Postgraduate Education Course
Infectious Diseases of pregnant women, fetuses and newborns
Bertinoro, Italy, October 2013

Diagnosis and management of fetal cytomegalovirus infection

The diagnosis of CMV fetal infection

Fetal US abnomalities

Maternal primary infection

What is the best sample for this diagnosis?

And what is the best timing?

Physiopathology of fetal CMV infection

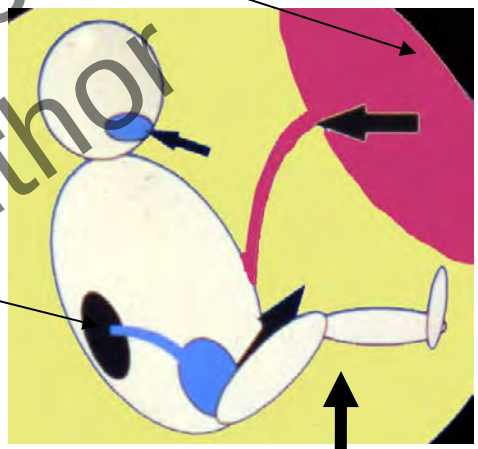
Maternal infection (maternal viremia)

1.Placenta infection

2. Fetal infection

Systemic infection
Fetal viremia

Replication in the kidney



Virus eliminated in fetal urine ≥ 16 weeks gestation

Virus accumulated in amniotic fluid

CMV detectable in AF usually at high level From ≥ 7 to 9 weeks after maternal infection

Fetal US abnomalities

Maternal primary infection

Do we have to check for the absence of maternal DNAemia ?

Amniocentesis at or after 20 WG
And at least 7 weeks after maternal primary infection

Is a positive CMV PCR in maternal blood a iatrogenic risk of amniocentesis?

•No increased risk to give birth to an infected baby when the mother had detectable DNA in blood by PCR at the time of prenatal diagnosis (MG Revello, JID, 2008)

•Therefore, the presence of CMV DNA in maternal blood did not seem to be a significant risk for iatrogenic transmission of CMV to the fetus

•However, most group would check for maternal viremia and avoid amniocentesis in cases of high viral load

CMV detection in amniotic fluid: culture or PCR?

Series	Culture		PCR	
	Sensitivity	Specificity	Sensitivity	Specificity
Lazzarotto et al, 1998, N=82	**50%**	100%	**100%**	**83%**
Nigro et al, 1999, N=117	57%	100%	100%	97%
Antsaklis et al, 2000, N=37	64%		79%	
Gouarin et al, 2001, N=97	72%	**98%**	**72%**	97%
Enders et al, 2001, N=176	79%	100%	81%	98%
Revello et al, 2002, N=88	**82%**	**100%**	93%	**100%**

CMV PCR in AF=
the gold standard for prenatal diagnosis

After maternal seroconversion three conditions are required for optimal sensitivity of prenatal diagnosis

1) **6 to 7 weeks' interval** between amniocentesis and maternal seroconversion

2) amniocentesis must be done **after 20 Weeks**

3) CMV detection in AF **with a performant PCR** test

In these optimal conditions, prenatal diagnosis has a specificity close to 100% and a sensitivity > 90%

However, even in these best conditions…

- **Rare false negative cases of prenatal diagnosis :**
  - with a negative PCR in amniotic fluid and a positive PCR in the urine at birth.
  - 8 cases in a serie from Revello et al (between 1990 and 2007), 4 cases between 2007 and 2013 in our center
- **Due to late passage of the virus :**
  - after 20 weeks
  - more than 7 weeks after maternal seroconversion: the interval between maternal seroconversion and fetal infection can be as long as 19 weeks[1]
- **Good prognosis?**
  - The 8 cases reported by Revello and al = 8 asymptomatic new-borns
  - The 4 cases in our center: 4 asymptomatic children (follow-up : 6 to 37 months)
  - More data are needed on the prognosis of these late infection cases

(1: Revello et al, JID, 2008)

Prenatal follow-up of an infected fetus

Positive CMV PCR in AF: infected fetus

Only 10 to 20%
of infected fetuses
will develop CMV disease
and be at risk of long term sequelae

Establishing fetal prognosis:
-imaging
-biology

Monthly ultrasound:
Extra cerebral fetal symptoms
Cerebral symptoms

Fetal brain MRI at 34 to 36 weeks

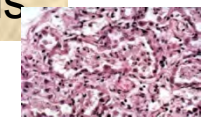
Fetal blood sampling

Pathophysiology of fetal CMV disease

Maternal infection

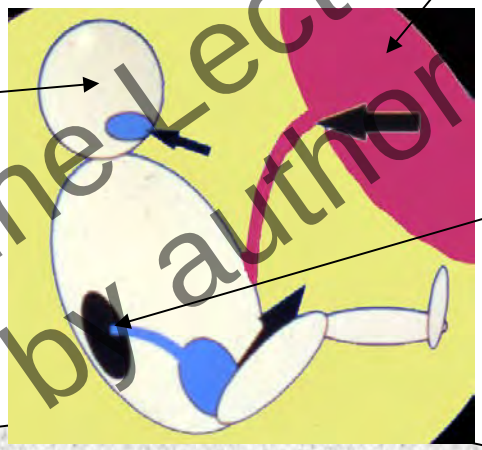
1.Placenta infection: placentitis

4 to 8 weeks



6. IUGR

7. Affection of the brain is late and unfrequent but of major concern



2. Replication in the kidney: oligohydramnios or polyhydramnios

3. Viral enterocolitis: hyperechogenic bowel

4. Viral hepatitis: Hepatomegaly

5. Liver insufficiency
Ascites
Pericardial effusion
Hydrops

Symptoms of systemic fetal infection:
often transient

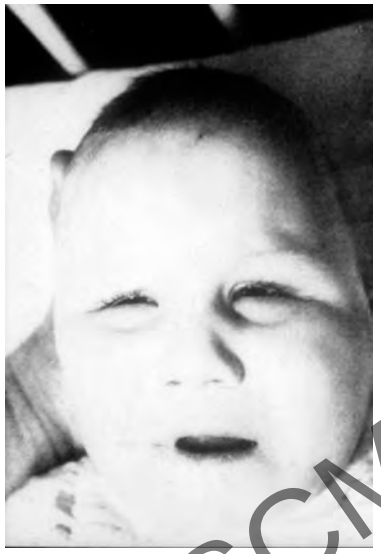
Extra-Cerebral US Features *(489 Cases in 43 series, 1983-2003)*

	N	(isolated abnormality)
Placentitis	9	(1)
Oligohydramnios	**17**	
Polyhydramnios	8	
Hyperechogenic bowel	**13**	**(2)**
Ascites	**32**	**(4)**
Liver & Spleen		
Calcifications	7	(1)
Hepatomegaly	7	
Pericardial Effusion	7	
Pleural Effusion	5	(1)
Cardiomegaly	5	(1)
Hydrops	9	(1)
IUGR	**37**	**(4)**

Series [n-N] 43 [1-189]
Any US Findings : 130 cases (26%)

Ville Y, Picone O, Leruez-Ville M,
From textbook of Perinatal Medecine. 2006

Brain Abn. / 304 infected fetuses: 1984 - 2003



Series [n-N] *	35	[1-189]
Any brain Abnormality	126	
Ventriculomegaly	32	(3)
Hydrocephaly	13	(2)
Microcephaly	21	(2)
Porencephaly	1	
Agenesis of the Corpus Callosum	6	
Abn. Gyration	14	
Subependymal cysts	3	(1)
Calcifications	20	(2)
Cerebellar Hypoplasia	13	
Pseudo- Dandy Walker	1	
Choroid Pl. cysts	2	

Prenatal prognosis markers in 73 infected fetuses

Multivariate analysis the best prognosis factors of poor outcome

Variable	*Adjusted OR*	*95% CI*	*p*
Cerebral ultrasound anomaly (if present)	**40.6**	[8.0;206.9]	$<10^{-4}$
Extra cerebral ultrasound anomaly	**4.4**	[1.3;15]	0.02

**(abnormal outcome =
clinical symptoms (including HL) at 6 mths
or histological lesions at TOP)**

Benoist BJOG 2008

All cerebral anomalies do not have the same prognosis value

- Some cerebral abnormalities have a very high positive predictive value for poor neurologic outcome: (Noyola et al, J Pediatr, 2001)
  - Microcephaly
  - Multiple intra cranial calcifications

- Whereas the prognosis value of more subtle or isolated brain abnormalities is less obvious (Candle Stick Images, Subependymal Cysts, Choroid Plexus Cysts, unilateral ventriculomegaly, isolated calcifications..))

Paris Follow-up Cohort of Asymptomatic Infected Neonates

131 infected children
with normal clinical examination at birth, 3 years follow-up

19 had

Abnormal Brain US at birth

- Candle Stick Images*: 11
- Subependymal Cysts: 6
- Choroid Plexus Cysts: 1
- Ventricular Asymmetry 1

7.6% of HL

- Profound Bilaterale 2.3%
- Severe unilatterale 3.0%
- Moderate unilaterale 2.3%

- All normal neurologic development
- Except one : Hyperexcitability

* Vasculatis of basal ganglia)

	OR	P
IUGR	5.1	0.05
IUGR+choroid plexus cysts	10	0.001

Dr Jean François Magny, IPP, 2013

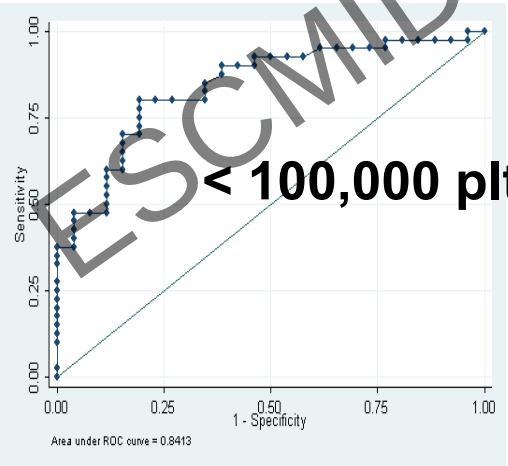
There is room for other predictive markers:
Notably to help establishing the prognosis of cases with mild US symptoms

Some fetal biological markers are studied

Fetal thrombocytopenia is associated with symptomatic infection

72 Cases of fetal infections
Benoist et al, BJOG, 2008

Variable	*Adjusted OR*	*95% CI*	*p*
Thrombocytopenia (for each decrease of 10000/mm$^{3)}$	**1.1**	[1.01;1.20]	0.05



< 100,000 plt/dL

39 cases of fetal Infections
Fabbri et al, BJOG, 2010

Platelets count ≤ 50 000/µl:
92%specificity
62% sensitivity
for the diagnosis
of a symptomatic
infection

:

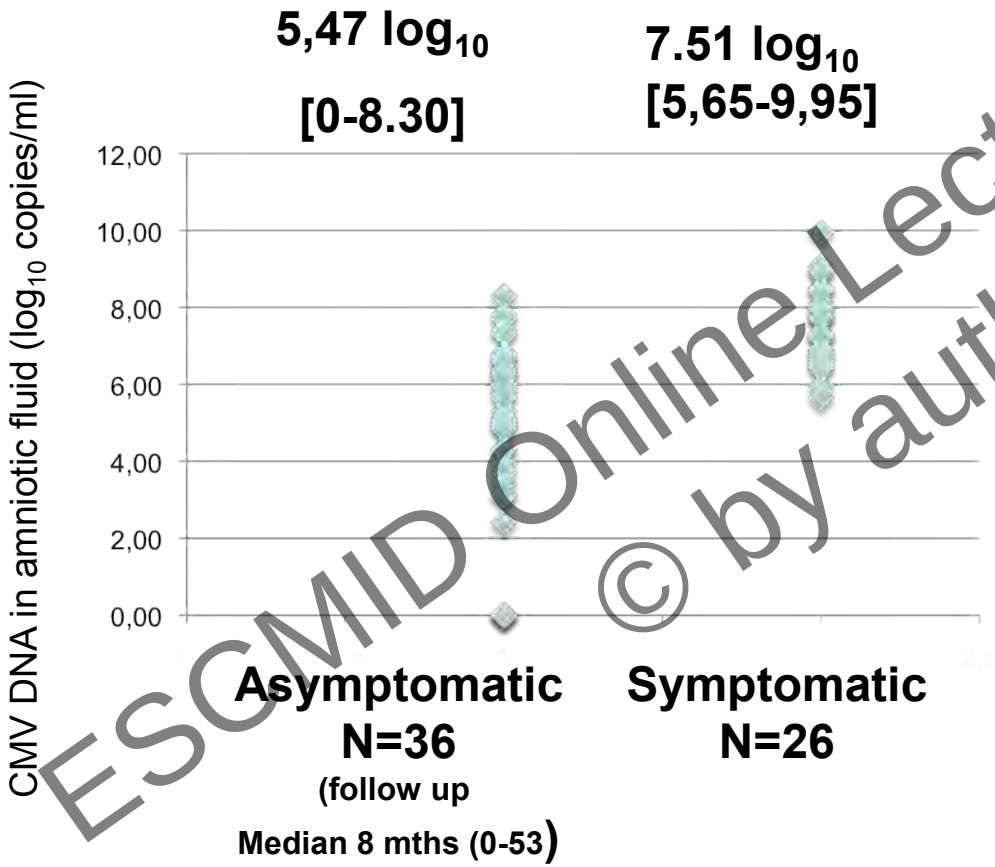
β2 microglobulinemia in fetal blood

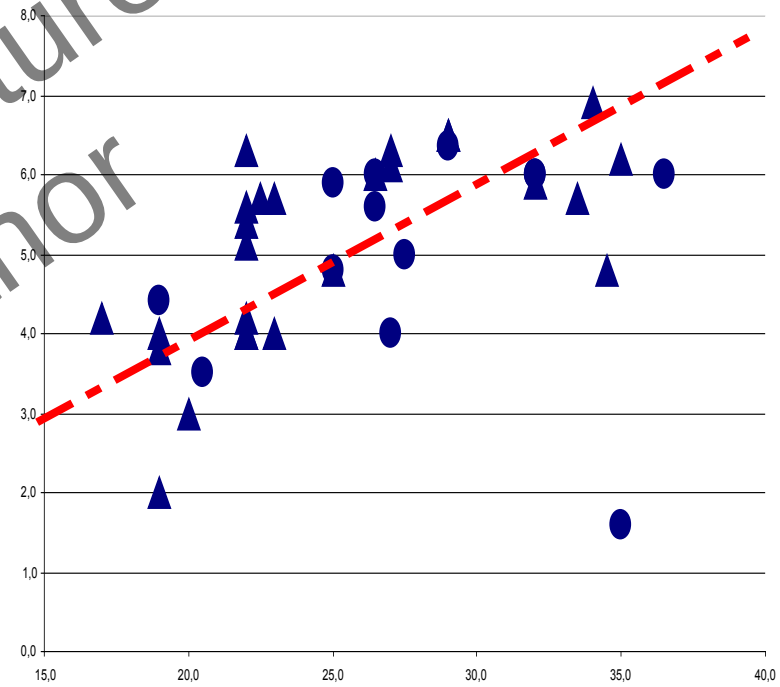
Fabbri et al, BJOG,2010

- 31 fetuses tested at 22 SG

- Level of β2 microglobulin ≥11.5 mg/L had a
  - 91.7% sensitivity to predict symptomatic disease at birth
  - 100% specificity to exclude asymptomatic infection at birth

VIRAL LOAD in AMNIOTIC FLUID
62 samples (2007-2013)



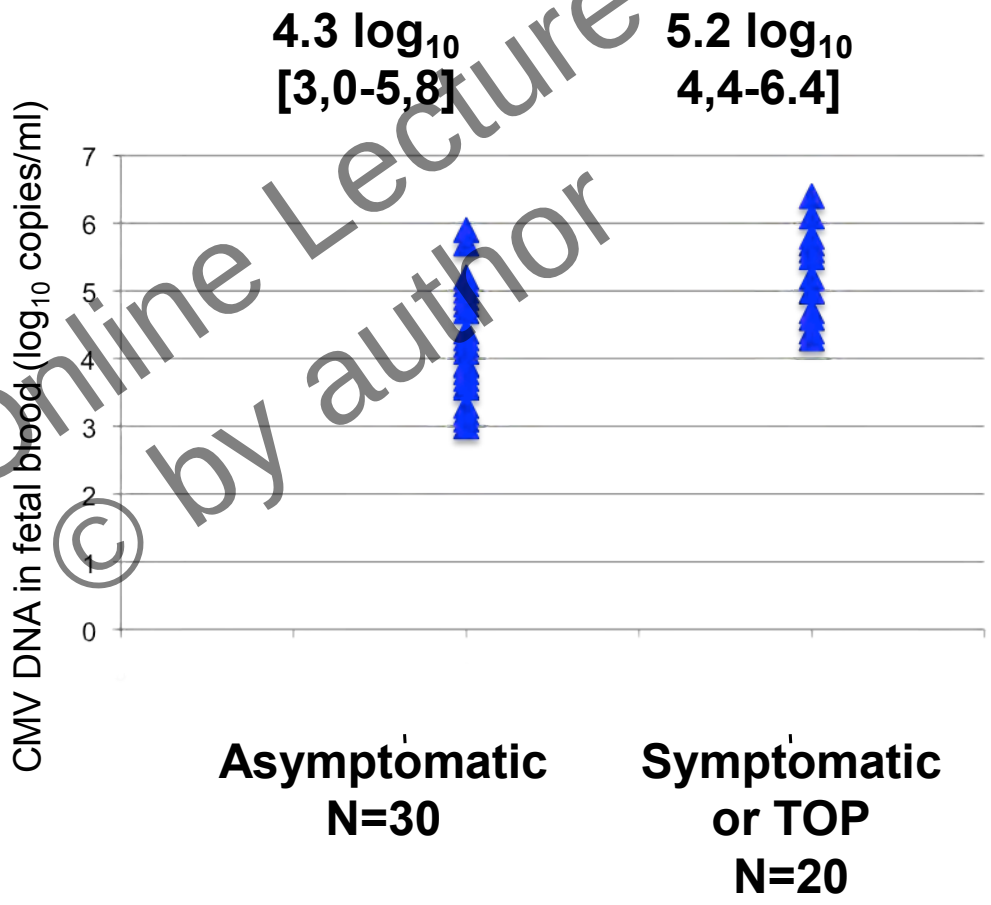


Gestational age at Amniocentesis

AF were obtained at a median of
23(20-37) WG in symptomatic cases
24 (17-37) in asymptomatic cases

VIRAL LOAD in FETAL BLOOD

50 fetal whole blood samples
tested prospectively
same extraction
and PCR protocol



Asymptomatic
Children followed
median of 8 mths (0-53)

Prenatal follow-up of an infected fetus

Positive CMV PCR in AF: infected fetus

Sceening for
•Extra cerebral fetal symptoms
•Cerebral symptoms

Monthly ultrasound
Fetal brain MRI at 34 to 36 weeks

Establishing fetal prognosis:
-imaging
-biology

Fetal blood sampling:
Platelets count
β2 microglobulinemia
Viral blood level?

What are the possibilities
for treatment of congenital CMV?

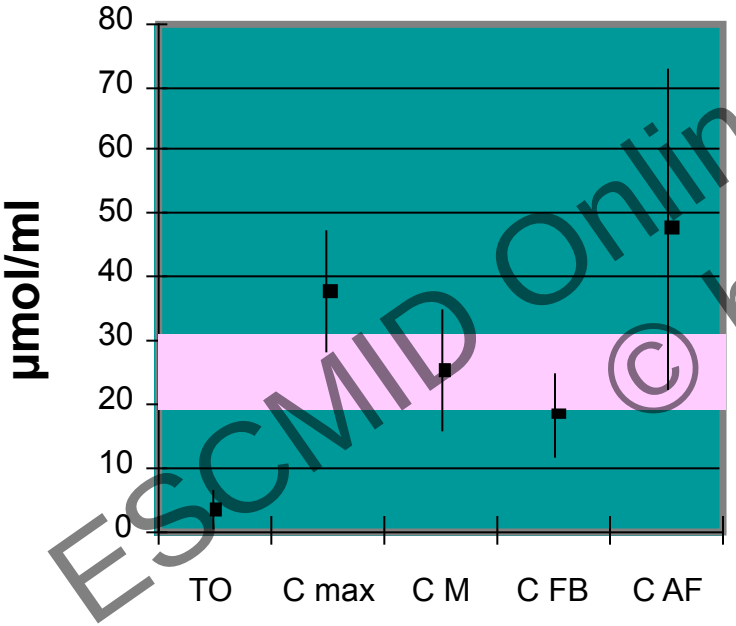
Two options are under evaluation:

-Hyperimmune globulin

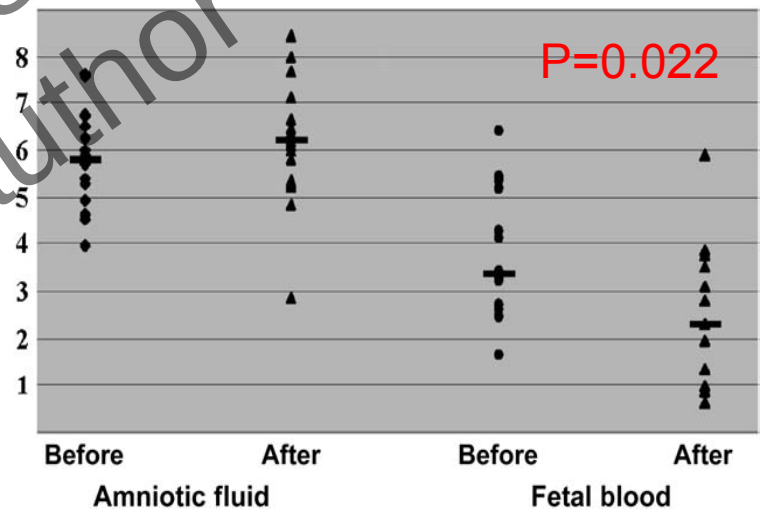
-Antiviral treatment

Pilot study : maternal administration of valacyclovir in symptomatic congenital CMV

20 women with 21 symptomatic infected fetuses were treated with 8 g/day of valacyclovir



Therapeutic concentrations achieved in maternal and fetal blood



Viral load in fetal bload decreased significantly after 1 to 12 weeks of treatment

Jacquemard et al, BJOG, 2008)

Cymeval II (Clinical trial NCT 01037712)

- Inclusion criteria: infected fetus WITH
  - Extra cerebral ou mild cerebral symptoms at US
  - or fetal thrombocytopenia <100 000
  - or DNAemia in fetal blood > 3000 copies/mL
- All enrolled mothers are treated with valacyclovir 2g/4/day until birth
- Results will be analysed based on the Simons´ method
  - Efficacity : ≥ 70% of asymptomatic babies (no clinical symptoms, normal imaging and biology)
  - 47 inclusions are needed
  - Intermediat analysis to be done after 11first cases
- First inclusion in January 2012
- Intermediate analysis in September 2012: 72% asymptomatic
- The study is ungoing with 25 inclusions so far

Conclusions

-Prenatal diagnosis is reliable

-Establishment of fetal prognosis remains difficult in some cases notably in those with mild symptoms

-The possibilities of treatment are still experimental

Merci …

à tous les praticiens,
aux patients et aux associations qui les aident…

Necker
ENFANTS MALADES
HÔPITAL UNIVERSITAIRE

Laboratoire de Virologie
Tiffany Guilleminot
Laurence Mascard
Yann Sellier

Maternité
Yves Ville
Laurent Salomon
Julien Stirnemann

Chanter Marcher Vivre
association reconnue d'intérêt général www.chantermarchervivre.org

Pédiatrie
Jean-François Magny
Muriel Nicloux
Nadia Bahi-Buisson
Laurence Laccourreye
Vincent Couloigner

Radio-pédiatrie
Pascale Sonigo
Marie-Elodie Millischer

2, rue Émile ZOLA
91120 PALAISEAU
Tél. : 01 60 14 99 89
Pour les yeux d'Emilie
Connaissance du C.M.V

Necker ENFANTS MALADES HÔPITAL UNIVERSITAIRE · ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS · UNIVERSITÉ PARIS DESCARTES