How I manage cold agglutinins

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Cold agglutinins have been one of the banes of blood group serologists since pretransfusion testing protocols were first implemented. Cold *auto*agglutinins interfere with ABO/Rh typing tests, yield unwanted positive tests for unexpected antibodies, and may mask the presence of concomitant, clinically significant alloantibodies. Cold *allo*agglutinins, such as anti-M, -P_1, or -Le^a rarely cause accelerated destruction of mismatched red blood cells (RBCs), and it is not necessary to detect examples of these antibodies that only react below body temperatures.

The extent to which cold agglutinins can interfere with the results of pretransfusion antibody detection and compatibility tests is evident from a study by Garratty[1] on the importance of anticomplement reagents in immunohematology. With a low-ionic-strength saline (LISS) method that included room temperature incubation and polyspecific (anti-IgG+ C3) antiglobulin serum, the rate of unwanted positive tests (due primarily to the detection of cold-reactive auto- and alloagglutinins) was on the order of 1.41 percent! Omitting the room temperature incubation phase and use of anti-IgG reduced the unwanted positive rate reduced to 0.1 percent.

AVOIDING COLD AGGLUTININS

Given these introductory comments, the proper handling of cold agglutinins in the transfusion service requires that pretransfusion antibody screening be performed utilizing methods that avoid their detection, namely:

1. No reading for direct agglutination, including immediate-spin tests and direct reading after 37°C incubation.
2. No microscopic examination of tests.
3. Use of anti-IgG instead of polyspecific antiglobulin reagent.

In these regards, polyethylene glycol or gel techniques[2,3] are ideal methods to use. Data supporting safety of the above recommendations are found in Trudeau and colleagues,[4] Laferriere and coworkers,[5] Judd and colleagues,[6-8] and Judd.[9]

The one problem that can arise from omitting readings for direct agglutination when screening for unexpected antibodies is the occurrence of a positive immediate-spin cross-match when the screening tests are negative. In this situation, before implementing an electronic cross-match, our approach was to verify that blood of the correct ABO type had been selected and to cross-match the units by the indirect anitglobulin test (IAT). If the units were compatible by IAT, they were released for transfusion. If not, an antibody identification study was initiated.

LABORATORY MANAGEMENT OF COLD AGGLUTININS

Despite implementation of methods to avoid their detection, potent cold agglutinins will continue to interfere with the results of pretransfusion tests. Their *appropriate* management entails:

1. Resolving blood typing discrepancies, with warm-washed (37°C saline) or 2-mercaptoethanol-treated RBCs, and "reverse" ABO tests at 37°C (control with group O RBCs) or with autoadsorbed or group O adsorbed serum-plasma.
2. Differentiating autoantibody from alloantibody, especially autoanti-I versus alloanti-I or alloanti-P. It is imperative to compare the reactivity of the autocontrol with reagent RBCs and (sometimes) with RBCs of the same ABO type as the patient (e.g., A_1 RBCs for recognition of anti-HI).
3. Excluding the presence of underlying potentially significant alloantibodies. This usually entails adsorption studies, although simple omission of

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potentiators with increased incubation at 37°C (to permit adequate detection for wanted antibodies) can often prove fruitful. An alternative approach is to look for IgG alloantibodies after inactivation of the IgM autoantibody with 2-mercaptoethanol.

Their *inappropriate* management entails application of prewarmed tests (see Judd,[10,11] Judd et al.,[12] Leger and Garratty[13] for an in-depth discussion).

SELECTING BLOOD FOR TRANSFUSION

Having differentiated alloagglutinins from autoagglutinins and ruled out the presence of underlying, potentially significant alloantibodies, the following policies can be helpful in the selection of blood for transfusion:

Alloanti-M, -P_1, and -Le and anti-HI (active at IAT)

1. Issue IgG IAT compatible units,
2. Avoid use of acidic LISS reagents, especially for anti-M, since many examples of anti-M give enhanced reactions at an acidic pH.[14]
3. Do not confirm the antigen-negative status of donor units for patients with anti-M, -P_1, or -Le.[15]
4. Cross-match A_1 units for anti-HI.

Anti-N

Rare examples of alloanti-N in individuals of African ethnicity deserve special mention, because these can be clinically significant when made by N– individuals who lack or have abnormal glycophorin B (Ss sialoglycoprotein). Such individuals (e.g., MS^u/MS^u or $MS^u/MsHe$) have RBCs that are completely devoid of N; they lack N on glycophorin A, and "N" on glycophorin B. Their serum may contain a potent alloanti-N that causes accelerated destruction of N– but S+/s+ RBCs (carrying "N"). Accordingly, whenever an anti-N is detected in pretransfusion testing, it is important to ascertain the ethnic background of the patient and, if African, test for "N" with *Vicia graminea* lectin after treatment of their RBCs with purified trypsin. If nonreactive, use of rare blood from N–U– individuals is indicated.[16,17]

Autoanti-I (active at IAT)

1. Confirm the ABO types of the donor units selected for transfusion and the intended recipient RBCs at time of assignment (reservation). It has already been determined that the anti-I is an autoantibody and therefore will not destroy transfused RBCs at a greater rate than untransfused RBCs. Accordingly, an antiglobulin cross-match is not required. Further, confirming the ABO types of both patient and donor RBCs is less laborious than performing prewarmed tests.
2. Alternatively, perform an immediate-spin cross-match with autoadsorbed serum. This will likely be available from earlier studies.
3. Alternatively, perform an IgG IAT cross-match on unadsorbed serum but without enhancement medium. Previously performed studies should indicate if compatible units will be obtained.
4 Alternatively, do an electronic cross-match.[18] One of its unsung advantages is that it does not yield unwanted positive tests!

Note: The use of blood warmers is rarely needed, except in florid cold agglutinin disease.[19]

Above antibodies, but inactive at IAT

1. Issue units compatible by IAT cross-match with anti-IgG.
2. Alternatively, do electronic cross-match.

Alloanti-I, -P, and so forth

Never prewarm! These antibodies have the potential to cause accelerated destruction of mismatched RBCs.[20,21] They should be managed in the same way as any potentially significant antibody to a high-prevalence antigen.

Cold agglutinins and heart surgery

There are conflicting opinions expressed in surgical journals.[22-24] Our approach is "don't screen for them; don't report them." We discourage requests for titration and thermal amplitude tests and recommend use of room temperature crystalloid instead of cold cardioplegia.

SUMMARY

In summary, although cold agglutinins can and have been a challenging, and perhaps even an annoying, phenomenon, the strategies described above can limit their effect on the transfusion service while helping to ensure maximal transfusion safety. Still, of greatest importance is the need to differentiate cold autoantibodies from cold alloantibodies and to exclude the presence of concomitant IgG alloantibodies.

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