# Cold Blood and Autoimmune Hematologic Disorders During Cardiopulmonary Bypass

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## Abstract

A discussion of clinical syndromes which can result from the disorders popularly known as "cold agglutinins" is presented. Current perfusion techniques may make it increasingly necessary to remain appraised of these potential complications of cardiopulmonary bypass. The pertinent specific disorders are *Cold Agglutinin Syndrome* and *Paroxysmal Cold Hemoglobinuria*. Mechanisms of the autoimmune systems are reviewed. The relationship between perfusionist, surgical team, and the hematology laboratory in approach to potentially complicated hypothermic procedures is discussed.

## Introduction

In the current practice of clinical cardiac surgery, most protocols routinely include systemic hypothermia during cardiopulmonary bypass. Recent emphasis upon cold cardioplegic protection of the ischemic myocardium has effected two aspects of bypass protocol: the delivery of blood cardioplegia solutions at temperatures as low as 4°C, and the maintenance of increasingly lower core blood temperatures during bypass. Increasing efficiency and convenience in the design of heat exchanging devices for the extracorporeal circuit has made the achievement of these regimens a reality.

At the same time, many perfusionists remain unaware of the prospect that cold-reactive antigen-antibody reactions may take place in the blood of cardiopulmonary bypass patients; or else assume that this fact is primarily a concern of the blood bank personnel, or perhaps the surgeon. However, it is often the case that the blood bank staff has no idea of the low temperatures to which blood in the extracorporeal circuit may be routinely subjected in these most current protocols. Even though the literature concerning manifestation of such reactions under surgical hypothermia is scarce, the perfusionist must know some fundamentals of these phenomena. This is necessary in order to provide the patient benefits of cold blood techniques, while at the same time preventing or managing any blood cryopathies which might be encountered.

It is thus pertinent to review and address the role of potential blood and circulation problems secondary to two different immuno-hematologic cryopathies, *Cold Agglutinin Syndrome* (CAS) and *Paroxysmal Cold Hemoglobinuria* (PCH); and to investigate what posture a cardiopulmonary bypass protocol might take toward them.

## Immunology Review

Antigen-antibody reactions such as CAS and PCH are classified as *primary* or *secondary*.[1] In the primary reaction, an antigen and antibody combine in serum to form an antigen-antibody complex:

$$Ag + Ab \longrightarrow AgAb$$

The part of an antigen molecule on a cell with which a specific circulating antibody can react is known as the antigenic site. For a high affinity molecular bond to take place at this site, the reaction requires the close physical approximation of the two oppositely charged ionic groups. The secondary antigen-antibody reaction produces, among other possible effects, agglutination and/or lysis of the various antigen-containing cells:

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$$AgAb \longrightarrow \begin{matrix} \text{cytolysis} \\ \text{agglutination} \end{matrix}$$

A designation of the secondary effect elicited by the antibody in an antiserum is used to name that antiserum. Thus the antibody (an immunoglobulin protein) in a serum that elicits agglutination is called an agglutinin; one eliciting hemolysis a hemolysin.[1]

Individuals with cold-reacting antigen-antibody disorders have circulating (auto)antibodies to antigens present on their own blood cells, primarily the erythrocytes.[1,2] These antigens are present for life on the surface of the cell membrane, similar to ABO blood group specificity distinctions and other genetic polymorphisms.[3] The antigens relating to CAS and PCH syndromes may be of more than one type, and it is not usually possible to definitively identify them. Sometimes they are antigens related to the Rh system or, more typically, the normally appearing I- (or in newborns, i-) antigen.[2,3,4,5,6,7] Ii antigens have been shown to be variously present on the surface of all formed elements of the blood, and also on the cells and body fluids of some other organ systems.[8,9]

The antigen and the antibody are thus both *endogenous*, as opposed to the case in other types of immune system blood reactions. Cold-reactive antibodies, however, are able to direct against any antigen-containing red cells, whether autologous *or* homologous.[1,10] The condition is considered by some as allergic, since the individual is sensitive to antigens on his/her own blood cells.

The disease conditions associated with these reactions typically result from the loss of erythrocyte function due to hemolysis, from effects of free plasma hemoglobin due to hemolysis, or from vascular obstruction due to hemagglutination.[2,3,6] The antibodies are immunoglobulins of the Ig series in the case of CAS (usually IgM, and to a lesser extent IgG and IgA); and of the D-L series in the case of PCH.[2,3,4,5,8,9] These two classes of immunoglobulins each have the capacity to activate *complement* when reacting with, and being attached in sufficient density to, an appropriately antigenic cell. They possess the specific "complement-fixing" ability to activate the series of blood protein interactions which will have the end effect of lysing the target cells. This process defines the action of the *complement system*.[3,5,7,11] A terminal component of the complement sequence weakens the cell membrane enough to permit puncture, loss of integrity, and subsequent lysis.[3] As few as 30 molecules of antibody attached to the erythrocyte may be sufficient to initiate hemolysis.[12]

While the activation of the complement system is typically an auto-immune reaction to the antigenic RBC, the type (or types) of cell reacted *upon* will determine the different possible clinical manifestations of the general disease condition: action upon erythrocytes to produce hemolysis and hemolytic anemia, upon leuckocytes to produce agranulocytosis and susceptibility to infection, upon platelets to produce thrombocytopenia and purpura, and upon vascular endothilial cells to produce fragile vasculature and vascular purpura.[1,2]

In addition to this cytolytic effect, the cold antibody disorder may cause the formation of RBC and/or platelet agglutinates with consequent obstruction of the peripheral vasculature and microcirculation.[1] One of these patients subjected to an environment of topical cold will present as suffering from acrocyanosis: Raynaud-like symptoms of poor peripheral circulation such as cold, numb, stiff, and often discolored fingers, toes, earlobes and nose. In severe cases gangrene may be present.[7,13]

Inherently characteristic of the two syndromes, the antibodies do not have the ability to bind to the cell surface and fix complement at 37 degrees C, but typically will bind and activate below 32 degrees C. Individuals with chronic hemolytic anemia at a sufficiently high antibody concentration, or titer (greater than about 1:1,000 assessed at 4°C — the titer varies with temperature), may exhibit some symptoms at a skin temperature of 32 degrees C; the condition rapidly worsens at further reduced temperatures. There may be a considerable difference in the temperature range over which the antibodies show maximal effect; they vary in *thermal amplitude*. The rare patient will show clinical signs at a body temperature very near 37 degrees C, and will be said to have an antibody with a high thermal amplitude.[7,14] This measure will often be as useful a clinical indicator as is the titer (which may reach 1:1,000,000).[8] Most adults in fact possess very low titers of cold antibody active at 4°C in their blood.[7,10,15,16] Titers below 1:64 are "normal," and such individuals are typically

symptom-free.[5]

CAS and PCH are often diagnosed in association with selected acute infections and viral disorders; but more typically exist in a chronic fashion without apparent cause, and are then termed idiopathic.[3] If such a "chronic" individual has never been exposed to a cold environment, then he/she may be unaware of possessing the syndrome until the first time that the individual may have reason to be blood typed and cross-matched — or acutely cooled to a blood temperature below that of an undetected thermal amplitude threshold value.

## Clinical Features

*Cold Agglutinin Syndrome*

CAS accounts for about one-third of all autoimmune hemolytic anemias.[6] The disease is relatively rare both in its idiopathic and in its infection related forms. The idiopathic form occurs mainly but not exclusively in individuals over the age of 50, and more frequently in women than in men.[16,17] Some pediatric and infant cases have been reported.[6,18,19] In addition to the standard profile of hemolysis/hematuria and intravascular agglutination/microcirculatory obstruction, erythrophagocytosis may occur in the spleen resulting in some enlargement of that organ. The liver may be slightly enlarged, jaundice may appear, the pallor of Raynaud's phenomenon may develop into the eventual presentation of gangrene. Hemoglobin may be as low as 7 gm%. Lymphocytes and leuckocytes which possess I-i antigens on their membranes may be lysed.[9,17] The leuckocyte count may be elevated.[17] Renal shutdown may occur in marked cases.[6] At 4°C the blood will be intensely agglutinated, as the titer rises rapidly with increasing cold.[6] Neurological features such as disorientation, Babinski sign, and EEG abnormalities may be present.[20] These and other CNS sequelae may or may not prove to be reversible.

The agglutination process evolves in two stages. The first involves the rapid interaction of the antigen and antibody on the surface of the particle (RBC or platelet). The second stage involves the cross-linking of adjacent particles or small agglutinates into large, easily visible aggregates. The condition is distinct from a situation of marked rouleau formation in that the "cold" agglutination is reversible upon rewarming.

While intravascular agglutination increases with the degree of cold, hemolysis activity declines under 10°C as lytic complement action falls off in magnitude.[6] In general, the degree of hemolysis is highly variable, and depends upon 1) the titer of the agglutinin, 2) the range (thermal amplitude) of its activity, and 3) the degree of cold and duration of exposure.[7,11,15,16] There is also, however, a certain amount of hemolysis which can be directly and perhaps proportionately attributed to concomitant agglutination; the effect is related to the mechanical stresses on agglutinated red cells.[6]

*Paroxysmal Cold Hemoglobinuria*

This condition, similarly found in association with various infections but frequently also idiopathic, is much more rare than CAS.[6] PCH D-L antibody binds to the erythrocyte in the cold (usually below 15 degrees C), coating the cell with an autohemolysin which subsequently lyses the cell by complement activation *upon rewarming to 37°C.*[21] There is some indication that the degree of hemolysis varies in proportion to the density of the coating on the RBC.[2,5,7] However, the D-L antibody is a very powerful hemolysin even in low concentrations.[6] As in CAS, episodes are highly variable in clinical effect depending upon the antibody's thermal amplitude.[2] Cold agglutinins may be concomitantly present.[17] In profile the paroxysm may display a shaking chill followed by a febrile episode. Dark red to brown urine may be passed for up to three days.[2] Acute renal failure may be present, along with mild forms of jaundice, hepatomegaly, and splenomegaly. Other observed effects may include neutropenia (due to the disappearance of granulocytes), leukopenia, leukocytosis, and erythrophagocytosis by leukocytes.[4,6,17,21] CNS manifestations may also be present in PCH.[20]

Occasional or intermittent acrocyanosis in PCH may mimic that feature of CAS, but a clear distinction is manifested by the dramatic darkness and suddeness of the hemoglobinuria, and by its occurrence being delayed until normothermia.

## Aspects of Detection and Screening

CAS and PCH are often confused with each other, even though each results from different

autoantibodies, and each reacts with different antigens on the blood cell.[6,17] Differential diagnosis of the two conditions is achieved in the Serology laboratory, following the appearance of clinical symptoms in the stricken individual or the detection of antibody abnormality by antibody screen in the blood of those patients having the occasion to be typed and cross-matched by the blood bank.

In the case of CAS, first indications might be seen at the bedside. At sufficient titer and/or thermal amplitude, the blood may clump upon being drawn into a room temperature blood sampling tube.[3,8,21,22] In the lab, clumping in the coulter counter or agglutinates observed in a routine smear may be the initial discovery.[3,6,8,22] Blood bank clinicians may simply hold the filled EDTA or citrated sample tube under a cold stream, or place it in momentary contact with the inside of a refrigerator wall and inspect for the presence of agglutination. A specific "cold antibody evaluation test" may be implemented, in which mixtures of patient and donor blood are incubated in various proportions at cold temperatures for purposes of detecting agglutination. In all positive cases, the clumps will then dissolve upon rewarming.

Following detection, the lab will proceed to confirm the antibody's identity, to determine its titer for purposes of inferring the degree of potential clinical effect, and to determine its thermal amplitude (as specific for that patient) for purposes of knowing at what temperature to anticipate antibody activation and clinical effect.[11]

The Coombs test, or antiglobulin test, identifies classes of antibodies on the RBC surface as being among those specifically responsible for CAS.[2,6] In "direct" application, the test demonstrates complement component attached by the antibody to the patient's washed red cell membranes; the "indirect" test establishes the presence of antibodies in the patient's serum which may react with either autologous *or* donor blood.[2] In CAS, the direct Coombs test is positive (whether the sample is warm or cold), and the indirect Coombs test is usually positive in a cooled sample.[2,6] This "routine" Coombs test is not unequivocal for CAS. Two variations known as the "gamma" Coombs test and the "non-gamma" Coombs test can be performed with different sera which are both more sensitive and specific.

The direct Coombs test is positive for PCH when applied at 4-12°C, but negative if warm. The indirect Coombs test is positive.[21] However, the definitive PCH diagnosis is a positive Donath-Landsteiner test, in which a blood sample hemolyzes upon rewarming from immersion in cold water.[1,2,6,17] A positive reaction to the Wasserman test for complement-fixing antibody is also typical.[2,6]

## Aspects in the Conduct of Perfusion

Numerous aspects of screening, prevention, and treatment concerning CAS and PCH will be readily apparent to the perfusionist. Ideally, clinical symptoms should never arise in the operating room if advance understanding, preparation, and communication between the laboratory and surgical teams has been adhered to.

### *Diagnosis*

A good communicative relationship with the blood bank is essential. We are obliged to keep them appraised of our evolving systemic hypothermia and cardioplegia regimens, especially where blood-based. We should be especially certain to keep them informed of any plans for profound hypothermia procedures if those procedures are unusual in the day-to-day profile of the institution's caseload. They should, in turn, provide us sufficient advance notification of the detection of cold antibodies in our patients, what the type of antibody may be, and what the critical temperature of the antibody will be for each of those individuals. Unanticipated clinical manifestation of cold antibody blood reactions *is* avoidable.

### *Prevention*

When surgery and perfusion are required in patients presenting with CAS or PCH potential, continued awareness of that fact by all, in conjunction with modified operative approaches, will eliminate or minimize any resultant difficulty.

A sufficiently warm operating room environment pre-induction will prevent the early appearance and discomfort of acrocyanosis in patients with antibodies of high thermal amplitude. Water recirculating "hypothermia blankets" under the patient should not be adjusted to cool, but warmed to insulate the patient from the cold table.

On bypass, normothermia may be permitted depending upon aspects of the operation.[10] Moderate systemic hypothermia might be permitted if the antibody has a sufficiently low critical temperature (e.g., below 30°C). If systemic core temperature is expected of necessity to go below this value, pre-operative exchange transfusion, plasma exchange, or plasmapheresis may be undertaken.[19,23,24] Such techniques can remove enough circulating agglutinin to lower the titer to a subclinical level.[24] Donor blood for anticipated transfusions may be prewarmed, and/or the RBCs washed free of plasma complement.[17,19] All these steps may be a requirement should deep hypothermia or cold blood cardioplegia be deemed a clinical necessity. Should the maintenance of systemic normothermia be chosen instead, compromise of any cold cardioplegic protection of the myocardium might be expected and minimized by various means: use of a posterior pericardial insulating pad and topical cooling device to insulate the heart from warm underlying tissues, snared caval venous cannulas to prevent warm systemic blood from entering the heart, and venting of the left heart to evacuate any warm bronchial and mediastinal return.

Blood temperatures within the extracorporeal circuit will be the pertinent index for comparison against the critical antibody temperature value; if the oxygenator used does not have built-in provision for measuring this, other arrangements will have to be made for a temperature probe somewhere in the circuit. Preferably this measurement should be made at the site of expected minimal blood temperature. An easily implemented protocol safeguard is to limit the minimum water temperature through the heat exchanger to several degrees above the antibody's critical temperature.

Intra-operative efforts at blood conservation and minimal clear prime volumes may be of crucial importance in these cases, as patients with an underlying idiopathic disorder and chronic hemolytic process may arrive with hemoglobin values reported as low as 7 gm% *before* perfusion and its hemodilution. An acute onset of further dilution by cardiopulmonary bypass will have the obvious effects upon oxygen-carrying capacity of the blood, and can lead to renal shutdown as well.[6]

The monitoring of pH and its temperature correction during bypass assume greater importance in these patients. As pH goes down in value, any cold agglutinin present will adsorb in greater amounts to the surface of the RBC.[15,25] Oxygen saturation and transfer values should be observed for signs of "A-V shunting" which may be indicative of capillary obstruction secondary to agglutination at those sites.

Even with the maintenance of systemic normothermia, *in vitro* agglutination in areas of the extracorporeal circuit is a concern in insufficiently diluted patients with antibodies of high titer or broad thermal amplitude. Once under bypass, any stasis of blood at room temperature in the cardiotomy system, and all blood within the extracorporeal circuit proximal to the heat exchanger must be closely observed and portions heated by radiant light or by immersion in a body temperature water bath.[24,26] Such a precaution assumes even more importance in perfusion systems where the venous blood passes through one or more components prior to the heat exchanger (i.e., the Sci-Med system[a]). Following termination of bypass, static residual volume in the perfusion circuit at room temperature must be carefully monitored for clumping if it is to be subsequently transfused or processed.

Cold cardioplegia presents an additional consideration in the perfusion management of these patients, as 1) coronary perfusate temperatures are expected to be much lower than systemic, and 2) any agglutinates occurring within the coronary circulation will disenhance the solution's distribution and thereby increase the chance of perioperative myocardial ischemic injury.

Should *blood-base* cardioplegia be desired, and the patient has undergone the necessary preoperative systemic plasmapheresis or exchange transfusion as described, added concern over the cardioplegic circulation will not be so important. However, it may still be pertinent to bear in mind that it is considered increasingly likely for even "normal" individuals to show idiopathic CAS symptoms the closer that their blood approaches 4°C — even though the titer may be low.[7,10,15,16] As such, users of cold blood cardioplegia should be routinely observing this circuitry for the early indication of any problems.

Safe *asanguineous* cold cardioplegic perfusion

[a]Sci-Med Life Systems, Inc. Minneapolis, MN 55441

of the heart essentially involves normothermic washout of the coronary circulation in order to rinse away susceptible blood elements prior to infusion of the cold solution. Berreklouw et al describe washout with normothermic cardioplegia solution until the coronary sinus return is clear, followed then by solution infusion at 4°C.[27] Prior to aortic unclamping and reperfusion of the heart, normorthermic crystalloid coronary perfusion of the isolated heart is again carried out to prewarm the myocardium, thus preventing subsequent cooling of intracoronary blood by the cold muscle tissues.[27,28]

*Therapy*

Unless concomitant intravascular (and/or extracorporeal) agglutination is present, PCH during bypass will not be evident until the observance of hematuria upon rewarming. Thus, in the idiopathic form at least, the condition is alleviated by no particular treatment — making therapy for PCH rather limited.

Unequivocal CAS features which may be observed and deemed clinically significant will give rise to a number of therapeutic considerations, based upon severity, prepared contingency regimen, and progress of the operation:

- Warming of the blood, of the room temperature, and of the water mattress blanket. Considered cessation of cold cardioplegia to be replaced if necessary by intermittent aortic cross-clamping and reperfusion. Warming of any donor transfusions.
- Cold agglutination reverses with rewarming, however at variable rates.[11,16] There is some evidence that CNS ischemia and dysfunction secondary to cerebral agglutinates may not entirely resolve.[20] Similar effects have been noted in the kidneys.[8] Blood viscosity increases during the syndrome as well.[29] For these reasons, microcirculatory flow enhancers such as Rheomacrodex may be helpful, as may intravenous peripheral dilators such as Nitroprusside or Nitroglycerin.
- Steroid therapy has been shown to be helpful in some cases of hemolytic syndromes.[2,6,7,12,20] The suggested mechanism is an alteration in macrophage complement-receptor function — the macrophages apparently cannot recognize erythrocytes in the presence of steroid.
- Immunosuppressive drugs may be helpful by lowering the antibody titer.[2,3,10,17] Azathioprene, Chlorambucil, and Cyclophosphamide have each been used with some success.
- Remembering how much core heat is radiated away from the open chest, the patient should be kept warm for the remainder of the procedure in the operating room; and following that in the post-operative care unit by means of adequate room temperature, warm bed blankets, warm water mattress blanket, and warming of any necessary donor transfusions.
- Continued awareness of the syndrome's potential manifestations is encouraged throughout the post-operative experience and beyond, as the underlying latent disease condition has been known to become more marked following cardiopulmonary bypass surgery.[10]

## Summary

The observing and reporting of clinically significant cold-reactive antibody hematologic disorders has been relatively infrequent. Experience with surgical patients subjected to cardiopulmonary bypass has been even rarer. Nevertheless, the growing number of such operations and the increasingly lower blood temperatures implemented within areas of the extracorporeal circuit require that perfusionists maintain some basic knowledge of the disorders, including diagnosis, prevention, and treatment. Therapy can be considerably diverse, given such variables as: critical temperature of the underlying condition; protocol temperatures within the systemic and cardioplegic extracorporeal circuits; palliative pre-operative precautions (if any); type, number, placement, and prime constituents of perfusion circuit components; preference for blood- or crystalloid-based cardioplegic solutions; and actual observance of clinical symptoms (if any).

In situations where patients receive blood cardioplegia at 4°C, idiopathic cold agglutination can even be considered "normal" (irrespective of titer). Wherever this regimen is employed, an extra and routine degree of caution and observation is required on the part of all concerned.

As clinical protocols and perfusion devices change over time, the prospects of concomitant

cold blood antibody disorders may have to be reconsidered with them. Looked at another way, devices and protocols (new or otherwise) may have to be evaluated in a different light *because* of the potentials for encountering CAS or PCH.

As a group, perfusionists should cultivate a relationship with clinical laboratory and blood bank personnel in order to ensure the safest possible perfusion procedures for our patients. Laboratory clinicians should be kept appraised of the changing ways in which we influence the circulatory system with cold temperatures; they in turn must be responsible for informing us of the detection of cold antibodies and their critical temperatures.

Finally, due to the deficiency in appropriate literature, this author would like to suggest that those with pertinent clinical experiences might share them via the *JECT* Letters to the Editor forum.

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