ABO HDFN
Are we doing right?

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Most Common but Given Less Importance

- Common cause of Hemolytic Disease of Newborn (HDN)
- Common cause of neonatal jaundice
- ABO incompatibility is the most common cause of re-admission
- No agreed criteria for the diagnosis

There are unresolved questions:

- Antenatal screening & newborn screening?
- Predictors of clinical severity
- Appropriate serological test
- Significance of a positive DAT

Clinical Spectrum and Morbidity

- Even the first child can be affected
- Characterized by a benign evolution; mild degree of hemolysis
- Anemia is rare with the main clinical problem being jaundice
- Mild to severe; 12-30% develop significant hyperbilirubinemia
- Early detection and treatment; to prevent bilirubin-induced encephalopathy
- Majority managed by phototherapy
- Rarely require exchange transfusion or/and IVIG

Aims and Objectives

- To assess the frequency of ABO HDFN
- To identify neonates at risk of developing severe hyperbilirubinemia
- To assess the predictive value of Immunohematological tests

Our Experience

- Prospective study ; October 2013 to May 2015
- 2856 mothers and their new born
- Clinical Assessment

Mother:

- Obstetric history
- Medical history
- Laboratory investigations

Newborn:

- General Condition
- Signs of HDN
- Treatment received
- Follow up

Laboratory Investigations

Mother's Sample

- ABO & Rh Blood Group
- Antibody Screening
- Antibody titration
- (Anti A, Anti B; IgG)

Newborn Sample

- ABO & Rh Blood Group
- Direct Antiglobulin test
- Elution test

- Other lab tests
- Tests on cord blood
- CBC
- Peripheral smear
- T.Bil & Direct Bil

Frequency of ABO Incompatibility

Race is a risk factor in ABO-HDN

Orzalesi et al; Positive DAT result was more common in Africans than in Caucasians

Toy et al.; Ethnic differences were detectable in the laboratory but not in clinical disease

Population	Authors	Frequency
Asian (Singapore) Pakistan	Han et al Irshad et al Toy et al	3.7% 22.2% 15%
Africans (Nigeria)	Oseni et al Toy et al	38% 17%
Caucasians	Klein et al & Toy et al	20% 14%
Hispanics	Toy et al	12%

ABO Incompatible Pregnancies - 34.34%

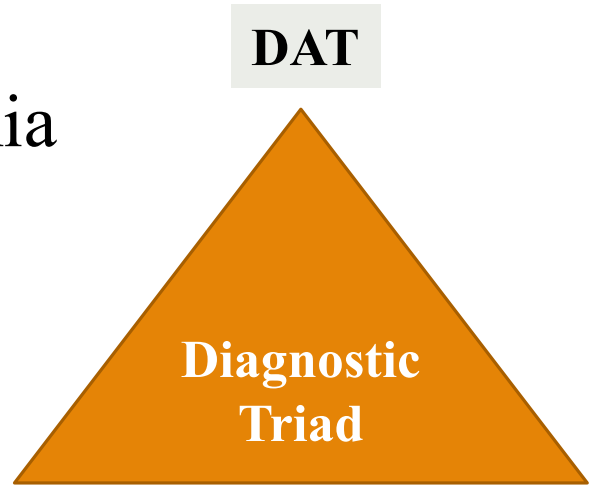
	Mother					
Baby	A	B	AB	O	Oh	Total
A	229	**83 (8.5%)***	30	**372 (37.9%)***	**1 (0.1%)***	715
B	**54 (5.5%)***	303	39	**355 (36.1%)***	-	751
AB	43 (4.4%)	53 (5.4%)	16	-	-	131
O	122	190	6	959	1 (0.1%)*	1278
Total	448	629	91	1686	2	2856

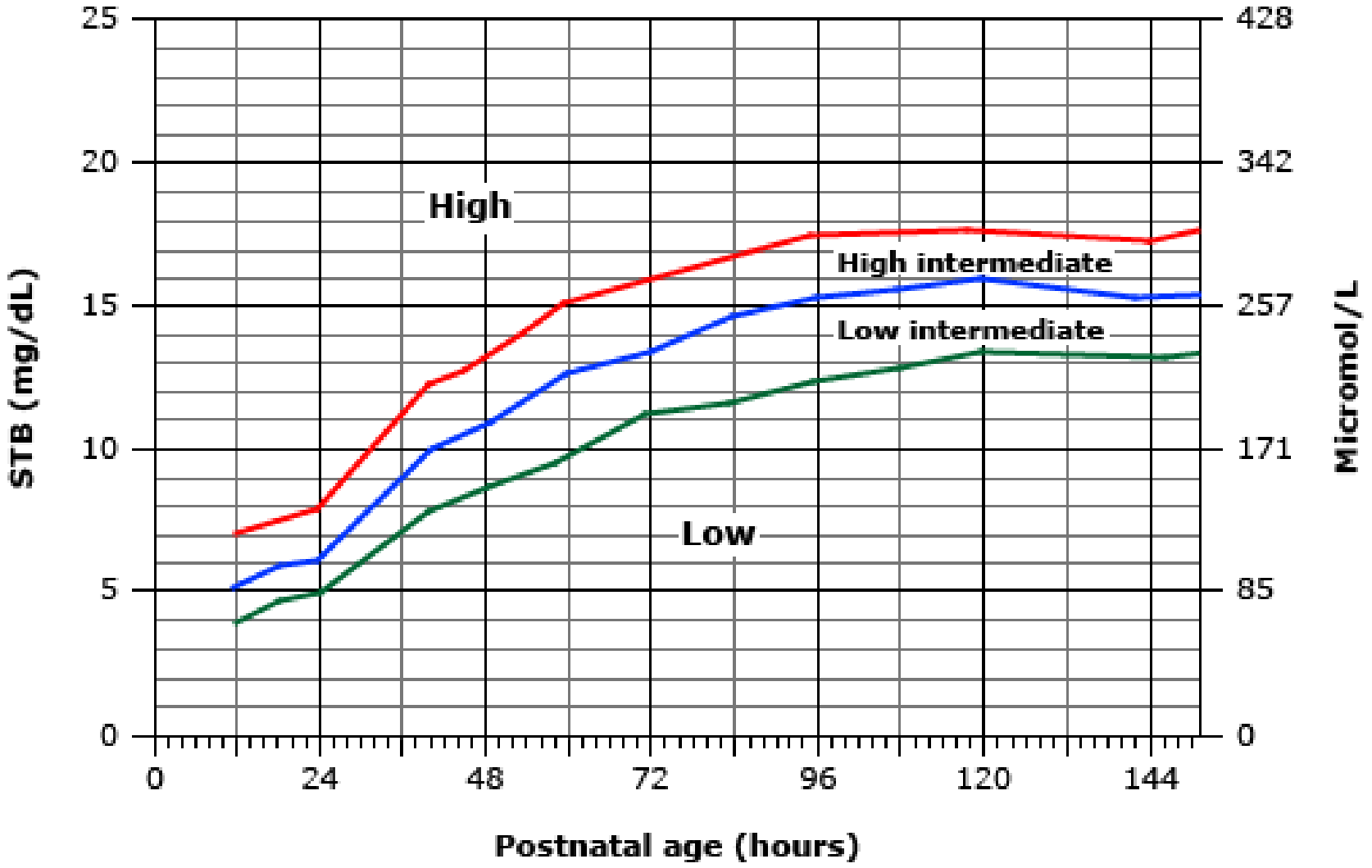
O-A and O-B incompatibility in 13% and 12% of cases respectively

When to Suspect ABO HDN?

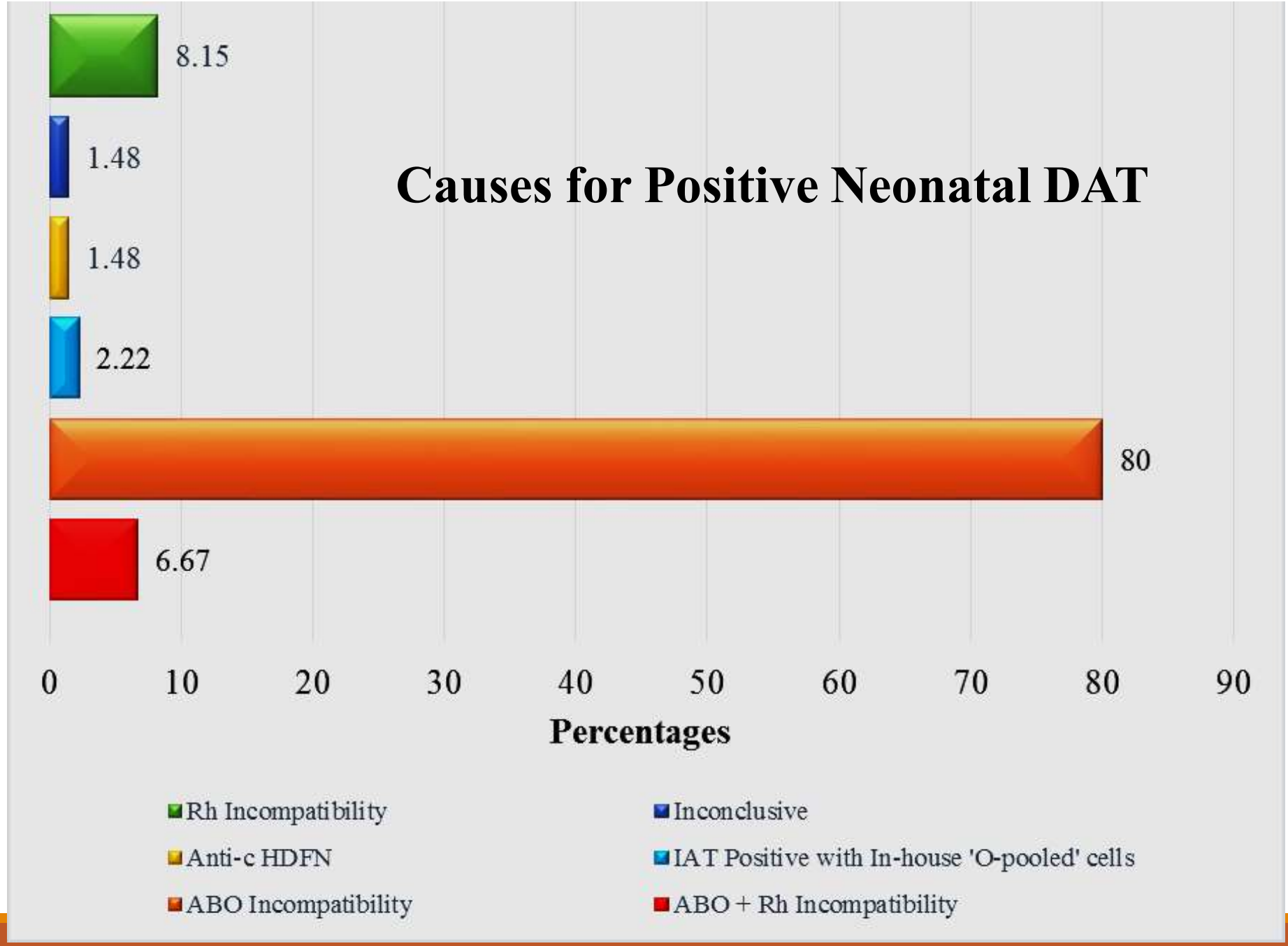
- ✓ Rapidly developing, early-onset hyperbilirubinemia not predicted by maternal antenatal antibody screening
- ✓ Negative antibody screening in mother and positive/negative DAT in the neonate
- ✓ Prolonged hyperbilirubinemia without significant anemia
- ✓ Hemolysis detected on blood film examination

  Large numbers of spherocytes with little or no nRBCs





Significant hyperbilirubinemia is defined as plasma total bilirubin (TB) concentrations in the high risk zone ($\geq 95^{th}$ percentile) as defined by the age specific Bhutani normogram


Causes for Positive Neonatal DAT
8.15
1.48
1.48
2.22
80
6.67
0
10
20
30
40
50
60
70
80
90
Percentages
Rh Incompatibility
Inconclusive
Anti-c HDFN
IAT Positive with In-house 'O-pooled' cells
ABO Incompatibility
ABO + Rh Incompatibility

Direct Antiglobulin Test – Column Agglutination Technique

Blood Group Combinations	DAT Strength							No. of Cases
	Strong Positive			Weak Positive				
	4+	3+	Total	2+	1+	WK+	Total	
O-A (No: 372)	2	13	15 (4%)	37	14	12	63 (17%)	78 (21%)
O-B No:355)	2	-	2 (0.6%)	10	13	4	27 (7.6%)	29 (8.2%)
Oh-A	-	-	-	-	1	-	1	1

Incidence of positive DAT in newborns; 3.8% & among ABO incompatible;14.6%

Direct Antiglobulin Test – Tube Technique

Blood Group Combinations	DAT by Tube	
	Positive	Negative
O-A (No: 372)	15 (4%)	357
O-B No:355)	2 (0.5%)	353
Oh-A	0	0

Incidence of positive DAT in newborns; 0.6% & among ABO incompatible;2.4%

DAT and Clinical Outcome

Clinical Outcome	DAT		P value
	Negative	Positive	
Onset of jaundice (hrs): Median (range)	48 (18-72)	30 (12 – 36)	0.005
Hyperbilirubinemia	3%	35%	<0.001
Duration of Phototherapy (hr)	48(24 – 120)	72 (16 – 216)	0.02

Predictive Value of Direct Antiglobulin Test

	CAT	Tube
Sensitivity	51%	11%
Specificity	93%	98%
Positive Predictive Value	34%	47%
Negative Predictive Value	96%	93%

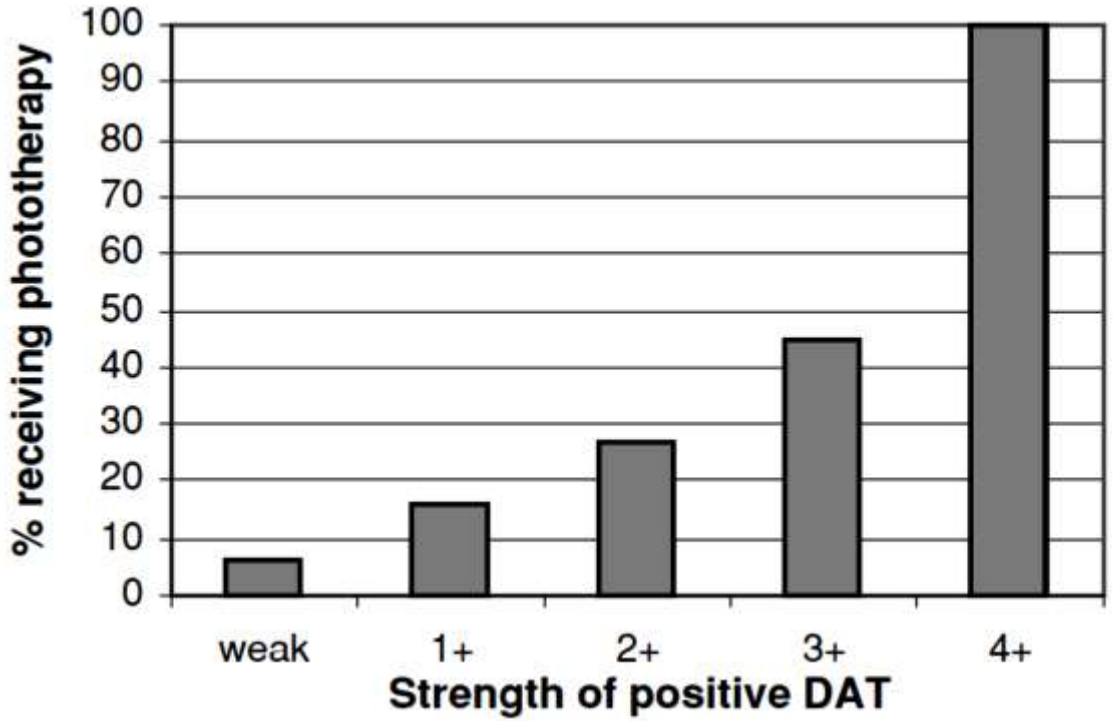
Predictive value of DAT

Positive predictive value of 12%–53% and a sensitivity of 15%–64% for the subsequent development of hyperbilirubinemia

Table 1: Use of the direct antibody test to predict the development of hyperbilirubinemia in newborns

Study	PPV (%)	NPV (%)	Sensitivity	Specificity
Meberg and Johansen[2]	12	96	64	65
Herschel et al[3*]	53	89	15	98
Dinesh[4†]	23	92	15	95

Predictive value of DAT…



- A positive DAT does not confirm ABO HDN
- A negative DAT does not rule out ABO HDN
- Methodology used influences the results and the sensitivity
- Mildly affected newborns – negative by tube
- Sensitive technique will be positive –

  in most ABO incompatible cases

Eluate

DAT Positive	Eluate Positive
Strong positive (4+ & 3+)	18 (100%)

Heat elution – DAT positive samples

Specificity of the antibody was confirmed

Negative DAT does not rule out HDN; When clinical suspicion is high, an eluate should be added following a negative DAT.

Predictive value of an eluate positivity: high

Investigators from Brazil: Quantitative elution, a titer of 1/16 was used as cut-off for the diagnosis

Table 1. *Serum bilirubin concentrations in ABO incompatible and control infants*

Group	*n*	Cord S/Bilirubin (μmol/l) (mean±S.D.)	S/Bilirubin (μmol/l) at 48 hours (mean±S.D.)	S/Bilirubin (μmol/l) at 96 hours (mean±S.D.)
A (DAGT (Coombs) positive)	18	49.9±29.7[a]	163.9±80.8[b, c]	170.6±102.9[b,
B (Eluate positive)	26	32.7± 9.5	112.0±56.7	122.9± 71.8
C (Eluate negative)	27	32.2± 8.8	118.7±49.5	118.7± 59.8
D (Control)	71	32.4± 9.8	114.5±55.5	113.2± 69.2

[a] $p<0.05$. [b] $p<0.01$. [c] Values modified by treatment.

Whyte et al..Scotland (*Acta Pzdiatr Scund 70)*

Table 3. *Effect of antibody status or capillary haemoglobin and reticulocyte counts (4th day)*

	A DAGT (Coombs) positive $n=17$	B Eluate positive $n=22$	C Eluate negative $n=22$	D Control $n=59$
Haemoglobin concentration (g/dl) (mean±S.D.)	17.97±3.24[a, b]	19.85±2.52	20.21±2.85	20.85±2.14
Reticulocyte count (%) (mean±S.D.)	3.05±2.22[a]	2.55±1.65	2.72±1.48	2.25±1.32

[a] $p<0.05$. [b] Includes two pre-exchange samples.

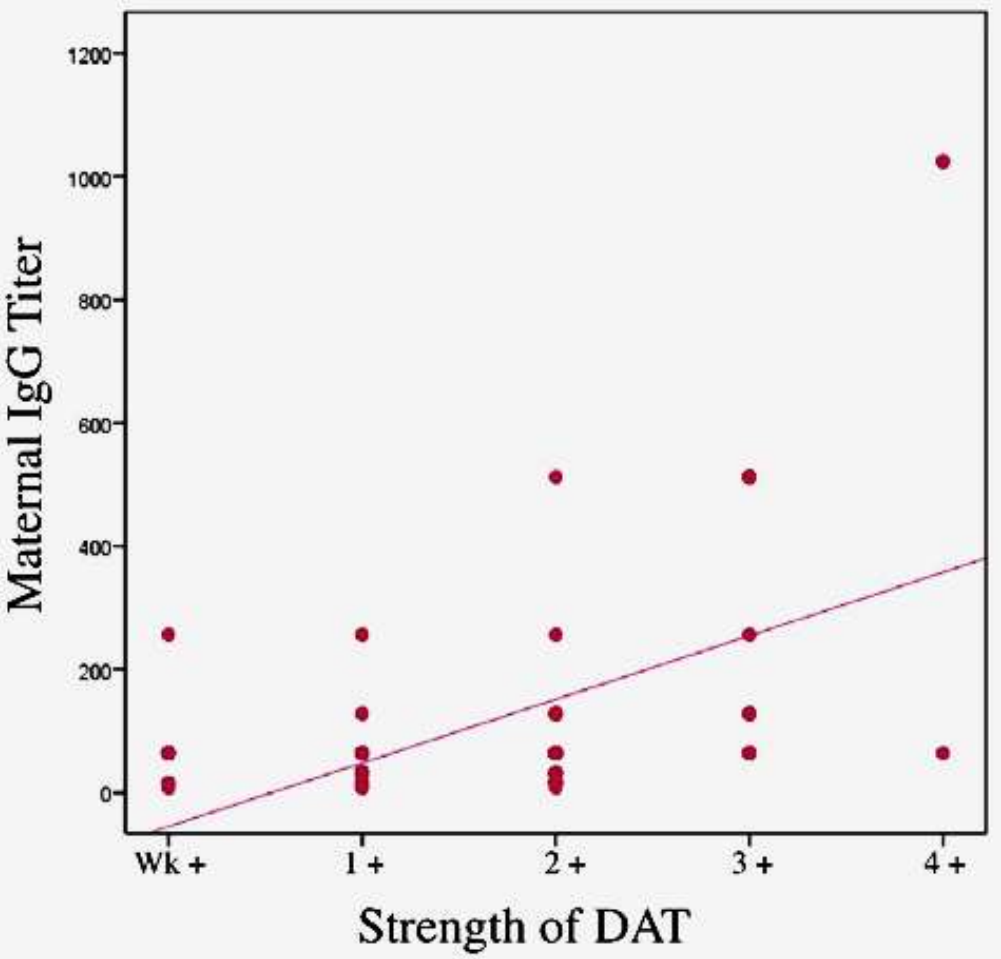
Useful for the early detection of infants requiring special observation and as a means of excluding ABO HDN from the differential diagnoses of heterospecific infants who develop jaundice.

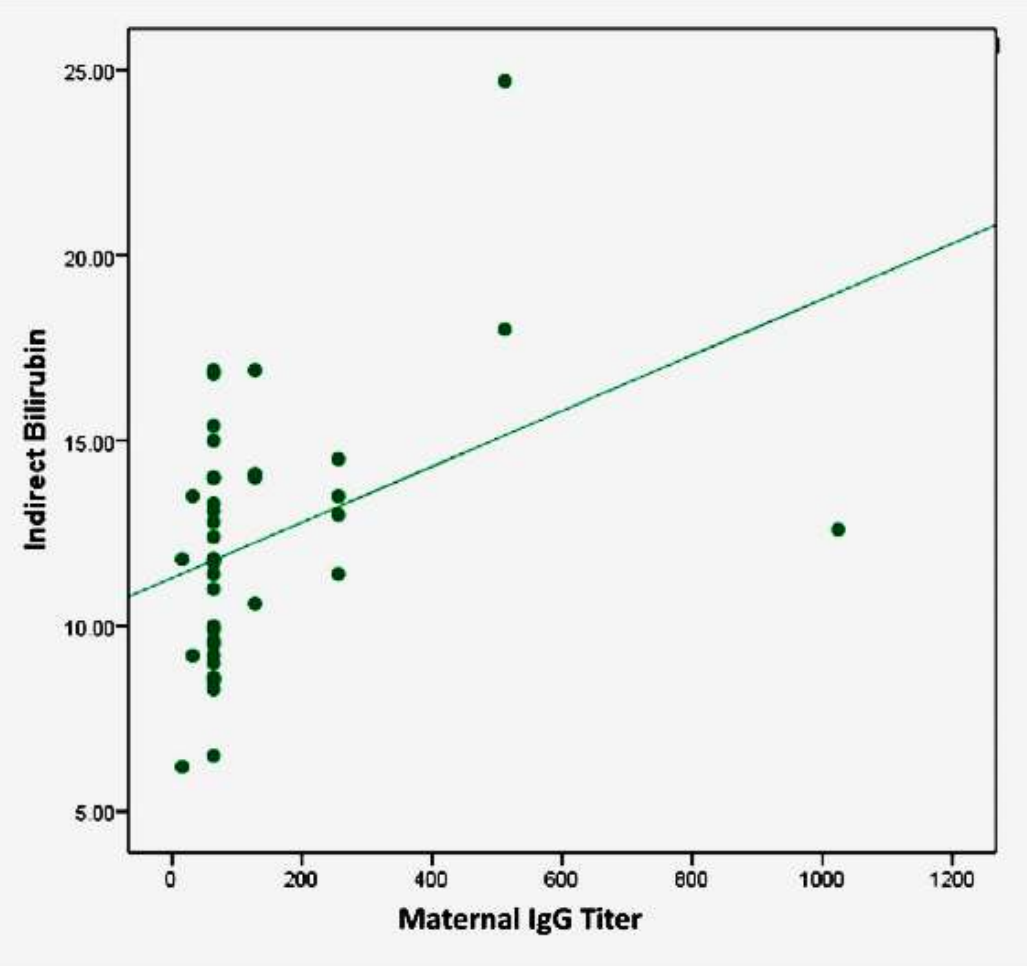
Combination of Tests – Improve Accuracy

Test	Sensitivity	Specificity	Positive predictive accuracy	Negative predictive accuracy
Quantitative elution	65%	90%	65%	90%
Bilirubin Concentration >4mg/dL	56%	90%	69%	85%
DAT – positive	65%	75%	42%	88%
Two positive tests	**77%**	**90%**	**70%**	**93%**

Eur. J of Pediatrics

Maternal Antibody Titer – Critical titer:64





R=0.408 and p-value = <0.001

(R=0.440; p=0.004)

Maternal Antibody Titer

- Titer of anti A is found to be higher compared to anti B (64 vs 32)
- 12% had critical titer or above in O-A group
- 6.7% had critical titer or above in O-B group
- Li et al. (*Fetal and Pediatric Pathology*, Early Online:1–10, 2015): IgG titer above 512 predict the need for the invasive treatment
- Bakkeheim et al; titres >512 had a sensitivity of 90% and a specificity of 72% for predicting immunoglobulin treatment and thus severe hyperbilirubinaemia
- Prenatal measurement of maternal haemolysin activity is time-consuming and of uncertain clinical value

O-A versus O-B Incompatibility

- Frequency of O-A & O-B were comparable
- DAT positivity is higher in O-A incompatibility
- Incidence of significant hyperbilirubinemia is higher in O-A group
- Lab evidence of hemolysis was higher among O-A group (65% Vs 45%)

Cellular Assay : Severity Prediction

The lytic effect of maternal IgG anti-A or anti-B antibodies can be measured in an antibody-dependent cell-mediated cytotoxicity (ADCC) assay

Strongly positive result
(more than 45% lysed target cells)

Correlates with the need for exchange transfusions

Weak or moderate lysis
(more than 10-45% lysed target cells)

Test to detect antigen density

ADCC negative

The Lancet; Brouwers et al

Summary & Conclusion

- ABO –HDN commonest cause of neonatal hypebilirubinemia
- Monitor at risk neonates for hyperbilirubinemia
- DAT alone is an unreliable marker to predict the development of HDN
- Strength of DAT is an important predictor
- Combined use of the DAT with clinical findings, red blood cell indices, and a blood smear would be more beneficial
- DAT should be reserved for diagnostic purposes in children with early or clinically significant hyperbilirubinemia
- Other Immunohematological tests are of questionable value

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Thank you