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Prenatal Monitoring Strategies for Lower-risk RBC Alloantibodies

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Faculty Disclosure

In compliance with CPD policy, Temerty Faculty of Medicine requires the following disclosures to the session audience

- This program has received no financial external support

- I have no potential conflicts of interest relevant to this talk

Objectives

- Discuss a plan to follow patients with alloantibodies less likely to cause HDN (e.g., M, N, Colton B, autoantibodies, inconclusive)
- Discuss suggestions for titration frequency for these antibodies
- Discuss titration and frequency if the antibody titer has been critical in a prior pregnancy, but the fetus was not affected by HDFN
- Review special laboratory techniques that might assist in assessment of clinical significance (e.g., DTT treatment of plasma)

Role of Serologic Monitoring

Serologically performed via titration to measure antibody strength and screen for pregnancies that require further non-serologic monitoring to assess for anemia (e.g., potential need for IUT)

- E.g., Identify cases where **fetus** is at risk (+/- neonate)
- Titre does not correlate well with HDFN severity

Some antibodies rarely or never require intrauterine intervention – but may cause mild HDN

- Due to antibody characteristics and timing of fetal expression
- Differing opinions on whether frequent serial titration is necessary in these cases

Case

32 year old South Asian female G2P1

Initial group & screen (12 weeks GA)

- O positive
- Newly identified anti-M in solid phase; titre 16
- No antibodies detected during 1st pregnancy

Defining Risk

1. What is the partner's antigen phenotype / zygosity?
   - (i.e., could the fetus express the cognate antigen)

2. What is the antibody specificity? Is it known to cause HDFN and/or require antenatal fetal intervention?

Most cases of fetal anemia are related to anti-D, -K or antibodies in combination with anti-D or -K.

- Few cases related to anti-E or -c.
- Very rare for lower risk non-Rh and non-K antibodies

Canadian Data

CBS Edmonton (2006-2010)
552 prenatal antibody cases

- 93 Jk/Fy (16.8%) antibodies

Table 4. Critical titres by antibody

Antibody	Severe fetal outcome*	Severe neonatal outcome*
Anti-D	64	8
Anti-C	—†	64
Anti-c	—	128
Anti-E	16	16
Anti-e	—	—
Anti-Fy^{a}	16	32
Anti-Fy^{b}	—	—
Anti-Jk^{a}	—	—
Anti-Jk^{b}	—	—

Severe fetal outcome indicated by IUT, maternal plasmapheresis or IVIg, intrauterine fetal death due to HDFN or delivery ≤ 32 weeks due to HDFN

Zwingerman et al. JOGC 2015;37:784-790

Canadian Data

MSH (1991 – 2014)
246 IUT cases

Variable	Mean/*n* (%)
Primary antibody	
D	188 (81.0)
Kell	32 (13.8)
Other	12 (5.2)

Snelgrove et al. Fetal Diagn Ther 2019;46:425-432

SBK (2010 – 2017)
128 alloimmunized pregnancies

	Cognate antigen positive			
	***Single* antibody**			
	D	**K**	**Other Rh antibody**	**Other alloantibody**
Mothers: (N, % 128 mothers)	16 (13)	2 (2)	25 (20)	11 (9)
Routine bloodwork only (n, %)	1 (6)	0 (0)	3 (12)	3 (27)
# Mothers with titration testing performed (n, %)	14 (88)	2 (100)	21 (84)	10 (91)
# Of titers/pregnancy (mean)[b]	1.4	0	3.0	1.2
Titer strength (median, IQR)[b]	128 (16-512)	N/A	32 (8-64)	16 (4-32)
Maximum titer >32 (n, %)[b]	13 (93)	0	11 (52)	2 (20)
Mothers with ultrasound (non-doppler) performed during pregnancy (n, %)	0	1 (50)	8 (32)	0
Mothers with Doppler ultrasound performed during pregnancy (n, %)	14 (88)	1 (50)	14 (56)	8 (73)
No. of Dopplers/patient (median, IQR)[c]	6 (3-13)	7 (0-13)	2 (0-6)	1 (0-6)
Abnormal MCA Doppler ultrasound (n, %)[c]	1 (7)	1 (100)	3 (21)	0
Intrauterine transfusion, (n, %)	0	0	0	0

Lieberman et al. Transfusion 2020;60:2537-2546

International Data

Country	Cases	Result
Netherlands Koelewijin. Transfusion 2008;48:941	1279 pregnancies with non-D Ab capable of causing HDFN → 567 at risk based on partner pheno	No "at risk" patients (n=155) with non-Rh or non-K antibodies required IUT or resulted in stillbirth
Ireland Walsh. Eur J Obstet Gyn 2013;171:235	102 pregnancies requiring 242 IUT from 1996 to 2011	No non-Rh or non-K antibodies implicated
USA Smith. Immunohematology 2013;29:127	264 pregnancies with Ab from 2007-2011	No non-Rh or non-K antibodies (n=37) required IUT* or resulted in stillbirth *2 IUTs included Anti-D in combination with S or Jkb Anti-M second most common Ab
UK Awowole. Eur J Obstet Gyn 2019;237:89	398 pregnancies with Ab from 2011-2016 29 IUTs	No non-Rh or non-K antibodies (n=190) required IUT or resulted in stillbirth Anti-M second most common Ab
China Li. BMC Preg and Child 2020;20:539	268 pregnancies with Ab from 2005-2019 → 92 IUTs	9 cases of fetal anemia (causing death or requiring IUT) → **7 anti-M, 2 -Mur**
Sweden Liu. Acta Obs Gyn Scand 2021;100:2216	1079 pregnancies at risk for HDFN from 1990-2016; 87 IUTs → 204 low risk Abs (excludes Rh, K, Fya, U)	Low risk: **1 case of IUT in anti-M**; no stillbirths Moderate-risk included IUT in **anti-Fya (1), -U (1)**

SUMMARY	**Overall, lower risk antibodies are unlikely to cause fetal anemia** **Rare exceptions do occur → risk of anti-M may be depend on race/ethnicity**

Anti-M

Anti-M rarely causes clinically significant HDFN and likely requires less follow-up if initial titres are low

North America / Netherlands:

- ~**800** cases of anti-M **without** clinically significant HDFN

Stetson et. al. algorithm:

- Critical titre of 64 (32 for all non-M abs)
- If initial titre ≥ 16 → q 4 weeks titres
- If initial titre < 16 → repeat at 28 weeks
  - Check for rapid rise in titre (≥ 32)

Netherlands

- Previously retested anti-M IgM at 24, 30 & 36 weeks for IgG conversion
- IgG conversion never observed

Stetson et al. Am J Perinatol Rep 2017;7:e205
de Haas et al. Vox Sang 2015;109:99

Anti-M can cause severe HDFN and may need to be followed closely in specific populations

> **110** published cases of severe HDFN:

- 104 cases from Asia (China 59, Japan 36)
- 11 other cases from non-Asian countries

- ➢ 21 antenatal intervention (IUT, PLEX, Ig)
- ➢ 9 fetal deaths
- ➢ Most IgG titres ≥ 32; lower titres of 1-16 also observed

Hypothesis: higher frequency of anti-M IgG in Asian populations (up to 80%), although most of the North American cases likely also contained IgG

Yasuda et al. Trans Med Rev 2013; 1
Li et al. Transfusion 2019;59:385
Li et al. Transfusion 2021;61:1908

Anti-M

Suggestions of an IgG component include:

- Reactivity at 37C in IAT with monospecific IgG AHG
  - Reactivity at RT does not rule out IgG, as many anti-M are present in combination IgG + IgM
- Reactivity in solid-phase

Confirmation of an IgG component +/- IgG titres via thiol reagents:

- Dithiothreitol (DTT)
- 2-Mercaptoethanol (2-ME)

Other Antibodies

Autoantibodies (~0.1%)

- Increase autoantibody production during pregnancy
- Two studies (n=142) demonstrated no harm to the pregnant patient or fetus for *pregnancy-induced* autoantibodies

Inconclusive / non-specific

- Depends on method (increased with solid phase: up to 1-2%)
- One study (n=88) did not identify clinical significance
  - 8.5% showed specificity on subsequent testing; 49% self-resolved
- BEST SRUS study underway

Surucu et al. Transfsus Med Hemo 2015;42:325
Hoppe et al. Transfusion 2001;41:1559
Van Winden et al. J Matern Fetal Neonatal Med 2016;29:2848

Back to the Case

Partner phenotyped as M+N- → 100% chance fetus will express the M antigen.

DTT treatment confirms an anti-M IgG titre of 8, which is below our lab-defined critical threshold.

Repeat sample requested in 4 weeks.

What is Optimal?

What is the optimal frequency of antenatal titration for lower risk RBC antibodies?

Several international guidelines exist → level of evidence is low, but collectively may help to inform

Optimal strategy may depend on:

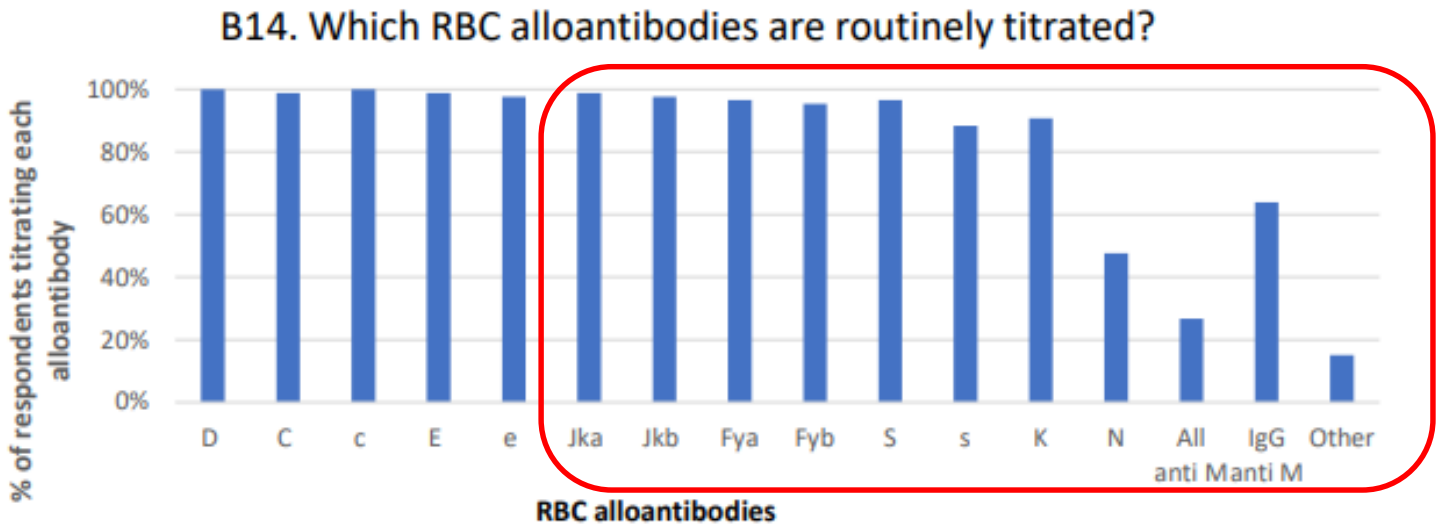
- Antibody factors (e.g., specificity, IgG vs IgM, initial titre, etc.)
- Patient factors (e.g., race/ethnicity)
- Clinician factors (e.g., avoiding overly complex sampling schedules)

International Recommendations

Country	Lower Risk Definition	Monitoring Strategy for Lower Risk
AABB (USA) 2005	Non-Rh antibodies	No recommended frequencies or critical titres due to limited evidence. Suggest differentiating anti-M (IgG vs. IgM).
ANZSBT (Australia & NZ) 2007	Cw, Fyb, Jk, S, s, M, Ge High: Rh, K, Fya	No recommended frequencies or critical titres due to limited evidence.
RCOG / BSCH (UK) 2016	Not anti-D, -K or –c *Rare cases of HFN in E, C, k, Fya, Jka, M, H	First trimester screen with follow-up screen at 28 weeks; critical titre of 32
Sweden 2015	Cw, f, Jk, M, Ss, Fyb, Lu, Kp, Yta, Co, Ge2,3 High/moderate: Rh, K/k, Fya, U	Titration every 8 weeks Critical titre same as other antibodies
ACOG (USA) 2018	N/A	Similar titration frequency (q 2-4 wks) as D Critical titre same as other Abs (8-32)

Canadian Context

Practice varies across Canada (COPTN survey 2018):



Balancing Act

Recheck at 28 weeks

- Reduced cost (lab, result follow up etc.)
- Reduced phlebotomy
- Risk of missing antibody requiring early or late antenatal intervention

Mimic high-risk (q 2-4 wk)

- Increased cost
- Increased phlebotomy
- Unlikely to miss a lower-risk antibody requiring antenatal intervention

Reduced frequency ⟷ Increased frequency

Balancing Act

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- Increased phlebotomy
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Alternative Monitoring

> q 4 wk or antibody/titre-specific

- Contain *at minimum* one extra early & late GA titration (vs. 28 week only)
- *Possibly* differentiate between high vs. low-risk for non-Rh/K antibodies
- Complex algorithm may cause confusion or error

Reduced frequency ← → Increased frequency

Critical Titre

Once critical titre (or 2 tube increase) is reached → fetal anemia assessment via Doppler monitoring should occur

Titre Caveats:

- Role of continued titration once Doppler monitoring has commenced has not been described or recommended
- Titrations have been shown to be unreliable if a past pregnancy has been affected by HDFN
- There is limited evidence if the same is true for low-risk antibodies that previously reached critical titre without HDFN
  - Guidelines remain silent

Moise et al. ACOG 2012;120(5):1132
Webb et al. Transfusion Med Rev 2018;213

Back to the Case

The patient had serial anti-M IgG titrations performed with titres fluctuating between 4 and 8.

The patient delivered a healthy neonate with no evidence of HDFN.

- Neonate was M+N+
- DAT negative

A repeat CBC performed 4 weeks later did not identify any late onset anemia.

Proposed Algorithm