# Transfusion-transmitted anaplasmosis from a leukoreduced platelet pool

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**BACKGROUND:** Human granulocytic anaplasmosis is an emerging tick-borne illness. *Anaplasma phagocytophilum* resides intracellularly, can cause asymptomatic infection, and can survive blood component refrigeration conditions for at least 18 days. To date, eight cases of transfusion-transmitted anaplasmosis (TTA) have been reported: seven attributed to red blood cell (RBC) units, five of which were prestorage leukoreduced using RBC leukoreduction filters, and one involving a process leukoreduced apheresis platelet (PLT) unit. Here, we report a case of TTA from a whole blood–derived PLT pool.

**STUDY DESIGN AND METHODS:** Donation segments from the 7 units of RBCs and two PLT pools transfused were examined. Fast protocol multiplex real-time *A. phagocytophilum* polymerase chain reaction (PCR) and serologic testing for immunoglobulin (Ig)M and IgG antibodies to *A. phagocytophilum* by enzyme immunoassay were performed.

**RESULTS:** Transmission was confirmed by positive *A. phagocytophilum* PCR and serology in one of 16 donors and by positive PCR and seroconversion in the recipient.

**CONCLUSION:** This is the first confirmed case of TTA from a whole blood–derived PLT pool prepared from PLT concentrates leukoreduced by in-line filtration of PLT-rich plasma.

Human granulocytic anaplasmosis (HGA) is an emerging tick-borne illness first described in the United States in 1994 and caused by the obligate intracellular Gram-negative rickettsial bacterium, *Anaplasma phagocytophilum*.[1] Since HGA became a reportable disease in 1999, cases have increased steadily, from 348 cases in 2000 to 2782 cases in 2013.[2]

The highest incidence of HGA has been reported in the Northeastern and North Central United States, but the disease has also been described in other parts of the United States, Europe, and China. In the Eastern and North Central United States, *A. phagocytophilum* is transmitted by the Eastern blacklegged or deer tick, *Ixodes scapularis*, while along the West Coast of the United States, it is transmitted by the Western blacklegged tick, *Ixodes pacificus*.

HGA can range from asymptomatic to life-threatening, particularly in the elderly and immunocompromised. Symptoms typically appear 5 to 21 days after a tick bite and include fever, headache, malaise, myalgias, dyspnea, cough, nausea, vomiting, diarrhea, neurologic disorders, and shock. Death occurs in less than 1% of clinical cases.[3-5] Laboratory abnormalities include leukopenia, thrombocytopenia, anemia, elevated serum hepatic transaminases, elevated

**ABBREVIATIONS:** HGA = human granulocytic anaplasmosis; IFA = immunofluorescence assay; PRP = platelet-rich plasma; TTA = transfusion-transmitted anaplasmosis; TTB = transfusion-transmitted babesiosis.

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Received for publication March 24, 2015; revision received September 21, 2015; and accepted September 21, 2015.

doi:10.1111/trf.13392



**TRANSFUSION** 2016;56;699–704

creatinine, and electrolyte abnormalities due to renal failure. The organism is phagocytosed by neutrophils, but rather than being killed in the phagosome, it instead can proliferate in the form of intracytoplasmic inclusions known as morulae.[1,3,4,6] It has also been suggested that HGA infection results in macrophage activation syndrome via excessive cytokine production, with inflammatory injury being caused by the host immune response rather than by the bacterial load itself.[5] Clinical diagnosis is suspected by the observation of morulae in the peripheral blood smear and confirmed by detection of *A. phagocytophilum* DNA by polymerase chain reaction (PCR) or by serologic evidence of a fourfold increase in immunoglobulin (Ig)G-specific antibody to *A. phagocytophilum* by indirect immunofluorescence assay (IFA) in paired serum samples (one taken in first week of illness and a second 2-4 weeks later).[4,5] Doxycycline is the treatment of choice, generally given for 7 to 10 days, and usually results in rapid clinical improvement and cure.[4]

Because *A. phagocytophilum* resides intracellularly, it can cause asymptomatic infection, and it can survive blood component refrigeration conditions for at least 18 days, it poses a potential risk for transmission via transfusion from an afebrile, asymptomatic donor.[7] Although most cellular blood components in the United States are leukoreduced before storage by either filtration or process leukoreduction, this does not appear to eliminate the risk of transfusion-transmitted anaplasmosis (TTA). To date, there have been eight reported cases of TTA, seven implicating red blood cell (RBC) units, five of which were leukoreduced before with RBC leukoreduction filters, and a single case involving a process leukoreduced apheresis platelet (PLT) unit.[8-14] Here, we present the first confirmed case of PLT-associated TTA from whole blood–derived prepooled PLTs (prepared from individual PLT concentrates) that were leukoreduced by in-line filtration of PLT-rich plasma (PRP).[15,16]

## CASE REPORT

In August 2014, a 78-year-old female with a past medical history of non–insulin-dependent Type II diabetes mellitus, hyperlipidemia, and diverticulitis status post–partial colectomy presented to a hospital in Providence, Rhode Island, after a ventricular fibrillation cardiac arrest (Day 0). The timeline of the following events as well as pertinent laboratory data are depicted in Fig. 1. On Day 3, the patient underwent coronary artery bypass grafting surgery. Perioperatively, 7 units of RBCs and 2 units of prepooled PLTs were transfused. One pooled PLT unit was prepared from five individual PLT concentrates, while the other was prepared from four PLT concentrates. All blood components (RBCs and pooled PLTs) were leukoreduced before storage by filtration. The patient was discharged to an inpatient rehabilitation facility on Day 10.

Three days later (Day 13), the patient was readmitted with a 1-day history of chills, fever (104°F), mild nonproductive cough, and hypoxia with an oxygen saturation of 84% on room air. Laboratory testing revealed a white blood cell count (WBC) of $7.4 \times 10^9$/L with 93.9% neutrophils, a hemoglobin (Hb) of 10.1 g/dL, a PLT count of $298 \times 10^9$/L, a creatinine of 1.11 mg/dL from a baseline of 0.74 mg/dL, and an aspartase aminotransferase (AST) of 30 IU/L (normal, 10-42 IU/L). Chest x-ray demonstrated atelectasis in the left lower lobe with an associated small pleural effusion. Intravenous vancomycin and piperacillin-tazobactam were initiated for empiric treatment of a presumed health care–associated pneumonia. On Day 14, the patient developed progressive hypotension and hypoxia prompting transfer to the intensive care unit for pressor support and high-flow nasal cannula oxygen supplementation. Repeat laboratory testing was notable for the presence of inclusion bodies in neutrophils consistent with morulae, a WBC count of $3.1 \times 10^9$/L with 81% neutrophils and 6% bands, a PLT count of $144 \times 10^9$/L, creatinine of 2.05 mg/dL, an AST (SGOT) of 101 IU/L, and a direct bilirubin of 0.5 mg/dL (normal, 0-0.3 mg/dL). The infectious disease service was consulted. The presence of morulae on blood smear and typical presenting symptoms and laboratory abnormalities prompted the consideration of HGA. Vancomycin and piperacillin-tazobactam were discontinued and empiric therapy with oral doxycycline 100 mg every 12 hours was initiated.

The patient's hypotension and hypoxia resolved. WBC and PLT nadirs were $2.4 \times 10^9$/L and $93 \times 10^9$/L, respectively, with normalization by Day 20. Liver function tests normalized on Hospital Day 18. Two leukoreduced RBC units were transfused on Day 24 for a Hb of 6.8 g/dL, with increase to 8.5 g/dL. Serum creatinine peaked on Day 21 at 8.48 mg/dL in the setting of acute tubular necrosis. A 10-day course of doxycycline was completed on Day 25, before discharge on Day 31. Follow-up laboratory testing 5 months later demonstrated a normal complete blood count and a creatinine of 0.97 mg/dL.

## INVESTIGATION

As the patient denied outdoor or animal exposure, and was confined to the inpatient rehabilitation center preceding readmission, TTA was suspected. Experienced, in-house hematopathologist review of peripheral blood smears from the case patient's initial hospitalization did not reveal morulae suggestive of anaplasmosis, but review of smears from her readmission did. Hematopathologist review of thin and thick blood smears from both the case patient's initial hospitalization and her readmission did not reveal intraerythrocytic parasites suggestive of *Babesia*. Serum testing for IgM and IgG antibodies by indirect immunofluorescence antibody (IFA) to *A. phagocytophilum* and *Ehrlichia chaffeensis*, as well as real-time PCR using primers targeting the *A. phagocytophilum* groEL

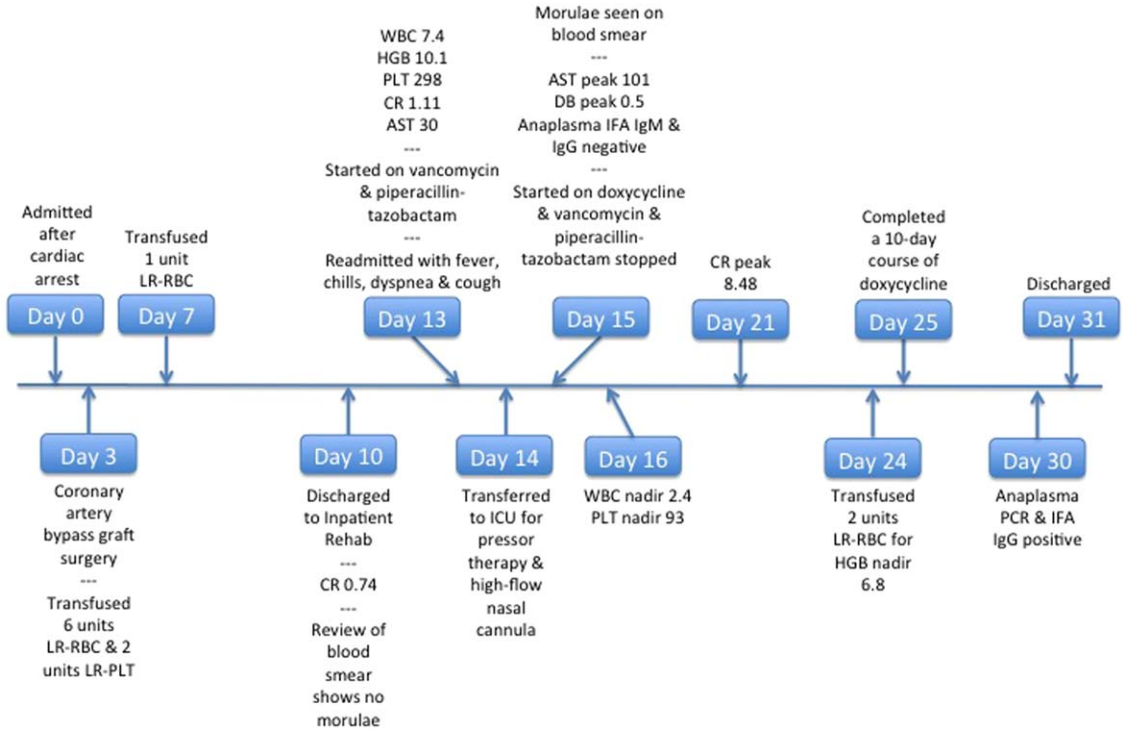


**Fig. 1. Case timeline of events with pertinent laboratory data. LR = leukoreduced; CR = creatinine; HGB = hemoglobin; AST = aspartate aminotransferase; DB = direct bilirubin; IFA = immunofluorescence antibody assay.**

gene were performed by Quest Diagnostic Laboratories (Chantilly, VA) and Focus Diagnostic Laboratories (San Juan Capistrano, CA), respectively. The PCR was performed pursuant to a license agreement with Roche Molecular Systems, Inc. (Indianapolis, IN).

The source blood center was notified. Approximately 0.5 mL from each of the 16 index whole blood donation segments was examined. Seven of these 16 index whole blood donations had been used to prepare the seven RBC concentrates that the case patient had received, while the other nine had been used to prepare the 2 units of pooled PLTs that she had received (four in one pool and five in the other). Serologic testing for IgM and IgG antibodies to *A. phagocytophilum* was performed by indirect enzyme-linked immunosorbent assay (ELISA) using proprietary recombinant fusion product antigen rErf-1 (Imugen, Inc., Norwood, MA).[17] Reactivity at four or more times the mean antibody concentration of four standard negative controls was considered a positive finding.[17] Fast protocol, multiplex, real-time PCR was performed by Imugen, Inc., to detect the presence of *A. phagocytophilum* DNA using primers and FAM-labeled probes targeting the *A. phagocytophilum* msp2 gene.[18] Detection of the expected 77-bp product in no more than 42 thermal cycles was considered a positive finding. Thin and thick blood smears from the donation segments were reviewed for the presence of intraerythrocytic parasites suggestive of *Babesia*, but none was found. Enzyme immunoassay (EIA) to *Borrelia burgdorferi* was performed by Quest Diagnostic Laboratories.

## RESULTS

Upon readmission, the patient's acute IFA serologies for *A. phagocytophilum* and *E. chaffeensis* were negative (IgM titer < 20 and IgG titer < 64), *B. borgdorferi* EIA was negative (≤0.9), and thin and thick blood smears revealed no intraerythrocytic parasites. PCR was positive for *A. phagocytophilum* DNA. Two weeks later, examination of the patient's convalescent IFA serologies for *A. phagocytophilum* returned positive with an IgG titer of 64.

The index whole blood segment of one of the sixteen whole blood donors who had contributed PLTs to the four-PLT pool tested positive for *A. phagocytophilum* DNA by PCR, as well as by serology with an ELISA IgM of 3.5 and an IgG of 5.1 (normal, <1.0 for each). The donor was a 19-year-old male, AB D+ who resides in an endemic area for *I. scapularis*. The sample was obtained from a segment of the original donation given August 29, 2014, and tested September 17, 2014, by PCR, and September 18, 2014, by ELISA. The RBC unit prepared from this donation was discarded. All other donation segments were negative by both PCR and serology. The implicated PLT pool had been transfused September 3, 2014, 10 days

**TABLE 1. TTA cases***

| Case characteristics | Cases | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Case 1, 1999[8] | Case 2, 2007[9] | Case 3, 2012[10] | Case 4, 2012[10] | Case 5, 2012[11] | Case 6, 2013[12] | Case 7, 2014[13] | Case 8, 2014[14] | Current, 2015 |
| Location | MN | MN | WI | WI | Slovenia | RI | CT | MA | RI |
| Age | 75 | 68 | 81 | 51 | 36 | 64 | 41 | 34 | 78 |
| Sex | Male | Male | Female | Female | Female | Male | Male | Female | Female |
| Comorbidities | Rheumatoid arthritis | Ankylosing spondylitis, steroid therapy, CKD | Rheumatoid arthritis, steroid and methotrexate therapy | Multiple myeloma, CKD, fungal sinusitis | Pregnancy | Gastritis, iron deficiency anemia | Gunshot wounds | Pregnancy, beta-thalassemia trait | Diabetes, coronary artery disease |
| Presenting symptoms | Fever, rigors, nausea | Fever | Fever, myalgias | Fever, chills, fatigue | Fever, respiratory distress | Fever, chills, headache, dyspnea, cough | Fever | Fever, nausea | Fever, chills, dyspnea, cough |
| Laboratory abnormalities | Anemia | Thrombocytopenia, multisystem organ failure | Pancytopenia, DIC, multisystem organ failure | Pancytopenia, elevated liver enzymes | Thrombocytopenia, elevated liver enzymes | Anemia, leukopenia, delayed hemolytic transfusion reaction | Thrombocytopenia | Anemia, leukopenia, thrombocytopenia, elevated liver enzymes | Pancytopenia, elevated liver enzymes, elevated creatinine |
| Incubation | 9 days | 7 days | 5 days | 7-14 days | 10 days | 4 days | 5 days | 11 days | 10 days |
| Blood component | RBC | RBC | LR-RBC | Suspected LR-RBC | LR-RBC | LR-RBC | Suspected LR-PLT | LR-RBC | LR-PLT |
| Donor | IFA+ | PCR+, IFA+ | PCR+, IFA+ | Not identified | PCR+, IFA+ | PCR+, EIA+ | PCR+, EIA+ | PCR+ | PCR+, EIA+ |
| Recipient | PCR+, IFA+ | PCR+, IFA+ | PCR+ | PCR+ | PCR+, IFA+ | PCR+ | PCR+ | PCR+, IFA+ | PCR+, IFA+ |

* Adapted from "AABB Fact Sheet" July 2013: update to Transfusion 2009;49(Suppl):169-71s.

CDC = Centers for Disease Control and Prevention; CKD = chronic kidney disease; CT = Connecticut; DIC = disseminated intravascular coagulation; LR = leukoreduced; MA = Massachusetts; MN = Minnesota; RI = Rhode Island; WI = Wisconsin.

before the occurrence of the febrile illness. The Rhode Island Department of Health was notified.

## DISCUSSION

We describe the first confirmed case of TTA from a whole blood–derived PLT pool prepared from PLT concentrates leukoreduced by in-line filtration of PRP. Diagnosis was confirmed by a positive serum *A. phagocytophilum* PCR and seroconversion in the recipient. The implicated blood component was a leukoreduced four-PLT pool that the case patient had been transfused 10 days before symptom onset. This was confirmed by a positive *A. phagocytophilum* PCR and ELISA in one of the whole blood donation segments that had contributed PLTs to the four-PLT pool. A limitation of this study is that different PCR and serology assays were performed on the donation segments and serum of the case patient at different laboratories. In addition, the age of the donation segments varied, and the potential relationship between segment age and test results remains unclear.

To date, there have been eight reported cases of TTA. Seven were attributed to RBC units, five of which were leukoreduced, and one case was attributed to a process leukoreduced apheresis PLT unit (Table 1).[8-14] This case differs from the previous cases in that the leukoreduction was performed by in-line filtration of PRP using PLT specific leukoreduction filters. PLT leukoreduction filters differ in their chemical composition and performance characteristics to RBC leukoreduction filters.[19] PRP derived from whole blood donations contains approximately 10% of the total WBCs, the majority of which are mononuclear cells (MNCs). Whole blood donations of 500 mL contain approximately $2 \times 10^9$ WBCs, and hence before leukoreduction, the PRP contains approximately $2 \times 10^8$ WBCs. After leukoreduction, the residual WBC content of the pooled product would be expected to be about $1 \times 10^5$ WBCs, and since the majority are MNCs, the residual neutrophil content would be about $1 \times 10^4$.[16,20] Hence, the inoculum size from one PLT donor would be approximately $2 \times 10^3$ to $3 \times 10^3$ neutrophils, which is very small and predicts that even more efficient leukoreduction filters would be unlikely to prevent TTA.

Currently, no FDA-licensed test exists for screening donors or their blood components for HGA. Inquiring about a history of tick bites or recent illness in donors may miss a significant proportion of HGA, since the majority of tick bites remain unnoticed and HGA can be asymptomatic. Additionally, not all individuals with HGA will develop visible morulae on blood smear, and PCR screening is unlikely to be cost-effective. Serologic screening is likely to be of limited benefit due to the potential that acutely infected donors who are tested during the diagnostic window may be missed. Leukoreduction could reduce but not eliminate the risk of TTA, possibly due to the presence of extracellular bacteria in blood components or the small inoculum size needed to cause symptomatic disease in vulnerable recipients.[7,21] A similarly small inoculum is considered to cause transfusion-transmitted babesiosis (TTB). In the case of TTB, the incubation period is longer than tick-borne cases, while in TTA the incubation period appears similar to tick-borne disease.[22]

Although TTA is of particular concern in endemic areas, shipment of blood components to nonendemic areas, or travel of donors to endemic areas, may result in a wider distribution of this disease. Consequently, an acute febrile illness in a recipient of an RBC or PLT unit with or without leukoreduction should prompt the consideration of TTA or TTB, regardless of the geographic location of transfusion or history of tick exposure in the recipient.

In conclusion, this represents the first reported case of a leukoreduced pre-pooled PLT component implicated in TTA. Other processing or testing approaches and a study of their cost-effectiveness will be required to prevent future cases of TTA.

### ACKNOWLEDGMENT

The authors thank Dr Carolyn Young of the Rhode Island Blood Center for her invaluable diagnostic contributions to the manuscript.

### CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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