Cord Blood Testing and HDFN
A Case Study

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Background

- KU Hospital policy: run ABO/Rh (front) type + Direct Antiglobulin test on cord bloods for neonates born to mothers who are:
  - Type O
  - Rh Negative
  - Have a history of a positive antibody screen for a clinically significant antibody
  - At the request of a practitioner/clinician
  - Policies vary by institution

Neonatal Hyperbilirubinemia

- AKA Neonatal jaundice
- Multiple possible pathways, predominantly (physiologically) either:
  - Increased bilirubin production (typically due to shortened RBC lifespan / intravascular hemolysis), OR
  - Decreased bilirubin clearance – more common in pre-term infants due to liver maturation
- Focus of our interest in transfusion service is ABO/Rh Incompatibility

Why do the DAT?

- About 2.6% of cord bloods have a positive DAT
- When there is an ABO incompatibility between mother and neonate, there is a large increase in likelihood of a positive neonatal/cord blood DAT
  - About 23% of cases where mother's type is O and newborn's type is A
  - About 13% of cases where mother's type is O and newborn's type is B
- (Valsami, Politou, Boutsikou, Briana, Papatesta, Malamitsi-Puchner, 2015).

Why do the DAT - continued

- Positive DAT isn't necessarily diagnostic of impending hyperbilirubinemia / jaundice.
- Possible (and obvious) increase in diagnostic value when considering an elevated bilirubin in conjunction with an ABO incompatibility.
- Still no direct guideline for specific testing algorithm to follow.
- (Peeters, Geerts, Mullem, Micalessi, Saegeman, 2015)

The patient

- Full Term Baby girl born to a mother with some unrelated complications
  - History of tobacco use
  - Pre-eclampsia
  - Type 2 Diabetes Mellitus
- Uncomplicated, healthy delivery.
- Routine hematocrit at birth: 42% (reference range 51-65%)

Mother's Blood Bank Testing

- Solid phase testing
- O Pos
- Negative antibody screen (3 cell)

- KU Policy – cord blood sample has to be run (front type + DAT)

Blood Bank Testing - Baby

ABO/RH	Cell Typing							Reverse Typing			
Interpretation	Phase	α - A	α - B	α - A, B	α - D	α - D	Mono Cont	A1 Cells	B Cells	A2 Cells	Auto Cont
B Pos	IS	0	4+		4+			ND	ND		

Direct Antiglobulin			
Poly	IgG	C3	Saline
ND	1+	ND	

- Blood Type: B Positive
- DAT: 1+ positive in tube
  - Due to ABO/Rh incompatibility

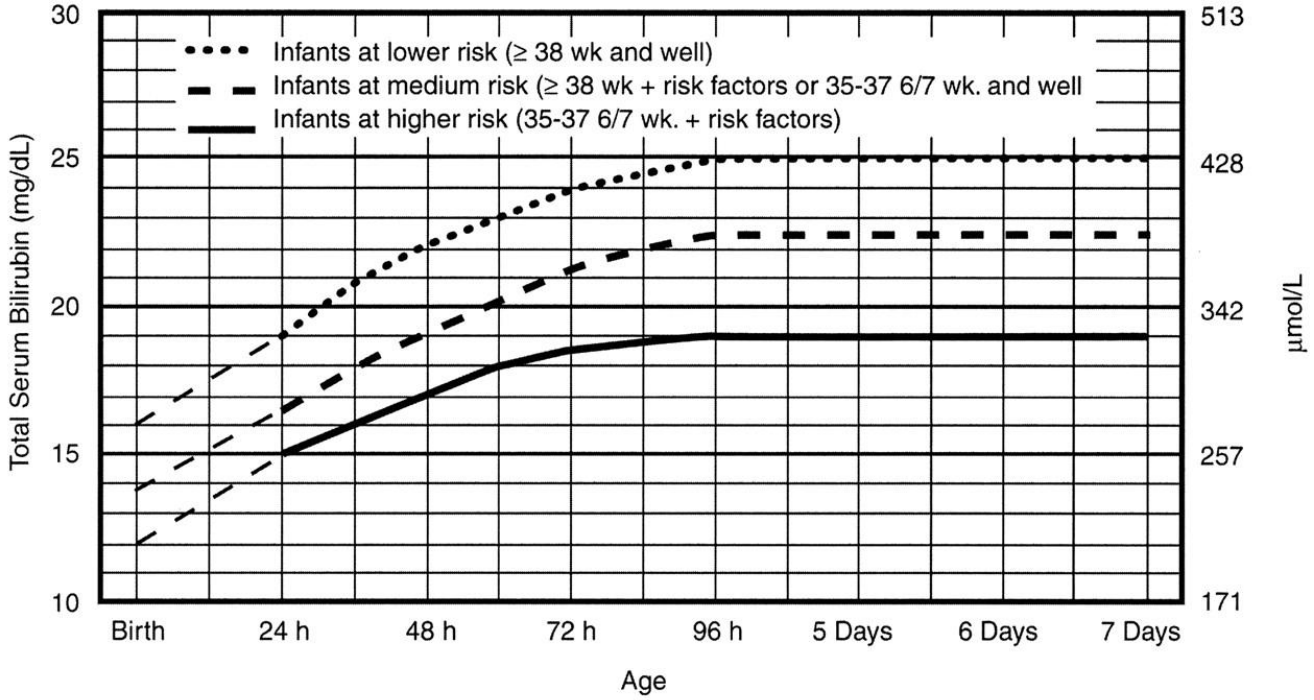
Day 2

- Normal behavior
- Slight feeding difficulties
- Jaundiced appearance, somewhat agitated demeanor
- Fairly unremarkable physical examination
- Total Bilirubin at 0935: 21.3 mg/dL, considered critically high (ref range for newborn = <8.0 mg/dL, anything ≥18.0 is considered critically high)
  - Panic value: close to indicated level for exchange transfusion of neonate by patient's gestational age.

Following up…

- Total Bilirubin at 0935: 21.3 mg/dL, considered critically high (ref range for newborn = <8.0 mg/dL)
- Re-check at 1046 (baby ~36 hours old): 20.7 mg/dL
- Re-check at noon, 20.8 mg/dL
  - Hemoglobin 12.2 g/dL (normal 17.5 – 22.5 g/dL)
  - Hematocrit 36.1% - ~6% decreased from previous day
- Repeat sample (Type and Screen) drawn, DAT repeated on Type and Screen sample
  - Same results

American Academy of Pediatrics guideline for exchange transfusion



- The dashed lines for the first 24 hours indicate uncertainty due to a wide range of clinical circumstances and a range of responses to phototherapy.
- Immediate exchange transfusion is recommended if infant shows signs of acute bilirubin encephalopathy (hypertonia, arching, retrocollis, opisthotonos, fever, high pitched cry) or if TSB is ≥5 mg/dL (85 µmol/L) above these lines.
- Risk factors - isoimmune hemolytic disease, G6PD deficiency, asphyxia, significant lethargy, temperature instability, sepsis, acidosis.
- Measure serum albumin and calculate B/A ratio (See legend)
- Use total bilirubin. Do not subtract direct reacting or conjugated bilirubin
- If infant is well and 35-37 6/7 wk (median risk) can individualize TSB levels for exchange based on actual gestational age.

Source: AAP July 2004

ELUATE

Antibody Detection Eluate						
Cell	Echo	Neo	Gel	L.W.	Eluate	
I				0	0	
II				0	0	
III						
AC						

Antibody Identification

Source Lot #	Phase: Eluate Gel					Source Lot #
A. Cells	0					
B Cells	3+					

Screening cells came back negative – so tested with A1 and B cells.

Determines antibody specificity against B cells alone (true anti-B).

Clinical Decision

- Initiate IVIg and Phototherapy
- Test Bilirubin to monitor
- Perform exchange transfusion if AAP Threshold exceeded
  - Blood bank was ready for exchange transfusion
- Repeated neurological examinations

Exchange Transfusion Guidelines

- RBCs <5-7 days old
  - CPDA-1 additive
  - Hgb S negative
  - Sometimes CMV negative is indicated
- Irradiated (just prior to transfusion, preferably)
- Unit hematocrit should be ~45-60%, reconstituted as whole blood with ABO compatible plasma (replaces clotting factors lost)

- *Source: AABB Technical Manual, 18th edition, Chapter 23, pages 579-580, 2014*

Exchange Transfusion Cont.

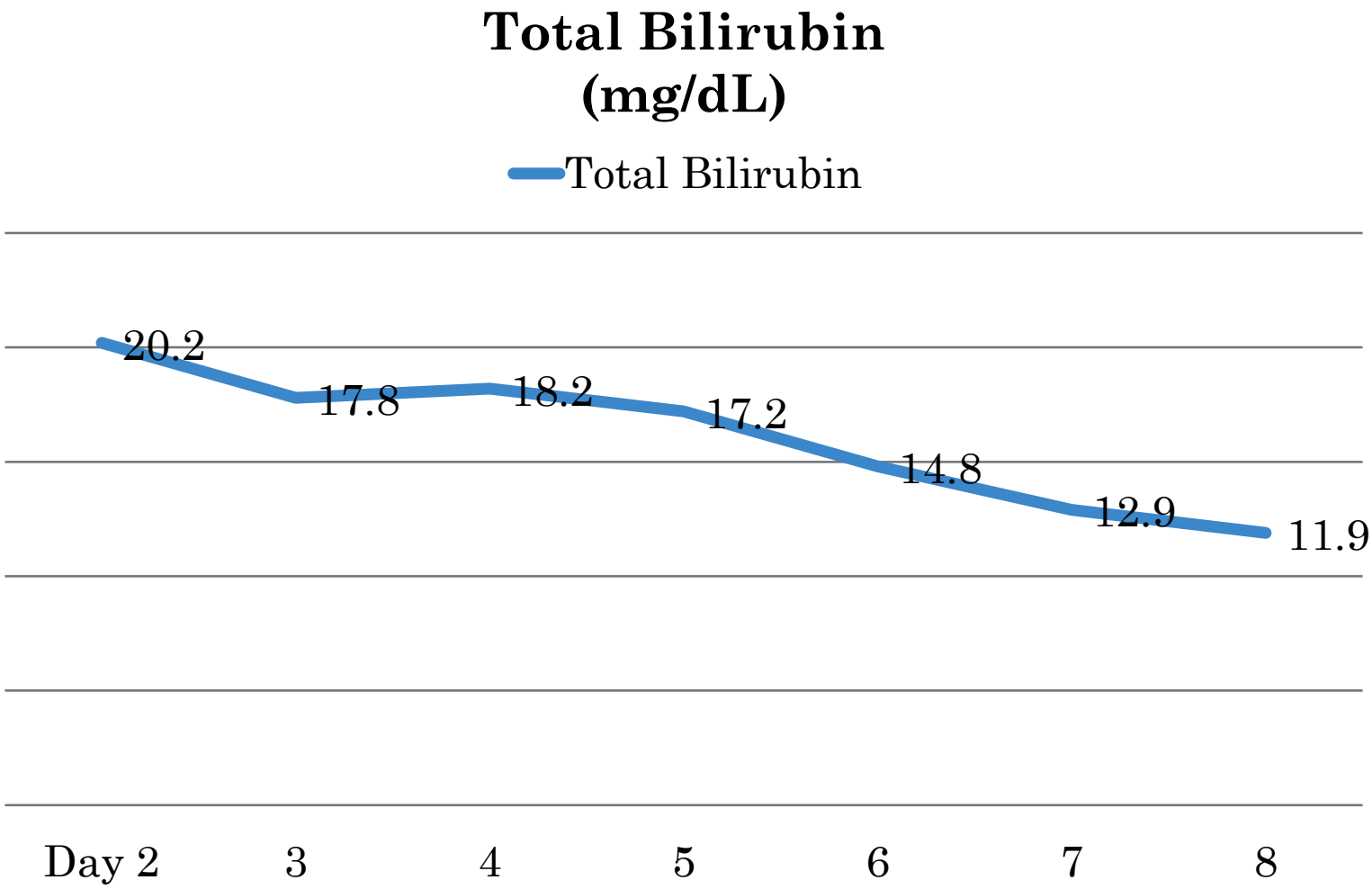
- Hospital specific policy: same as AABB guidelines, except desired hematocrit of unit is 50-60%.
- Simple C1V1=C2V2 equation.
  - (initial volume)(initial HCT) = (final volume)(desired HCT)
- Aliquot made and tested in Hematology to get original and reconstituted HCT.
- End product resembles whole blood

- Viewed as last-ditch effort (rarely performed).

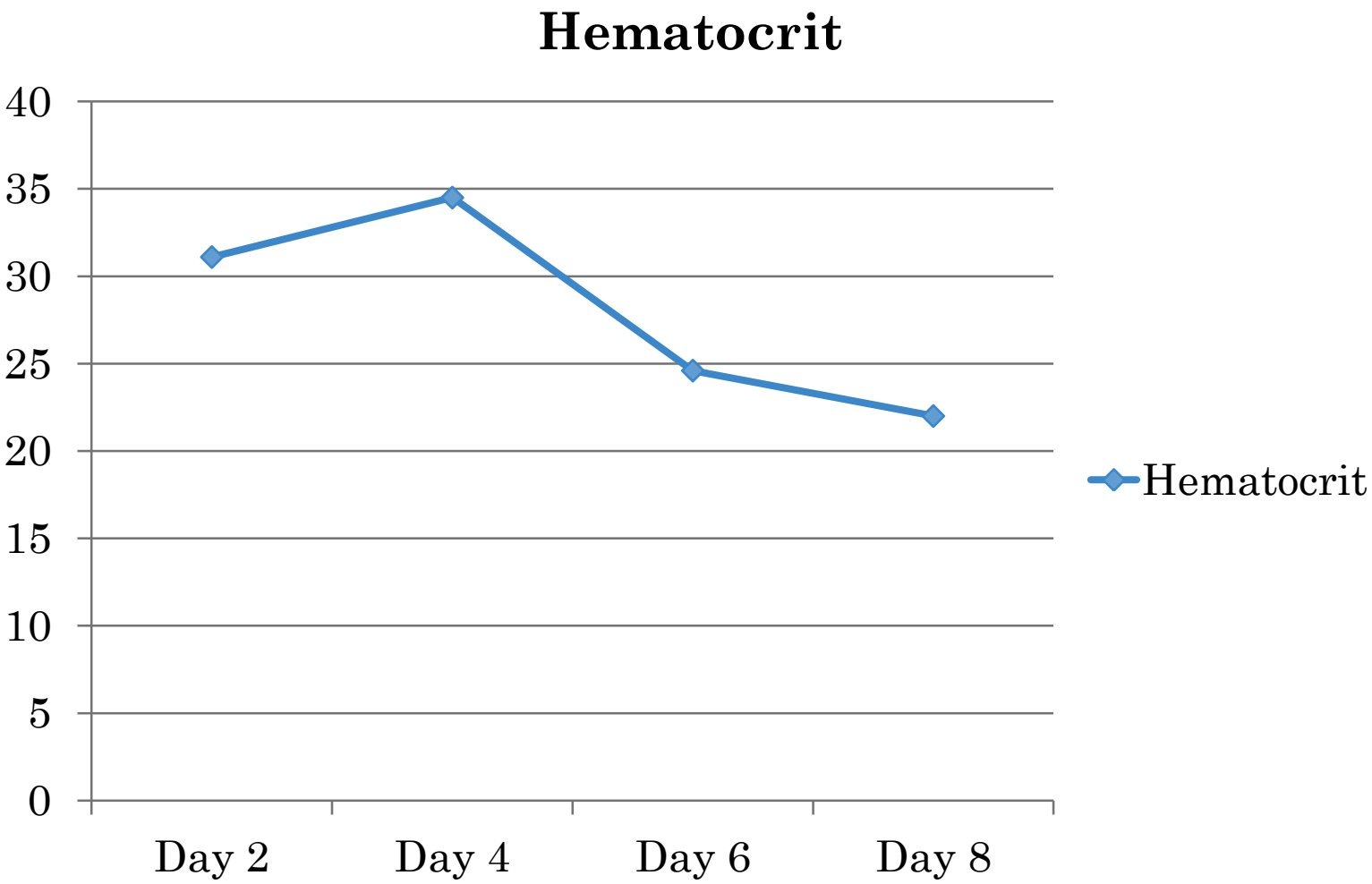
Patient's course of treatment

- Remaining day 1 labs (after initiation of therapy)
  - 1500: 20.0 mg/dL
  - 1900: 20.4 mg/dL
  - 2300: 18.1 mg/dL
    - Hemoglobin 10.9 g/dL (normal 17.5 – 22.5 g/dL)
    - Hematocrit 31.1%
- Average bilirubin declining significantly (see graph on next slide)

Average Daily Total Bilirubin, days 2-8



Improvement on total bilirubin, but…



Patient Outcome

- Labs slowed down to conserve blood:
- Day 10: Total Bilirubin: 12.8 mg/dL (no supportive care – last inpatient level 12.2 mg/dL on day 8)
- Day 12: Total Bilirubin: 9.6 g/dL
- Patient discharged to home with outpatient follow up
- Day 22: Hematocrit 27.1% (up from 22.9% on Day 12)
- Day 27: Hematocrit 25.6%, Total Bilirubin 0.9 mg/dL (within normal limits)

Patient Outcome, Cont.

- Baby at home
- Recovery from anemia slowed due to feeding mechanism defect + normal growth & dip in hemoglobin/hematocrit.
- If needed, we would transfuse the baby with a CPDA unit (KU keeps stock units for neonates as needed, and can designate a unit for a neonate if necessary).

Blood Bank / Lab Role

- This case is a good argument for utilizing cord blood screening – early detection of ABO incompatibility could trigger a need for enhanced surveillance and laboratory analysis
- Some thought that mother's titer could be important.
  - Small cohort study in which a titer of $\geq$ 512 for anti-A or anti-B was considered significantly high risk (90% sensitivity, 72% specificity for predicting need for therapy).
  - (Bakkeheim, Bergerud, Schmidt-Melbye, Akkok, Liestol, Fugelseth, Linemann, 2009).

References

- Bakkeheim, E., Bergerud, U., Schmidt-Melbye, A., Akkok, C. A., Liestol, K., Fugelseth, D., & Lindemann, R. (2009). Maternal IgG anti-A and anti-B titres predict outcome in ABO-incompatibility in the neonate. *Acta Paediatrica*. doi:10.1111
- Peeters, B., Geerts, I., Van Mullem, M., Micalessi, I., Saegeman, V., & Moerman, J. (2016). Post-test probability for neonatal hyperbilirubinemia based on umbilical cord bilirubin, direct antiglobulin test, and ABO compatibility results. *European Journal of Pediatrics*.
- Neonatal Transfusion Guidelines. (2014). In *Technical Manual* (18th ed., pp. 579-580). AABB.
- Valsami, S., Politou, M., Boutsikou, T., Briana, D., Papatesta, M., & Malamitsi-Puchner, A. (2015). Importance of Direct Antiglobulin Test (DAT) in Cord Blood: Causes of DAT ( ) in a Cohort Study. *Pediatrics & Neonatology,* (56), 256-260.

Questions?

- Special thanks to the Blood Bank staff at KU Hospital and our manager, Laurie Wolf, for allowing me time to research this case study.