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Peripheral Blood Stem Cell Transplant Related *Plasmodium falciparum* Infection in a Patient with Sickle Cell Disease

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Abstract

Background—Although transmission of *Plasmodium falciparum* (Pf) infection during red blood cell transfusion from an infected donor has been well documented, malaria parasites are not known to infect hematopoietic stem cells. We report a case of Pf infection in a patient 11 days after peripheral blood stem cell transplant for sickle cell disease.

Study Design and Methods—Malaria parasites were detected in thick blood smears by Giemsa staining. Pf HRP2 antigen was measured by ELISA on whole blood and plasma. Pf DNA was detected in whole blood and stem cell retention samples by real-time PCR using Pf species–specific primers and probes. Genotyping of 8 Pf microsatellites was performed on genomic DNA extracted from whole blood.

Results—Pf was not detected by molecular, serologic or parasitologic means in samples from the recipient until day 11 post-transplant, coincident with the onset of symptoms. In contrast, Pf antigen was retrospectively detected in stored plasma collected 3 months prior to transplant from the asymptomatic donor. Pf DNA was detected in whole blood from both the donor and recipient post-transplant, and genotyping confirmed shared markers between donor and recipient Pf strains. Look back analysis of red blood cell donors was negative for Pf infection.

Conclusions—These findings are consistent with transmission by the stem cell product and have profound implications with respect to the screening of potential stem cell donors and recipients from malaria-endemic regions.

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Keywords

Plasmodium falciparum; Sickle Cell Disease; Peripheral Blood Stem Cell Transplant; Real-Time PCR; PfHRP2 Antigen ELISA

Introduction

Malaria is endemic in 108 countries worldwide and is estimated to cause symptomatic infection in nearly 250 million people yearly (1). Commonly acquired during the bite of an infected *Anopheles* mosquito, malaria parasites can also be transmitted by transfusion of infected red blood cells (RBC) (2, 3) or in solid organ transplants (4). Acute malaria has also been reported following bone marrow transplantation from malaria-infected donors (5–9), although the route of transmission in these cases is uncertain since many of the transplant recipients also received blood transfusions from the infected donor or underwent transplant in a malaria-endemic region where natural infection cannot be excluded and screening donors may not be systematic (10).

Sickle cell disease (SCD) is caused by a single nucleotide mutation in the β-globin gene that leads to increased polymerization of the abnormal hemoglobin (HbS) and formation of sickle-shaped RBC. Characteristic clinical manifestations include anemia, hemolysis, and recurrent vaso-occlusive crises. Life expectancy of affected individuals is dramatically decreased. The only cure is allogeneic stem cell transplantation (11), an approach that has been used successfully in children (12) and, more recently, in adults (11).

Approximately 300,000 children with SCD are born each year, up to 70% of which reside in malaria-endemic regions of sub-Saharan Africa (1). This high level of co-endemicity is attributed to genetic pressure resulting from the association of HbS with decreased susceptibility to malaria infection (13, 14). In the U.S., potential blood donors with a recent history of travel to a malaria-endemic area are excluded from donation. Laboratory testing for malaria is not routinely performed. Using this strategy, the estimated incidence of malaria transmission is less than 1 case per million RBC units transfused (15). Exclusion of stem cell donors based on travel history is more difficult, since HLA-matched siblings are preferred donors independent of travel history. This presents a unique problem in the context of stem cell transplantation for SCD, where disease impact is greatest in malaria-endemic settings.

We describe a case of Pf infection in an African patient with SCD and auto-splenectomy who underwent peripheral blood stem cell transplant (PBSCT) from an asymptomatic HLA-matched sibling donor with three negative thick smears for malaria. Serologic and PCR analysis was consistent with transmission of Pf in the stem cell product. Implications for pre-transplant screening are also discussed.

Case Report

A 25-year-old woman was admitted for non-myeloablative allogeneic PBSCT for SCD (HbSS). Originally from Freetown, Sierra Leone, she had lived in the U.S. for more than two years before transplant without foreign travel during this period. The HLA-matched sibling donor, who has sickle trait, arrived in the U.S. from Sierra Leone three months prior to donation. He was asymptomatic, denied a recent history of malaria infection, and had three negative thick smears. He was mobilized with 900 micrograms filgrastim for six days prior to two consecutive 25-liter peripheral stem cell collections.

The recipient was conditioned with alemtuzumab, total body irradiation, and sirolimus. Prior to stem cell infusion, she underwent a 5-unit RBC exchange to reduce HbS% to below 30%. She also received two simple RBC transfusions. Eleven days post-transplant, she became febrile and was treated for neutropenic fever with vancomycin, piperacillin/tazobactam, and anidulafungin. Prophylactic acyclovir was continued. Her white cells began to recover during this period with 96% donor myeloid cells at day 19. On day 13, during review of a routine peripheral blood smear, intracellular ring forms consistent with malaria (3.5% parasitemia) were identified (Figure 1) and the patient was started on atovaquone and proguanil. Real-time PCR subsequently confirmed Pf infection (Figure 2). Parasitemia peaked at 5.2% before clearing on day 3 of treatment. The patient completed 7 days of anti-malarial treatment without complication but continued to have fevers to 40°C. These resolved with rejection of the transplant and were ultimately attributed to engraftment syndrome.

The donor, who was PCR and *Plasmodium falciparum* Histidine-rich protein 2 (PfHRP2) positive for Pf (Figure 2), was treated with a 3-day course of atovaquone and proguanil prior to remobilization and apheresis. The recipient received a second transplant from the same donor without reconditioning two months after the initial transplant, but failed to engraft. She did not develop recurrent fever or Pf parasitemia and eventually had autologous recovery of blood counts.

Methods

The donor and recipient were admitted on a protocol to study stem cell transplantation for sickle cell disease (NCT00977691) and provided informed consent for all clinically indicated analysis.

Pf parasitemia was quantified on Giemsa-stained thin blood smears. A minimum of 300 RBC was examined. Percent parasitemia was calculated as: (parasitized RBC/total RBC) × 100.

Genomic DNA was extracted from 200 microliters of whole blood and stem cell preparation using QIAamp DNA Mini Kit (Qiagen, Maryland), and real-time PCR was performed using species-specific primers and probes for Pf 18S rRNA (16,20). Blood from a subject with smear-positive Pf malaria served as a positive control (see Extended Methods in the Supplementary Appendix).

Pf HRP2 antigen was measured in plasma and whole blood using the SD Malaria Antigen Pf ELISA kit (Standard Diagnostics, Inc, Suwon, Korea) modified to allow quantification with a standard curve (17) (see Extended Methods in the Supplementary Appendix).

Polymorphic Pf microsatellite regions were identified in donor and recipient samples by fluorescent-labeled PCR typing (18). Specific primers targeting DNA repeats were used to amplify and quantify polymorphisms (see Table 1 and Extended Methods in the Supplementary Appendix).

Results

Recipient

Blood smears obtained to assess neutropenia were available from days 13 and 14 post-transplant and were retrospectively found to be positive for Pf. Stored whole blood samples were available beginning 2 days before transplant and plasma sample 14 days pre-transplant but were PCR-negative until day 11, coincident with the onset of fever, after which the

inverse of cycle threshold (1/CT) for detection of Pf rRNA increased with increasing parasitemia (Figure 2). PfHRP2 antigen testing was performed on stored whole blood and plasma beginning day 2 post-transplant, and on plasma obtained 2 weeks before transplant. Antigen was detected in both whole blood and plasma at all post-transplant time points, albeit at lower levels in plasma samples (Figure 2). Pre-transplant plasma was antigen-negative.

RBC donors

All seven RBC donors were screened prior to donation according to standard blood bank procedures, which include questions about travel to malaria-endemic areas. Once malaria was diagnosed in the transplant recipient, the donors were recontacted to confirm their travel histories and to provide blood samples for indirect fluorescent antibody testing (CDC, Atlanta, GA). Anti-malarial antibodies were not detected in any of the donors.

Stem cell donor

Although the donor had been malaria smear-negative and was asymptomatic, his blood was reassessed using PCR and PfHRP2 antigen ELISA at day 17 post-transplant (Figure 2). PCR testing was negative; however, antigen testing in blood and plasma was positive. A stored plasma sample obtained 3 months before transplant was also antigen-positive. Repeat testing at day 40 post-transplant, prior to remobilization of his stem cells, was Pf positive by both PCR and ELISA. Follow up PCR on day 45, after treatment for malaria, was negative. Post-treatment PfHRP2 ELISA remained positive, consistent with published data demonstrating persistence of antigen for more than two weeks after clearance of parasitemia (19). The stem cell products from both mobilizations were examined by PCR and supernatants from these stem cells were assessed by PfHRP2 antigen ELISA. PCR results were positive only for the first product (before treatment for malaria); whereas, the supernatants from both mobilizations were positive for PfHRP2 antigen (Figure 2).

Genotyping

Genotypes were assignable based on seven of eight Pf microsatellite markers. Pf DNA from both the donor and recipient showed multiple genotypes (Table 1 and Supplementary Appendix Figure 1) consistent with mixed infection. Four markers, B5M5 (210), C1M4 (200), C13M13 (135), and C14M17 (189), were shared between the donor and recipient.

Discussion

With continued increases in travel and immigration and better access to medical care, the incidence of tropical infections in transplant patients is likely to rise. In this regard, malaria diagnosis presents a number of challenges. As illustrated by our patient, the timing, signs and symptoms of post-transplant malaria overlap with those of neutropenic fever. Furthermore, routine blood smears are insensitive for malaria diagnosis, and thick smears -- while more sensitive -- require considerable time and expertise for accurate interpretation (20). Finally, rapid diagnostic tests are being used increasingly in malaria-endemic countries (21) and are FDA approved in the United States. These tests, while available, are still not the standard of care at most academic hospital centers and only recommended for symptomatic patients and not for screening purposes. Negative RDT results still have to be confirmed with serial blood smears to completely rule out infection.

Although pre-transplant screening for malaria does not obviate the need for clinical vigilance, it could decrease the incidence of transplant-related malaria. In the setting of stem cell transplantation, clinical malaria can arise from pre-existent subclinical infection in the donor exacerbated by immunosuppression, or from parasite transmission during transfusion

of blood or blood products. We explored each of these possibilities using quantitative species-specific PCR and PfHRP2 antigen ELISA to detect active infection in the stem cell donor and recipient. Malaria antibody testing was used as a sensitive measure of exposure in the RBC donors.

Species-specific real-time Pf PCR is a quantitative assay based on detection of 18S rRNA and is reported to be more sensitive than microscopy, detecting 0.7 parasites per microliter of blood, and 100% specific (16, 20). This was confirmed in our study, in which smear-negative samples from both recipient and donor were PCR-positive, and the species was confirmed by genotype analysis. PfHRP2 is a water-soluble Pf membrane protein released during schizont rupture (22). In clinical samples, the PfHRP2 ELISA is as sensitive as microscopy, but has better reproducibility for Pf with a lower coefficient of variation (17). Although the sensitivity of the assay is optimal in whole blood, PfHRP2 can be detected in plasma and culture supernatants. The PfHRP2 ELISA cannot be used to calculate parasite burden, since the protein can be bound or free in plasma and has multiple epitopes that can bind differing levels of detector antibodies, giving variable results (22).

Unlike other malaria species, Pf lacks a persistent liver or blood stage. Consequently, late relapse or recrudescence does not occur. Our patient had not traveled to a malaria-endemic area for more than two years. Although the onset of clinical Pf infection has been reported up to 4 years after exposure in a patient with sickle cell disease (23), the timing of onset of symptoms in our patient and the negative PfHRP2 antigen and PCR prior to transplant are most consistent with an exogenous source of parasitemia, implicating either the RBC transfusions or the stem cell product. Transmission of malaria during RBC transfusion was excluded by the look-back investigation of the donors. In contrast, PCR and PfHRP2 antigen ELISA were positive in the stem cell donor, consistent with subclinical infection. Furthermore, the donor stem cell product transfused 13 days prior to the development of clinical malaria was also positive by both methods.

Genotyping studies, while inconclusive, were also suggestive of Pf transmission from donor to recipient. Multiple genotypes were detected in samples from both the donor and the recipient, which is not surprising since the majority of malaria patients in Africa are infected with multiple Pf strains (24). This technology is limited in detecting minor alleles (less than 10% of total malaria burden) and these parasitic alleles may have been missed with this genotype method, but could have contributed to the final parasitic transplant infection (18). Furthermore, although the genotype match between donor and recipient strains was imperfect (~57%), this would be expected in the setting of person-to-person transmission given the highly polymorphic nature of microsatellites, the amplification dynamics of PCR, and the possibility of preferential transmission of selected parasite strains.

PBSCT stem cells are collected by apheresis, processed and frozen at temperatures below −180 C°. Some RBC are preserved in this process, and thus, Pf-infected RBC from the donor could have been the source of infection in the transplant recipient. Alternatively, since similar freezing conditions have been used to successfully store free Pf merozoites for *in vitro* culture (25), viable extracellular parasites may have been present in the stem cell product. Finally, other hematopoeitic cells, including monocytes and neutrophils, can ingest parasitized RBC and may be present in small numbers in PBSCT stem cells (26). In any case, transfused merozoites (free or released from infected cells) could infect new RBC as in a natural infection. In this regard, it is noteworthy that the patient received an exchange transfusion before transplant, providing a source of normal RBC for infection.

One of the major limitations of our study was the lack of availability of whole blood samples from either the donor or recipient prior to transplant. This precluded direct

comparisons of the different malaria diagnostic tests at most time points. A surprising result was detection of PfHRP2 antigen in the whole blood of the stem cell donor on day 17 post-transplant despite negative smears and PCR. The microsatellite sequencing data was useful in this regard, confirming the presence of Pf in the donor.

Taken together, the PCR, sequencing and serologic results are consistent with transmission of malaria by PBSCT from an infected asymptomatic donor with negative blood smears. Not only does this case demonstrate that malaria parasites can survive freezing under appropriate conditions, but it raises questions as to the most appropriate screening method for donors and recipients from malaria-endemic regions. Although adverse outcomes were not reported in previously published cases of transplant-associated malaria (4–10), the introduction of malaria infection at the time of stem cell infusion could have played a role in transplant rejection in our patient. In conclusion, we propose that pre-transplant screening include both multi-species PCR and PfHRP2 antigen ELISA, and that any positive test prompt malaria treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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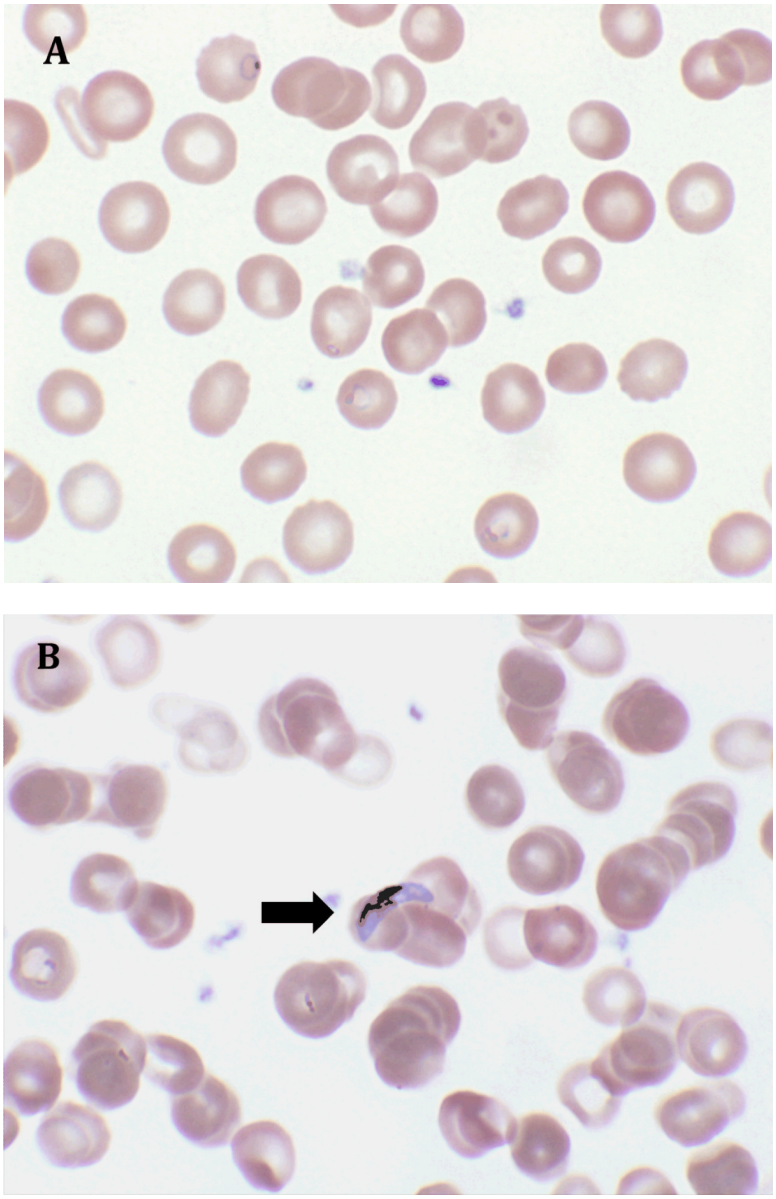

Figure 1.
Giemsa stained blood smears from the transplant recipient (1000×) showing erythrocytes infected with *Plasmodium falciparum*. (A) Immature ring form trophozoites at 5.2% parasitemia (day 13 post-transplant) (B) Crescent-shaped gametocyte (arrow) three days after initiation of treatment (15 days post-transplant).

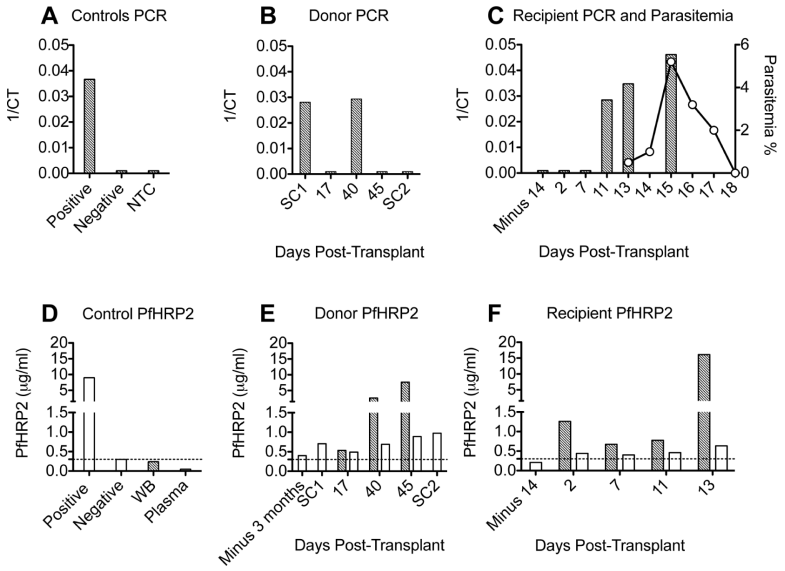

Figure 2.

Documentation of Pf infection in the donor and recipient by microscopy, real-time PCR and PfHRP2 antigen ELISA. Results of Pf testing are shown for controls (A, D), the donor (B, E) and the recipient (C, F). Percent donor parasitemia is indicated by the solid line and open circles in panel C. Donor parasitemia was undetectable at all time points tested. Panels A–C show Pf specific real-time PCR results expressed as 1/cycle threshold (1/CT). The bars represent mean results from triplicate measures of whole blood (shaded bars) and plasma (white bars). NTC = no template control. Positive and negative = whole blood from a normal blood bank donor and a patient with smear-positive Pf malaria, respectively. SC1 and SC2 = stem cell retention samples from the initial transplant and the second transplant (after treatment for malaria), respectively. SC1 was markedly positive for Pf. Panels D–F show PfHRP2 ELISA results expressed as micrograms/ml. SC1 and SC2 supernatants were both positive for PfHRP2. Positive and negative = serum controls provided by the manufacturer. WB and plasma = whole blood and plasma samples from a normal blood bank donor. The dashed line represents the threshold for a positive result, calculated using the negative control sample per the manufacturer's instructions.

Table 1

Genotyping of parasite strains from the donor and recipient.

Market	A BM17	B B5M5	C C1M4	D C1M67	E C4M62	F C13M13	G C13M63	H C14M17
Donor	246/210	**210**	243/**200**/185/140	218	194/165/131/121	258/**135**212	N/A	**189**/92
Recipient	232/220/213	**210**	**200**	189	151/138	161/**135**	N/A	**189**

The numbers indicate the allele sizes (in base pairs) corresponding to the major and minor peaks for 8 polymorphic microsatellite regions for the donor and recipients' malaria strains. Marker C did not produce a signal. Peaks shared between the donor and recipient are shown in bold.