Hemolysis: A positive agglutination reaction while studying titration of anti A/B antibody for ABO-incompatible solid organ transplants

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For a long time, it has been thought that matching of ABO blood groups is an absolute requirement for successful solid organ transplants (SOT). In the last few years, however, several centers have started to perform ABO-incompatible SOT with encouraging results. Today, transplant physicians[1] are confronted with ABO-incompatible grafts in for kidney and liver from living donors. Initial results of these kidney transplants (KT) achieved low graft survival due to high anti A and anti B isoagglutinin in the recipients but recently developed desensitization protocols have improved survival to levels that are comparable to ABO-compatible KT. However, isoagglutinin is still regarded as a major obstacle[2] to ABO-i KT.

Request for baseline isoagglutinin levels was received by the department of transfusion medicine for a patient planned for ABO-incompatible renal transplant. The patient was suffering from chronic renal failure for many years. ABO and D blood typing was performed routinely by both manual tube and column agglutination technology (CAT). Screening of irregular antibody was done using surgiscreen, ortho clinical diagnostics (USA) with polyspecific anti-human globulin (AHG) cards. Isoagglutinin titration was done in two phases: One was immediate spin (IS) using reverse diluent cards and the other was at AHG phase using CAT cards containing polyspecific AHG (Anti-immunoglobulin G [IgG] and anti-C3d). The antibody titration (isoagglutinin levels) was performed by doubling dilution technique of the patient's plasma and reagent cells used were A1 and B pooled cells (3-5% suspension) for anti A and anti B, respectively. The blood group of the patient was O Rh (D) positive. Antibody screen and auto control of the patient was negative.

On titration of both anti A and anti B, the strength of reaction of neat (N), 1:2 and 1:4 dilution showed reddish tinge in the reaction chamber [Figure 1]. The plasma was negative for any free hemoglobin. These reddish tinges were due to the hemolysis caused by the high antibody strength in the patient. The antibody titers were anti-A (IS - 1:64, AHG - 1:512) and anti-B (IS - 1:32, AHG - 1:512). On further dilution, the strength of the reaction decreased, and the reddish tinge disappeared [Figure 2].

The ABO antibodies are predominantly IgM, activate complement, and react at room temperature or colder.[3] The predominant Ig class of antibodies in group O serum is IgG.[3] Knowledge of the amount of IgG anti-A and anti-B in patients planned for ABO-incompatible SOT allows prediction of the graft survivals.[4] Both Ig classes of ABO antibodies react preferentially at room temperature (20-24°C) or below and efficiently activate complement at 37°C.

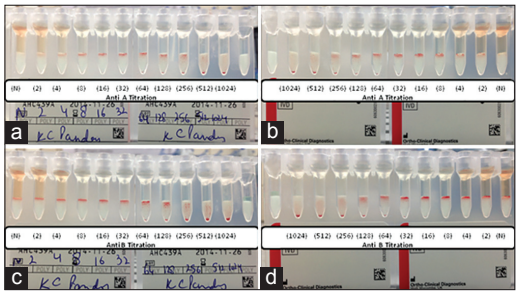

Figure 1: Anti A and Anti B antibody titration (front side of cards a, c; back side b, d)

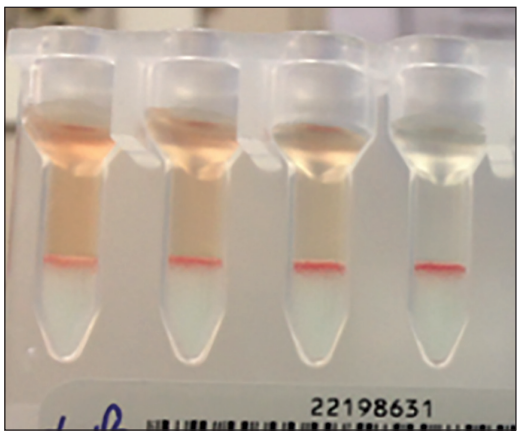

Figure 2: Reddish tinge depicting hemolysis

This illustration highlights the importance of keeping in mind that hemolysis must be regarded as a positive sign of an antigen-antibody reaction if the pretest serum was not hemolyzed and no hemolytic agent was added to the test. This phenomenon is particularly observed when dealing with anti-A and anti-B antibodies as both of them cause rapid *in vitro* lysis of the incompatible cells.

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