## Hematopoietic mixed chimerism after allogeneic BMT

Chimerism after BMT has been documented by various genetic markers, RBC antigens, and, less frequently, by immunoglobulin allotypes. These markers are less sensitive than short tandem repeats (STRs), which provides a powerful tool for analysis of human polymorphisms. Application of STR analysis in patients undergoing allogeneic BMT has been used to detect mixed or complete chimerism, recurrent leukemia, and endogenous repopulation of marrow in early and late phases after transplant. It can detect minor populations of DNA in the period after transplant with adequate sensitivity, whereas traditional immunohematologic evaluation is limited because of the necessity of transfusing RBCs.[1]

We describe a 21-year-old man with acute lymphoblastic leukemia who received an allogeneic unrelated BMT from a female donor after achieving complete remission. The patient blood group was A1/C–c+D–E–e+, and the donor's was O/C–c+D+E+e–; that is, there was a minor ABO mismatch. The marrow was treated to remove RBCs and plasma. The conditioning treatment was cyclophosphamide plus total body irradiation and rabbit antilymphocyte globulin (ATG) (rabbit ATG, Fresenius, Bad Homburg, Germany) (60 mg/kg cyclophosphamide on Days –3 and –2, 8 Gy on Day –1 in a single dose, and 3 mg ATG/kg/day on Days –6 to –2). GVHD prophylaxis was cyclosporine A (CsA) plus short-course methotrexate: CsA was given at a starting dose of 3 mg per kg and adjusted to maintain blood levels of 150 to 250 µg per mL. The patient had moderate GVHD (skin, 3; liver, 2; gastrointestinal tract, 0). He received transfusion in the early phase after transplant with no more than 6 units of WBC-reduced and irradiated RBCs and 6 WBC-reduced and irradiated plateletpheresis units, all blood group O.

After 4 months without RBC transfusions, his Rh phenotype was C–c+D+E+e+. His RBCs typed as group O with mixed-field reactions with anti-A,B and anti-A sera. In tube tests, the mixed field was more evident with monoclonal antisera compared with polyclonal antisera. The mixed field with anti-A,B and anti-A sera was more evident with the gel method than with the tube method. The patient's ABO blood group was studied monthly after transplantation. Weak reactivity with monoclonal anti-A,B and anti-A antisera was observed consistently. Twelve months after BMT, the patient was in complete hematologic remission, but mixed-field ABO typings persisted, more evident on gel than tube testing.

We also applied molecular methods to study the blood group and the DNA polymorphisms of peripheral blood and marrow (see Table 1). The sixth and seventh exons of the ABO gene were amplified and sequenced with specific primers.[2] The result of sequence-based typing of the blood group was *ABO* 0101*, *0201*, and it was confirmed that granulopoiesis came from the donor. Furthermore, with nine STR-specific primers (AmpFLSTR Profiler, Applied Biosystems, Foster City, CA), we amplified the patient's DNA before and after BMT and also the donor's DNA. The analysis was performed with an automatic sequencer (ABI PRISM 310, Applied Biosystems). Results showed a complete donor reactivity allele pattern in five of nine loci, whereas in the other four there was a sharing of alleles between the donor and the patient before BMT in both peripheral blood and marrow. The

**TABLE 1. RBC typings and DNA polymorphisms demonstrating mixed chimerism in the glycophorin A+-enriched population**

| | Results of laboratory tests | | | | | |
|---|---|---|---|---|---|---|
| | | Patient | | | | |
| | | | After BMT | | | |
| Laboratory tests | Donor | Before BMT | 1 year | 2 years 8 months | 2 years 8 months, glycophorin A+ population | 3 years 6 months |
| ABO blood group (peripheral blood) | | | | | | |
| Anti-A,B | 0 | 4+* | ± (MF) | ± (MF) | NT | ± (MF) |
| Anti-A | 0 | 4+ | ± (MF) | ± (MF) | NT | ± (MF) |
| Anti-A1 | 0 | 4+ | 0 | 0 | NT | 0 |
| Anti-B | 0 | 0 | 0 | 0 | NT | 0 |
| Rh blood group (peripheral blood) | | | | | | |
| C | 0 | 0 | 0 | 0 | NT | 0 |
| c | 4+ | 4+ | 4+ | 4+ | NT | 4+ |
| D | 4+ | 0 | 4+ | 4+ | NT | 4+ |
| E | 4+ | 0 | 4+ | 4+ | NT | 4+ |
| e | 4+ | 4+ | 4+ | 4+ | NT | 4+ |
| STR (marrow) | | | | | | |
| D3s1358 | 15, 18 | 15, 18 | 15, 18 | 15, 18 | 15, 18 | 15, 18 |
| VWA | 14, 16 | 17 | 14, 16 | 14, 16 | 14, 16, 17 | 14, 16 |
| FGA | 22 | 22 | 22 | 22 | NE | 22 |
| TH01 | 6, 7 | 9, 9.3 | 6, 7 | 6, 7 | 9, 9.3 | 6,7 |
| TPOX | 8, 11 | 9, 12 | 8, 11 | 8, 11 | NE | 8, 11 |
| CSF1P0 | 12 | 10, 11 | 12 | 12 | 12 | 12 |
| D5S818 | 11, 13 | 12, 13 | 11, 13 | 11, 13 | 11, 13 | 11, 13 |
| D13S317 | 8, 13 | 8, 12 | 8, 13 | 8, 13 | NE | 8, 13 |
| D7S820 | 8, 9 | 10 | 8, 9 | 8, 9 | NE | 8, 9 |
| Amelogenin | XX | XY | XX | XX | XY | XX |

* 4+ = agglutination score.
† MF = mixed field.
‡ NE = not evaluable.

absence of mixed chimerism in the patient after BMT was confirmed also by studying the amelogenin gene located on the sex chromosome.

At 2 years 8 months after BMT, the immunohematologic features were stable. We studied erythroid precursors obtained by cell sorting from marrow, with anti-glycophorin A MoAb. The enriched erythroblastic population was studied by STR, and mixed chimerism was shown at three loci (vWA, TH01, and amelogenin), whereas in the other two evaluable and discriminatory loci, the allele pattern was that of the donor only.

At 3 years after BMT the pattern was unchanged. The patient remains in complete remission with limited chronic GVHD, which was first diagnosed on Day 134.

Mixed erythrocyte chimerism has been described previously in BMT recipients. In our case, the absence of development of anti-D could indicate that the immune system converted completely to donor's group O, whereas a hematopoietic group A clone persisted after BMT for a long time. In patients who received transplantion for severe combined immunodeficiency, erythrocyte chimerism was linked to a failure of engraftment of donor pluripotent PBPCs or to a differentiation of PBPCs along the erythroid lineage.[3] In a case similar to ours, persistently weak group A reactivity was found 3.5 years after a group O marrow transplant in a group A patient.[4] The authors proposed that the recipient residual serum A transferase attached the *N*-acetylgalactosamine to substance H on the RBC membrane to form blood group substance A.

By studying the enriched erythroblastic precursor population, were we able to demonstrate that mixed chimerism was due to a persistent clone of recipient's RBC precursors. This case, as has been seen with studies on lymphocytes,[5] underlines the limitation of assaying for chimerism with a whole-blood sample compared to enriched RBCs.

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## PLT transfusions from D+ blood donors to D– patients with hematologic diseases: an update

We update the results of our previously published study of the risk of alloimmunization after PLT transfusions from D+ blood donors to D– patients with hematologic diseases.[1] In that report, the median follow-up of patients after transfusion was only 8 weeks. After 2 additional years of study, we now extend our median follow-up to 15.5 weeks.

Between April 1999 and July 2003, 8581 patients on our service received transfusions, of whom 1344 (15.7%) were D–. Seventy-six (5.7%) D– patients received PLT transfusions and 36 (47.4%) had a hematologic disease. Of these, 4 (11.1%) received D– (matched) PLT transfusions and 32 (88.9%) received D+ (mismatched) PLT transfusions. There were 21 men and 11 women with a median age of 56.5 years (range, 6-80 years). Seventeen of these patients (53.1%) had acute leukemia; 9 (28.1%), non-Hodgkin's lymphoma; 3 (9.4%), myelodysplastic syndromes; 2 (6.3%), chronic myelogenous leukemia in blast crisis; and 1 (3.1%), multiple myeloma. Twenty-nine (90.6%) patients received chemotherapy and 8 (25%) patients underwent autologous PBPC transplantation.

The patients received 284 pooled PLT transfusions, and 210 (73.9%) of these PLT units were D-mismatched. Of the D-mismatched PLT units, 178 (84.8%) were ABO-compatible (major and minor). Major ABO mismatch was present in 19 (9.0%) D-mismatched PLT transfusions and minor ABO mismatch was present in 12 (5.7%). ABO mismatch (major and minor) was present in 1 (0.5%) D-mismatched PLT transfusion.

By July 2003, 12 (37.5%) patients had expired after a

median follow-up of 15.5 weeks (range, 1-96 weeks). Twenty patients were alive after a median follow-up of 13.5 weeks (range, 1-175 weeks). None of the 32 patients with hematologic diseases developed anti-D after a median follow-up of 15.5 weeks (range, 1-175 weeks). These results confirm our previous finding of a low risk of alloimmunization in D– hematologic patients who received transfusion with PLTs from D+ blood donors. Randomized, well-defined, and well-conducted studies are necessary to clearly define guidelines for this still unresolved aspect of PLT transfusion therapy.

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## Identification of two new single-nucleotide polymorphisms in FUT3 associated with the Lewis-null phenotype

Like ABH and other carbohydrate antigens, the synthesis and expression of Lewis blood group antigens requires sequential action of multiple glycosyltransferases. Two distinct fucosyltransferases ultimately determine $Le^a$ and $Le^b$ expression: Lewis or FUT3, an α1,3/4-fucosyltransferase required for both $Le^a$ and $Le^b$ synthesis, and Secretor (FUT2), an α1,2-fucosyltransferase required for $Le^b$ and type 1 chain ABH synthesis. Mutations in FUT3 gene result in the Lewis-null phenotype (le).[1]

To date, 10 single-nucleotide polymorphisms (SNPs) in FUT3 have been published, of which 7 are known to disrupt enzyme activity or Golgi retention (Fig. 1).[1-4] Individual FUT3 SNPs are typically paired, resulting in multiple *le* alleles containing two or more mutations, which often differ in frequency between ethnic and geographic populations.[1] In the US, mutations at nucleotides (nts) 202, 314 ($le^{202,314}$), 59, 508, and 1067 ($le^{59,508}$ and $le^{59,1067}$) are common, with the $le^{202,314}$ allele accounting for 60 percent of all Le(a–b–) phenotypes in whites.[2] Among South African blacks and Asians, $le^{59,508}$ and $le^{59,1067}$ are the predominant *le* alleles (30%-40%), whereas $le^{202,314}$ is relatively uncommon.[3,4] Five additional FUT3 SNPs (C304>A, G370>T, G484>A, G667>A, and 808>A) were recently identified among South African Xhosans, leading to two new *Le* ($Le^{304}$ and $Le^{370}$) and three *le* alleles ($le^{484,667}$, $le^{202,314,484}$, and $le^{484,667,808}$).[4] We now report the identification of two new FUT3 SNPs, associated with up to six variant African *le* alleles, in African Americans.

Whole-blood samples from group O volunteer donors were a gift of T. Copeland (Immucor, Norcross, GA). Washed RBCs were serologically typed for Lewis antigens with monoclonal anti-$Le^a$ and anti-$Le^b$ according to the manufacturer's instructions (Gamma Biologicals, Houston, TX). Genomic DNA was isolated from a total of 15 Le(a–b–), 5 Le(a+b–), and 5 Le(a–b+) donors (Gentra Systems, Minneapolis, MN). Primers for both PCR amplification and sequencing were designed to amplify FUT3 exon 3, which contains the protein-coding region (forward primer, 5′-GTGAGGTCCCAGGTAAGAGAGAG-3′; reverse primer, 5′-AATAGCAGCTCCTCTCAGGACTC-3′). PCR was performed for 30 cycles to yield a 1291-bp product, which was isolated by gel electrophoresis, followed by direct sequencing PCR with the same primers. DNA sequence data were analyzed with the target gene sequence in GenBank as a reference (Accession Numbers NT_011255, AF131913, and U27326).

As shown in Table 1, nine SNPs were identified, including two new SNPs at nts 13G>A and 1022G>T. A 10th SNP was also observed at nt 61 (61G>A), which was silent. No mutations were observed at nts 304, 370, or 808.[4] The 13G>A mutation was present in 50 percent of Lewis-null individuals in conjunction with mutations at nts 484 and 667 ($p < 0.000005$; $\chi^2 = 25.11$, d.f. = 29), giving rise to three, and potentially five, new *le* alleles ($le^{13,484}$, $le^{13,484,667}$, $le^{13,59,484,667}$, $le^{13,59,667}$, and $le^{13,59,508}$), with relative frequencies ranging from 0.17 to 0.03. The G1022>T was identified in only one individual and paired with a mutations at nts 59 and 1067 ($le^{59,1022,1067}$). Neither 13G>A nor 1022G>T SNP were identified in Le(a+b–) and Le(a–b+) donors, including five African American persons (data not shown).

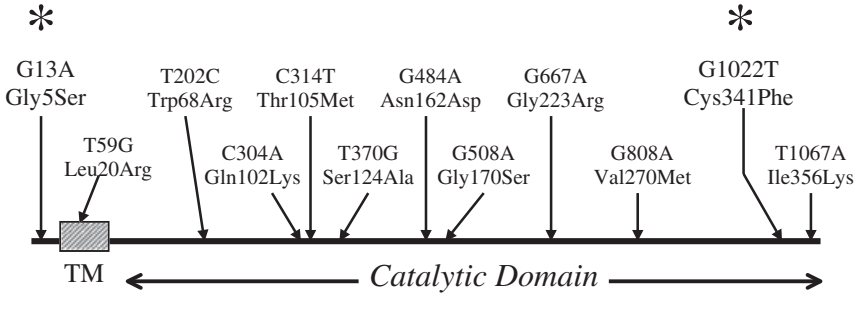


**Fig. 1. Location and relationship of SNP mutations in FUT3 (Lewis). Asterisks (*) indicate the two new SNPs identified in this study.**

Both 13G>A and 1022G>T SNPs result in missense mutations in the translated protein (Fig. 1). The 13G>A

**TABLE 1. FUT3 genotype in Lewis-null donors**

| | | SNPs and amino acid changes in FUT3 (Lewis gene)* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample | Race† | G13>A Gly5Ser | T59>G Leu20Arg | T202>C Trp68Arg | C314>T Thr105Met | G484>A Asn162Asp | G508>A Gly170Ser | G667>A Gly223Arg | G1022>T Cys341Phe | T1067>A Ile356Lys |
| S12 | U | WT | ++ | WT | WT | WT | ++ | WT | WT | WT |
| S18 | B | + | ++ | WT | + | + | + | + | WT | WT |
| S19 | B | + | ++ | WT | + | + | + | + | WT | WT |
| S20 | U | + | ++ | WT | + | + | WT | + | WT | WT |
| S43 | B | WT | + | WT | WT | WT | WT | WT | WT | WT |
| S45 | W | WT | WT | ++ | ++ | WT | WT | WT | WT | WT |
| S47 | B | WT | ++ | WT | + | WT | WT | WT | + | ++ |
| W3 | U | + | WT | + | + | + | WT | + | WT | WT |
| W6 | U | ++ | WT | WT | WT | ++ | WT | + | WT | WT |
| W7 | U | + | WT | WT | WT | + | WT | + | WT | WT |
| W13 | B | + | WT | WT | WT | + | WT | + | WT | WT |
| W14 | U | + | WT | WT | WT | + | WT | + | WT | WT |
| W15 | U | WT | ++ | WT | WT | WT | ++ | WT | WT | WT |
| W16 | U | WT | ++ | WT | WT | WT | ++ | WT | WT | WT |
| W21 | B | WT | ++ | WT | WT | WT | ++ | WT | WT | WT |
| Heterozygous | | 7 | 1 | 1 | 5 | 7 | 2 | 8 | 1 | 0 |
| Homozygous | | 1 | 8 | 1 | 1 | 1 | 4 | 0 | 0 | 1 |
| Total alleles | | 9 | 17 | 3 | 7 | 9 | 10 | 8 | 1 | 2 |

* WT = wild type or no mutation; + = heterozygous; ++ = homozygous.
† B = African American black; W = white; U = unknown.

polymorphism results in a Gly5Ser substitution within the cytoplasmic domain of the enzyme. Although the latter is unlikely to effect enzyme activity, serine residues have been shown to modulate sialyltransferase activity via post-translational phosphorylation.[5] In contrast, the G1022>T mutation, resulting in a Cys341Phe in the catalytic domain of the molecule, is probably an enzyme-inactivating mutation. The mutation lies just proximal to the mutation at Ile356Lys, which has been shown to inactivate Lewis enzyme activity.[1] Furthermore, the loss of a cysteine residue, particularly within the catalytic domain, could profoundly affect enzyme activity and tertiary structure owing to a theoretical loss of disulfide bonds.

In summary, we have identified two new FUT3 SNPs and four to six new *le* alleles in le(a–b–) African Americans. The G13>A is linked to mutations common in native Africans (G484>A, G667>A) and may represent a western variant of African *le* alleles.[4] Given the ethnic diversity in the US, these results illustrate the difficulty in the use of only restriction enzyme or allele-specific PCR methods for Lewis genotyping.[2,3]

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## Role of the Hct in the treatment of thrombocytopenic patients

This letter is written to comment on the publication of Heddle and associates.[1] This article reviewed the clinical measures that have been used to assess the nonsurgical bleeding diathesis in anemic thrombocytopenic patients with hematologic and oncologic disorders who received

prophylactic transfusion of allogeneic PLTs to maintain PLT counts ranging from 5,000 to 20,000 per μL.

The authors do not report the quality of the allogeneic prophylactic PLTs that were transfused, the Hct values of the patients before the prophylactic transfusion, the occurrence of PLT refractoriness, and the indications for RBC transfusion that were administered to these patients.

The viability and function of the allogeneic compatible PLTs and the Hct of the patients at the time of the prophylactic transfusion are important factors that may influence the bleeding disorder in these thrombocytopenic patients. Allogeneic previously frozen washed PLTs have been shown to be more effective in reducing nonsurgical blood loss and reducing the need for allogeneic RBCs and FFP after cardiopulmonary bypass surgery than liquid-preserved PLTs stored at 22°C for a mean of 3.4 days.[2]

In our studies of patients subjected to cardiopulmonary bypass surgery, blood loss was measured intraoperatively after neutralization of the heparin and postoperatively during the 24-hour postoperative period. In our study the blood loss not related to surgical causes, referred to as nonsurgical blood loss, was associated with an increase in the bleeding time and a decrease in the Hct 2 hours after cardiopulmonary bypass surgery.[3] The transfusion of allogeneic RBCs in anemic thrombocytopenic patients after cardiopulmonary bypass surgery to a Hct of 35 vol percent reduced the nonsurgical blood loss and reduced the need for allogeneic PLTs and FFP.

Several investigators have reported that RBCs have an important hemostatic effect and that the RBC and PLT interactions in vivo reduce the bleeding time.[4-6] Clinical studies in anemic uremic patients have shown that increasing the Hct with either RBC transfusion or EPO corrects the prolonged bleeding time and reduces the bleeding diathesis observed in these patients.[7-9] In addition, treatment with EPO or RBCs to correct anemia in uremic patients has been shown to improve PLT function.

Blajchman and associates[10] evaluated the effect of the Hct on the bleeding time in both normal and thrombocytopenic rabbits. Nonthrombocytopenic rabbits with Hct levels above 35 percent had significantly shorter bleeding times than rabbits with Hct values lower than 35 percent. These investigators reported that anemia contributed significantly to the prolonged bleeding time in both thrombocytopenic and nonthrombocytopenic rabbits and that RBC transfusions were capable of shortening the bleeding time.

RBCs have been shown to have a beneficial effect on PLT function. By dispersing PLTs from the center of the blood vessel toward the vessel wall, RBCs concentrate PLTs near the endothelial cells of the vessel wall where they are poised to respond to injury. Several studies by Valles and associates[11] have reported that RBCs are prothrombotic and activate PLTs. Allogeneic compatible PLTs produce only a transient increase in PLT count because of their short life span, whereas the allogeneic compatible RBCs produce a prolonged effect on the Hct value because of their long life span.

Studies are needed to determine how both the PLT count and the Hct affect hemostasis in thrombocytopenic and anemic patients with hematologic and oncologic disorders. RBC transfusions may prove to be safer and cheaper than prophylactic PLT transfusions to minimize the bleeding diathesis in these patients.

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*The above letter was sent to Heddle et al.: Dr Heddle offered the following reply.*

We thank Dr Valeri and colleagues for their letter and for raising some interesting points for discussion. It is important to emphasize that the purpose of our article was to explore methodologic issues when using bleeding as an outcome measure. To find examples of the different ways that bleeding had been analyzed, we identified studies in the literature that used bleeding as an outcome measure. The goal of this review was not to summarize the results of the studies but to review and critique the methods used. Although it would have been relevant for the authors of the original studies to provide information about the "quality of the allogeneic prophylactic PLTs that were transfused, the Hct values of the patients before the prophylactic transfusion," the occurrence of PLT refractoriness, the indications for the RBC transfusions, and any other factors that might have an effect on the frequency or severity of bleeding, this information was not relevant to our discussion of the measurement and analysis of bleeding and in many cases was not reported in the original articles.

Nevertheless, Dr Valeri and colleagues have emphasized several important observations that have been reported in the literature and may be the key to understanding a complex interaction between physiologic factors that contribute to an increased risk of bleeding in some patients. It is likely that there are many factors that increase bleeding risk of both thrombocytopenic and nonthrombocytopenic patients. These risk factors likely include Hct as well as other factors such as uremia, hypoalbuminemia, recent BMT, and recent hemorrhage.[1] Further studies are needed to examine the effects of these or other factors on the frequency and severity of bleeding as well as intervention (treatment) studies that could influence the frequency of these events. If and when these studies are performed there must be careful consideration given to the methodologic issues involved when bleeding is used as the outcome measure. Our article was designed to identify and describe these methodologic considerations.

We agree with Valeri and colleagues that "studies are needed to determine how both the PLT count and the Hct affect hemostasis in thrombocytopenic and anemic patients with hematologic and oncologic disorders." In fact, several of the authors (N.H., R.C., K.W.) are investigators in a pilot clinical trial designed to investigate the effect of Hct on the frequency of bleeding in anemic, thrombocytopenic patients. Well-designed studies of this nature will provide evidence to guide the practice of RBC and PLT transfusions in these patients. Only when data from these studies are available will we know whether Dr Valeri and colleague's hypothesis is correct.

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## HBV DNA in plasma pools for fractionation

We tested for HBV DNA by NAT in 200 archived plasma pools for fractionation that had been sent to our institute for laboratory testing. We present the following findings as indirect evidence of the residual risk of transfusion-transmitted HBV infection and of the potential for decreasing the risk by testing for HBV DNA by NAT.

Sixty of the plasma pools originated in the US and 140 were from Italy. Pool sizes ranged from 2000 to 4000 individual collections. Samples from all pools had previously tested negative by the Institute's standard assays for HBsAg, HCV RNA, anti-HIV, and anti-HCV. HBV DNA NAT was performed with two different assays, an in-house method, and an HBV detection test (COBAS Ampliscreen, Roche Diagnostic Systems, Branchburg, NJ). Both methods were validated by the WHO HBV DNA (97/746) International Standard (genotype A, subtype HBsAg *adw2*). In compliance with European guidelines for NAT validation,[1] we tested four series of six half-log dilutions, ranging from 316 to 0.316 IU per mL, in six independent assays, obtaining a total of 24 results for each dilution. The detection limit (95% cutoff) for each NAT method was calculated by probit analysis (SPSS Version 11.0.1, SPSS Italia, Milan, Italy) (Fig. 1).

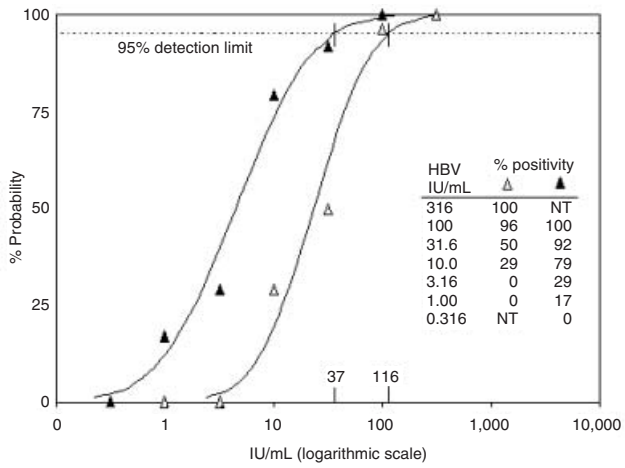


**Fig. 1. Probability of detecting HBV DNA at different concentrations with the in-house method (△) or the COBAS Ampliscreen HBV test (▲). Concentrations represent half-log dilutions and rates of positive reactions (%). The 95 percent cutoff was 116 IU per mL for the in-house method and 37 IU per mL for the COBAS Ampliscreen HBV test. The 95 percent CIs were 73 to 257 and 21 to 95, respectively.**

With our in-house NAT method, we detected HBV DNA in 2 of 200 pools (1%), 1 pool from Italy and 1 from the US. Both pools tested negative when diluted 1:10, reflecting very low viral loads. Amplification was conducted with primers targeting the S region of the HBV genome (nucleotides 256-279 and 540-561 for the outer primers and nucleotides 303-324 and 516-537 for the inner primers). Although this region is not the most conserved, it has the advantage of detecting possible HBsAg escape mutants. Sequence analysis of the first round of amplification of the two positive samples allowed us to exclude such viral mutants. The HBV+ samples were identified to be genotype A, subtypes *ayr* (US pool) and *adr* (Italian pool).[2]

With the COBAS Ampliscreen HBV test, we confirmed the presence of HBV DNA in the same two pools and in no others. Having excluded the presence of HBV mutants, we interpret these results to reflect blood collections during the early HBV-seronegative window period or deviations from good laboratory practice at blood centers.

Our finding that 1 percent of plasma pools tested positive for the presence of HBV DNA provides indirect evidence of the residual risk of HBV infectivity in donated blood. Although this risk is a serious concern for the transfusion of blood components, plasma derivatives do not appear to be at risk of transmitting HBV.[3] Adding NAT to current laboratory testing has the potential for detecting residual HBV infectivity in pools of plasma for fractionation.

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## Human herpesvirus type 8 among Brazilian blood donors

The seroprevalence of human herpesvirus type 8 (HHV-8) varies considerably among blood donor populations in different geographic regions. Caterino-de-Araujo and coworkers[1] detected antibodies to latent nuclear HHV-8 antigens among 7.4 percent of blood donors in Brazil, but there are no studies evaluating HHV-8 DNA among Brazilian blood donors. HHV-8 DNA was detected among 22.5 percent of 49 blood donors in Central Africa,[2] but not among 100 blood donors in southeastern Texas where there is a relatively high (23%) seroprevalence of HHV-8.[3] We report the results of testing for HHV-8 antibodies and HHV-8 DNA among 400 randomly selected donors from a large public blood center in São Paulo, Brazil.

Samples were tested for antibodies against HHV-8 by indirect immunofluorescent assay.[4] Antibodies against HHV-8 lytic or latent antigens were detected in 4 percent (16/400), and only 0.5 percent (2/400) had detectable antibodies to both antigens. A chi-square test of linear trend analysis was computed to evaluate the association of age, sex, and educational status, independently, with HHV-8 infection. ORs were calculated with 95 percent CIs using computer software (Epi Info, Version 6.4, CDC, Atlanta, GA). The presence of HHV-8 antibody was associated with female sex (Table 1).

**TABLE 1. Distribution of blood donors reactive to HHV-8 antigens (latent or lytic) according to demographic characteristics**

| | | Reactive samples to HHV-8 antigens (latent or lytic) | | | |
|---|---|---|---|---|---|
| Characteristics | Number | Number (%) | OR | 95% CI | p value |
| Age (years) | | | | | |
| 18-29 | 183 | 8 (4.3) | 1.00 | | |
| 30-39 | 128 | 5 (3.9) | 0.89 | 0.22-3.17 | |
| 40-49 | 55 | 1 (1.8) | 0.41 | 0.01-3.15 | |
| Over 50 | 34 | 2 (6.5) | 1.37 | 0.14-7.29 | p trend = 0.9 |
| Gender | | | | | |
| Male | 220 | 4 (1.8) | 1.00 | | |
| Female | 180 | 12 (6.7) | 3.86 | 1.14-16.65 | p = 0.012 |
| Educational status | | | | | |
| Incomplete high school | 118 | 6 (5.1) | 1.00 | | |
| Complete high school | 191 | 7 (3.7) | 0.71 | 0.20-2.63 | |
| College graduate | 91 | 3 (3.3) | 0.64 | 0.10-3.09 | p trend = 0.5 |
| Total | 400 | 16 (4.0) | | 2.30-6.41 | |

The 16 HHV-8-seroreactive blood donors were also tested for HHV-8 DNA in plasma and PBMCs using nested PCR.[2] The outer primers were KS1 5′-AGCCGAAAGGATTC CACCAT-3′ and KS2 5′-TCCGTGTTGTCTACGTCCAG-3′, and the inner primers were WH-KS-1 5′-GTGCTCGAATC CAACGGATT-3′ and WH-KS-2 5′-ATGACACATTGGTGG TATAT-3′. PCR detection sensitivity was evaluated by serial dilutions of a DNA standard containing a known quantity of HHV-8 DNA and was able to detect fewer than five copies of HHV-8 genome in a background of 200,000 human cells. HHV-8 DNA was detected in plasma and PBMCs of 1 of the 400 (0.25%) donors who also had antibodies to both latent and lytic antigens. Our finding of HHV-8 DNA in our blood donor population indicates that HHV-8 is likely to be transmitted by blood transfusions in this region.

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## SUBMISSION OF LETTERS

Instructions for submission of letters can be found in the Detailed Instructions for Authors published on pages 128 to 133 of the January issue. Submit letters to:

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