Antibody Investigation Case Studies

BCSLS
30 September 2010
Sidney, BC

Kathy O’Shea – Patient Services
CBS / BC & Yukon
Patient Services Laboratory at Canadian Blood Services in Vancouver

Antibody Investigation

- hospital referral cases
- prenatal antibody cases
- platelet / HLA antibody cases
Two cases seen in reference laboratory

**Case 1**
Hemolytic Disease of the Fetus and Newborn

**Case 2**
Multiple Antibodies in a Surgical Patient
Case Study – HDFN

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30 September 2010
Sidney, BC

Kathy O’Shea – Patient Services
CBS / BC & Yukon
Patient Services reference laboratory received samples from large teaching hospital, Vancouver area

“Query antibody to low-incidence antigen”

Hospital’s results on mother’s sample:
- O Positive
- antibody screen negative
Baby girl born with severe HDFN
Pathologist: “Baby was flat”

Full term infant
Birth weight 2790 grams
(Within normal reference range)
Hemoglobin 76 g/L
Reference range 145 - 225 g/L (for infants 0-3 days old)

pH decreased,
Bilirubin 248 µmol/L
Spherocytes were seen on blood smear

Treated for sepsis but culture was negative
Baby required a double exchange transfusion

Received 2 units of O Negative, leukocytes reduced (LR) Irradiated red cells
Hospital’s results on baby’s sample:

- B Positive
- direct antiglobulin test 1+ with anti-IgG AHG reagent

Samples from mother and baby sent to CBS Patient Services reference laboratory in Vancouver
Mother: Mrs A-O, 41 years old

Gravida 6, para 2
She had history of multiple pregnancy losses

This pregnancy was result of in vitro fertilization (IVF)

Began as a twin pregnancy, one twin died early in pregnancy
CBS test results

Mother
O Positive, DAT negative
All panel cells negative, PEG antiglobulin method

Tested her plasma for “lows”
Antibodies against low incidence antigens
Mother’s plasma tested against the prevalence antigens: following cells possessing low

Cw, Lu:14, Kpa, Wra, Cob, Dia, V, VS, Jsa, He, Mg, Mta, Sta, Mi III, Mur, Vw, Mia, Bea, Cx, Ew, Goa, FPTT, JAL, Rh32, Rh33, Evans, Targett, Sc:2, SARAH, Ula, Dantu, Rd, Rba

All test cells negative, PEG antiglobulin method
Baby A-O

B Positive
DAT: 1+ with anti-IgG reagent

Baby’s sample very small
- no eluate made at this time

(Rationale: wait until we had some idea of what antibody we were working with.)
Fresh samples received from mother and father

Father was B Positive, DAT negative

Full phenotypes done for mother and father
Mother
D+ C- E- c+ e+; M- N+ S- s+; P1+; K-
Fy(a-b-)  Jk(a+b-)

Father
D+ C- E- c+ e+; M+ N+ S- s+; P1+; K-
Fy(a-b-)  Jk(a+b+)

Phenotype suggests mother and father are black
- both were Ror and Fy(a-b-)

Canadian Blood Services
it's in you to give
New York Blood Center

- enzymes
- ethnicity

Query: is this a “low” found in black population?

Query: do father and baby possess this low and does mother have antibody against it?
Continued to look for low: tested mother’s plasma against additional rare test cells possessing low incidence antigens

**Result**
All test cells were negative using a PEG antiglobulin method

See test worksheet from CBS National Reference Laboratory – list of many lows
# CBS National Reference Laboratory

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<th>Antigen</th>
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<td>Rh40 (Tar)</td>
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<td>Di^a</td>
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<td>Rh43 (Crawford)</td>
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<td>Rh48 (Jal)</td>
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Is there any chance that we could be looking at ABO incompatibility?

Mother:  O Pos  
Father:   B Pos  
Baby:     B Pos
“With the widespread use of RhIG, ABO incompatibility has become the most common cause of HDFN.”

“Despite the prevalence of ABO HDFN, destruction of fetal red cells only rarely leads to severe anemia, probably because fetal ABO antigens are poorly developed and antibody is neutralized by tissue and soluble antigens.”
“After birth, hyperbilirubinemia generally can be successfully treated with phototherapy; rarely, exchange transfusion may be required.”

“IgG ABO antibodies of high titer are most frequent in group O mothers. Therefore, group A and B infants of group O mothers are more severely affected”
“In populations of European ethnicity, group A infants are most commonly affected; in those of African ethnicity, group B infants are likely to be affected.”

“The overall incidence of ABO HDFN is higher in populations of African ancestry than in those of European ancestry.”
Titre mother’s plasma for anti-A and anti-B

- plasma vs. A1 cell: 256
- plasma vs. B cell: 1024

Method: SIAT, 37 °C, 30 minutes, anti-IgG AHG reagent, commercial reagent red cells (Immucor)

Are these values high?
Tested 6 random group O prenatal samples vs. B cells

- prenatal #1: 256
- prenatal #2: 256
- prenatal #3: 64
- prenatal #4: 32
- prenatal #5: 16
- prenatal #6: 16
- Mrs A-O: 1024

Method: Prewarm SIAT, 37 C, 30 min
“In Whites, titres of anti-A are often higher than that of anti-B.”
“In one study anti-B levels in group A and group O people were found to be comparable” Thomsen et al. 1929

“In another, anti-B was found to be higher in group O than in group A persons” Ichikawa 1959
In Blacks, anti-A and anti-B levels are generally higher than in Whites, and anti-B levels often equal those of anti-A”

Grundbacher 1976
“In studying Nigerian blood donors, Worlledge et al. found that anti-A agglutinin levels often exceed those of anti-B, but that hemolysins were more common among the anti-B.”

Worlledge et al. 1974
Titration values are difficult to interpret

The “normal ranges” of titres of anti-A and anti-B are in the order of 8 to 2048, and 8 to 256 respectively.

However some normal healthy individuals have titres higher than those listed.

Mollison, Engelfriet and Contreras, 1993
Call to hospital for ethnic origin of mother and father

Both were Nigerian
Baby: B Positive     DAT: 1+ with anti-IgG

Eluate
B cell 1   3+
B cell 2   3+
B cell 3   3+
O cell 1   0
O cell 2   0
O cell 3   0
O cell 4   0
Case Reports

“ABO incompatibility due to immunoglobulin G anti-B antibodies presenting with severe fetal anemia”

Ziprin, et al. Transfusion Medicine, 2005
Case #1

30 yr old Ghanian lady, O Negative
Baby’s father B Positive, twin pregnancy

Previous pregnancy: infant death due to HDFN
Mother’s postpartum anti-B titre: 1024
Both twins showed increased velocity in MCA Doppler

Twin 1 (male), B Positive - needed intrauterine transfusion, transfusion at birth plus 2 top-up transfusions

Twin 2 (female), B Positive – needed intrauterine transfusion and transfusion at birth
Case # 2

Nigerian woman with sickle cell disease
She was O Positive

1 previous miscarriage

Mother needed exchange transfusion due to sickle cell crisis
Spontaneous delivery of B Positive baby with HDFN

Needed transfusion at birth and another transfusion at day 6

Mother’s anti-B titre: 32,768
In Summary

Severe HDFN can be caused by ABO discrepancy between mother and baby

Especially, high titre anti-B in black population
Question

How to deal with future pregnancy?

- letter to patient’s doctor
- communication to patient
- wallet card for patient
- recommend referral to high-risk fetal assessment program
Acknowledgements

Kelly Bizovie, Laila Robinson
Royal Columbian Hospital

Gail Stillwell, Lhevinne Ciurcovich
CBS / BC & Yukon
Questions / Comments / Suggestions
Case Study
Multiple Antibodies in a Surgical Patient

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Kathy O’Shea – Patient Services
CBS / BC & Yukon
Started with phone call from large hospital

Patient needs heart surgery in very near future

Patient has multiple antibodies

Will need 8 – 10 units of blood on hand

(May transfer patient to another hospital!)
This patient had a wallet card stating her antibodies and full phenotype.

**Phenotype**

D+  C+  E-  c+  e+  M+  N+  S+  s-  Le(a-b-)

P1+  K-  Fy(a+b+)  Jk(a-b+)
Female patient, 67 years

Her blood group was A Positive

She had the following antibodies:

- anti-E
- anti-Cw
- anti-K
- anti-Jka
- anti-s
Letter from cardiovascular surgeon:

“She requires a third time redo cardiac procedure, including mitral valve repair or replacement, tricuspid replacement, and ascending aortic aneurysm resection. This is a formidable undertaking in view of her transfusion problems.”
“I expect that I would have to have 10 units of blood available prior to undertaking such an operation. I wonder if you could see her and advise me of possible strategies”
What’s going through our minds?

How can we provide blood for this patient?

Plan A           Plan B          Plan C

What blood groups can we look at:
A Pos
A Neg
O Pos
O Neg
What do we know about these antibodies? Are they clinically significant?

- anti-E: Yes
- anti-Cw: Yes
- anti-K: Yes
- anti-Jka: Yes
- anti-s: Yes
How easy will it be to find antigen negative units?

- anti-E 70% donors compatible
- anti-Cw 99%
- anti-K 91%
- anti-Jka 23%
- anti-s 12%

Calculation: 1.7 units per 100 tested
Plan A
Gather as much blood as possible

Blood in Vancouver – screen liquid supply

Units from other CBS Centres across Canada

Liquid units, frozen units
Sent a request to all CBS Blood Centres across Canada

Response
- 1 unit from CBS Saskatoon
- 1 from CBS Hamilton
- 2 from CBS Winnipeg

Also – 2 units frozen, could be shipped to Vancouver in frozen state
Frozen blood - keep in mind

- it takes 1 hour to deglycerolize blood, so about 2 hours before gets to hospital

- once blood is thawed, it expires in 24 hours
One idea: start by transfusing deglycerolized blood

Use at beginning of surgery saves the liquid units for a later (less predictable) time.

- urgent situation
- post operative use
- during evening or weekend
Plan B

Consider “dropping” the requirement for antigen negative blood for one or more of the antibodies

This is always a medical decision made by hematopathologist and surgeon.
Look at a recent antibody investigation to determine

- which antibodies are presently reacting
- reaction strength of each antibody
Information from hospital

- anti-E: 1+ reaction strength
- anti-Cw: N/A
- anti-K: 2+
- anti-Jka: 0
- anti-s: weak / 1+
Considerations

Anti-Jka (Kidd system) known to cause intravascular hemolytic transfusion reactions

Clinical significance of anti-s
- transfusion reaction: no to mild
- HDFN: no to severe

Plan C

If patient bleeding profusely in OR, consider transfusing antigen positive blood

Return to antigen negative blood once patient stabilizes

This is always a medical decision made by hematopathologist and surgeon.
Problem: when is surgical date?

Hospital: “no date for OR yet, waiting to hear from CBS as to availability of units”

CBS: “we are stockpiling units, but exact number of units available depends on surgical date”
Patient received 2 doses of EPO while she waited

Finally – surgical date was chosen
Pre-op hemoglobin was 119 g/L
12 liquid units sent to hospital
(2 were deglycerolized)
Antigen negative for all 5 antibodies
Additional units standing by at CBS

7 liquid and 15 frozen

18 more: E- Cw- K- Jk(a-) but s+
Surgery was successful

Patient was stable

Total use

  9 RBCs (including 2 deglyc’d)
  15 platelets
  10 frozen plasma
  10 cryoprecipitate
In summary

Nothing special about this case

Exercise in practical considerations when patient has multiple antibodies

Importance of communication between hospital and blood supplier
Questions / Comments / Suggestions