IVIG-Associated Hemolysis

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“Blood Matters”

Objectives

- to provide background information on intravenous immune globulin (IVIG)
- to present a case of hemolysis related to IVIG administration in Nova Scotia
- to describe the pathophysiology of IVIG-associated hemolysis
- to briefly review the available literature on IVIG-associated hemolysis

Intravenous Immunoglobulin (IVIG)

- sterile solution of human immunoglobulins for intravenous administration
- manufactured from large pools of human plasma by a combination of cold ethanol fractionation, precipitation, filtration and anion exchange chromatography
- plasma collected in Canada from volunteer donors

IVIG: Indications For Clinical Use

- IVIG being used to treat an increasing number of clinical conditions

- **Primary Immune Deficiency**

  - congenital agammaglobulinemia + hypogammaglobulinemia
  - common variable immunodeficiency
  - severe combined immunodeficiency
  - Wiskott-Aldrich syndrome

IVIG: Indications For Clinical Use

- **Secondary Immune Deficiency**
  - hematological malignancies with infections due to hypogammaglobulinemia (eg. chronic lymphocytic leukemia)
  - pediatric HIV infection

- **Neurological and Autoimmune Conditions**
  - Guillain Barre Syndrome (GBS)
  - chronic inflammatory demyelinating polyneuropathy (CIDP)
  - multifocal motor neuropathy
  - myasthenia gravis
  - acute disseminated encephalomyelitis
  - immune thrombocytopenic purpura (ITP)

IVIG: Indications For Clinical Use

- **Rheumatological Conditions**
  - Juvenile Idiopathic Arthritis
  - Juvenile Dermatomyositis
  - Kawasaki Disease
- **NAIT (neonatal alloimmune thrombocytopenia)**
- **Hemolytic Disease of the Newborn**

Unlabeled and Non-Indications for IVIG

Hematology	Neurology
• AML with Thrombocytopenia • Aplastic anemia • Aplastic Anemia with Pancytopenia • CD5 Leukemia • Disseminated intravascular coagulation • Hematopoeitic stem cell transplantation (unless patient is on a multinational protocol that recommends IVIG) • Heparin-induced thrombocytopenia • Leukemia • Mantle Cell Lymphoma • Myelodysplastic Syndrome with Thrombocytopenia • Non-Hodgkins Lymphoma with Sepsis Sickle Cell Anemia • Thrombocytopenia (unless patient has ITP)	• Adrenoluekodystrophy • Amyotrophic Lateral Sclerosis • Anti-NMDA receptor encephalitis • Autism • Bell's Palsy • Brainstem encephalitis • Critical Illness Neuropathy • Devics Disease • Diabetic Neuropathy • Encephalomyelitis • Hashimoto's Encephalopathy • IgM Paraproteinemic Neuropathy • Inclusion Body Myositis • Limbic encephalitis • Myelitis • Myelopathic Process • Nerve Impairment Similar to MS • Opsoclonus (involving eye movement) • Paraneoplastic Cerebellar Degeneration • Paraneoplastic Neuropathy • Paraneoplastic Subacute Cerebellar Degeneration • POEMS Syndrome • Post Polio Syndrome • Recurrent Demyelination of the Optic Nerve • Sensory Neuropathy • Transverse Myelitis

IVIG: Dosing

- **Immune replacement dose**:

  0.4-0.6 g/kg every 3-4 weeks based on trough IgG levels

- **Immunomodulatory dose**:

  1 g/kg per day for 2 days every 3-4 weeks

IVIG: Mechanism of Action

- IVIG supplies a broad spectrum of IgG antibodies against bacteria, viruses, and toxins that are effective in the prevention or attenuation of infections

- > 98% of the protein contained within IVIG has the electrophoretic mobility of gamma globulin with a similar distribution of IgG subclasses to that found in normal serum

IVIG: Adverse Events

- allergic + anaphylactic reactions
- fever + rigors
- hypotension + respiratory distress
- renal insufficiency + osmotic nephrosis
- venous thromboembolism
- infectious agent transmission (viruses, vCJD)
- aseptic meningitis

IVIG: Adverse Events

- RBC hemolysis due to passively transfused antibodies to blood group antigens (ABO) contained within the IVIG product

  - usually anti-A or anti-B isohemagglutinins primarily of the IgG_4 subclass

  - rare adverse event (~1%)

  - hemolysis may range from mild to severe

  - transfusion may be necessary to treat symptomatic anemia

CASE

- 45 year-old male admitted to ICU with paralysis secondary to CIDP (chronic inflammatory demyelinating polyneuropathy)

- Past Medical History:
  - schizophrenia
  - hypothyroidism

- Medications: levothyroxine, zantac, gabapentin, quetiapine, meropenum, heparin

CASE

- given IVIG (Privigen) 40 g per day for 6 days (total dose 240 g)
- two different lot numbers of Privigen were used

Laboratory Values

	Hemoglobin/HCT	LDH	Haptoglobin	Total Bilirubin	Cr
• March 3	115 (0.359)	103		5	133
• March 4	101			4	
• March 5	97				
• March 7	86			14 (direct bili 3)	
• March 10	83	550			
• March 11	83			18 (direct bili 3)	221
• March 13	68 (0.210)	663	< 0.06	14	
• March 14	77 (post 2 units RBC)	574	< 0.06	13	
• March 16	70	426			
131					
• March 31	90	197			

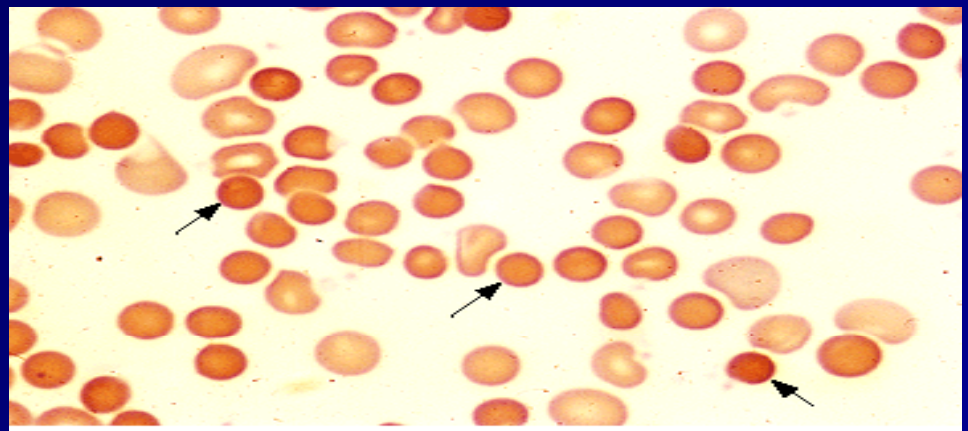
Laboratory Data

- Pre-transfusion testing:
  - blood group AB+
  - antibody screen negative

- Post-transfusion testing:
  - direct antiglobulin test (DAT) 3+ IgG
  - eluate demonstrated passive anti-A1 and nonspecific reactivity

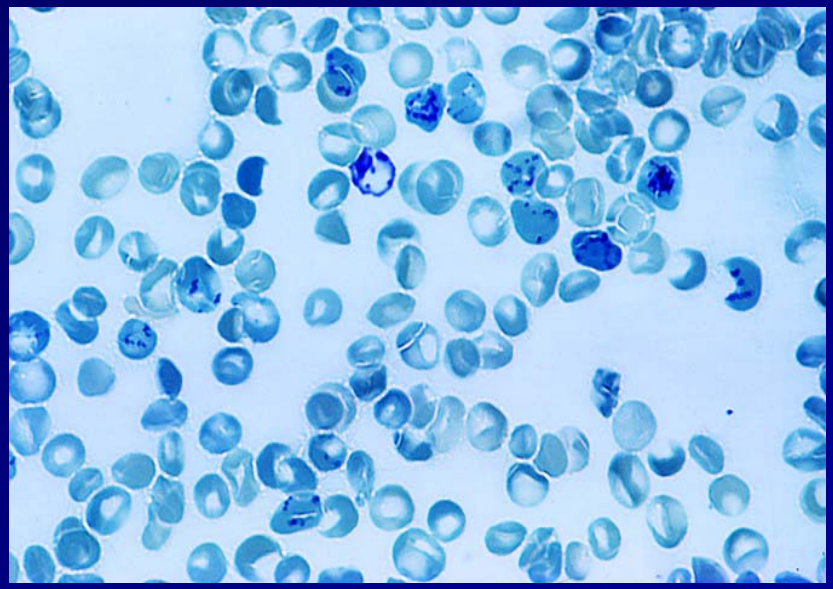
- Peripheral blood film:
  spherocytes, polychromasia, nucleated RBC

Spherocytes



Spherocytes Peripheral blood smear shows multiple spherocytes which are small, dark, dense hyperchromic red cells without central pallor (arrows). These findings are compatible with hereditary spherocytosis or autoimmune hemolytic anemia. Courtesy of Carola von Kapff, SH (ASCP).

Reticulocytes
(new methylene blue stain)



CASE

- transfused 1 unit of AB+ RBC on March 12 and 1 unit of AB+ RBC on March 13
- hemolytic anemia recognized 6 days after the end of the IVIG course (March 14)

CASE

- **Diagnosis**:

- “Delayed hemolytic transfusion reaction secondary to IVIG”

- transfusion reaction reported to the following:
  - Canadian Blood Services
  - Health Canada
  - product manufacturer (CSL Behring)

IVIG-Associated Hemolysis Pathophysiology

- antibodies to blood group antigens (A and B) result from type O individual donors with high titres of these blood group antibodies
- all commercial preparations of IVIG contain isohemagglutinins to A and B antigens
- the titres of the blood group antibodies (anti-A and anti-B) vary between lots of IVIG because the product is pooled from thousands of blood donors

IVIG-Associated Hemolysis Pathophysiology

- IgG Antibodies:
  - activate complement poorly
  - recognized by Fc receptors on phagocytes of the reticuloendothelial system
  - extravascular hemolysis occurs in the spleen

IVIG-Associated Hemolysis Pathophysiology

- industry standards exist which limit the titre of anti-A and anti-B (and anti-D) isohemaglutinins in IVIG products (should be 1:16 or less)
- HOWEVER...even at low titre there is still a risk of hemolysis secondary to these antibodies
- hemolysis is usually seen with high-dose IVIG therapy (immunomodulatory dose)

IVIG-Associated Hemolysis Pathophysiology

- hemolysis can range from mild to severe
- mild hemolysis may go unrecognized
  - clinically insignificant decreases in hemoglobin and hematocrit occur in 64% of patients (Stangel 2003)
- rate of clinically significant hemolysis is estimated to range from 1.6-6.7% (Wilson 1997, Daw 2008)

IVIG-Associated Hemolysis

- IVIG must be recognized as a potential cause of hemolysis:
  - CBC should be performed 48-72 hours following IVIG infusion

- IVIG recipients should be monitored for clinical signs and symptoms of hemolysis:
  - pallor
  - weakness
  - dyspnea
  - chest pain
  - dark urine
  - jaundice

IVIG-Associated Hemolysis

- recommended laboratory workup in the setting of hemolysis due to IVIG:
  - CBC
  - reticulocyte count
  - peripheral blood film
  - total and indirect bilirubin
  - serum haptoglobin
  - direct antiglobulin test (DAT) + eluate if DAT positive
  - lactate dehydrogenase (LDH)

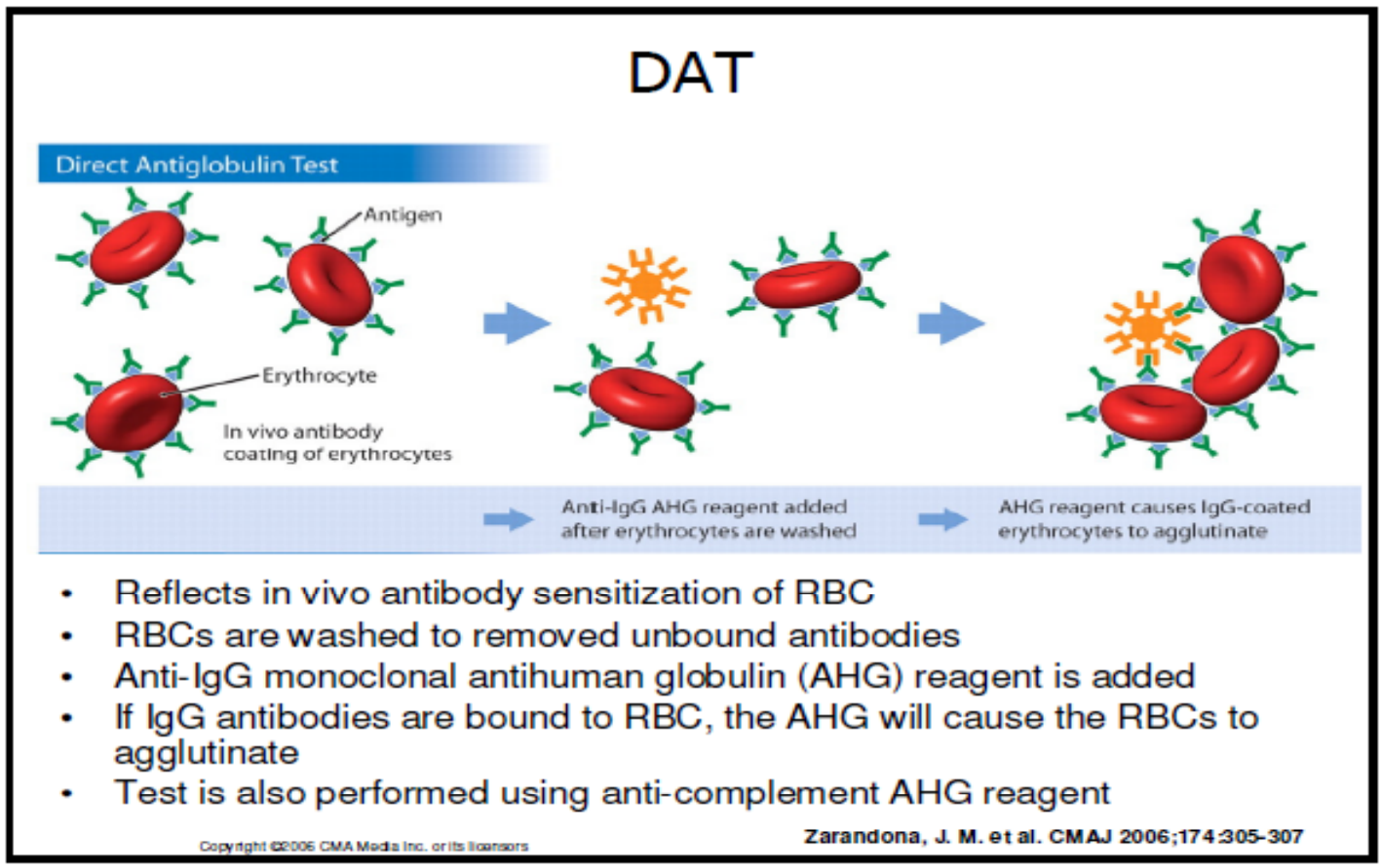
IVIG-Associated Hemolysis

Direct Antiglobulin Test (DAT)

- test for IgG and/or complement (C3d) on the surface of patient red cells using antihuman globulin reagent (AHG):
  - polyspecific (detects both IgG and C3d)
  - monospecific (detects either IgG or C3d)

- DAT usually positive for IgG, negative for C3d in the setting of IVIG-associated hemolysis

Direct Antiglobulin Test



IVIG-Associated Hemolysis: Laboratory

- Elution: dissociation of antibodies from the surface of the patient's RBC enables testing against a panel to identify the specificity of auto- or alloantibodies

- eluate positive for anti-A, anti-B (or anti-D) in the setting of IVIG-associated hemolysis

IVIG-Associated Hemolysis: Laboratory

- antibody screen is negative
- can perform antibody titres (anti-A or anti-B) on the lot of IVIG or on the eluate but this process is difficult to standardize

IVIG-Associated Hemolysis: Management Strategy

- the presence of anti-A or anti-B in the plasma of non-group O patients should be excluded prior to issuing blood group-specific units

- give group O RBC if transfusion required due to symptomatic anemia

- if clinically-significant hemolysis occurs:
  - switch the lot number of IVIG as continued hemolysis is unlikely if this is done
  - discontinue IVIG therapy

Literature Review of Studies Related to Hemolysis Following Treatment with IVIG

- IVIG is increasingly recognized as a potential cause of hemolysis

- Hemolysis after treatment with intravenous immunoglobulin due to anti-A. Morgan *et al*. Transfusion Medicine 2011. 21:267-270

- Hemoglobinuria and acute kidney injury requiring hemodialysis following intravenous immunoglobulin infusion. Welles *et al*. Am J Kidney Dis. 2010; 55(1):148

- Acute hemolysis after high-dose intravenous immunoglobulin therapy in highly HLA sensitized patients. Kahwaji *et al*. American Journal of Nephrology. 2009

- Hemolytic transfusion reactions after administration of intravenous immune (gamma) globulin: a case series analysis Daw *et al*. Transfusion 2008. 48:1598-1601

- Acute hemolysis in a patient with CMV pneumonitis treated with intravenous immunoglobulin Coghill *et al*. Biol Blood and Marrow Trans. 2006, 12:786-788

Literature Review of Studies Related to Hemolysis Following Treatment with IVIG

- Side effects of intravenous immunoglobulin in neurological autoimmune disorders. A prospective study. Stangel *et al*. Journal of Neurology 2003. 250:818-821

- Hemolytic anemia associated with intravenous immunoglobulin. Wilson *et al*. Muscle & Nerve. 1997, 20:1142-1145

- Hemolysis after high dose intravenous Ig. Thomas *et al*. Blood. 1993, 82:3789

- Immune hemolysis, disseminated intravascular coagulation, and serum sickness after large doses of immune globulin given intravenously for Kawasaki disease. Comenzo *et al*. Journal of Pediatrics. 120:926-928

- Hemolysis induced by intravenously-administered immunoglobulin. Nicholls *et al*. The Medical Journal of Australia. 1989, 150:404-406

- Hemolytic anemia following intravenous gamma globulin administration. Brox et al. The American Journal of Medicine. 1987. 82:633-635

HEMOLYTIC ANEMIA ASSOCIATED WITH INTRAVENOUS IMMUNOGLOBULIN

JOHN R. WILSON, MD,[1*] NIRMALA BHOOPALAM, MD,[2] and MORRIS FISHER, MD[1]

Muscle & Nerve 1997. 20: 1142-1145

- 45 patients who received IVIG over a 13 month period
- IVIG given for both immune replacement and immunomodulatory indications

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Muscle & Nerve 1997. 20: 1142-1145

- DAT results were available for 42 patients:
  - no patient had a positive DAT prior to IVIG therapy
  - 12 patients had a positive DAT post-IVIG therapy (8 patients received IVIG for neurologic disorders)

- 11 out of 12 patients with a positive DAT developed hemolysis severe enough to lower hemoglobin by at least 1 g/L
  - 3 out of 12 required blood transfusion
  - 1 out of 12 had a shortened IVIG treatment course due to hemolysis

Wilson et al. Muscle & Nerve 1997

Table 1. Patients developing hemolysis after receiving IVIg.

Patient	Indication	Blood type	Hgb pre-IVIg	Hgb post-IVIg	DAT	Antibody identified
1	Hypogammaglobulinemia	A+	15.6	11.9	+	Anti-A, anti-D
2	Myasthenia gravis	A+	14.0	10.2	+	Anti-A
3	Chronic lymphocytic leukemia (CLL) with hypogammaglobulinemia	A+	7.1	6.1	+	Anti-A, anti-D
4	CLL with idiopathic thrombocytopenic purpura	A+	8.4	6.8	+	Anti-A
5	Acute myelogenous leukemia with HSV infection	O+	8.8	6.7	+	Anti-D
6	CIDP	A+	12.2	9.3	+	Anti-A
7	CIDP	A+	15.5	14.1	+	Anti-A
8	CIDP	A+	8.6	6.7	+	Anti-A
9	GBS	A+	13.9	10.9	+	Anti-A
10	CIDP	A+	15.9	14.1	+	Anti-A
11	CIDP	A+	14.6	13.6	+	Anti-A
12	CIDP	A+	15.0	14.1	+	Anti-A

Hemolytic transfusion reactions after administration of intravenous immune (gamma) globulin: a case series analysis

Zohra Daw, Ruth Padmore, Doris Neurath, Nancy Cober, Melanie Tokessy, Diane Desjardins, Bernhard Olberg, Alan Tinmouth, and Antonio Giulivi

Transfusion 2008; 48:1598-1601

- 16 cases of hemolytic transfusion reactions associated with the administration of IVIG over a 2.5 year period
- hemolysis was observed from 12 hours to 10 days after first dose of IVIG
- mean decrease in hemoglobin was 32 g/L (range 8-52 g/L)
- nadir hemoglobin level occurred from 1 day to 2 weeks after the last dose of IVIG

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- positive DAT was seen in 14 of 16 patients, mostly 1+ IgG
- spherocytes were present on peripheral blood film in 12 of 16 cases
- anti-A or anti-B antibodies were detected in the patient's plasma and/or eluate in 10 of 16 cases

Daw *et al*. Transfusion 2008

TABLE 1. Individual patient characteristics

Case	Age (years), sex	Diagnosis	IVIG total amount (g)	Brand of IVIG	Blood group	DAT	Eluate and/ or plasma	Decrease in Hb (g/L)	Hemolysis requiring RBC transfusion	Number of inflammatory markers*
1	27, F	Gestational ITP	50	Gammagard	AB–	Negative	Anti-A	14	No	1
2	50, F	Guillain-Barré syndrome	200	Gamunex	A+	Negative	ND	36	No	1
3	36, F	Sepsis (*Streptococcus pyogenes*)	120	Gamunex	O–	Positive polyspecific	See text	43	Yes (2 units)	1
4	66, F	Guillain-Barré syndrome	100	Gamunex	AB+	Weak+ IgG	Negative	36	No	3
5	61, F	Postoperative necrotizing fasciitis	120	Gammagard	B+	1+ IgG	Anti-B	†	No	4
6	51, F	Postoperative necrotizing fasciitis	100	Gamunex	A+	Weak+ IgG, 1+ complement	Anti-A	32	No	4
7	44, F	HIV+, CAH, sepsis	315	Gamunex + Gammagard	B+	Weak+ IgG	Anti-B	34	No	6
8	71, M	Rhabdomyolysis	200	Gamunex	A+	Weak+ IgG	Anti-A	30	No	1
9	19, F	Viral meningitis with postinfectious Guillain-Barré syndrome	180	Gamunex	A+	Weak+ IgG	Anti-A	47	No	1
10	23, F	ITP	210	Gamunex	B+	Weak+ IgG	Anti-B	51	No	0
11	60, M	Guillain-Barré syndrome	200	Gamunex	A+	Weak+ IgG	Anti-A	50	No	2
12	18, F	Viral encephalitis	120	Gamunex	B+	Weak+ IgG	Negative	24	Yes (1 unit)	3
13	60, M	Postoperative necrotizing fasciitis	350	IGIVnex	A+	3+ IgG	Anti-A	8	No	1
14	55, M	Guillain-Barré syndrome	300	Gammagard	B+	Weak+ IgG	Negative	52	No	2
15	22, M	Systemic lupus	285	Gamunex	B+	Weak+ IgG	Auto‡	13	Yes (3 units)	4
16	49, M	Guillain-Barré syndrome	295	Gamunex	A+	1+ IgG	Anti-A	30	No	3

* Inflammatory markers include haptoglobin, ferritin, fibrinogen, D-dimer, erythrocyte sedimentation rate, C-reactive protein, or decreased serum albumin.
† Ongoing RBC transfusions resulted in an increase of 19 g per L, but this increment is less than expected.
‡ Eluate demonstrated warm auto-antibodies.
CAH = cold agglutinin hemolysis; ITP = immune thrombocytopenic purpura; ND = not done.

Daw *et al*. Transfusion 2008

TABLE 2. Summary of patient characteristics predisposing to IVIG-related hemolysis

Characteristics seen in >50% of cases	Number of patients (%)
High cumulative dose of IVIG	15/16 (94)
Non-O blood group	15/16 (94)
Female	10/16 (63)
Positive inflammatory serologic marker	15/16 (94)

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- the titres of isohemagglutinins in the IVIG products used (Gammunex, Gammagard, and IVIGnex) were found to be within recommended levels:
  - anti-A titre of 4 to 8
  - anti-B titre of 2 to 4

Acute Hemolysis After High-Dose Intravenous Immunoglobulin Therapy in Highly HLA Sensitized Patients

Joseph Kahwaji,* Eva Barker,† Sam Pepkowitz,‡ Ellen Klapper,‡ Rafael Villicana,* Alice Peng,* Robert Chang,* Stanley C. Jordan,* and Ashley A. Vo*

Journal of the American Society of Nephrology 2009

- IVIG used in the setting of renal transplantation for desensitization and treatment of antibody-mediated rejection
- 18 cases of hemolysis seen in 16 patients who received IVIG from 2003-2008
- all patients with hemolysis were non-O blood types

Acute Hemolysis After High-Dose Intravenous Immunoglobulin Therapy in Highly HLA Sensitized Patients

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Journal of the American Society of Nephrology 2009

- all patients received IVIG at a dose of 1 to 2 g/kg
- average drop in hemoglobin was 38 g/L
- 83% of patients with IVIG-associated hemolysis required blood transfusion for symptomatic anemia

Kahwaji et al. 2009

Journal American Society of Nephrology

Table 1. Cases of hemolysis (2003–2008)

Case	Age (yr)	Race	Gender	Blood Type	IVIG Product	Hemoglobin Decrease (g/dl)	DAT	Blood Transfusion (Number of Units)
1	52	H	Female	A+	Gamunex	5.3	IgG+	Yes
2	23	AA	Male	A+	Gamunex	4.7	IgG+	No
3	56	H	Female	A+	Gamunex	5.6	IgG+	Yes (4)
4	27	AA	Female	B+	Gammagard	4.9		Yes (2)
5	36	AA	Male	AB+	Gamunex	5.8	IgG+	Yes (4)
6	37	AS	Female	A+	Gammagard	5.7	IgG+	Yes (2)
7	46	W	Male	A+	Gamunex	3.3		Yes
8	45	H	Female	A+	Gamunex	2.4		Yes
9	66	W	Male	A−	Privigen	3.1		Yes
10	42	W	Female	A+	Gamunex	4.0	IgG+	Yes (2)
11	56	W	Female	A+	Gamunex	3.6	IgG+	No
12	47	W	Female	A−	Gamunex	2.1	IgG+	Yes (2)
13	47	W	Female	A−	Gamunex	2.2		No
14	46	W	Male	A+	Gamunex	2.8	IgG+	Yes (2)
15	73	W	Male	B+	Gamunex	5.3	IgG+	Yes (2)
16	73	W	Male	B+	Gamunex	1.9	IgG+	Yes (2)
17	28	W	Male	A+	Gamunex	2.6	IgG+	Yes (2)
18	21	H	Male	A+	Gamunex	3.0	IgG+	Yes (1)

H, Hispanic; AA, black; AS, Asian; W, white.

Kahwaji et al. 2009
Journal American Society of Nephrology

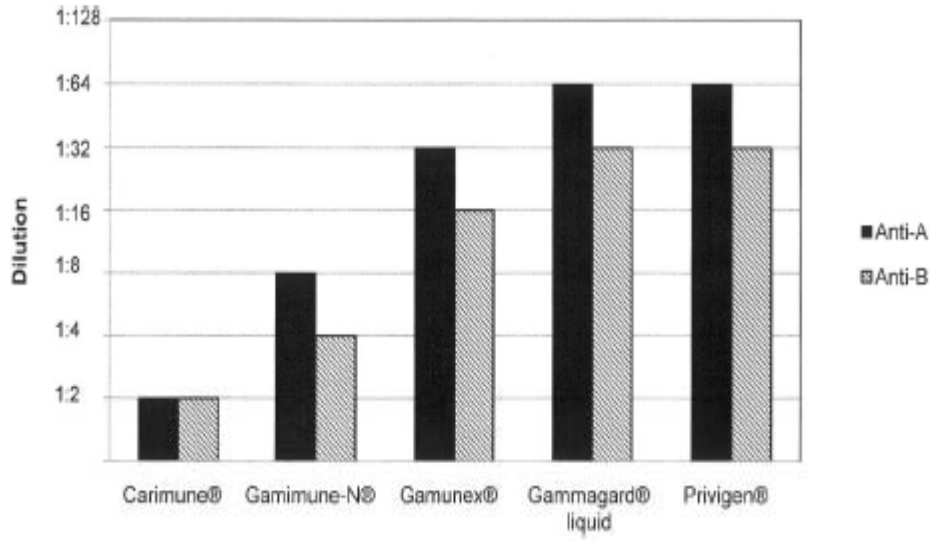

Figure 1. Isohemagglutinin titers in IVIG products. Carimune, the lyophilized product, had the lowest titers. The four liquid preparations tested had higher titers.

- isohemagglutinin titres (anti-A and anti-B) ranged from 1:2 to 1:64 in the IVIG products

Kahwaji et al. 2009
Journal American Society of Nephrology

Table 3. Hemolysis by specific IVIG product and blood type (2007–2008)

	Gamunex (n = 139)	Privigen (n = 5)	Gammagard[a] (n = 10)	Carimune (n = 71)
Total hemolysis	11 (8%)	1 (20%)	1 (10%)	0
Blood type				
A	8 (6%)	1 (20%)	1 (10%)	0
B	3 (2%)	0	0	0
AB	0	0	0	0
No hemolysis	128 (92%)	4 (80%)	9 (90%)	71 (100%)

[a]Gammagard liquid.

IVIG-Induced Hemolytic Reactions Reported to the Nova Scotia Provincial Blood Coordinating Program

- April 1, 2008 to March 31, 2011:
  - 11 hemolytic reactions secondary to IVIG therapy
  - Privigen (5 cases)
  - Gamunex (3 cases)
  - IVIGnex (2 cases)
  - Sandoglobin (1 case)

- 4 cases of IVIG-associated hemolysis due to Privigen were reported between January 1, 2011 and March 31, 2011
  - all patients were blood group A or AB

IVIG-Associated Hemolysis Key Points

- must be aware of the potential hemolytic complications of IVIG, especially when immunomodulatory doses are given (1-2 g/kg) to patients with non-O blood groups
- if hemolysis is diagnosed, change IVIG lot number or discontinue IVIG therapy
- if transfusion for symptomatic anemia is required, give group O RBC if anti-A or anti-B is present in a patient's plasma or the eluate
- must report these transfusion reactions appropriately

Thank you

Questions or comments?