C, human body temperature, the temperature of the second water bath was decreased one degree at a time until the temperature reached 22°C. At each temperature reading, testing for agglutination was conducted in the following manner:

A 3–5% red blood cell suspension was prepared from O positive blood. For each dilution, two drops were placed in a test tube along with two drops of plasma from each dilution incubated at 37°C. These test tubes were then placed into the second water bath, and temperature was equilibrated for 15 minutes at each desired temperature setting. Following this each test tube was immediately placed in an Adam-Serofuge for 20 seconds and the contents then examined microscopically for agglutination. This procedure was repeated for 3 individual master dilutions of the cold agglutinin antibody.

The criteria used to determine the degree of agglutination are: a) No agglutination–100% free red blood cells; b) Slight agglutination–very few red blood cell agglutinates almost 100% free red blood cells; c) Moderate agglutination–several red blood cell aggregates but some free red blood cells; d) Severe agglutination–almost 100% agglutination and very few free red blood cells; e) Very severe agglutination–100% agglutination and no free red blood cells.

Results

Figures 1–6 show the effects of hemodilution on cold agglutinins in combination with hypothermic conditions. Figure 1 represents an undiluted sample of plasma containing a cold agglutinin antibody. As the temperature of the water bath was lowered from 37°C to 22°C, the degree of severity of agglutination increased. With no hemodilution present, agglutination was not observed until the temperature decreased to 35°C. At 34°C there was slight agglutination shown for Trial 1. Slight agglutination was also present at 33°C and 32°C for all trials performed. At 31°C, moderate agglutination occurred for Trials 1 and 3 and slight agglutination for Trial 2. As the temperature was lowered from 30°C to 28°C, moderate agglutination persisted. At 27°C, severe hemagglutination resulted in Trials 2 and 3 and moderate agglutination in Trial 1. Severe agglutination occurred at 26°C and 25°C, and at 24°C agglutination was seen in Trials 1 and 2 and very severe agglutination was seen in Trial 3. At 23°C very severe hemagglutination resulted in Trials 1 and 3, and severe agglutination was shown in Trial 2. Very severe agglutination was seen for all trials at 22°C.

Figure 2 represents a 3 to 4 ratio of hemodilution of a plasma sample containing cold agglutinins. Hemagglutination was not present until 32°C for trials 1 and 3, and this was slight agglutination. Slight agglutination was also seen at 31°C for all 3 trials, at 30°C 1 and 2, at 29°C for all trials, and at 28°C, 27°C, and 26°C for Trial 1. Moderate agglutination occurred at 30°C for Trial 3, at 28°C, 27°C, and 26°C for Trials 2 and 3, and at 25°C for Trials 1 and 3. Severe agglutination was seen at 25°C for Trial 2, at 24°C and 23°C for all three trials, and at 22°C for Trials 1 and 2. Very severe hemagglutination only occurred at 22°C for Trial 3.

Figure 3 shows the effects of a 2 to 3 ratio of hemodilution of the cold agglutinin antibody. Slight hemagglutination was first present at 27°C for Trial 2 at 26°C–24°C for all trials, and at 23°C for Trial 1. Moderate agglutination accurred at 23°C for Trials 2 and 3 and at 22°C for Trials 2 and 3. No degree of severe or very severe agglutination was present for this amount of hemodilution.

Figure 4 shows the degree of agglutination occurring when the cold agglutinin antibody was diluted to a 1 to 2 ratio. Slight hemagglutination first occurred at 26°C for Trial 2, at 25°C for Trials 1 and 2, at 24°C and 23°C for all 3 trials, and at 22°C for Trial 2. Moderate agglutination was observed at 22°C for Trials 1 and 3. Again no degree of severe or very severe agglutination was present for this amount of hemodilution.

Figure 5 represents a 1 to 3 ratio of hemodilution of the cold agglutinin antibody. No degree of aggluti-

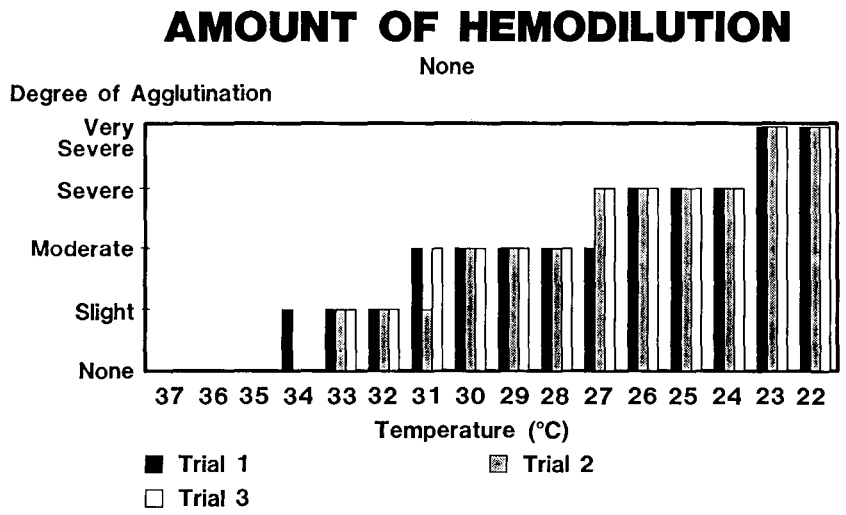

Figure 1

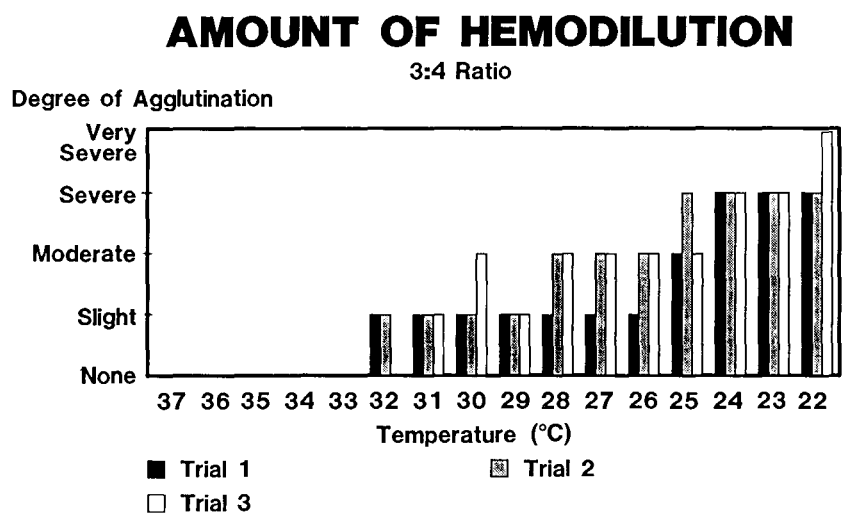

Figure 2

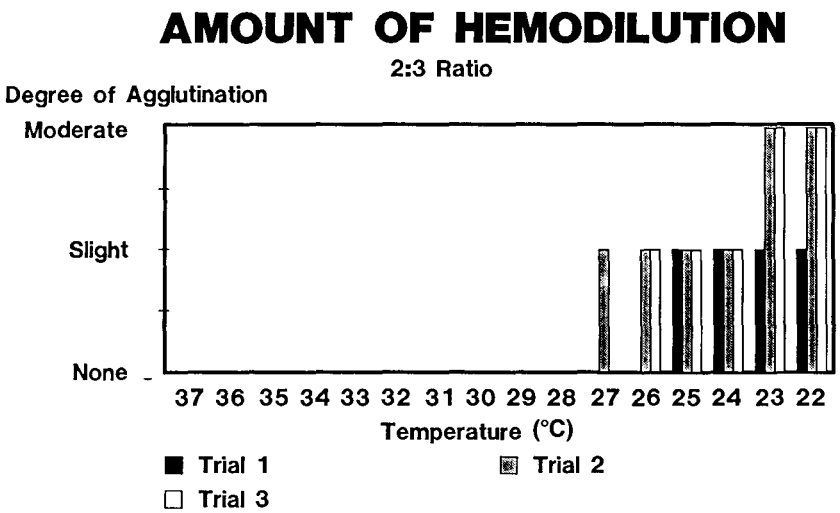

Figure 3

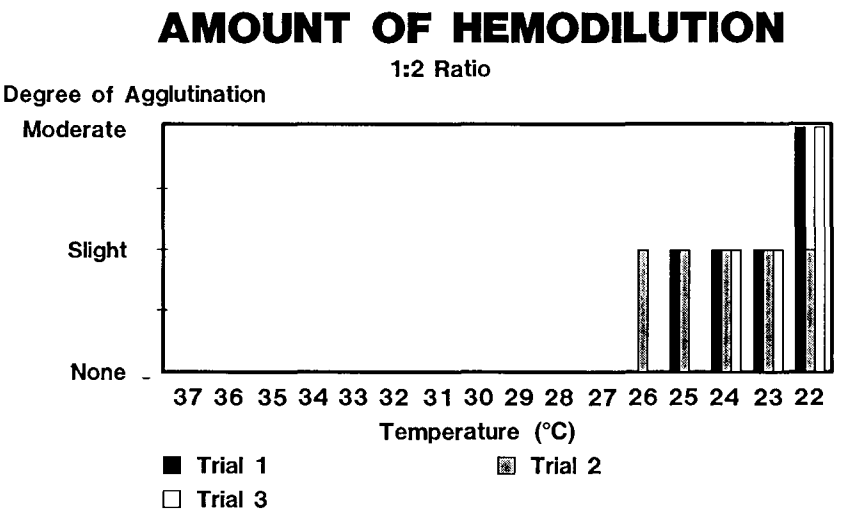

Figure 4

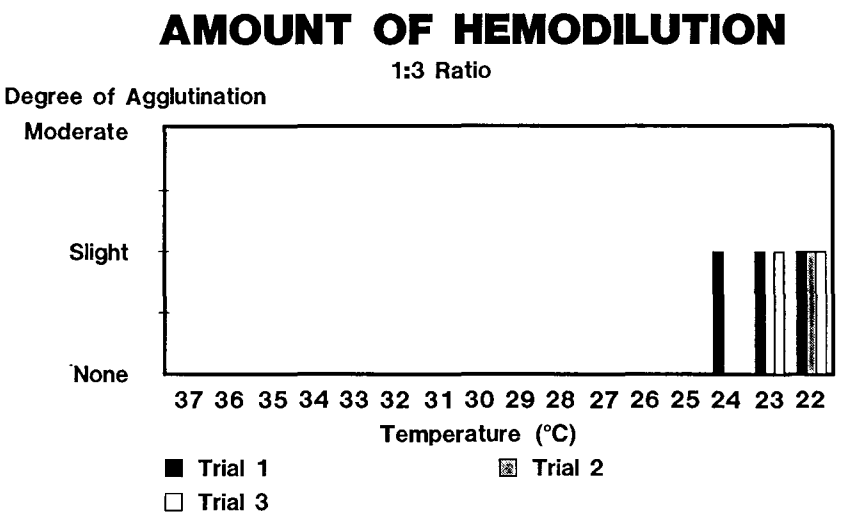

Figure 5

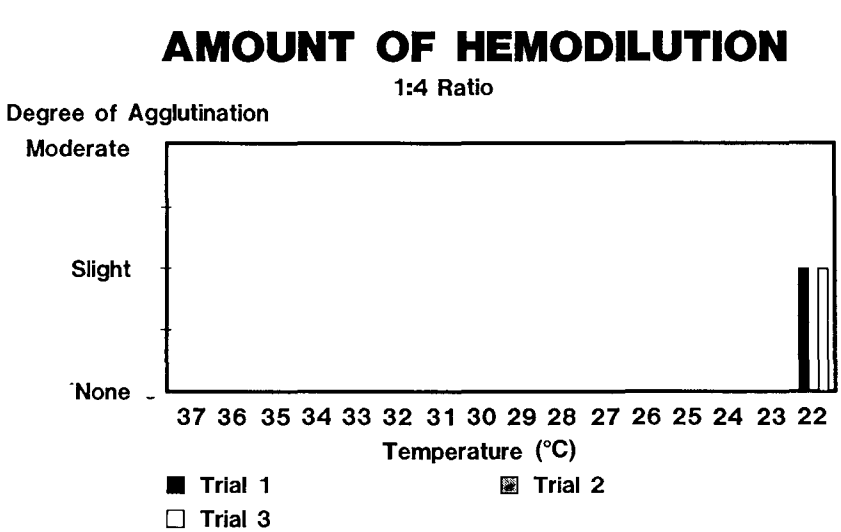

Figure 6

nation was present until the temperature of the antibody was lowered to 24°C. Slight agglutination occurred at this temperature for Trial 1 only, at 23°C for Trials 1 and 3, and at 22°C for all three trials. No moderate, severe, or very severe hemagglutination was present for this ratio of hemodilution.

Figure 6 represents the degree of agglutination occurring at a 1 to 4 ratio of hemodilution of the cold agglutinin antibody. For this ratio slight hemagglutination occurred at 22°C for Trials 1 and 3 only.

Discussion

The Cold Agglutinin Syndrome is a rare immunologic disorder which occurs in one-third of all autoimmune hemolytic anemias. It normally is idiopathic and occurs mainly in patients over 50 years of age. This disease occurs more frequently in females than males and is present in some infants and pediatrics.[7] Cold agglutinins, both normal and pathological, are auto-antibodies usually of the IgM class but occasionally of the IgA and IgG classes. The auto-antibodies are directed against the I antigen or related antigens on erythrocytes of either autologous or homologous blood. They are referred to as cold-reactive antibodies or cold agglutinins. The antigens of the cold agglutinin syndrome are usually not identifiable and can be of more than one type. They are complement-fixing antibodies which can cause severe hemolysis and red blood cell agglutination at temperatures below 37°C. Cold agglutinins have a certain range of thermally mediated immunologic activity. Antibody activation occurs below the thermal amplitude which may be narrow (only a few degrees Celsius) or broad and approach normal body temperature. A very rare patient, such as the one used in this study, will exhibit clinical signs of the disease at body temperature. These patients are said to have an antibody with a high thermal amplitude. There is a maximum temperature, which is always less than body temperature, above which the antibodies activity as agglutinates and/or hemolysins ceases. This maximum temperature is called the critical temperature of the antibody. Cold agglutinins when cooled below the thermal amplitude exhibit an exponential increase in activity (titer) and rapid reversal of red cell agglutination and antibody activity on rewarming.[3,13] Most adults possess very low titers of cold antibodies active at 4°C in their blood.[4,14] These cold auto-antibodies active at 4°C may be demonstrated in the serum of normal healthy subjects and usually the titer is less than 1 in 64. Pathologic cold auto-antibodies commonly have a titer greater than 1 in 1000, but the antibody usually does not react above 30°C.

The hematological test for the significance of cold agglutinins to cause in vivo hemolysis consists of the following: 1) Red blood cell agglutination in saline at 20°C is sought; 2) If there is no agglutination in saline at 20°C after incubation for 30–60 minutes, there is little risk of in vivo significant hemolysis; 3) If there is agglutination at 20°C, then red blood cell agglutination at 30°C is sought in albumin; 4) If absent, then the cold agglutinin is unlikely to cause hemolysis in vivo at any temperature; 5) If both tests are positive, the cold agglutinin present is of clinical significance; 6) An antibody which agglutinates at 20°C in saline but not at 30°C in a protein-enriched medium is of much reduced clinical significance.[12]

Hemodilution of blood containing cold agglutinins reduces the incidence of the cold agglutinin antibody in serum binding with its antigen located on the red blood cell membrane which reduces the opportunity for hemagglutination. We found as the amount of hemodilution was increased the temperature at which hemagglutination was first observed decreased. Also, with an increasing amount of hemodilution the degree of severity of agglutination decreased at corresponding temperatures. This indicates that with increasing amounts of hemodilution hemagglutination will occur at lower temperatures, and this agglutination will be at a lesser degree than if no hemodilution is used.

Several techniques have been proposed for providing myocardial protection in a patient with cold autoagglutinins. The first technique consists of mildly cooling the patient to just above the critical temperature after the aorta has been cross-clamped. The coronaries are then perfused with a normothermic potassium crystalloid cardioplegia solution to remove the blood from the coronaries before 4°C cold crystalloid cardioplegia is started.[1,2] The heart is rewarmed with a normothermic cardioplegia solution prior to removal of the aortic cross-clamp and reperfusion with blood. Instead of removing all the cold agglutinin antibody from the circulation, blood is only removed from the coronary circulation. Cold cardioplegia is primarily relied upon for myocardial preservation. Systemic hypothermia is not used below the critical temperature of the cold agglutinin. The use of normothermic crystalloid cardioplegia for initial coronary washout requires immediate subsequent infusion of cold crystalloid cardioplegia to limit myocardial oxygen extraction.

A second technique for managing the cardiac surgical patient with cold agglutinins involves maintaining the patient with normothermic cardiopulmonary bypass without the use of cold cardioplegia, giving the patient plasma exchange transfusions by continuous flow centrifugation preoperatively to remove the cold agglutinin and then conducting surgery under hypothermia.[1,9,10] A third technique utilizing systemic normothermia and continuous coronary perfusion of a fibrillating heart without aortic cross-clamping has

been used but this method has proven to be very ineffective.[8]

In a study by Moore, et al., 1984, the effects of hypothermic cardiopulmonary bypass were observed in five patients with strongly positive cold agglutinins at 4°C and in 10 controls.[11] The characteristics of the cold agglutinins in the experimental group had a low thermal amplitude (28°C or less), a low 4°C agglutination titer (1:32 or less) and nonspecificity (non-anti-I and non-anti-i). They concluded that a 1:4 dilution with pump prime or a 2:4 dilution with cardioplegic solution and pump prime has little effect on hemolysis or agglutination. However, these results are valid only for those patients with cold agglutinins who are asymptomatic with a low titer, nonspecific antibody.[11] On the other hand, the results of our study indicate hemodilution of the cold agglutinin antibody greatly affects the degree of agglutination, even in symptomatic patients with a high titer, specific antibody.

There remains some controversy as to the degree of hemodilution that can be tolerated during cardiac surgery and still provide adequate oxygen delivery to the tissue. Although some degree of hemodilution occurs in all cardiac surgery in which cardiopulmonary bypass is employed, further hemodilution may be needed in patients with cold agglutinins. With an average normal hematocrit of 38% to 42%, a 3 to 4 ratio of hemodilution will produce a post-dilution hematocrit of approximately 30%, a 2 to 3 ratio a hematocrit of 27%, a 1 to 2 ratio a hematocrit of 20%, a 1 to 3 ratio a hematocrit of 13%, and a 1 to 4 ratio a hematocrit of 10%.

Hemodilution and hypothermia make it possible to dissolve more oxygen in the blood which may increase oxygen delivery to the tissues. A combination of hemodilution and hypothermic conditions are possible in a patient with cold agglutination disease which can still provide adequate oxygenation to the tissues.

This study was limited to a patient with one of the most severe forms of cold agglutinin disease who required weekly plasmaphoresis and exhibited agglutination at normal body temperatures. Further study, under clinical conditions of hypothermic cardiopulmonary bypass, is being considered.

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Questions from the Audience

Question: Aaron Hill, Falls Church, VA: Very nice presentation. We have experienced this problem with patients during cardiopulmonary bypass. One particular case was a patient who came from the cathlab, had not been coldscreened before, and we used blood cardioplegia. In preparation of a batch type blood cardioplegia, we had a settling out and had to break that cardioplegic circuit down, and use crystalloid. One caution I would give everyone, even with an extreme amount of hemodilution: I would suggest that these patients be approached very, very cautiously. For any emergency patients that you are doing, I would be quite concerned about using blood cardioplegia for those patients without previous coldscreens. I would recommend people try to avoid the use of hypothermia in those patients and go with the crystalloid regimen. Early in our experience, back at the University of Rochester, we ran into a situation, lost a pediatric patient in the cooling process and the pump perfusate turned into the consistency of jello. And that was it. It was all over. We could never get the patient back.

Response: Especially if you are using blood cardioplegia, your blood bank should be aware of the possibility that you are going to be taking this blood down to 4°C and should screen a lot of these patients at that temperature. If you have two different regimens, normally the cold agglutinin can be picked up in a screen in most patients when they draw it from the patient in the room. It is put into the tube and it starts to agglutinate if that patient has a very severe form of the disease. And since most type and crossmatches are done at room temperature they are normally picked up by most of your blood banks. If this patient has one that is really active at 4° and you are using blood cardioplegia, it is recommended by the manufacturer, I believe, that screens be done at that temperature. I think Shiley sent a mailing out to the blood banks dealing with that subject.

Question: Sue Bastian, Fort Wayne, IN: How long have you been doing screens?

Response: At our institution we've been doing coldscreens, not down to 4°, but coldscreens since about 1981. Since about 1983, when we started using blood cardioplegia instead of crystalloid cardioplegia, we have started doing coldscreens at 4° with the physicians that use blood cardioplegia.

Question: What kind of frequency have you seen with cold agglutinin and what thermal amplitude?

Response: That has been very, very infrequent and almost nonexistent at our institution in patients who exhibit clinical signs of cold agglutinin syndrome, because you need a higher titer to really affect your surgical outcome. A patient with 1:1000 and above titer will cause you some problems. We've seen some at 1:164 but we really haven't been too concerned. And you don't see agglutination in these patients. But there are quite a few of those.

Question: So you're saying that a titer of 1:1000 or higher is only significant for pump patients?

Response: I think that for normal hemodilution during the cardiopulmonary bypass, I would really consider those patients that you would want to maintain above the thermal (critical temperature) and use crystalloid cardioplegia as opposed to blood cardioplegia.

Question: We've been doing cold agglutinin screening on our patients in the last year. We just do a screen on 4°. We've seen a fairly significant number of positives, however. They are usually just reactive up to 10°.