

A novel method for the laboratory workup of anaphylactic transfusion reactions in haptoglobin-deficient patients

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BACKGROUND: Patients with congenital haptoglobin deficiency can develop anti-haptoglobin antibodies after exposure to blood products, and they can suffer from life-threatening anaphylactic transfusion reactions. Here, we present a case of a 57-year-old Chinese male with myelodysplastic syndrome who manifested an anaphylactic transfusion reaction during the transfusion of platelets. The only abnormality detected during his reaction laboratory workup was an undetectable haptoglobin level in the absence of evidence of hemolysis.

STUDY DESIGN AND METHODS: Surface plasmon resonance (SPR) was explored as a method to be able to detect the presence of anti-haptoglobin antibodies in serum. First, haptoglobin was immobilized to the surface of an SPR sensor chip. The patient's serum sample was injected, and the binding response was monitored in real time. Serum samples from five healthy volunteers were used as negative controls. Binding specificity was assessed in competition experiments using soluble haptoglobin. Anti-IgG, -IgA, -IgM, -IgD and -IgE antibodies were used to identify the antibody isotype.

RESULTS: An IgG anti-haptoglobin antibody was detected in the patient's serum with SPR.

CONCLUSION: SPR provided a rapid, readily available method for the detection of an IgG anti-haptoglobin antibody in an anhaploglobinemic individual. This confirmed the underlying etiology of the anaphylactic nonhemolytic transfusion reaction and justified the necessity of stringently washed cellular products for all future transfusions and strong caution for future use of plasma-containing products.

Haptoglobin is a plasma glycoprotein synthesized by the liver that is uniquely capable of irreversibly binding free hemoglobin. It is a tetrameric protein composed of two alpha chains and two beta chains, and there are three major phenotypes: Hp1-1, Hp2-2, and Hp2-1. These phenotypes are encoded by two autosomal codominant alleles, Hp¹ and Hp², which are located on chromosome 16.^{1,2}

While certain pathological states such as acute hemolysis and severe liver disease can lead to acquired hypohaptoglobinemia and anhaploglobinemia, congenital haptoglobin deficiency can also result in these conditions.^{2,3} Of note, an approximately 28-kb deletion in the haptoglobin gene, Hp^{del}, has been described.⁴ Homozygous individuals (Hp^{del}/Hp^{del}) do not produce any haptoglobin, and heterozygous individuals (Hp1/Hp^{del} or Hp2/Hp^{del}) produce very low levels of the protein. Anhaploglobinemia has been observed in many different populations around the world.³ However, no other definitive genetic cause has been described, and the Hp^{del} allele has only been observed in East Asian populations.⁵

ABBREVIATIONS: CMD = carboxymethyl-dextran; EDAC = 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride; ELISA = enzyme-linked immunosorbent assay; PBST = phosphate buffered saline with TWEEN 20; SPR = surface plasmon resonance; sulfo-NHS = N-hydroxysulfosuccinimide.

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Although the absence of haptoglobin is not detrimental to the general health of the individual, it can prove problematic in the transfusion setting. If patients with congenital anhaploglobinemia are exposed to haptoglobin-containing blood products, they can develop allogeneic antibodies with haptoglobin specificity, and they may experience anaphylactic transfusion reactions upon future exposure to blood products. Here, we report a case of an anaphylactic transfusion reaction in an anhaploglobinemic individual, and we describe a novel surface plasmon resonance (SPR) assay to detect clinically significant antibodies in the patient's serum that can be used to guide future transfusion management.

CASE REPORT

A 57-year-old Chinese male, O Rh(D) + blood type, was diagnosed with refractory cytopenia myelodysplastic syndrome 5 years before presentation. He initially presented with complaints of dyspnea on exertion and a profound anemia with a hemoglobin of 3.3 g/dL. The patient chose to proceed with supportive care only, and he was transfusion dependent with red blood cells (RBCs) over the next several months. Approximately 1 year after diagnosis, he was treated with two cycles of 5-azacytidine but he chose to discontinue chemotherapy due to side effects including increased transfusion dependency and neutropenic fevers. Over the next 4 years, he chose supportive care only, which consisted of frequent RBC transfusions. All transfusions were tolerated without complication at our institution.

The patient experienced a significant worsening of his clinical symptoms, and his disease was reclassified as refractory anemia with excess blasts-2 myelodysplastic syndrome. The patient was started on decitabine, and after Cycle 3 he presented to our institution with an episode of fever and epistaxis. His platelet count was 6000/ μ L, and frank blood was observed to be oozing from his left nostril, for which a unit of platelets was ordered. The patient was premedicated with 100 mg of hydrocortisone, 50 mg of diphenhydramine, and 650 mg of acetaminophen due to a history of a "hypoxia and hypotension" after his first platelet transfusion at an outside hospital, which occurred 1 month earlier. Before transfusion, the patient's vital signs were as follows: blood pressure, 133/70 mm Hg; heart rate, 100 bpm; temperature, 37.1°C; respiratory rate, 16; pulse oximetry, 100% on room air.

Approximately 15 minutes after the platelet transfusion had started, the patient developed labored breathing, hypotension, and tachycardia. The patient's vital signs were as follows: blood pressure, 78/39 mm Hg; heart rate, 140 bpm; temperature, 37.1°C. The patient was placed on 7 L of oxygen by face mask to maintain pulse oximetry of 95% to 100%. He was also treated with two intramuscular injections of epinephrine (0.5 mg) and 3 L of normal saline. The patient's blood pressure improved to baseline, and he was eventually weaned back to room air.

A transfusion reaction workup was performed by the transfusion service. There was no laboratory evidence of hemolysis: hemoglobinemia, negative; direct antiglobulin test, negative; total bilirubin, 1.0 mg/dL (0.0-1.0 mg/dL); lactate dehydrogenase, 133 U/L (120-246 U/L); urinalysis, clear yellow; urobilinogen, 1.0 EU/dL (0.1-1.0 EU/dL). The case was categorized as an anaphylactic transfusion reaction according to the Centers for Disease Control and Prevention National Healthcare Safety Network Biovigilance Component Hemovigilance Module Surveillance Protocol criteria for an allergic reaction.⁶ Haptoglobin and IgA protein levels were ordered to test for common inherited causes of anaphylactic reactions. The IgA was within normal levels (IgA = 226 mg/dL [40-350 mg/dL]), but the haptoglobin was below the reportable range of the assay (haptoglobin <8 mg/dL [40-240 mg/dL]). At our institution, haptoglobin is measured by an immunoturbidimetric assay (Architect C16000, Abbott Laboratories) with a lower limit of quantitation of 8 mg/dL. The raw signal from the analyzer indicated that there was little to no haptoglobin in the sample, although a concentration could not be determined at these levels. Based on this finding, we hypothesized that this patient had congenital anhaploglobinemia, and we used SPR to determine if the patient had developed anti-haptoglobin antibodies.

MATERIALS AND METHODS

Reagents

Purified haptoglobin from human serum was purchased from Sigma Aldrich. SPR sensor chips (CMD200m), 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDAC), N-hydroxysulfosuccinimide (sulfo-NHS), 1 M ethanolamine (pH 8.5), 10 mM glycine, and acetate buffers were obtained from BiOptix Analytical. Phosphate buffered saline was purchased from Bio-Rad Laboratories. Antibodies specific for human IgG, IgM, IgA, IgD, and IgE were obtained from Sebia Inc.

Patient samples

Waste clinical specimens from the patient (gold-top serum-separator tubes) were obtained from the Clinical Chemistry Laboratory in the Department of Laboratory Medicine at Memorial Sloan Kettering Cancer Center. Serum samples from five healthy volunteers were also obtained and used with approval from the Institutional Review Board.

SPR experiments

All experiments were performed on an SPR instrument (BiOptix 404pi, BiOptix Analytical) held at 20°C. Haptoglobin was immobilized to a CMD200m sensor chip using standard amine coupling. For immobilization, the running buffer consisted of phosphate buffered saline, pH 7.4, 0.05% TWEEN 20 (PBST). The surface was activated using a 1:1 mixture of EDAC to sulfo-NHS (250 nM each), which was injected at a

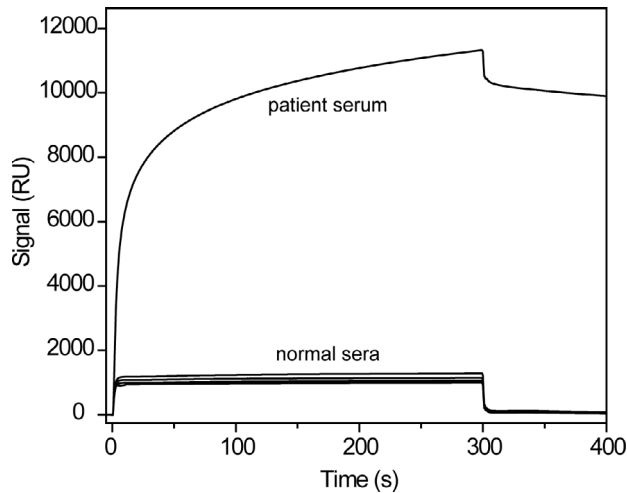


Fig. 1. Binding sensorgrams to the haptoglobin-immobilized surface. Serum from the patient and five healthy volunteers were injected for 300 seconds over the haptoglobin-immobilized surface. The large increase in signal in the patient sample indicates that there is binding to haptoglobin.

flow rate of 20 $\mu\text{L}/\text{min}$ for 420 seconds. Following activation, haptoglobin (5 $\mu\text{g}/\text{mL}$ in 10 mM sodium acetate, pH 4.5) was injected at a flow rate of 5 $\mu\text{L}/\text{min}$ for 600 seconds to achieve an immobilization density of about 4000 response units. The surface was deactivated by injecting 1 M of ethanolamine, pH 8.5 (flow rate, 20 $\mu\text{L}/\text{min}$ for 600 sec). Following deactivation, the running buffer was switched to PBST with 1 mg/mL soluble carboxymethyl dextran (PBST-CMD). All serum samples were diluted 1:4 in PBST-CMD. Samples were injected at a flow rate of 5 $\mu\text{L}/\text{min}$ for 300 seconds. After each injection,

the surface was regenerated with 10 mM of glycine, pH 1.5 (flow rate = 20 $\mu\text{L}/\text{min}$ for 15 s) to dissociate any protein that bound the immobilized haptoglobin. Binding sensorgrams were processed and analyzed using computer software (Scrubber 2.0c, BioLogic Software).

Assessing binding specificity

The specificity of binding was evaluated by competition experiments. Soluble haptoglobin was added to the patient sample to compete with binding to the immobilized haptoglobin on the SPR surface. First, the patient serum was mixed with serum from a healthy volunteer, which contained normal levels of haptoglobin. Specificity was also assessed by spiking the patient sample with 50 or 500 $\mu\text{g}/\text{mL}$ of purified haptoglobin. All samples were diluted 1:4 in PBST-CMD and were injected as described above.

Determining antibody isotype

Antibodies specific for human IgG, IgM, IgA, IgD, and IgE were used to identify the isotype of the anti-haptoglobin antibody. Samples were injected at a flow rate of 5 $\mu\text{L}/\text{min}$ and were injected in series over a blank reference surface and the haptoglobin-immobilized surface. The reference surface was used to monitor for nonspecific binding. Sample injections consisted of three sequential steps. First, diluted patient serum was injected for 300 seconds to allow the antibody to bind the immobilized haptoglobin. Next, running buffer (PBST-CMD) flowed over the surface while the instrument prepared the next injection. Finally, anti-IgG (diluted 1:2 in PBST-CMD) was injected for 300 seconds as a secondary reagent to bind to the haptoglobin antibody. The surface was then regenerated with 10 mM of glycine, pH 1.5 as described above. The three-step

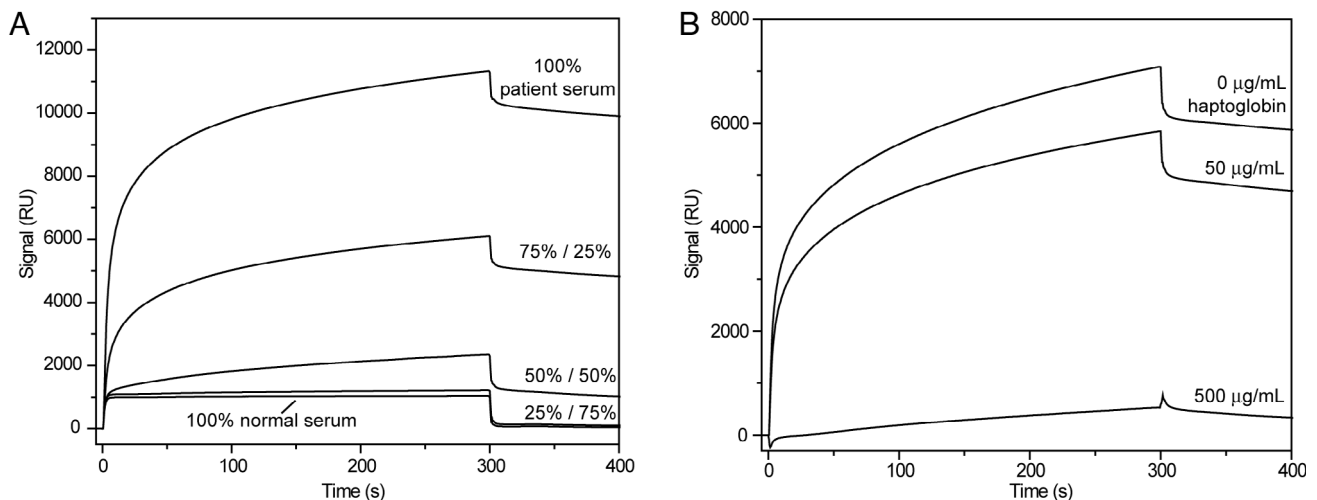


Fig. 2. Competition experiments to test specificity of binding. (A) The patient sample was mixed with serum from a healthy volunteer in different ratios (% patient/% healthy volunteer) before SPR analysis. (B) The patient sample was spiked with various amounts of haptoglobin before SPR analysis.

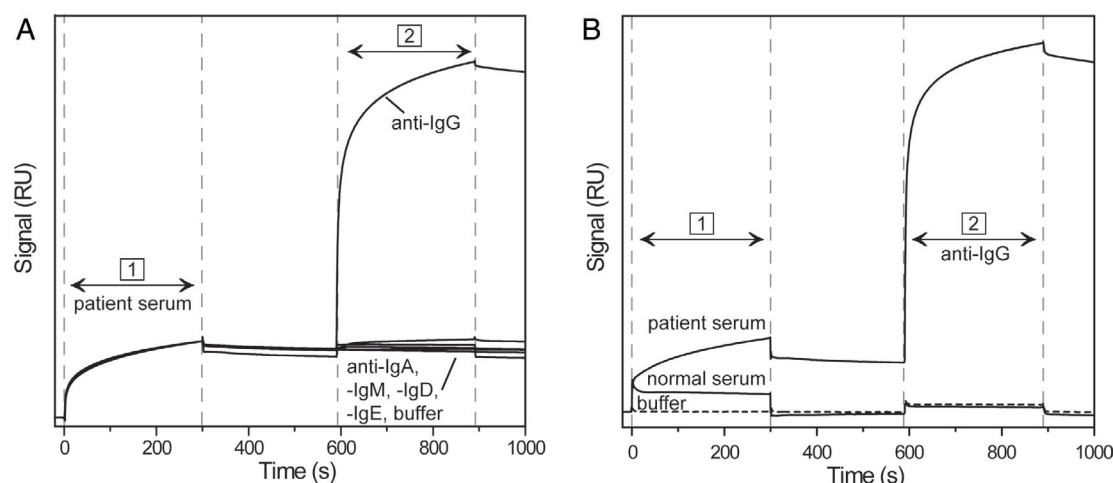


Fig. 3. Determining antibody isotype. (A) The isotype of the haptoglobin antibody was identified using a two-step injection. In Step 1, patient serum was injected to allow the antibody to bind the immobilized haptoglobin. In Step 2, anti-IgG antibody was injected as a secondary reagent to bind to the haptoglobin antibody. After regenerating the surface, the two-step process was repeated using anti-IgM, -IgE, -IgA, or -IgD antibodies in Step 2. Sensorgrams have been normalized to the signal at 300 seconds and referenced to the blank surface. (B) Normal serum from a healthy volunteer was injected in Step 1 followed by anti-IgG antibody to assess nonspecific binding of the anti-IgG to serum proteins. PBST-CMD buffer was injected in Step 1 followed by the anti-IgG antibody to check for nonspecific binding to the haptoglobin-immobilized surface. Sensorgrams have been referenced to the blank surface.

process was repeated with the patient sample in Injection 1, followed by buffer and anti-IgE, anti-IgA, anti-IgM, anti-IgD antibodies or PBST-CMD (negative control) in injection 2. For additional controls, we used serum from a healthy volunteer or PBST-CMD buffer in Injection 1, followed by anti-IgG antibody in Injection 2. These controls were used to check for nonspecific binding between the anti-IgG antibody and serum proteins or the haptoglobin surface, respectively. For the isotyping experiments, all sensorgrams were referenced to the blank surface to correct for nonspecific binding.

RESULTS

When the patient sample was injected over the haptoglobin-immobilized surface, we observed a large increase in signal, indicating that a component of the serum was binding to haptoglobin. This response was not observed in the sera of five healthy volunteers (Fig. 1). We found that this binding was specific to haptoglobin, as we observed a concentration-dependent decrease in signal when we mixed the patient serum with soluble haptoglobin, either from normal serum (Fig. 2A) or a purified stock of haptoglobin (Fig. 2B). Finally, we found that the component in the patient sample that was binding to the haptoglobin was an IgG antibody. Injection of the patient sample followed by anti-IgG antibody produced a large signal that was not observed with the other anti-Ig antibodies or with the negative control (PBST-CMD buffer) (Fig. 3A). We did not observe a signal increase when the anti-IgG antibody was injected after normal serum or PBST-CMD

buffer (Fig. 3B), indicating that the signal observed with the anti-IgG antibody was not due to nonspecific binding to serum proteins or to the haptoglobin surface.

DISCUSSION

Patients who are homozygous $\text{Hp}^{\text{del}}/\text{Hp}^{\text{del}}$ are at significant risk of nonhemolytic transfusion reactions that are characterized by severe allergic symptoms, ultimately leading to anaphylaxis.⁷⁻¹² These patients, who typically have a history of prior transfusions, are found to be haptoglobin deficient only after manifesting a severe nonhemolytic transfusion reaction, and they have IgG and usually IgE^9 (Table S1, available as supporting information in the online version of this paper) anti-haptoglobin antibodies in serum. All of the previously reported cases have occurred in East or Southeast Asia, where the incidence of anhaploglobinemia due to the Hp^{del} homozygous genotype is estimated to be 1 in every 1500 Koreans, 1 in every 4000 Japanese, and 1 in every 1000 Chinese.⁵

To our knowledge, this is the first reported case of an anhaploglobinemia-related anaphylactic transfusion reaction in the United States, which occurred in a Chinese individual. Because testing for the $\text{Hp}^{\text{del}}/\text{Hp}^{\text{del}}$ genotype or anti-haptoglobin antibodies is not available in the United States, we developed an SPR assay to detect anti-haptoglobin antibodies in our patient. SPR is an optical technique that measures binding interactions in real time and in a label-free manner.¹³ In most case reports, enzyme-linked immunosorbent assay (ELISA) and western blotting are used to detect

anti-haptoglobin antibodies (Table S1, available as supporting information in the online version of this paper). We chose SPR over these other techniques because it requires fewer biologic reagents that do not need to be labeled. Therefore, method development is simplified. In addition, because measurements are made in real time, results are available much faster compared to ELISA and western blotting, which have several wash and incubation steps.

Unlike some of the previous case reports, we did not detect IgE anti-haptoglobin antibodies in the patient sample. It is possible that haptoglobin-specific IgE antibodies were present but were missed by our assay because they are much less concentrated than IgG antibodies. One strategy to detect anti-haptoglobin IgE antibodies is to deplete IgG from serum before analysis.⁸ Due to limited sample volume, we were unable to perform this additional testing and cannot conclude about the presence or absence of haptoglobin-specific IgE antibodies in our patient.

Our case also differs from previous reports because we were unable to perform genetic testing to determine if our patient was homozygous for the haptoglobin gene deletion (Hp^{del}). This test is not available at any reference laboratory in the United States, and we did not have the expertise or resources to develop this test in-house. However, this test may not be necessary in the workup of a transfusion reaction. While the homozygous Hp^{del} genotype is associated with an increased risk for developing anti-haptoglobin antibodies, it is not a foregone conclusion that a patient will have a future anaphylactic transfusion reaction.⁹ In addition, transfusion reactions and anti-haptoglobin antibodies have been reported in an individual who was not haptoglobin deficient.¹⁴ Therefore, detection of an anti-haptoglobin antibody is a critical factor in diagnosing a haptoglobin-related transfusion reaction.

In this case, SPR provided a rapid, readily available method for the detection of anti-haptoglobin antibodies in an anhaploglobinemic individual. The methodology was able to determine that IgG anti-haptoglobin antibodies were present. This confirmed the underlying etiology of the anaphylactic nonhemolytic transfusion reaction for the clinical team and justified the necessity of stringently washed cellular products for all future transfusions. The patient had no additional reactions with the use of washed products and premedication with steroids and antihistamines. The patient was informed of the diagnosis and the importance of washing all future cellular blood products should he need transfusion at other institutions. Considering the large population of East Asians in some areas of the United States and the prevalence of anhaploglobinemia in these groups, clinicians should be aware of the transfusion-related risks associated with this condition. With the growing diversity of the US patient population as well as use of transfusion as a therapeutic modality, it may be advantageous for reference laboratories or blood centers in the United States to offer such assays for the workup of these cases.

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CONFLICT OF INTEREST

STA, VK, and CCG have disclosed no conflicts of interest. KLT received research support in the form of equipment from BiOptix Analytical.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Table S1. Summary of case reports.

Supplemental Table 1: Summary of case reports

Reference	Number of patients in report	Number of patients with IgG antibody	Number of patients with IgE antibody	Methods used to detect anti-haptoglobin antibodies
Shimada et al, 2002 ⁹	7	7	7	IgG: ELISA and Western blot analysis IgE: ELISA and Western blot analysis after depletion of IgG from serum
Shimode et al, 2006 ¹¹	1	1 ^a	unclear if tested	ELISA and Western blot analysis
Kim et al, 2012 ⁷	1	1	unclear if tested	ELISA
Iwamoto et al, 2014 ¹²	1	1	0	IgG: ELISA and Western blot analysis IgE: ELISA and IgE crosslinking-induced luciferase expression (EXiLE)
Muta et al, 2009 ¹⁴	1 ^b	1	0	ELISA
Morishita et al, 2000 ⁸	1	1	unclear if tested	ELISA
Nishiki et al, 2002 ¹⁰	1	1	unclear if tested	ELISA and Western blot analysis

^aantibody isotype not specified in case report, likely IgG

^bnon-haptoglobin-deficient patient