Detection of homologous blood transfusion by flow cytometry: a deterrent against blood doping

Blood doping has the explicit goal of increasing the level of circulating hemoglobin in the bloodstream to enable greater oxygen transport during exercise and therefore to improve endurance performance. Tests have been developed that are capable of detecting athletes using either recombinant human erythropoietins or hemoglobin-based oxygen carriers to boost hemoglobin levels, but to date there are no standard methods for the detection of blood transfusion in an athlete intent on achieving increased oxygen-carrying capacity through the traditional form of blood doping.[1]

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Previous studies have shown that a greater than 5% increase in circulating hemoglobin is necessary to improve performance[2] suggesting that athletes would need to infuse at least one unit of blood to obtain a surreptitious performance advantage. Assuming a blood volume of 4-5 L for an adult, transfusing a single unit of blood would result in approximately 10% of the red cell population being of donor origin. The antigenic profile of cells from any individual is under genetic control, and except in certain rare malignancies, hematopoiesis in the bone marrow produces red cells which have an identical and specific spectrum of blood group antigens.

In a clinical setting, the course of red cell engraftment after allogeneic bone marrow or peripheral stem cell transplantation may be followed by quantification of antigenically distinct donor and recipient red cells using flow cytometry.[3] This technology has been used to determine the original phenotype of multi-transfused patients[4] and to determine zygosity in paternity testing.[5] Since the flow cytometer has a sensitivity of approximately 0.07%,[6] this technology should be capable of detecting the transfusion of a single unit of blood, provided there is at least one antigen unmatched between donor and recipient. It should be noted that sibling transplant donors matched for histocompatibility A, B and DR antigens are rarely identical with regard to blood group antigens. Antibodies suitable for flow cytometry should be of class IgG, and some blood group antigens, notably A and B, M and N, stimulate a predominantly IgM response. It must also be assumed that blood intended for transfusion to athletes shortly before a competition would be matched for at least the groups ABO and Rh(D).

We report here the results from preliminary trials of phenotyping packed red cell samples, some of which were deliberately spiked with 10% red cells from another donor matched only for ABO and Rh(D). These samples were intended to mimic blood doping through transfusion. Both series were tested blind, the first using a panel of 10 antibodies (Table 1), and the second a panel of 12 antibodies (Table 2). Cells were labeled for flow cytometry through use of a secondary antibody, a fluorescein-conjugated sheep anti-human immunoglobulin.

In the first series, 6 of the 7 spiked samples and the 3 unadulterated samples were correctly identified. One spiked sample was not detected, and on closer examination the mixed bloods were found to be identical except for expression of the antigen S, for which no antibody was available at the time of testing. For the second series, there was clear evidence for 7 spiked samples and 3 unadulterated samples.

The chance of two blood samples matched for ABO and Rh(D) being identical for the panel of 12 blood group antigens is less than 1:500, the probability based on the most common phenotypic frequency of each antigen in a Western European population.[7] Other populations may have a very different antigenic pattern and require a modified panel for testing.

Flow cytometry appears to be a technique capable of detecting homologous transfusion with a high degree of sensitivity. False positive results do not appear to present a problem. The half-life of transfused red cells is about 55 days[8] and blood samples are stable for some weeks if refrigerated at 4°C. The technology thus seems well suited for application as a test method to detect homologous blood doping even 2-3 weeks after infusion.

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Table 1. An initial trial of the analysis of 10 blood samples, some of which had been deliberately contaminated with approximately 10% red cells from a second sample matched for ABO and Rh(D) antigens. The percentage of red cells positive by flow cytometry for 10 blood group antigens are listed. Certain samples (*) showed distinct differences in expression of the antigen depending on whether homozygous or heterozygous, since the intensity of expression of the antigen (mean log fluorescence 1, MFL1) was dependent on the number of antigenic sites on the surface of the red cell.

	Sample number									
Antibody to blood group antigen	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Rh(c)	9.1	86.6	100	8.9	100	100	100	100	0	8.7
Rh(E)	0	87.3	0	9.7	100	100	11.4	100	0	9.7
Rh(e)	100	10.6* 89.4*	100	100	100	100	100	100	100	100
K1 (Kell)	0	0	0	0	0	0	0	0	0	0
K2 (Cellano)	100	100	100	100	100	100	100	100	100	100
Fy[a] (Duffy[a])	89.4	100	100	100	100	100	100	100	100	0
Fy[b] (Duffy[b])	100	0	100	100	100	100	11.2	100	0	100
Jk[a] (Kidd[a])	100	100	100	100	100	100	100	100	85.9* 11.2*	8.7
Jk[b] (Kidd[b])	0	0	0	100	100	100	89.0	100	9.3* 90.7*	100
s	100	87.9* 12.5*	0	100	100	100	12.3	100	100	100

Table 2. A second trial involving analysis of a further 10 blood samples, some of which had been deliberately contaminated with red cells from a second sample matched for ABO and Rh(D) antigens. The percentage of red cells positive by flow cytometry for 12 blood group antigens are tabled. Certain samples (*) showed distinct differences in expression of the antigen depending on whether homozygous or heterozygous, since the intensity of expression of the antigen (mean log fluorescence 1, MFL1) was dependent on the number of antigenic sites on the surface of the red cell.

Antibody to blood group antigen	*Sample number*									
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Rh(C)	100	88.3	0	100	100	100	87.5	100	100	100
Rh(c)	100	100	100	100	91.2	0	100	100	100	90.3
Rh(E)	100	90.4	0	0	91.3	92.8	87.4	0	0	87.9* 8.9*
Rh(e)	100	100	100	100	89.6	7.1	100	100	100	90.6* 9.9*
K1 (Kell)	0	89.4	0	0	0	0	0	0	0	0
K2 (Cellano)	100	100	100	100	100	100	100	100	100	100
Fy^a ($Duffy^a$)	0	100	100	100	100	100	100	100	100	100
Fy^b ($Duffy^b$)	100	100	100	88.7	90.1	0	100	100	9.0	100
Jk^a ($Kidd^a$)	100	100	100	100	88.7	100	100	0	100	100
Jk^b ($Kidd^b$)	0	0	0	0	100	0	0	0	100	100
S	0	0	0	9.0	100	0	0	0	0	100
s	100	100	100	90.6	100	100	11.7	100	8.7	90.5

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Enlargement of hepatoduodenal ligament lymph nodes in β thalassemia children receiving multiple transfusions: a common observation

Of 37 thalassemic major patients receiving regular blood transfusion and chelation therapy, 12 (32.4%) had enlarged hepatoduodenal ligament nodes. Of these 12 patients, 9 (83%) had a ferritin level > 2,500 ng/mL. At the 24-month follow-up 8 patients showed persistent lymphadenopathy. It is important to recognize hemosiderin lymphadenopathy and avoid unnecessary investigation in asymptomatic thalassemic patients.

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Despite modern iron chelation therapy, iron overload and secondary hemochromatosis are still major clinical problems for thalassemia patients. Thalassemic patients are frequently referred for abdominal ultrasonography for the detection of abnormalities such as liver cirrhosis and gallstones. Incidental findings of hemosiderin-laden lymph nodes have been reported in thalassemic patients. Visualization of these nodes by plain radiography,[1] abdominal lymphography[2] and computed tomography[3,4] is feasible.

We conducted a study using ultrasonography to determine how often hepatoduodenal ligament (HDL) nodes were detected in these patients and to evaluate whether their presence had any clinical significance or relationship with ferritin levels.

Thirty-seven thalassemic patients were recruited (20 boys and 17 girls, age 2.75 to 21 years, mean 11.3 years). These patients were receiving regular blood transfusion and chelation therapy. Abdominal ultrasonography was performed by the same pediatric radiologist (WCWC) and double-checked by the second investigator (CM).

The lengths of the long and short axes of the largest HDL node identified in each patient were measured. Three measurements were made for each axis and the mean value was calculated. Enlargement of the node was diagnosed when either the mean

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