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U.O.C. Clinica Ginecologica ed Ostetrica
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Isoimmunizzazione materno-fetale

Specializzanda: Dr.ssa Cristiana Nardi

CASO CLINICO

- Femmina M.J. 23 anni
- PARA 0000
- Non eseguiti accertamenti in gravidanza
- 32 sg come U.M.

- Accesso al PS per prurito ⟶ RICOVERO

Ricovero

- EE: Hb 103 g/l, indici di fx epatica e renale nella norma, Sali biliari neg
- Assetto emoglobinico: Delta Talassemia

- Sierologia: HBsAg neg, HCV neg, Lue neg, Rubeo immune, Toxo immune, CMV pregresso, HSV 1-2 recettiva, Parvo IgM e IgG pos,

- Emogruppo: A neg
- TCI: positivo titolo 1:128 Ac irregolari incompleti a specificità anti D.

Ricovero

- Ecografia ostetrica (32+3 sg): DBP 87 mm (36+1sg), CC 322,3 (36+3sg), CA 293 mm (33+3 sg), FL 69,1 mm (35+4 sg).
- Biometria fetale al 92° (?)
- Emodinamica fetale nella norma
- Emodinamica materna con PI medio>95°
- PS MCA medio 64,4 cm/sec Hb fetale calcolata 9,2 g/dL (0,85-0,64 MoM)
- Non versamenti,
  non tachicardia fetale,
  LA regolare,
  placenta posteriore

Ricovero dopo 3 gg

- Parvovirus B19 DNA: non rilevabile
- TCI ripetuto a 3 gg: positivo titolo 1:256 . Ac irregolari incompleti a specificità anti D
- Ecografia ostetrica:
  emodinamica fetale nella norma,
  PS MCA medio 65,3 cm/sec Hb fetale calcolata 9,6 g/dL (0,85-0,64 MoM)
  Non versamenti,
  non tachicardia fetale,
  LA regolare, placenta posteriore

Ricovero dopo 10 gg

- TCI ripetuto a 7 e 10 gg: invariato (positivo titolo 1:256 . Ac irregolari incompleti a specificità anti D)

- Ecografia ostetrica:
  emodinamica fetale nella norma
  PS MCA medio 62,9 cm/sec Hb fetale calcolata 10,15 g/dL (lieve anemia)
  Non versamenti, non tachicardia fetale, LA regolare, placenta posteriore

Dimissione con
controlli ecografici seriati
ed esami di laboratorio

Controllo ad un mese

- TCI : positivo titolo 1:512. Ac irregolari incompleti a specificità anti D

- Ecografia ostetrica:
  emodinamica fetale nella norma
  PS MCA medio 62,9 cm/sec Hb fetale calcolata 10,15 g/dL (lieve anemia)
  Non versamenti, non tachicardia fetale, LA regolare, placenta posteriore, PP podalica

TAGLIO CESAREO

TAGLIO CESAREO

- Nascita di Femmina 2910 gr, Hb nella norma
- Gruppo da sangue funicolare: A Rh Pos (si segnala presenza di anticorpi anti D)
- TCI materno dopo il parto: 1:512 Ac irregolari incompleti a specificità anti D)
- EI placentare: ndp

Isoimmunizzazione materno-fetale
Anti Rh (D)

- Assenza dell' antigene Rh (D) sui GR
- Varianti :
  il gene è presente ma non viene tradotto/espresso o espresso in modo parziale (weak Du)
  Queste pazienti possono risultare positive o negative ai reagenti per RH e sono comunque potenzialmente a rischio di sviluppare anticorpi antiD.

- Sistema Rhesus consiste di numerosi antigeni C, c, E, e, G
- Per cui è possibile che un immunizzazione avvenga anche con gli altri antigeni per i quali non è possibile fare prevenzione

A partire da 30 gg di gestazione Ag Rh(D) viene espresso sui GR fetali.

Isoimmunizzazione materno-fetale
Non Anti Rh (D)

- Antigene Kell
  - 10% dei casi di anemia fetale severa
  - 2 meccanismi d'azione: 1. emolisi cellulo-mediata
    2. inibizione dell'eritropoiesi

- Antigene Duffy
  - Sono di 2 tipi Fy(a) e Fy (b)
  - solo anti Fy (a) causano anemia severa
  - 82% africani sono Fy a-b-: l'assenza dell'antigene è protettiva per invasione da Plasmodium Vivax

- MNS system: antigeni M,N,S,U
  - Anti N : lievi casi di anemia fetale
  - Anti M : severa anemia solo con anticorpi ad alto titolo a 37°
  - Anti S e Anti U: severa anemia se ad alto titolo

Isoimmunizzazione materno-fetale Non Anti Rh (D)

- P System: antigeni P1 e P2
  - Solitamente sono IgM che non attraversano la placenta
  - Se madre è fenotipo p può produrre anti P1 : severa anemia

- Lewis : antigeni Le (a), Le (b)
  - Gli anticorpi anti Lewis non causano anemia fetale perché sono IgM che non attraversano la placenta e non sono espressi sulle emazie fetali

- I antigene
  - Altamente espresso su emazie fetali antigene i
  - Adulto esprime antigene I
  - Raramente dopo infezione da EBV si osserva transitorio riscontro di anti i

Table 1. Atypical Antibodies and Their Relationship to Fetal Hemolytic Disease

Blood Group System	Antigens Related to Hemolytic Disease	Hemolytic Disease Severity	Proposed Management
Lewis	*		
I	*		
Kell	K	Mild to severe†	Fetal assessment
	k	Mild	Routine obstetric care
	Ko	Mild	Routine obstetric care
	Kp^a	Mild	Routine obstetric care
	Kp^b	Mild	Routine obstetric care
	Js^a	Mild	Routine obstetric care
	Js^b	Mild	Routine obstetric care
Rh (non-D)	E	Mild to severe†	Fetal assessment
	C	Mild to severe†	Fetal assessment
	c	Mild to severe†	Fetal assessment
Duffy	Fy^a	Mild to severe†	Fetal assessment
	Fy^b	‡	Routine obstetric care
	By^3	Mild	Routine obstetric care
Kidd	Jk^a	Mild to severe	Fetal assessment
	Jk^b	Mild	Routine obstetric care
	Jk^3	Mild	Routine obstetric care
MNSs	M	Mild to severe	Fetal assessment
	N	Mild	Routine obstetric care
	S	Mild to severe	Fetal assessment
	s	Mild to severe	Fetal assessment
	U	Mild to severe	Fetal assessment
	Mi^a	Moderate	Fetal assessment
MNSs	Mt^b	Moderate	Fetal assessment
	Vw	Mild	Routine obstetric care
	Mur	Mild	Routine obstetric care
	Hil	Mild	Routine obstetric care
	Hut	Mild	Routine obstetric care
Lutheran	Lu^a	Mild	Routine obstetric care
	Lu^b	Mild	Routine obstetric care
Diego	Di^a	Mild to severe	Fetal assessment
	Di^b	Mild to severe	Fetal assessment
Xg	Xg^a	Mild	Routine obstetric care
P	$PP_{1}P^k$ (Tj^a)	Mild to severe	Fetal assessment
Public antigens	Yt^a	Moderate to severe	Fetal assessment
	Yt^b	Mild	Routine obstetric care
	Lan	Mild	Routine obstetric care
	En^a	Moderate	Fetal assessment
	Ge	Mild	Routine obstetric care
	Jr^a	Mild	Routine obstetric care
	Co^a	Severe	Fetal assessment
	Co^{1-b}	Mild	Routine obstetric care
Private antigens	Batty	Mild	Routine obstetric care
	Becker	Mild	Routine obstetric care
	Berrens	Mild	Routine obstetric care

(continued)

Anticorpi atipici e relazione con Anemia emolitica fetale

EZIOLOGIA

1. Emorragia transplacentare feto-materna durante ogni gravidanza

- Aborto
- IVG
- Gravidanza ectopica
- Procedure invasive intrauterine (amniocentesi, villocentesi)
- MEU
- Trauma addominale
- Gravidanze non clinicamente riconosciute (compresi vanishing twins)
- Manovre di Rivolgimento esterno

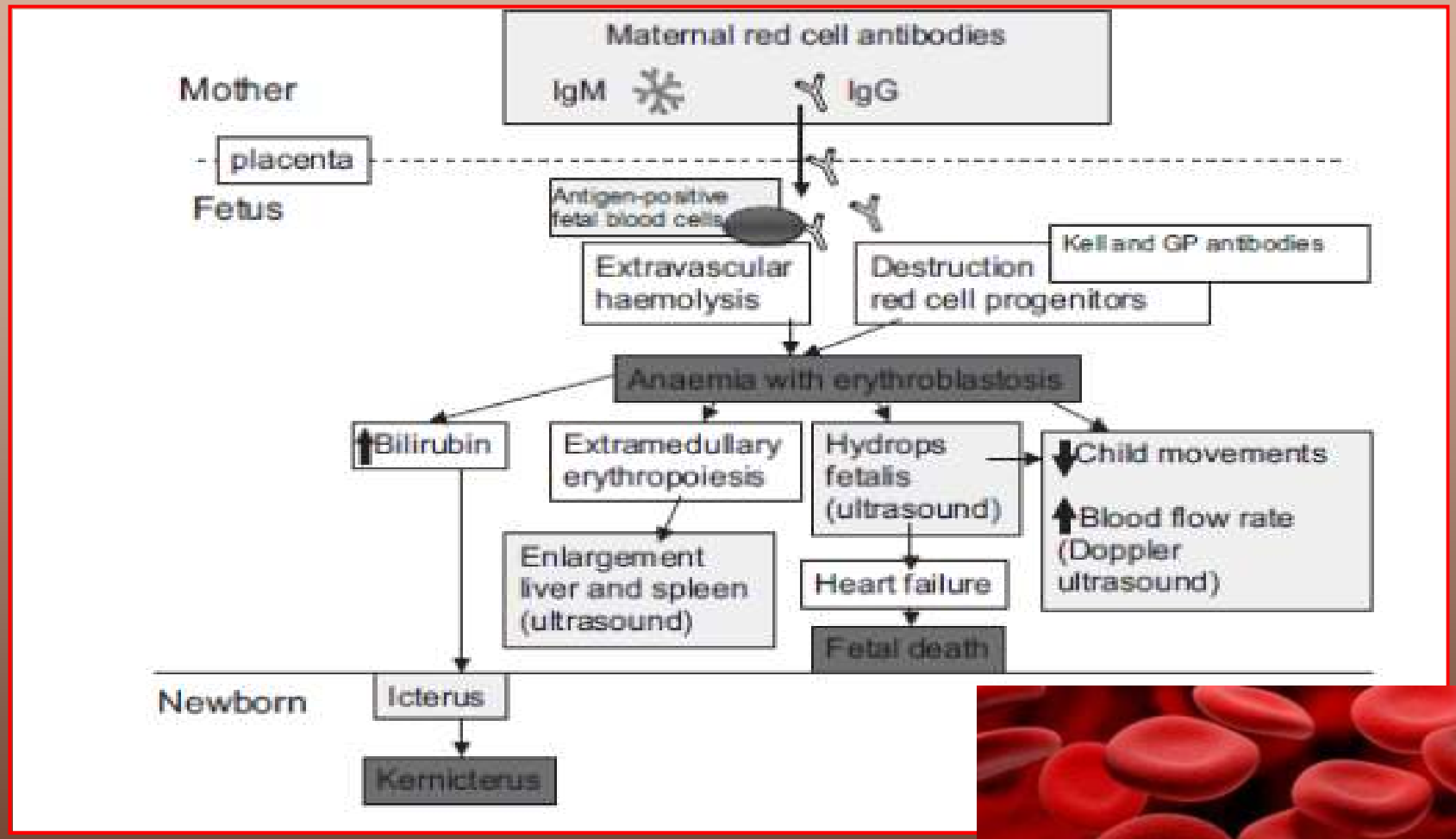
2. Emotrasfusione con emazie contenenti l'antigene o contaminazione con sangue positivo per l'antigene (ad es compatibilità Kell non viene considerata)

3. Inibizione eritropoiesi fetale (anti-Kell)

Volume di sangue fetale capace di causare isoimmunizzazione: 0,1 mL

- **Anticorpi nel siero materno dopo 5-15 sg**

PATOGENESI



Haemolytic disease of the fetus and newborn

Vox Sanguinis (2015) **109**, 99–113

Diagnosi

- TCI positivo: tipizzazione e titolo
- Test positivo = feto a rischio NON feto che sta sviluppando anemia.

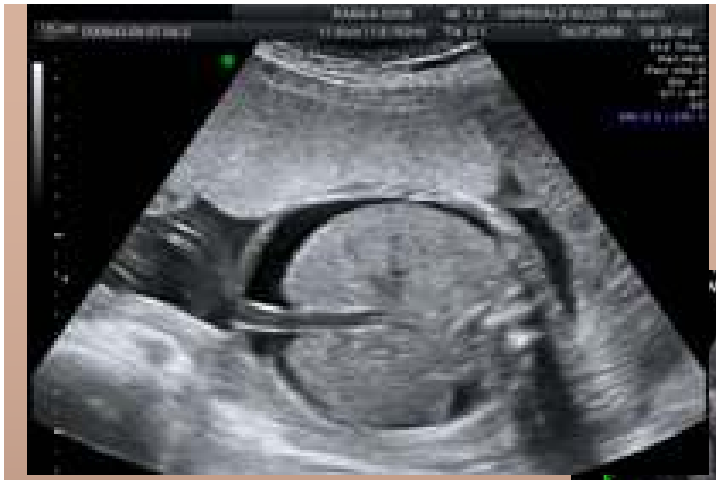
ANEMIA → Tipo e Concentrazione Anticorpale

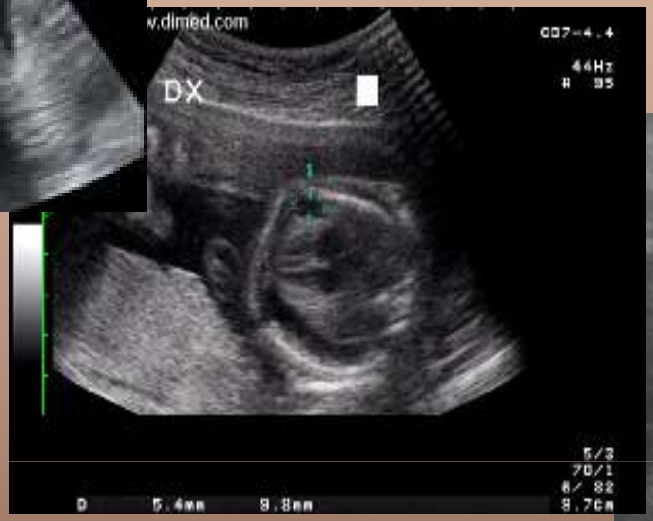
Altri fattori:

- Sottoclassi e glicosilazione degli anticorpi materni
- IgG 1 attraversano placenta nelle prime sg: alta morbidità prenatale
- IgG 3 atraversano la placenta nel III trimestre: severe complicanze neonatali
- IgG 2 e IgG 4 : scarso impatto
- Struttura , maturazione e distribuzione degli antigeni sulle emazie fetali
- Efficienza del trasporto placentare di IgG
- Maturità della milza fetale

Anemia fetale severa

IDROPE FETALE





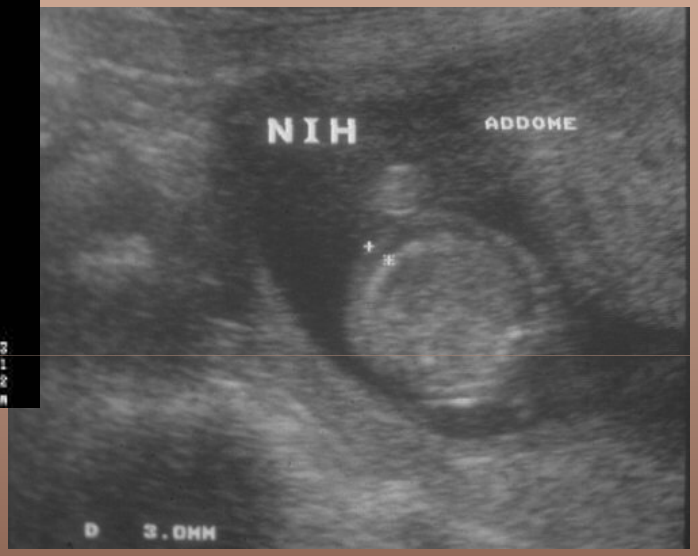


Table 1 Ultrasound findings in a progressive order predicting and demonstrating fetal anemia.

Liver/spleen dilatation
Omphalic vein dilatation
Increased placental thickness
Hydramnios
Ascites
Hydrothorax
Pericardial fluid
Generalized hydrops (fetal ascites)

72 Papantoniou et al., Therapeutic management of fetal anemia

Management

1. Determinazione gruppo e TCI materno seriato ad ogni gravidanza
- Donna con anamnesi di pregressa isoimmunizzazione fetale non indicato TCI seriato
- Titolo critico fra 1:8 e 1:32
- Se titolo iniziale è < 1:8 controllo ogni 4 sg

of more than one dilution is significant. A *critical* titer is that titer associated with a significant risk for severe erythroblastosis fetalis and hydrops, and in most centers this is between 1:8 and 1:32. If the initial antibody titer is 1:8 or less, the patient may be monitored with titer assessment every 4 weeks. For patients with alloimmunization involving antigens other than D, similar titer levels should be used to guide care except in Kell-sensitized patients because Kell antibodies do not correlate with fetal status (19).

460 ACOG Practice Bulletin *Alloimmunization During Pregnancy*

Management

Determination of Paternal Genotype

The initial management of a pregnancy involving an alloimmunized patient is determination of the paternal erythrocyte antigen status. If the father is negative for the erythrocyte antigen in question (and it is certain that he is the father of the fetus), further assessment and intervention are unnecessary. In cases of Rh-D alloimmunizatio.. in which the father is Rh positive, the probability that he

antibody with the corresponding antigen. If the father is homozygous for the D antigen, all his children will be Rh positive; if he is heterozygous, there is a 50% likelihood that each pregnancy will have an Rh-negative fetus that is not at risk of anemia. Given that the genes coding for the

Determinazione del gruppo sanguigno paterno

se padre negativo: nessuna indagine

se padre positivo:

Eterozigosi =50% di possibilità che il feto sia a rischio

Omozigosi = 100% di possibilità che il feto sia a rischio

Management

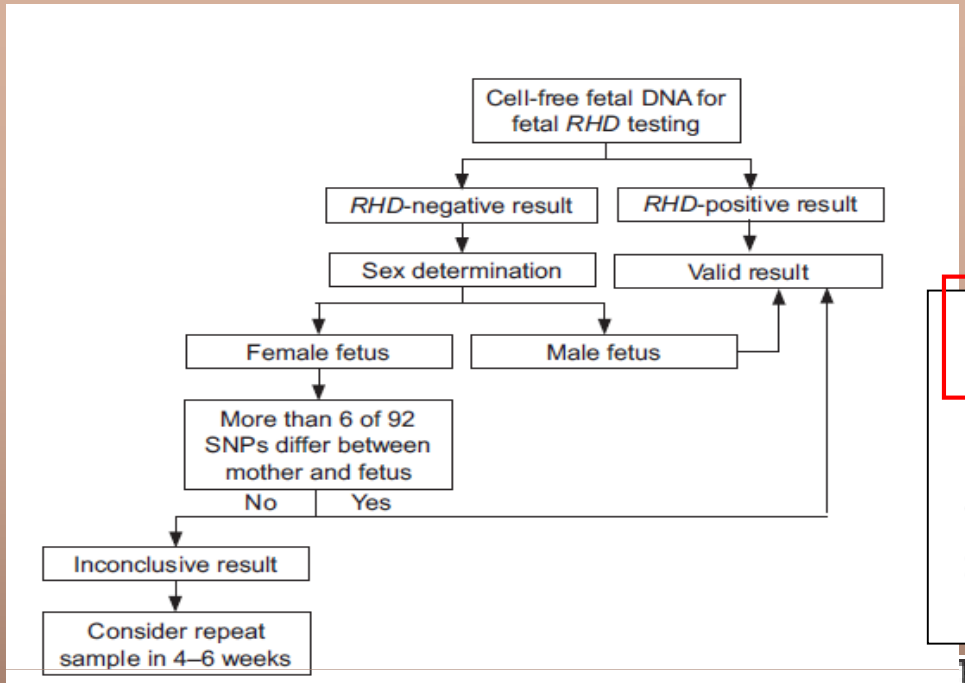


Fig. 1. Algorithm for determining the results of cell-free fetal DNA testing to determine the fetal *RHD* status. SNP, single-nucleotide polymorphism.

Moise. Red Cell Alloimmunization in Pregnancy. Obstet Gynecol 2012.

The decoding of the human genome showed that the responsible loci for the determination of Rh are on chromosome 1 (OMIM 111680) [57]. This knowledge allowed us to modulate modern management of the Rh-affected pregnancies with the determination of fetal *RhD* genotype with the use of cell-free fetal DNA and the follow-up of antigen-positive fetuses [33, 60, 77]. The latter is successfully carried out with the

Therapeutic management of fetal anemia: review of standard practice and alternative treatment options

J. Perinat. Med. 41 (2013) 71–82 •

- Determinazione dell'antigene fetale

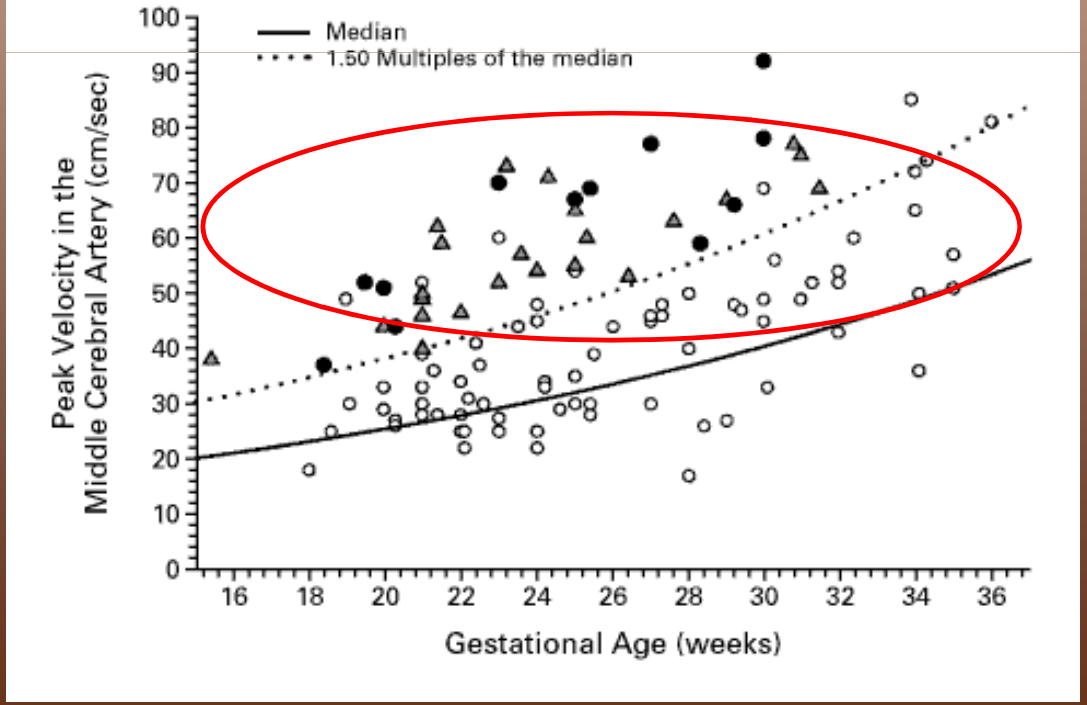
- ❑ Amniocentesi (PCR sensibilità 98,7% e specificità 100%)
- ❑ Villocentesi (non consigliata: possibile emorragia feto materna e isoimmunizzzazione)
- ❑ DNA fetale (a partire dal II trimestre specificità 99%)

Arteria Cerebrale Media

Before 2000, techniques to detect fetal anemia were either invasive (cordocentesis, amniocentesis), unreliable (antibody assays level in maternal blood, fetal cardiac rhythm), or late (sinusoidal fetal cardiac rhythm, signs of hydrops on ultrasound examination). Mari and coworkers demonstrated in 2000[5,6] that moderate to severe anemia can be detected noninvasively by Doppler ultrasonography from an increase in the peak velocity of systolic blood flow in the middle cerebral artery (MCA-PSV) to more than 1.5 multiples of the mean (MoM) in fetuses at risk of maternal RBC alloimmunization. Other studies[7-13] over the past decade have confirmed these results: there is a strong correlation between the Doppler measurement of MCA-PSV and fetal anemia: $R^2 = 0.55$ in the study by Mari and colleagues[6] and 0.654 in the one by Carbonne and colleagues[8] ($p < 0.001$). The threshold for intervention in Mari's study was 1.5 MoM.

Cerebral Doppler velocimetry to predict fetal anemia after more than three intravenous fetal exchange transfusions

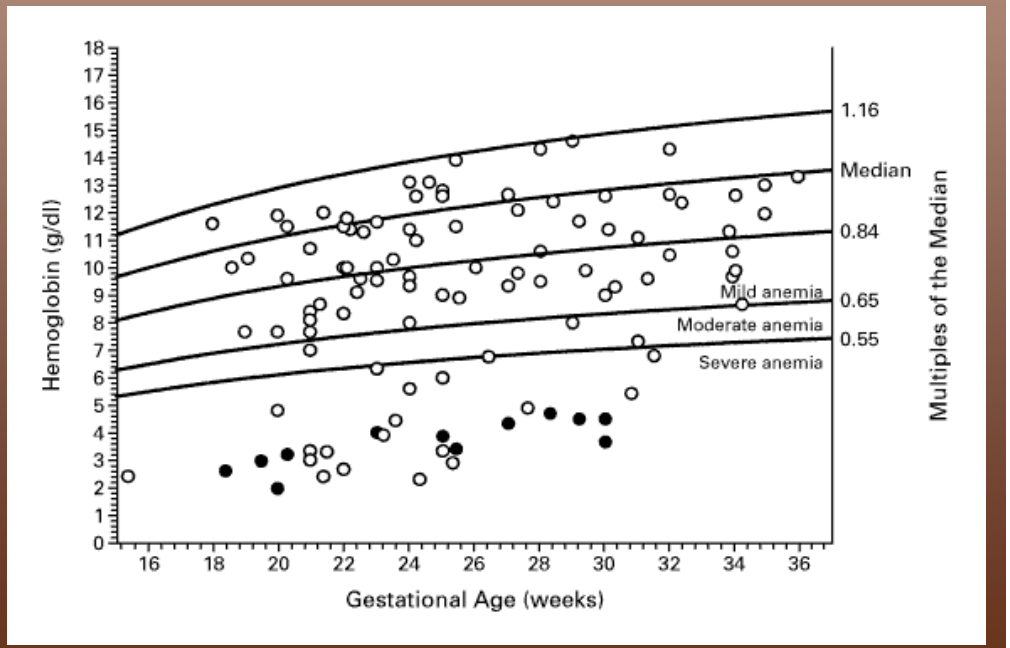
2968 TRANSFUSION Volume 54, November 2014



Arteria Cerebrale Media

- Anemia moderata/severa : valori al di sotto di 1,5 multipli della mediana per età gestazionale
- Sensibilità 100%, falsi positivi 12%
- Alto tasso di falsi positivi dopo 34-35 sg
- Procedura per esperti (angolo di insonazione non superiore a 30°, feto in stato quiescente)
- A partire da 16-18 sg, ogni 1-2 sg

pared with non-hydropic fetuses [81]. Hydrops is characterized by generalized skin edema and fluid collection in more than one area, such as pericardial, pleural, or ascitic effusions. It develops when the Hgb deficit is >6 SD below the mean for gestational age (i.e., Hct 15%, Hgb 5 g/dL) [55], whereas

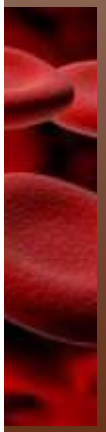


460 ACOG Practice Bulletin *Alloimmunization During Pregnancy*

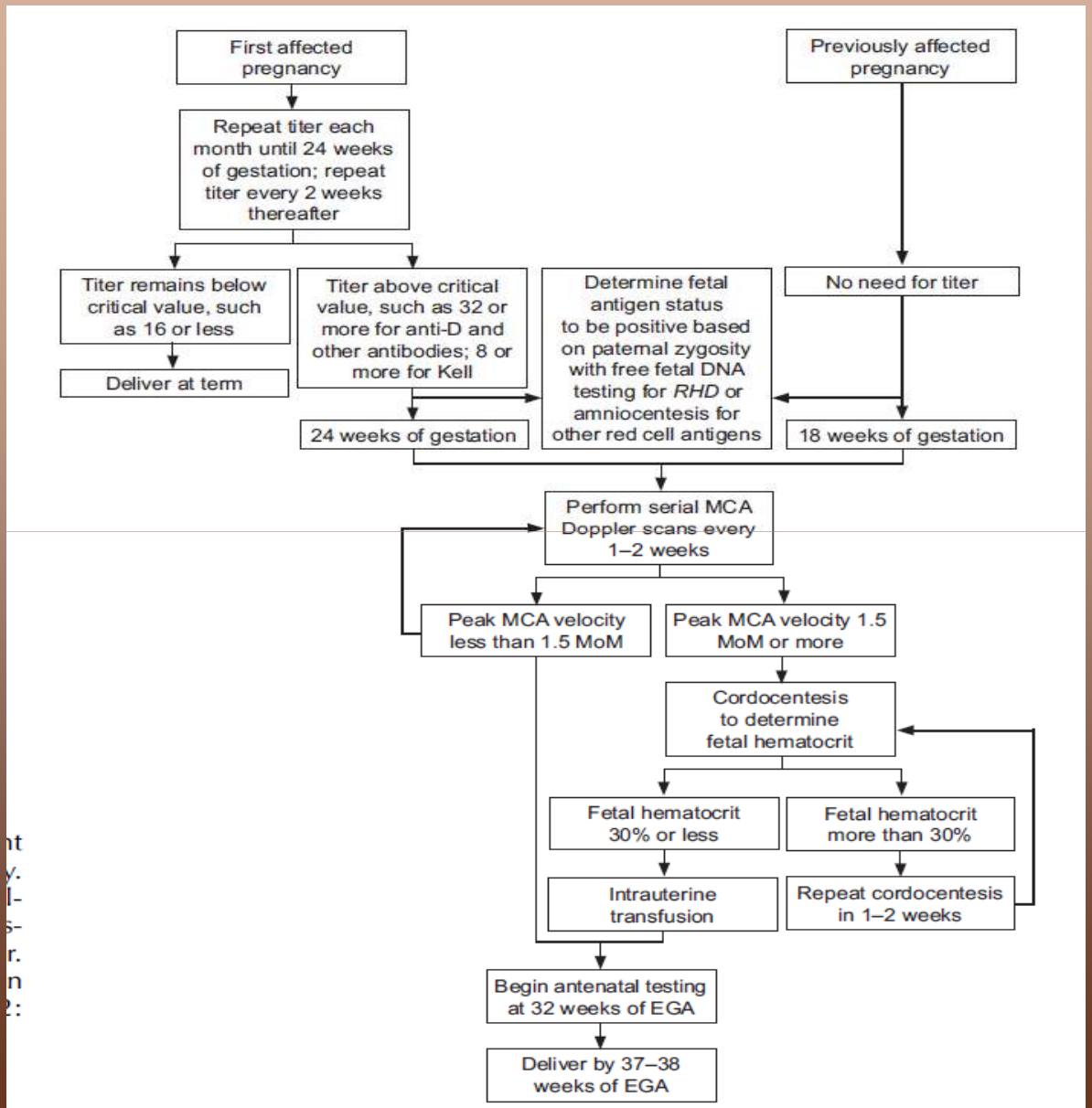
TABLE 1. REFERENCE RANGES FOR FETAL HEMOGLOBIN CONCENTRATIONS AS A FUNCTION OF GESTATIONAL AGE.*

WEEK OF GESTATION	MULTIPLES OF THE MEDIAN				
	1.16	1.00 (MEDIAN)	0.84	0.65	0.55
	grams per deciliter				
18	12.3	10.6	8.9	6.9	5.8
20	12.9	11.1	9.3	7.2	6.1
22	13.4	11.6	9.7	7.5	6.4
24	13.9	12.0	10.1	7.8	6.6
26	14.3	12.3	10.3	8.0	6.8
28	14.6	12.6	10.6	8.2	6.9
30	14.8	12.8	10.8	8.3	7.1
32	15.2	13.1	10.9	8.5	7.2
34	15.4	13.3	11.2	8.6	7.3
36	15.6	13.5	11.3	8.7	7.4
38	15.8	13.6	11.4	8.9	7.5
40	16.0	13.8	11.6	9.0	7.6

*The hemoglobin values at 0.65 and 0.55 multiples of the median (cutoff points for mild and moderate anemia, respectively) are also shown. The values at 1.16 and 0.84 multiples of the median correspond to the 95th and 5th percentiles, respectively (the normal range).



Management



Management and prevention of red cell alloimmunization in pregnancy
Moise K., Argoti P. Obstetrics and Gynecology Vol 120, n° 5, november 2012

Time for Delivery

1. Lieve anemia → 37-38 sg
2. Anemia severa:
valutare rischi correlati a trasfusioni ripetute e cordonocentesi / rischio di prematurità

NB= Tasso di sopravvivenza > 32 sg è 95%
(induzione maturità polmonare fetale)

Review article

Therapeutic management of fetal anemia: review of standard practice and alternative treatment options

Trasfusione Intrauterina: Cordonocentesi

It develops when the Hgb deficit is >6 SD below the mean for gestational age (i.e., Hct 15%, Hgb 5 g/dL) [55], whereas a transfusion is performed when the fetal Hgb level is 4–6 SD below the mean gestational age, which corresponds to an Hgb deficit of more than 6 g/dL. Hydropic signs will nor-

Intrauterine transfusion (IUT) for fetuses with severe anemia is the main therapeutic intervention in affected pregnancies and represents one of the greater achievements of fetal therapy

associated with survival rates of more than 90% in non-hydropic fetuses [47, 49]. Fortunately, only 10% of affected pregnancies will require transfusion *in utero*, while the remaining 90% will be well-monitored with MCA-PSV [44]. Doppler usltrasonography has also been very useful in monitoring posttransfusion fetuses and determining the next IUT.

total oxygen carrying capacity [88]. Subsequent IUTs can be performed on fixed intervals that vary among different centers (2 weeks between the second and the third procedure and every 3 weeks for the following IUTs) based on an estimated drop in Hct of 1% per day [55]. Alternatively, a calculated

Complicanze:
- MEU 2% (aborto 5% se < 20 sg)
- Bradicardia fetale transitoria 8%
- Distress fetale da ematoma rottura o spasmo del cordone, corioamnionite = TC emergente

Ig vena e/o Plasmaferesi

Table 2 Clinical case reports and case series about the use of plasmapheresis and maternal and fetal administration of IVIG in pregnancies complicated with fetal anemia due to red cell alloimmunization.

Authors and year [reference]	Number of cases	Antibodies present	Treatment period	Therapeutic scheme	IUT (IV/IP)	Pregnancy outcome
Berlin et al., 1985 [7]	1	Anti-D Anti-C	25 weeks	0.4 g/kg/day for 5 days (single dose)+plasmapheresis		Live birth
Scott et al., 1988 [68]	1	Anti-D	20 weeks to delivery	0.4 g/kg/day for 5 days Four subsequent fetal IVIG dose (10 mL)	IV	Live birth
De La Camara et al., 1988 [15]	2	Anti-D	24 and 30 weeks to delivery	0.4 g/kg/day for 4 days every 14 days	–	2 Live births
Chitkara et al., 1990 [9]	5	Anti-D Anti-Kell	15–27 weeks to delivery	1 g/kg/week	IV	1 Fetal death 4 Live births
Margulies et al., 1991 [43]	24	Anti-D	<20 weeks (n=8) 20–28 weeks (n=7) >28 weeks (n=9)	0.4 g/kg/day for 4–5 days every 15–21 days	–	3 Fetal deaths 21 Live births
Dooren et al., 1994 [20]	10	Anti-D	20–31 weeks to delivery	85.7±11.6 mg/kg of EFW after every IUT (fetal administration)	IV	3 Fetal deaths 7 Live births
Alonso et al., 1994 [1]	1	Anti-D	28 weeks to delivery	0.4–0.5 g/kg EFW at 3-week intervals	–	Live birth
Gottvall and Selbing, 1995 [29]	5	Anti-D	22 weeks if fetal Hb <100 g/L or anti-D >2.0 µg/mL to delivery	100 g over 4–5 days and repeated after 6 weeks if fetal lung maturity was not ascertained and fetal Hb was >70 g/L	–	No perinatal birth reported
Deka et al., 1996 [16]	6	Anti-D	16–18 weeks (n=5) and 13 weeks (n=1) to IUT or delivery	0.1 g/kg 3–4 times/week	IV (n=2)	6 Live births
Voto et al., 1997 [85]	30	Anti-D	<20 weeks to delivery	0.4 g/kg/day for 5 days every 15–21 days	IV	22 Live births 6 Fetal and 2 Neonatal deaths
Porter et al., 1997 [61]	1	Anti-D	14 weeks to delivery	1 g/kg/week	IP+IV	Live birth
Palfi et al., 2006 [58]	1	Anti-D	12 weeks to delivery	100 g/week+plasmapheresis	IV	Live birth
Kriplani et al., 2007 [38]	4	Anti-D	22–34 weeks to delivery	1 g/kg EFW at IUT	IV	4 Live births
Ruma et al., 2007 [66]	9	Anti-D Anti-Kell	6 to 30 weeks	1 g/kg/day (2 doses) followed by 1 g/kg/week+plasmapheresis	IV	9 Live births
Novak et al., 2008 [54]	1	Anti-D	16+5 weeks to delivery	1 g/kg/week+plasmapheresis	–	Live birth
Fox et al., 2008 [25]	4	Anti-D Anti-Kell	14 weeks to IUT	0.8 g/kg/week	IP+IV	4 Live births
Connan et al., 2009 [12]	6	Anti-D Anti-K Anti-C	8 weeks to delivery	1 g/kg/week	IP+IV (4) IP (1) IV (1)	6 Live births
Isojima et al., 2011 [34]	1	Anti-D	15 weeks to delivery	Plasmapheresis+400 mg/kg/day for 5 days (single dose)	IV	Live birth
Lakhwani et al., 2011 [39]	1	Anti-Kell	28 weeks to delivery	Plasmapheresis	IV	Live birth

Review article

Therapeutic management of fetal anemia: review of standard practice and alternative treatment options

severe red alloimmunization with early onset. In the following years, the directed maternal immunomodulation may be a realistic therapeutic option to delay the development of fetal hydrops and decrease or eliminate the need for IUTs. Clinical indications for use of IVIG are derived from isolated case reports or small case series, which might be because severe fetal anemia before 15 weeks is exceptional or unsuccessful cases are not published. Strict protocols should be standardized and should specify several aspects of alternative treatment modalities such as the onset and intervals between interventions, the optimal dosage of IVIG administration, the indications for combination with plasmapheresis, and the end point of the therapy.

Delivery of an RhD positive infant*

Abortion

- therapeutic termination of pregnancy
- spontaneous abortion followed by instrumentation
- spontaneous complete or incomplete abortion after 12 weeks' gestation

- threatened abortion before 12 weeks
  when bleeding is heavy or repeated or is associated with abdominal pain; in particular, if these events occur as gestation approaches 12 weeks

- threatened abortion after 12 weeks
  when bleeding continues intermittently, anti-D immunoglobulin should be given at approximately 6-week intervals, and the volume of fetomaternal hemorrhage should be assessed

Invasive prenatal diagnosis

- chorionic villus sampling
- amniocentesis
- cordocentesis

Other intrauterine procedures

- evacuation of the uterus because of mola hydatiforma
- multifetal reduction
- fetal therapy (insertion of shunts etc.)

Antepartum hemorrhage
when bleeding continues intermittently, anti-D immunoglobulin should be given at approximately 6-week intervals, and the volume of fetomaternal hemorrhage should be assessed

External version of the fetus
Abdominal trauma
Ectopic pregnancy
Intrauterine fetal death
Stillbirth

Dose:

before 20 weeks gestation	50 µg (250 IU)
after 20 weeks gestation**	100 µg (500 IU)

Timing:
as soon as possible, but no later than **72 hours** after the event.

Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2010 Mar; 154(1):3–8

PREVENTION OF RhD ALLOIMMUNIZATION IN RhD NEGATIVE WOMEN

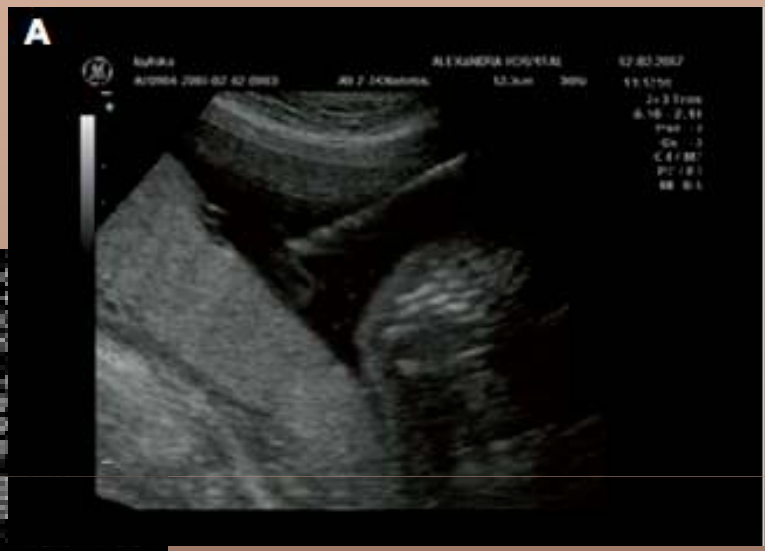
Prevenzione

28 sg in gravida Rh negativa

Follow up dopo somministrazione di IgG anti D

TCI positivo fino a 6 settimane dopo la somministrazione
(effetto terapeutico)
Monitoraggio del titolo

ISOIMMUNIZZAZIONE MATERNO-FETALE



Dipartimento della Salute della Donna e del Bambino

Dr.ssa Cristiana Nardi