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CASE REPORT

A case of extravascular hemolysis with Tk-activation

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Key Clinical Message

A 50-year-old female with ovarian cancer for 4 years presented with abdominal pain. She started antibiotics for possible infection, and developed extravascular hemolysis. All antigen detection tests were negative, but lectin panel suggested Tk-activation. Additional laboratory testing in conjunction with blood bank is essential to investigate rare cause of hemolysis.

Keywords

Extravascular hemolysis, polyagglutination, Tk-activation.

Introduction

Polyagglutination is the condition in which RBCs are agglutinated by almost all normal adult ABO compatible human sera, but not by cord blood sera or newborn's sera [1]. It occurs by alterations in RBC membrane glycoprotein structure by (a) the action of bacterial enzymes leading to cryptantigen exposure such as T, Tk, Th, or Tx, (b) somatic mutation of an enzyme essential for formation of normal RBC membrane creating antigen such as Tn, (c) inheritance of rare alleles for formation of RBC membrane creating antigen such as Cad, Nor, or Hyde Park, (d) undetermined cause in Va or Tr, or alternatively (e) antibodies to some bacteria or their thermostable products that adhere to RBCs causing agglutination with or without antiglobulin serum [1–3].

Many antigenic determinants caused by bacterial enzymes have been discovered, among which T cryptantigen is the most common. Antibodies to those cryptantigens are usually naturally occurring IgM antibodies that are found in all normal adult sera but not in sera from cord blood or newborns. Therefore, when cryptantigens are exposed, polyagglutination can occur.

Most commonly, T-activation is a transient phenomenon associated with bacterial infection such as pneumococci, streptococci, staphylococci, clostridia, Escherichia coli, and Vibrio cholera [3]. Neuraminidase produced by these bacteria removes *N*-acetyl neuroaminic acid residues from RBC membrane sialoglycoproteins and exposes β-linked galactosyl residues, the so-called T cryptantigen (Fig. 1) [4]. T-activation in children is not uncommon with some diseases. About 11–27% of infants with necrotizing enterocolitis are reported to have T-activation [5, 6], but hemolysis is rare. On the other hand, T-activation in adults is rare and only 0.5% of hospitalized patients have T-activation and less patients, 0.01% in the same population, show polyagglutination [7]. Patients with malignancies, especially gastrointestinal malignancy, and/or sepsis are at higher risk and cryptantigens are detected in 7.6% of those patients [8, 9]. Hemolysis is common in adults with this condition and 70% of them have elevated free serum Hgb. It is also associated with hemolytic uremic syndrome with T-activation on renal endothelium, red cells, and platelets.

Tk-activation is less common than T-activation and is also a transient phenomenon associated with infection. Bacteroides fragilis, Escherichia freundii, Escherichia coli, Flavobacterium keratolyticus, Clostridium welchii, Group D streptococci, and Pseudomonas species have been reported as causative organisms [3, 10]. β-galactosidase of bacterial origin cleaves galactose residue from paraglobo-side on RBC membrane leading to N-acetylglucosamine

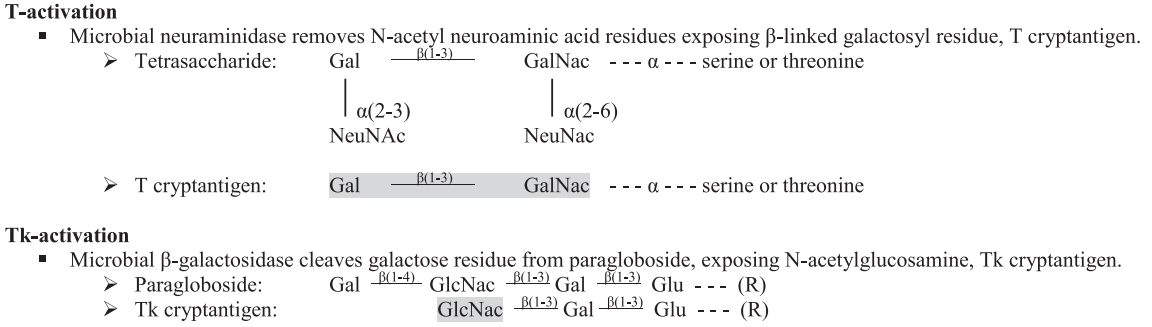


Figure 1. Cryptantigen exposure.

exposure, the so-called Tk cryptantigen (Fig. 1) [4]. Tk-activation is also reported in patients with necrotizing enterocolitis, but the association with acquired B antigen is suggested in patients with infection.

In most reported cases of cryptantigen activation in adults, patients experienced intravascular hemolysis with positive polyagglutination tests and positive agglutination with selected lectins specific for each cryptantigen. However, the mechanism of hemolysis is not fully understood. We here report a case with Tk-activation with negative polyagglutination in the setting of extravascular hemolysis.

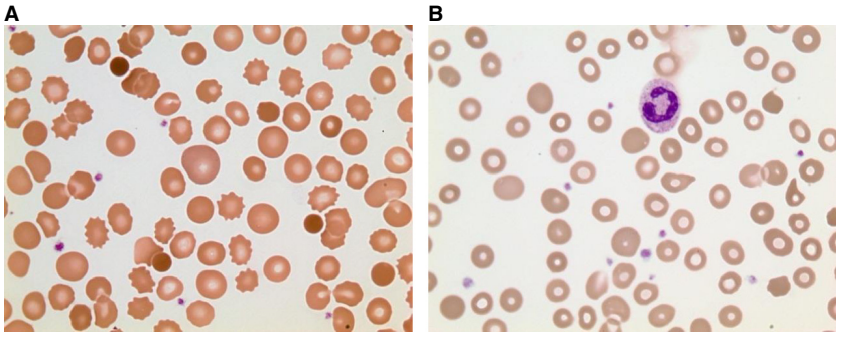
Case Presentation

A 50-year-old female presented to emergency department (ED) at University of Michigan Health System in September 2012 complaining of increasing right lower quadrant abdominal pain, malaise, nausea, vomiting, fever and chills for 1 day, clear vaginal discharge for 2 weeks, and recent lower leg swelling extending up to the right upper thigh. The patient had a history of hysterectomy for a benign indication in 1999, and bilateral salpingo-oophorectomy, omentectomy, and tumor debulking for stage IV high-grade papillary serous ovarian cancer in 2008. Subsequently, she received multiple courses of chemotherapy for extensive metastasis, most recently completed etoposide 1 month prior to presentation. Other past medical history was significant for hypertension, hypothyroidism, mitral valve prolapse, and supraventricular tachycardia. Medications included nifedipine, levothyroxine, phenothiazine for nausea and acetaminophen/oxycodone for pain control.

Physical examination demonstrated stable vital signs with known tachycardia and regular rhythm, clear lung fields, a known palpable-fixed mass in right lower quadrant, and a nodular firm mass on anterior vaginal wall. Edema in the extremities was not present. Laboratory data on presentation showed only mild anemia (Hgb 10.3 g/dL), mildly decreased WBC count with relative neutrophilia ($1.8 \times 10^3/mm^3$, 87.2%), and platelet count was within reference range. Metabolic panel and urinalysis were normal. A test for glucose-6-phosphate dehydrogenase (G6PD) deficiency was negative.

Chest X-ray showed an apparent cavitary lesion (2.1×2.3 cm) projecting over the posterior left eighth rib in the region of the left hilum. A vague opacity suspicious for an infectious process was also found in the lingula of left lung. Abdominal ultrasound showed hepatic metastatic lesions and enlarged hepatic lymph nodes. Chest computerised tomography (CT) showed pulmonary nodules and enlarged lymph nodes and a small pericardial effusion. No pleural effusion was observed. Abdominal CT revealed significant worsening of metastatic disease with peritoneal carcinomatosis, urinary bladder wall thickening suggestive of metastatic disease, multiple enlarged abdominal and pelvic lymph nodes, hepatic lesions, and small volume of ascites. Bilateral lower extremity venous study showed no evidence of deep vein thrombosis.

An infectious process was suspected, and Zosyn® (piperacillin/tazobactam) and vancomycin were administered. On the next day, the patient developed hemolytic anemia with Hgb of 7.6 g/dL, lactate Dehydrogenase (LDH) of 1135 IU/L, haptoglobin of <10 mg/dL. Antibiotics were held for possible drug-induced immune hemolytic anemia. At this point, she had mild renal insufficiency (Cr 1.6 mg/dL) with aspartate amino transferase (AST) 60 IU/L and alanine amino transferase (ALT) 16 IU/L, and total/direct/indirect bilirubin 7.3/0.6/6.7 mg/dL. Ferritin was 16,500 ng/mL, iron was 106 μg/dL, total iron-binding capacity was 206 μg/dL, and transferrin saturation was 51.5%. Prothrombin time (PT)/partial thromboplastin time (PTT) were slightly increased to 14.1/34.9 sec, respectively, and international normalized ratio was 1.3. Fibrinogen was slightly elevated to 453 mg/dL and D-dimer was also elevated to 4.01 mg/L on the following day. Reticulocyte count and percentage were taken on hospital day 5 and day 8, which were 69.55 B/L and 2.39%, and 124.27 B/L and 4.17%, respectively. A stool occult blood test was negative. A peripheral smear showed numerous spherocytes, burr cells, large platelets,



A: Admission day 5: spherocytes, Burr cells, large platelets
B: Admission day 12: a few spherocytes and large platelets remained

Figure 2. Findings in peripheral smear.

Table 1. Lectin panel interpretation.

		Polyagglutinable cells			
Lectin	Normal cells	T	Tk	Tn	Cad
Arachis hypogea	–	+	+	–	–
Salvia sclarea	–	–	–	+	–
Salvia horminum	–	–	–	+	+
Glycine soja	–	+	–	+	+/–

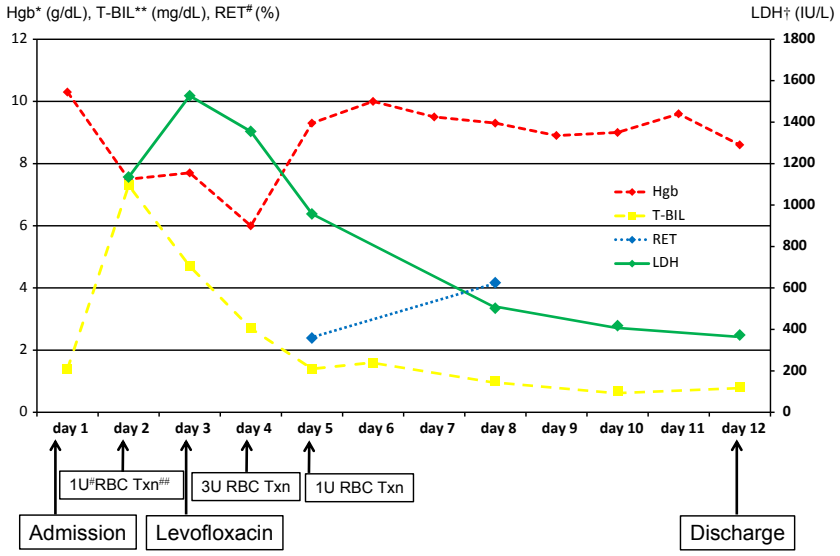
and some Howell–Jolly bodies, but schistocytes were not observed. (Fig. 2)

In blood bank testing, antibody detection tests including low-ionic strength saline antibody screening, polyethylene glycol-enhanced antibody screening, and acid glycine elution were all negative. Cold agglutinin studies were negative. Polyspecific, monospecific, and enhanced direct antiglobulin tests (DAT) and enhanced DAT with patient's untreated and ficin-treated cells were also negative. Polyagglutination testing done as a part of our enhanced DAT protocol with five ABO compatible adult sera and two cord sera were negative, however, a lectin panel (Table 1, HBS-Lectin Kit, Hemo bioscience, Durham, NC) revealed positivity (3+) with only Arachis hypogea suggesting Tk-activation.

Microbiology tests showed a few vancomycin-resistant Enterococcus species in feces, and levofloxacin was started from hospital day 3. The culture of ascites was not tested because the ascites was most likely associated with her condition with extensive pelvic metastasis of her ovarian cancer. She was transfused with a total of 5 units of RBCs (1 unit on hospital day 2, 3 units on day 4, 1 unit on day 5, Fig. 3) for her low Hgb. Her hemolysis subsided 2 days after levofloxacin administration started supported by stable Hgb, decreasing LDH and total bilirubin (Fig. 3). Chest X-ray (CXR) on day 4 revealed development of patchy right basal opacity and streaky left basilar opacities suggestive of pneumonia. The patient's hemolysis and other symptoms subsided and she was discharged in stable condition 9 days after levofloxacin was started.

Discussion

Our case developed hemolysis supported by decreased Hgb and haptoglobin with increased reticulocyte count, indirect bilirubin, and LDH. Moreover, it showed evidence of extravascular hemolysis in the peripheral smear. At the time of hemolysis, the peripheral smear showed numerous spherocytes and some burr cells, Howell–Jolly bodies, Dohle bodies, and large platelets, but schistocytes were not observed. In nonhereditary conditions, extravascular hemolysis is the most common cause of spherocytes, whereas schistocytes indicate intravascular hemolysis. Other possible causes of nonhereditary spherocytosis include severe hypophosphatemia, acute alcoholism, septicemia with some bacteria, or following severe burn injury. Of note, G6PD testing on this patient was normal. Burr cells are mostly artifacts, but it can occur with some medications, uremia, or mild hemolysis in hypomagnesemia or hypophosphatemia. Howell–Jolly bodies can be seen in megaloblastic anemia or postsplenectomy. Dohle bodies in neutrophils are seen in burns, infections, trauma, or neoplastic diseases. Our patient's peripheral smear findings are best explained by extravascular hemolysis. Large platelets can be observed in the condition with immune thrombocytopenia, disseminated intravascular coagulopathy (DIC), myeloproliferative disorders, preeclampsia, or recovery from transient hypoplasia from chemotherapy. This may also support a possibility of DIC. However, this patient had completed chemotherapy for her cancer 1 month prior to the presentation. Therefore, large platelets may be due to recovery from chemotherapy. The fact that her peripheral smear on the day of discharge was still showing large platelets supports this theory.



Hgb*: Hemoglobin, T-BIL**: Total bilirubin, RET#: reticulocyte count, LDH†: lactate dehydrogenase, U#: unit, Txn##: transfusion

Figure 3. Hospital course in laboratory tests.

Table 2. Reaction of lectins with cryptantigens.

		Polyagglutinable cells											
Lectin	Normal cells	T	Tk	Tn	Cad	Th	Tx	Nor	HEMPAS	HbM	Acquired B	VA	Tr
Arachis hypogea	–	+	+	–	–	+	+	–	–	w	+/–	–	+
Salvia sclarea	–	–	–	+	–	+	–	–	–	–	–	–	+
Salvia horminum	–	–	–	+	+	–	–	–	–	w	–	–	+
Glycine soja	–	+	–	+	+/–	–	–	NT	NT	+	NT	NT	NT
Glycine max	–	+	–	+	–	–	–	–	–	+	–	–	NT
Dolichos biflorus	–	–	–	+	+	–	–	–	–	–	w/–	–	–
Griffonia simplicifolia I	–	–	–	+	–	–	–	–	–	–	+	–	NT
Griffonia simplicifolia II	–	–	+	–	–	–	–	–	–	+	–	–	+
Helix pomatia	–	+	–	+	+	NT	NT	–	+	+	+	+	NT
Leonurus cardiaca	–	w/–	–	–	+	–	–	–	–	–	–	–	NT
Medicago disciformis	–	+	–	–	NT	+	–	NT	NT	+	NT	NT	NT
Phaseolus limensis	–	–	–	–	–	–	–	–	–	NT	+	–	NT
Polybrene	–	–	+	–	+	+	+	NT	NT	NT	NT	NT	NT
Vicia cretica	–	+	–	–	–	+	–	–	–	w	–	–	NT
Vicia hyrcanica	–	+	+	NT	–	+	NT	NT	NT	+	NT	NT	NT
Vicia villosa	–	+	–	NT	NT	–	NT	NT	NT	+	NT	NT	+

HEMPAS, congenital dyserythropoietic anemia, type II; HbM, hemoglobin M-Hyde park; w, weakly reactive; NT, not tested.

Cryptantigen exposure associated with infection is rare but well developed concept of condition. Quite a few cryptantigens have been described, and each antigen agglutinates with some lectins in specific combination as showing in Table 2 [1, 2, 11]. Our laboratory used a commercially available lectin kit containing four lectins shown in Table 1 (Arachis hypogea, Salvia sclarea, Salvia horminum, Glycine soja). As shown in Table 2, reactivity to only Arachis hypogea among these four lectins gives rise to a possibility of Tx cryptantigen in addition to Tk cryptantigen (same results in gray in Table 2). However, Tx activation is most commonly seen in children and reported to associate with infection by Streptococcus pneumonia [11]. Therefore, Tx activation is less likely in our case. Furthermore, I hypothesize that it may be possible to suspect which organism is causing the problem in some cases depending on the type of exposed cryptantigen that patient has.

The most commonly reported case scenario of hemolysis with cryptantigen activation in adults is that of a patient with intravascular hemolysis in the setting of malignancy and/or sepsis, or hemolytic uremic syndrome,

mostly in conjunction with infection. Our patient had only a few Enterococcus species in feces with relative neutrophilia but not absolute neutrophilia, and vague evidence of infection on the chest X-ray. Blood and urine cultures did not show any organisms. However, elevated D-dimer with slightly high PT/PTT raised a possibility of DIC. Fibrinogen was also slightly elevated, however, increased fibrinogen does not rule out DIC because fibrinogen is an acute phase reactant which is synthesized in the liver and is often elevated in inflammatory conditions. Therefore, it gives rise to a possibility that this patient had mild acute DIC caused by infection. The patient complained swollen leg at presentation, but it was not observed in the hospital, and deep vein thrombosis (DVT) scan of legs were negative. Thus, the high fibrinogen and D-dimer are not related to DVT, which may support possibility of DIC. Additionally, the fact that the hemolysis subsided after levofloxacin was administered also supports possibility of infection. Meichenin et al. reported that Tk cryptantigen could be a colorectal carcinoma-associated antigen in rats [12]. Similarly, the possibility that ovarian cancer cells may express Tk cryptantigen cannot be fully excluded, although it has not been proven. Additionally, our case had been having high-grade papillary serous ovarian cancer with extensive metastasis for as long as 4 years prior to development of hemolysis. Therefore, the infection may be taking at least some roles to cause her new condition of cryptantigen exposure.

The mechanism of hemolysis in the setting of cryptantigen exposure is still not fully understood. The suggested mechanisms include (a) cell destruction caused by antigen–antibody reaction, (b) direct hemolytic action of bacterial enzymes and toxins [13], (c) altered interaction of complement components with desialyated red cells [14], (d) shortened survival of RBCs due to reduced membrane sialic acid content [15, 16]. Furthermore, it is questionable if cryptantigen exposure causes hemolysis. Many reported cases with cryptantigen exposure did not have hemolysis, but some cases showed intravascular hemolysis [17–22]. And one report by Moores et al. showed a patient with severe extravascular hemolysis associated with T-activation [23]. Our patient had evidence of extravascular hemolysis supported by peripheral smear findings without any specific causes other than Tk-activation. Other laboratory results including elevated AST and normal ALT, and undetected urinary hemosiderin and hemoglobin also support extravascular hemolysis. Therefore, intravascular red cell destruction due to complement activation resulted from anti-Tk is unlikely as a mechanism of hemolysis in this case. Negative DAT also argues against antibody or complement mediated hemolysis, although the DAT can be negative when cryptantigens absorb the corresponding antibodies and RBCs with those fail to agglutinate [3]. Additionally, Crookston et al. question a cause-and-effect relationship between anti-T and hemolysis based on in vitro and in vivo studies and clinical reports [17]. Direct action of bacterial enzymes and toxins might play a role in hemolysis. However, our case showed only a vague evidence of infection. Therefore, in our case, the mechanism of extravascular hemolysis is most likely a shortened survival of RBCs because of altered RBC membrane glycoprotein structures due to bacterial enzymes or toxins.

Lastly, the number of reports of hemolysis with Zosyn® (piperacillin/tazobactam) is increasing lately. Antibodies to piperacillin or piperacillin/tazobactam have been reported to cause both intra and extravascular hemolysis [24–27]. Our patient received one dose of Zosyn® on hospital day 1. However, it was held because of the consideration of drug-induced immune hemolysis (DIIH). Although DIIH almost always demonstrates positive DAT, Pierce et al. say that piperacillin does not bind strongly to RBC membranes, such that drug-coated RBCs may be difficult to create for in vitro testing. However, they also state that these drug-dependent antibodies provoke precipitous intravascular hemolysis due to complement fixation by interacting with a neoantigen variably composed of RBC membrane proteins and drug epitopes [28]. Our case showed negative DAT even at the time of hemolysis, which is not common for DIIH. Therefore, the possibility of DIIH cannot be ruled out completely, but hemolysis from Tk-activation may be a better explanation, especially because the positive lectin panel was demonstrated in our case.

Conclusion

We reported a case of extravascular hemolysis most likely due to Tk-activation. In our case, the mechanism of hemolysis from Tk-activation is probably due to shortened RBC survival by altered RBC membrane sialic acid content which can cause extravascular hemolysis rather than due to complement activation by an antigen–antibody reaction which often causes intravascular hemolysis. Additionally, when routine antibody detection tests are negative in cases with clinically proven hemolysis, it is important to investigate further by performing cold panel, polyagglutination tests, and a lectin panel to detect rare cause of hemolysis in conjunction with blood bank.

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Conflict of Interest

None declared.

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