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Policlinico S. Orsola-Malpighi

Prognostic blood markers of brain damage in fetuses with congenital CMV infection

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Management of congenital CMV Infection

TOPICS

1. Diagnosis of maternal infection
2. Diagnosis and prognosis of fetal infection
3. Diagnosis of infection in newborn

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CONGENITAL CMV INFECTION
Prenatal diagnosis

Invasive prenatal diagnosis

Amniocentesis/ Cordocentesis

+

Non invasive prenatal diagnosis

Ultrasound examination

+

COUNSELLING

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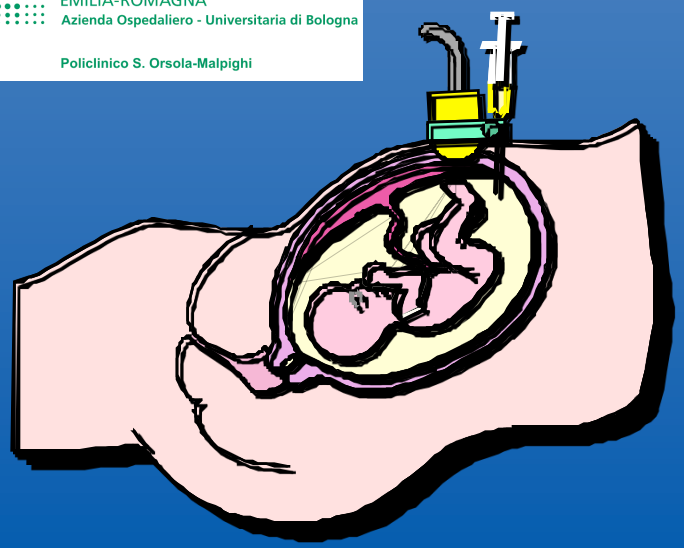

Amniocentesis: 20-21 weeks gestation

Primary Maternal infection
(the first half of gestation)

Observation of ultrasound abnormalities

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CONGENITAL CMV INFECTION
Prenatal diagnosis

When should prenatal diagnosis be performed?

- **after 6-8 weeks from the onset of maternal infection**

CMV is a slow replication virus and 6-8 weeks is the time required from maternal infection to virus detection in AF

- **amniocentesis is performed not before 20-21 weeks gestation**

Fetus excretes CMV via urine into the AF and a sufficient amount of fetal diuresis is produced only after 20-21 WG

Which tests?

- Virus isolation (shell vial)
- Viral DNA by Real Time PCR

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Virus isolation and Real Time PCR in 796 samples of amniotic fluids of primarily infected women and congenital CMV infection

		CONGENITAL INFECTION Yes	No	Total	SENS %	SPE %	PPV %	NPV %
Virus isolation	Pos	140	0	140	74.0	100	100	92.5
	Neg	49	607	656				
	Total	189	468	796				
Real Time PCR Copies/mL	Pos	150	0	150	79.4	100	100	94.0
	Neg	39	607	646				
	Total	189	607	796				

(Lazzarotto T et al. CMV San Francisco 2012)

PCR is more sensitive than virus isolation and the specificity of the two tests is the same.

Among the 646 cases with negative result: No congenital CMV infection was identified in 607 cases, while 39 newborns were infected but were asymptomatic at birth and during subsequent monitoring (NPV 94%)

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Literature reports since 2000. Sensitivity, specificity, and predictive values of PCR tests for CMV detection in amniotic fluid

Author	Publication year	SENS %	SPE %	PPV %	NPV %
Goegebuer et al.	2008	85	100	nd	nd
Revello et al.	2004	90.2	100	100	90.4
Revello et al.	2002	92.7	100	100	92.7
Enders et al.	2001	81.1	97.9	93.8	93.1
Gouarin et al.	2001	72	96.9	91.3	89
Azam et al.	2000	71	100	nd	nd
Liesnard et al.	2000	80	100	nd	nd

nd: not done

Diagnosis of fetal CMV infection

20-21 weeks gestation

Amniotic fluid is an appropriate material

for diagnosis of fetal CMV infection

at 20-21 weeks gestation

What about fetal blood?

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Diagnosis of fetal CMV infection

20-21 weeks gestation

Fetal blood samples taken at 20-21 WG does not permit accurate results for diagnosis

- IgM ⇒ sens 40-80%

- DNAemia ⇒ sens 40-90%

100% sensitivity ⇒ cordocentesis is performed after 30 weeks gestation.

(Benoist et al. AJOG 2008)

Diagnosis of fetal CMV infection

Fetal blood samples taken at 20-21 WG

1993-1995

FETAL BLOOD	Test	Results	Congenital infection YES	Congenital infection NO	SNS %	SPE %	VPP %	VPN %
	PCR	pos	4	8	66.6	81.8	33.3	94.7
		neg	2	36				
	pp65 agemia	pos	1	1	16.6	97.7	50	89.5
		neg	5	43				
	IgM	pos	1	0	48	100	100	89.7
		neg	5	44				

Lazzarotto et al. J Clin Microbiol 1998

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Diagnosis of fetal CMV infection

Fetal blood samples taken at 20-21 WG

2012-2014

Serological and virological markers in fetal blood of 15 CMV-infected fetuses with high viral load (10^5 copies/ml) in the amniotic fluid.

Viral markers on fetal blood	15 infected fetuses	SENS (%)
CMV IgM +	5	33.3
CMV qPCR +	14	93.3

Fetal blood was collected from the umbilical cord after elective termination of pregnancy

IgM detection and PCR on fetal blood taken at 20-21 WG have a sensitivity for the diagnosis of fetal CMV infection lower than that obtained with amniotic fluid.

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Diagnosis of fetal CMV infection

20-21 weeks gestation

Amniotic fluid is the most appropriate material for the diagnosis of fetal CMV infection

Fetal blood does not give any additional diagnostic value because the tests used are not sensitive enough to detect directly or not directly the virus.

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Diagnosis of fetal CMV infection

20-21 weeks gestation

Why is it important to offer

a comprehensive (invasive and non invasive)

prenatal diagnosis?

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It is currently accepted that ultrasound examinations do not identify more than ~20% of infected fetuses and the sensibility is further reduced if the ultrasound is performed when the state of the fetal CMV infection is unknown.

AJOG American Journal of Obstetrics & Gynecology

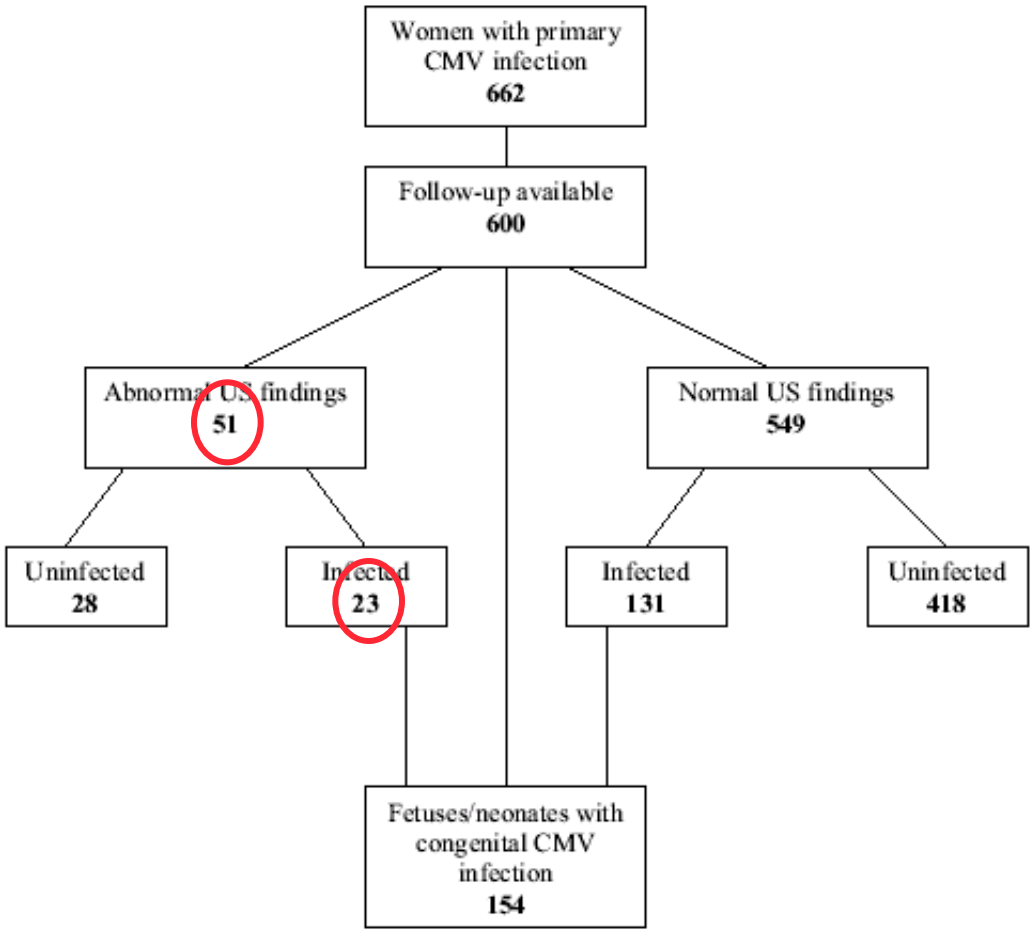
Guerra, et al. 2008

Out of 600 pregnant women who underwent ultrasound examinations with complete follow up, there were 51 abnormal ultrasound findings.

From these 51 findings, only 23 fetuses were actually infected.

SENS 14.9%
SPEC 93.7%
PPV 45.0%
NPV 76.1%

FIGURE 1
Ultrasound findings in pregnant women with CMV primary infection with respect to the presence or absence of CMV infection in fetus or neonate



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Prognosis of fetal CMV infection

20-21 weeks gestation

What can we say about the fetal markers for the prognosis of congenital CMV infection?

What about amniotic fluid?

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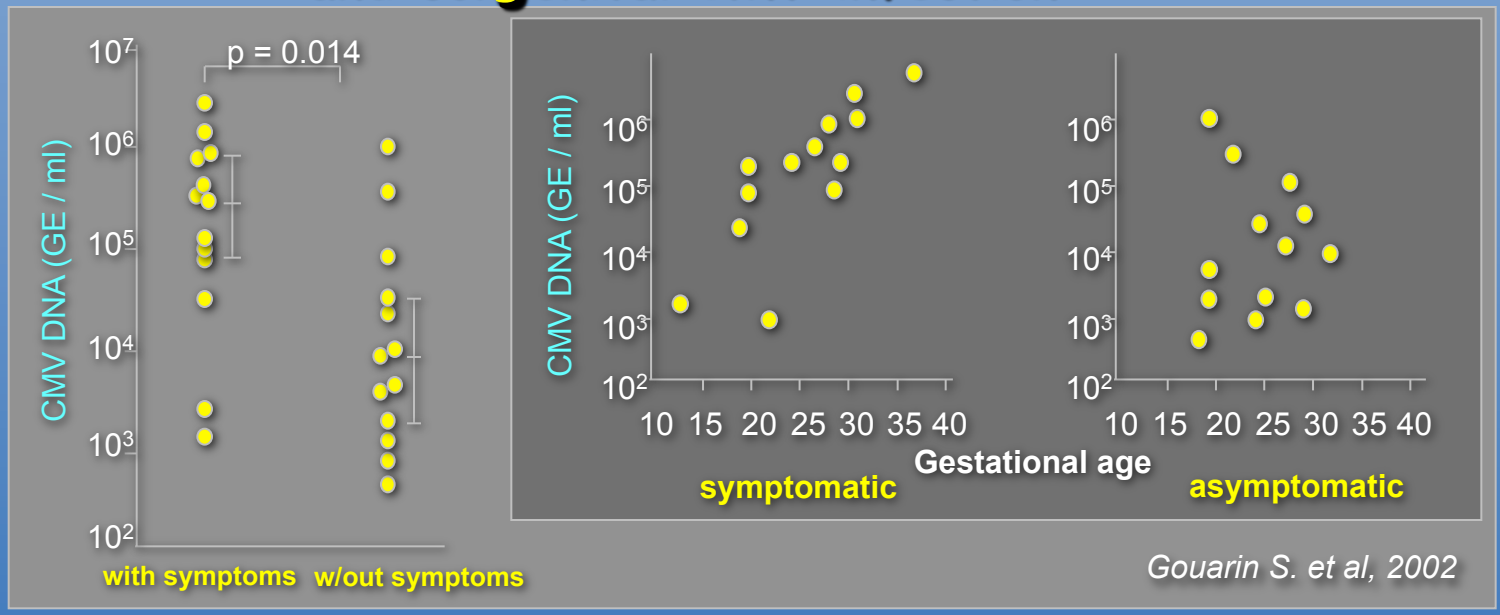
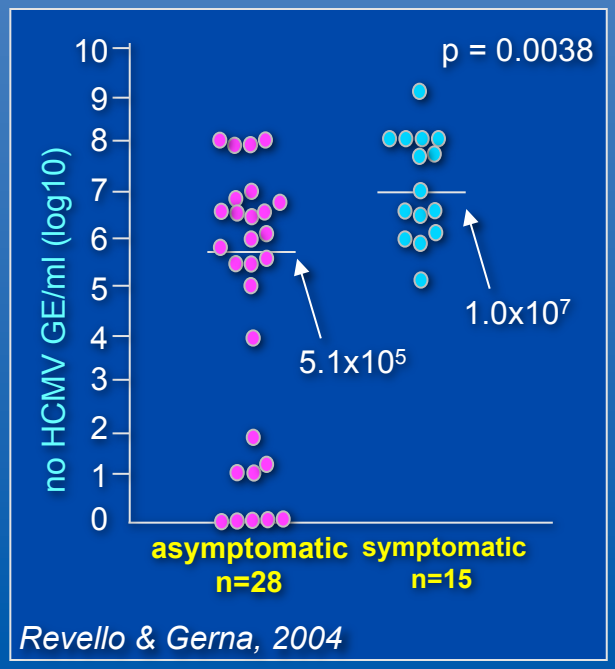
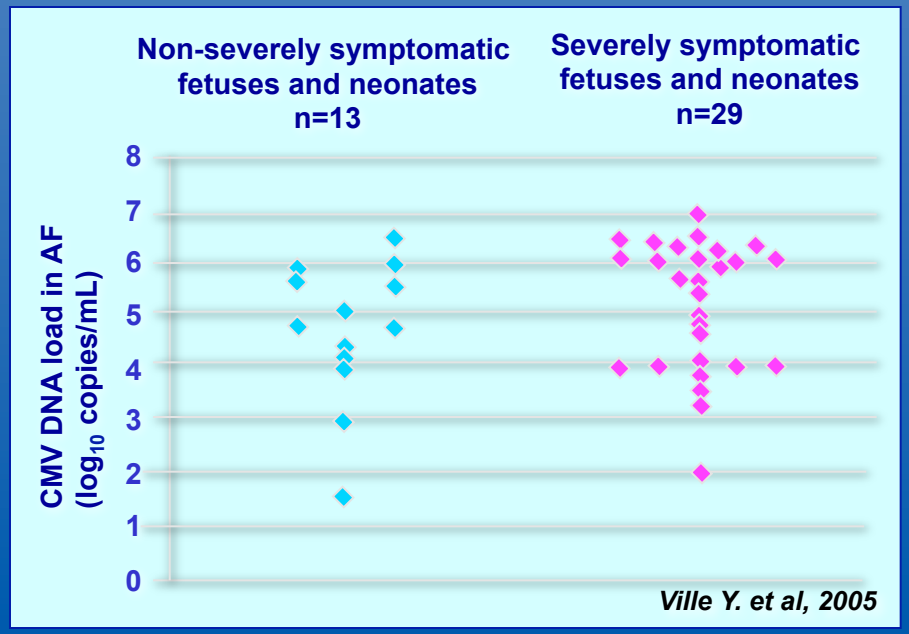
CMV load in amniotic fluids of primarily infected women and congenital CMV infection



Gouarin S. et al, 2002



Revello & Gerna, 2004



Ville Y. et al, 2005

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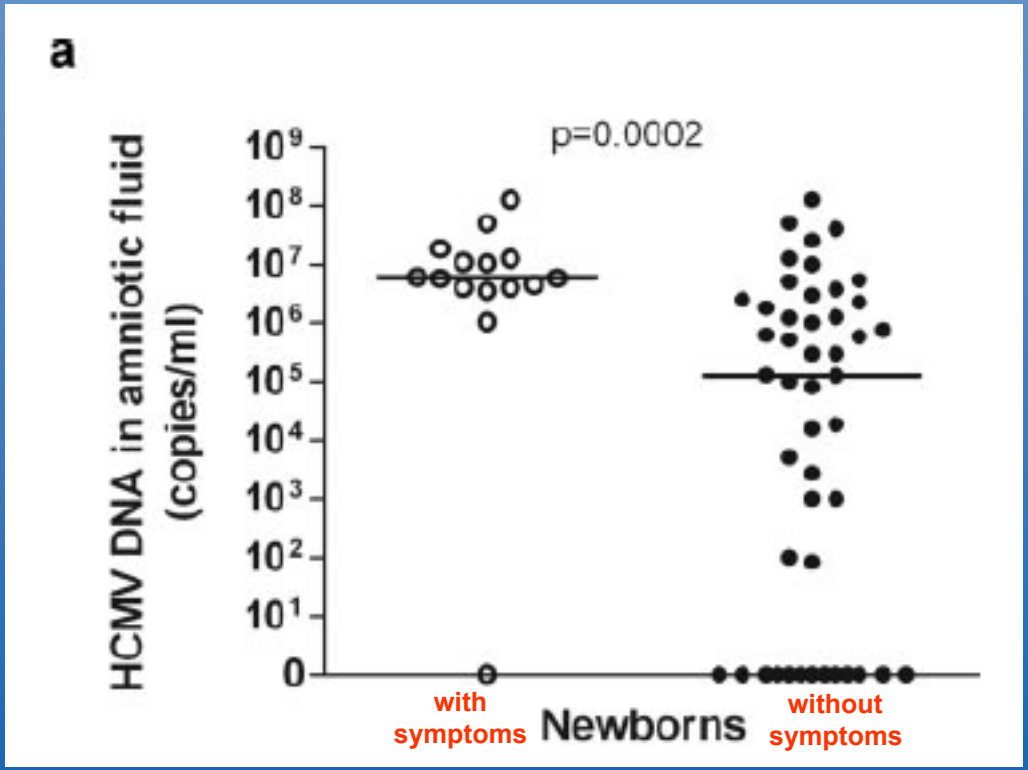
CMV viral load in amniotic fluids of primarily infected women and congenital CMV infection

Zavattoni et al. J Med Virol 2014



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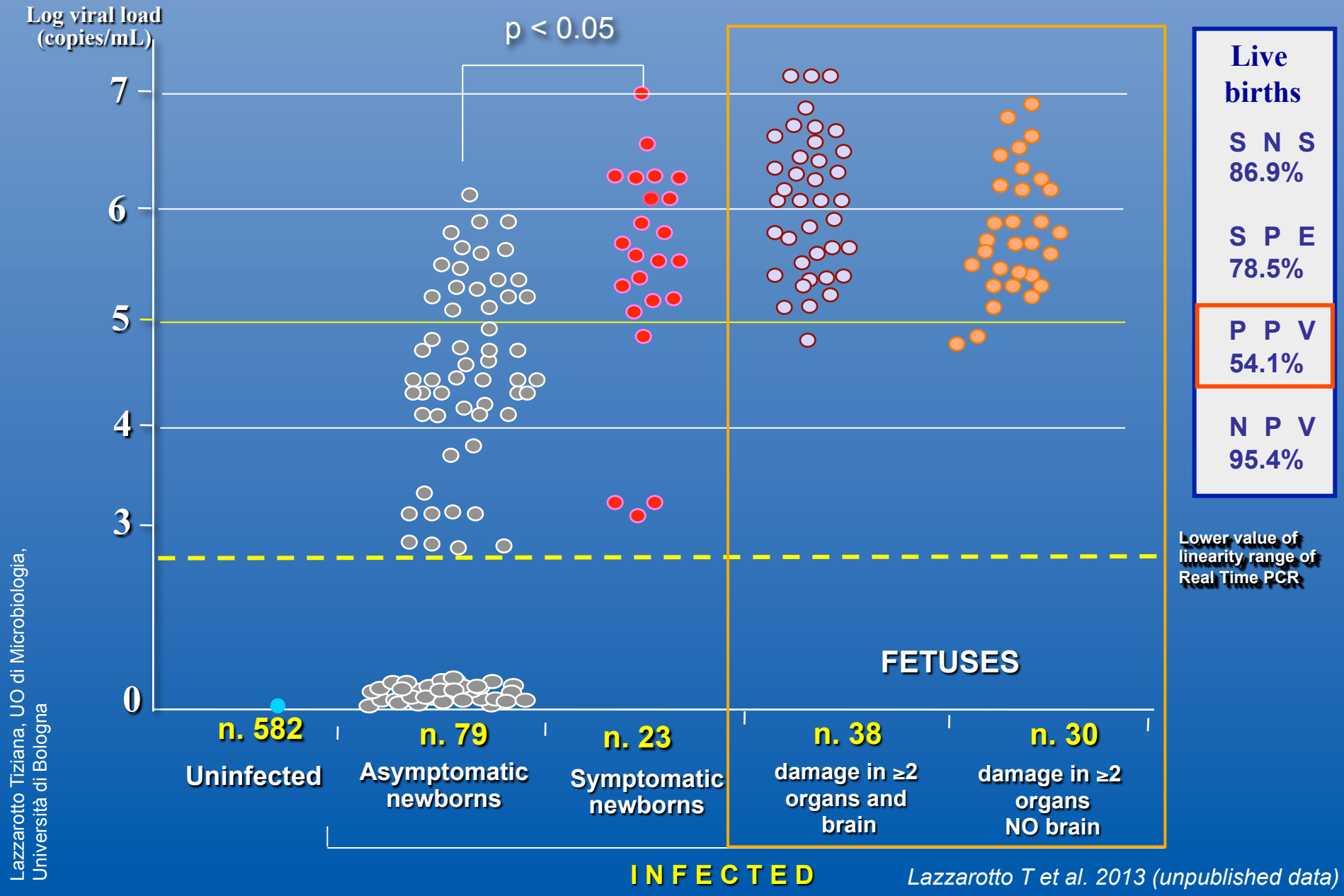
The results of qPCR in 772 amniotic fluids from primarily infected mothers & the relationship with pregnancy outcome



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Lazzarotto T et al. 2013 (unpublished data)

Prognosis of fetal CMV infection

20-21 weeks gestation

High viral loads in amniotic fluids may be associated with symptomatic or asymptomatic congenital infections

→ however the highest values tend to indicate a more severe infection.

(literature reports: Gouarin et al. 2002 and Zavattoni et al. 2014)

Therefore, is cordocentesis useful for prognostic purposes in fetuses with CMV infection?

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Prognosis of fetal CMV infection

20-21 weeks gestation

The level of specific IgM and viral blood load is not correlated with a poor outcome. It has been proposed that platelet count gives a better indication.
(Benoist et al. AJOG 2008)

The determination of multiple markers (haematological, biochemical and virological markers) in fetal blood following virus detection in AF, is predictive of perinatal outcome in fetuses with CMV infection.
(Fabbri et al. BJOG 2011).

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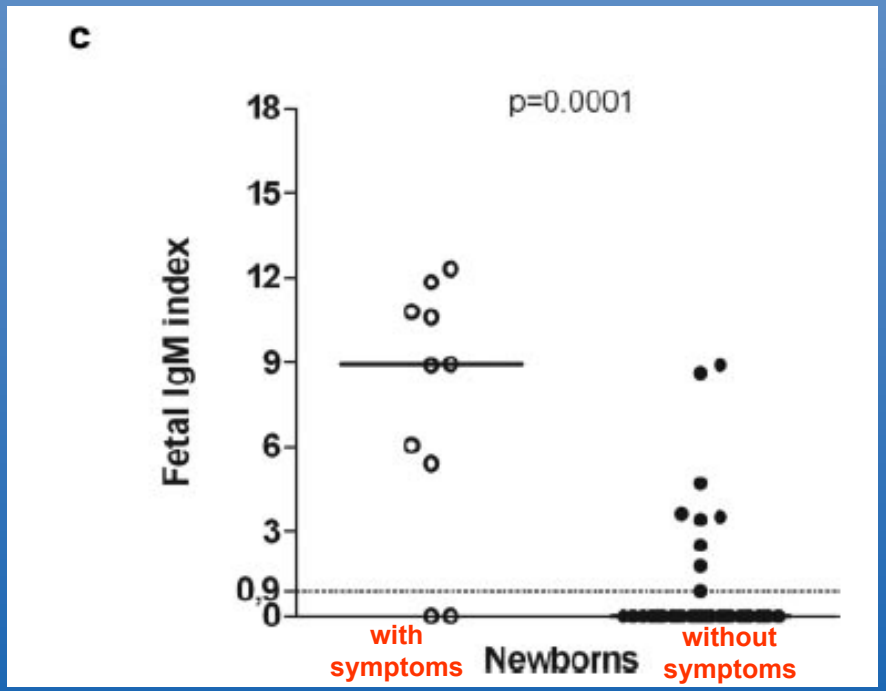
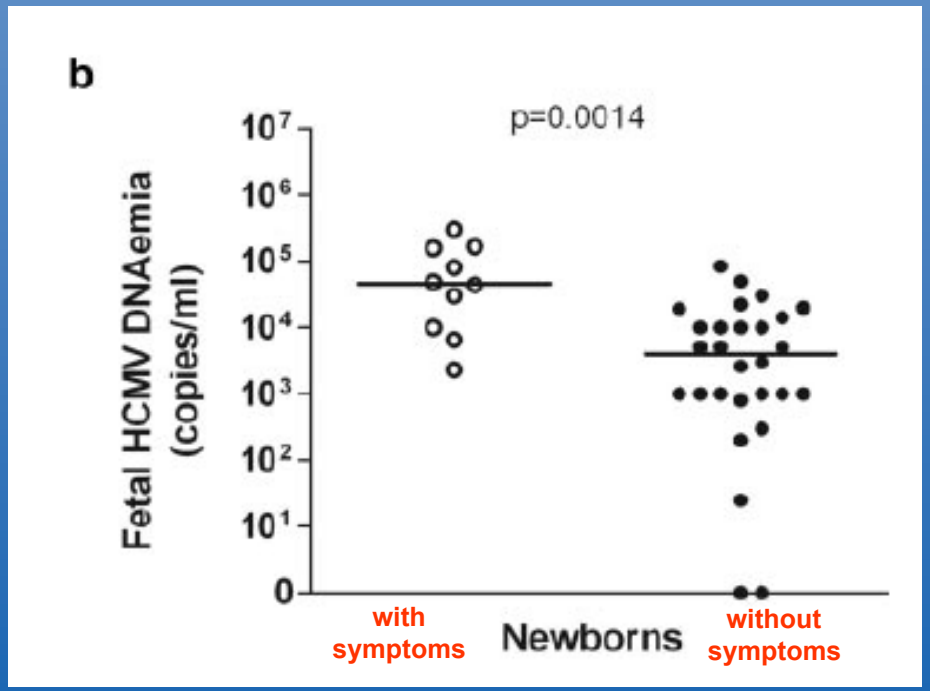
CMV-IgM index and CMV load in fetal blood of primarily infected women and congenital CMV infection

Zavattoni et al. J Med Virol 2014





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Study population

15 pregnant women at 20-21 WG with diagnosis of CMV fetal infection.
In all cases: amniotic fluids were positive for viral isolation and qPCR with a high viral load (> 10^5 copies / mL).
At the moment of amniocentesis all pregnant women underwent ultrasound examinations.

As negative control we studied 4 CMV-seronegative pregnant women at 20-21 WG.

Study design

- After elective pregnancy termination, 2 mL of fetal blood were collected from the umbilical cord.

- Virological, haematological and biochemical markers in fetal blood were studied.

This study (SA43GABR) was approved by the Ethical Committee of St. Orsola-Malpighi General Hospital, Bologna.

RESULTS

19 pregnant women

electively terminated their pregnancy at 20-21 WG

15 CMV infected fetuses

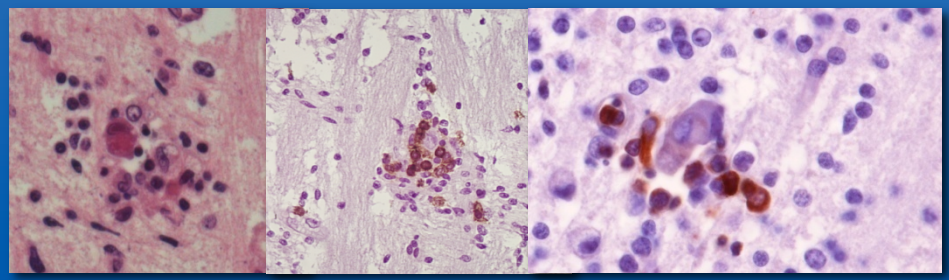
4 fetuses non CMV infected

histological evaluation

12 fetuses
CMV-pos brain (IHC)
brain histological damage

3 fetuses
CMV-neg brain (IHC)
no brain histological damage

1 fetus cardiac malformations
2 spina bifida
1 trisomy 21



CMV antigen expression and inflammatory response were studied in all fetal tissues using immunohistochemical staining procedures.

Gabrielli et al. J Clin Virol 2009
Gabrielli et al. CMI 2012

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Prognosis of fetal CMV infection

Case #	ULTRASOUND at 20-21 WG	AUTOPSY	
1	Negative	Brain damage	12 CMV POSITIVE BRAINS (IHC) with histological damage
3	Negative	Brain damage	
4	Negative	Brain damage	
12	Negative	Brain damage	
18	Negative	Brain damage	
23	Negative	Brain damage	
0	Microcephaly	Brain damage	
2	Cerebral periventricular echogenicity, hyperechogenic bowel	Brain damage	
16	Microcephaly, intraventricular septa, hyperechogenic bowel	Brain damage	
19	Cerebral ventriculomegaly	Brain damage	
20	Hyperechogenic bowel	Brain damage	
24	Cerebral periventricular echogenicity	Brain damage	
7	Negative	Negative	3 CMV NEGATIVE BRAINS (IHC) without histological damage
8	Negative	Negative	
9	Negative	Negative	

Findings detected by ultrasound and correlation with or without histological brain damage in the 15 fetuses studied

6/12 fetuses with histological brain damage have a pathological US (50%).

Sens: 50%
Spec: 100%
PPV: 100%
NPV: 34%

US examinations were performed by one experienced ultrasonographer working in S. Orsola-Malpighi Hospital in Bologna, Italy (Dr. G. Simonazzi).

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Prognosis of fetal CMV infection

Fetal blood samples taken at 20-21 weeks gestation

Serological markers detected in fetal plasma of CMV-infected fetuses and correlation with fetal brain damage

VIDAS® ToRC panel	12 fetuses with brain damage	3 fetuses without brain damage
CMV IgM +	5	0
CMV IgM -	7	3
P	*0.245* *Fisher's exact test*	

When comparing the blood of infected fetuses with and without brain damage, we observed no statistical difference in detection of CMV specific IgM antibodies.

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Prognosis of fetal CMV infection

Fetal blood samples taken at 20-21 weeks gestation

Virological markers detected in fetal whole blood of CMV-infected fetuses and correlation with fetal brain damage

HCMV ELITe MGB kit	Median values (range)	
	12 fetuses with brain damage	3 fetuses without brain damage
Real time PCR	104,408 (1,470 – 1,993,500)	1,428 (0 – 16,335)
P	*0.02* *Wilcoxon test*	

On the contrary, when comparing the blood of infected fetuses with and without brain damage, we observed a statistical difference in median values of CMV-DNAemia levels.

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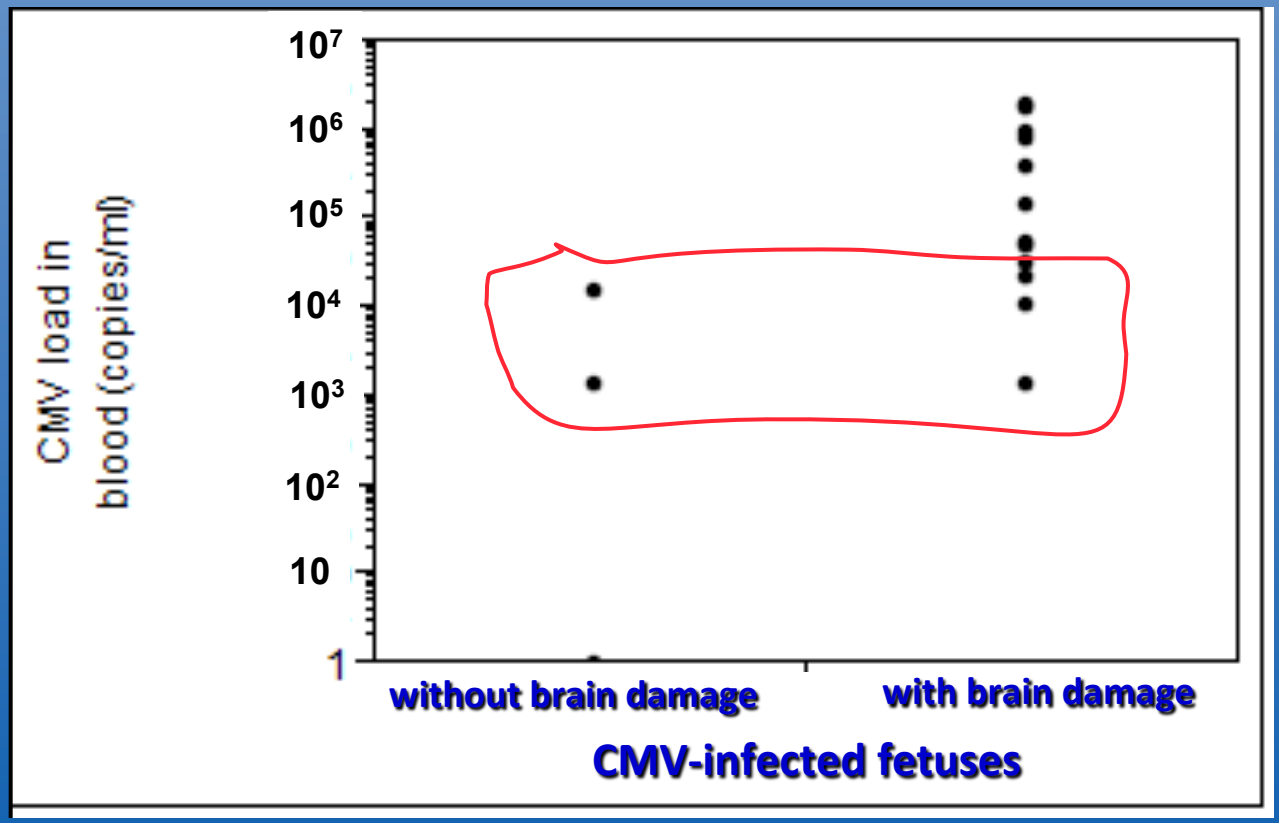
Prognosis of fetal CMV infection

Fetal blood samples taken at 20-21 weeks gestation

Virological markers detected in fetal whole blood of CMV-infected fetuses and correlation with fetal brain damage



CMV DNA load in fetal blood samples is significantly higher in fetuses with brain damage.

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Prognosis of fetal CMV infection

Fetal blood samples taken at 20-21 weeks gestation

In conclusion:

1) CMV IgM markers detected in fetal blood are not prognostic of symptomatic congenital CMV infection;
2) viral DNA levels in fetal blood can be misleading.

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Prognosis of fetal CMV infection

Fetal blood samples taken at 20-21 weeks gestation

Fetal blood parameters	Median values (range) 12 fetuses with brain damage	3 fetuses without brain damage	*P value*	Median values (range) 3 fetuses no CMV-infected
White blood cells	9.05x10^3/µL (2.6-16.4)	8.1x10^3/µL (3.8-10.1)	NS	10.1x10^3/µL (2.9-11.5)
Erythroblasts	10.2x10^3/µL (3.4-32.8)	1.8x10^3/µL (0.6-12.0)	NS	4.6x10^3/µL (0.2-56.4)
Hemoglobin	11.8 G/dL (8.0-12.5)	11.7 G/dL (11.0-11.7)	NS	8.5 G/dL (7.7-11.5)
T lymphocytes	3 469/mmc (666-8582)	3 334/mmc (1801-3818)	NS	1 719/mmc (1632-4814)
CD4/CD8	0.6 (0.2-2.3)	0.8 (0.7-1.4)	NS	3.5 (2.7-6.1)
Natural killer	1 821/mmc (112-6012)	1 429/mmc (240-1530)	NS	1 079/mmc (104-1256)
ß2- Microglobulin	18.9 ng/mL (9.7-39.0)	7.7 ng/mL (4.1-9.7)	0.02	5.25 ng/mL (4.8-6.3)*
Platelet count	93x10^3/µL (18-161)	265x10^3/µL (126-315)	0.04	145x10^3/µL (126-154)

* statistical values obtained from 4 uninfected fetuses

Wilcoxon test

The parameters that showed a statistically significant difference between the two groups are the ß2-microglobulin and the platelet count .

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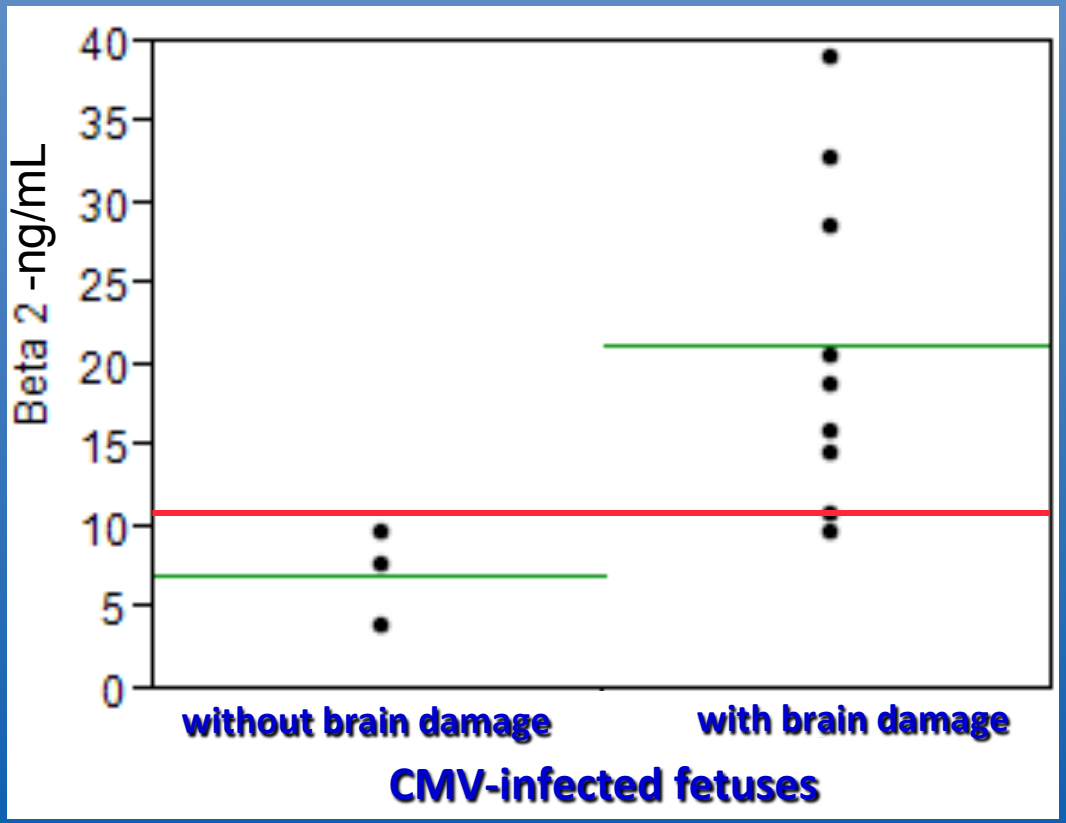
Prognosis of fetal CMV infection

Fetal blood samples taken at 20-21 weeks gestation

ß2- microglobulin detected in fetal blood of CMV-infected fetuses and correlation with fetal brain damage



Cut-off > 11 ng/mL
Prevision of severe disease
(Roc curve)

Sensitivity = 78%
Specificity = 100%

PPV = 100%
NPV = 60%

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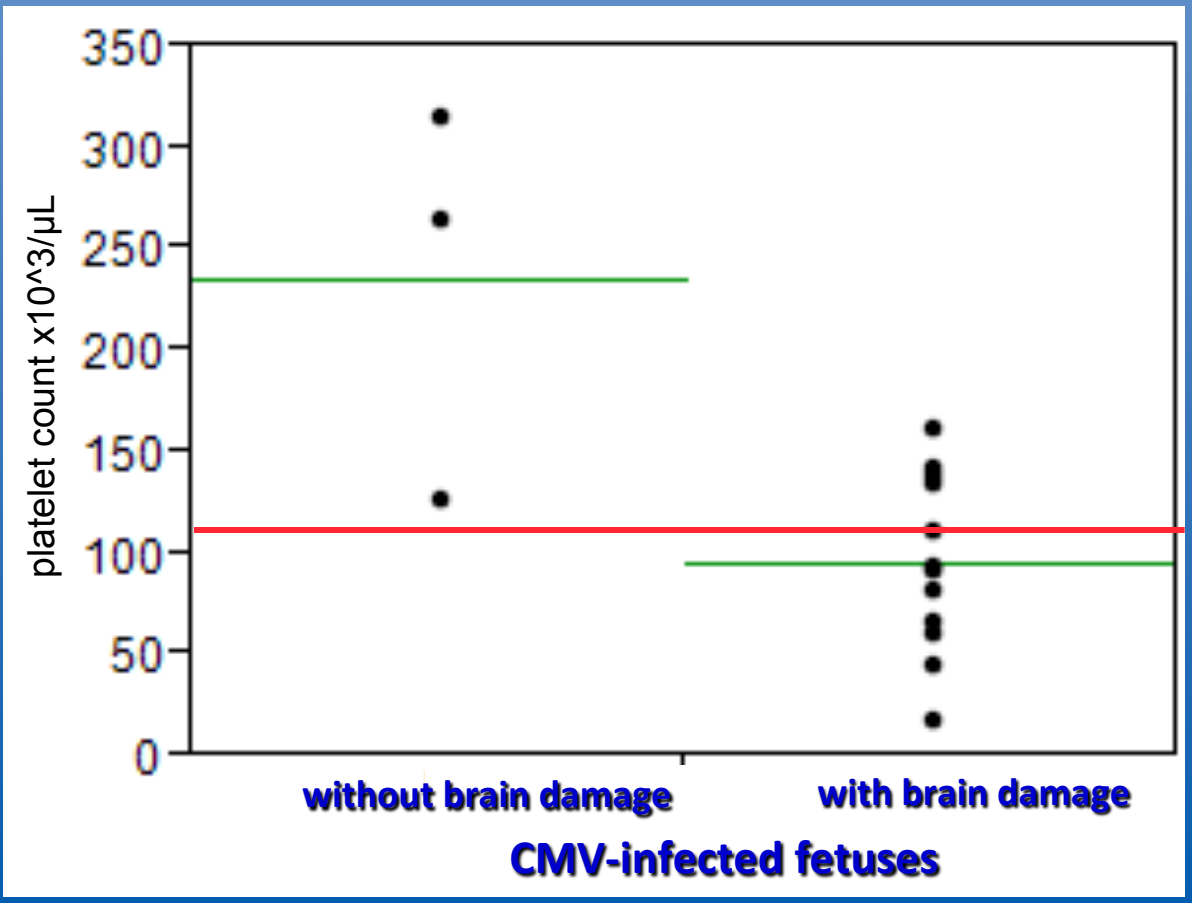
Prognosis of fetal CMV infection

Fetal blood samples taken at 20-21 weeks gestation

Platelet count in fetal blood of CMV-infected fetuses and correlation with fetal brain damage



Cut-off ≤ 110x10^3/µL
Prevision of severe disease
(Roc curve)

Sensibility = 67%
Specificity = 100%

PPV = 100%
NPV = 43%

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Prognosis of fetal CMV infection

Fetal blood samples taken 20-21 weeks gestation

Prognostic markers in compliance of severe fetal CMV disease

1) ß2- microglobulin > 11 ng/mL
2) Platelet count ≤ 110x10^3 /µL
3) CMV DNAemia levels > 5.000 copies/mL (Zavattoni 2014)

Markers Agreement	9 fetuses with brain damage	3 fetuses without brain damage	*total*	*SENS* %	*SPE* %	*PPV* %	*NPV* %
YES	4	1	5	44	67	80	29
NO	5	2	6				
total	*9*	*3*	12				

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Therefore, prenatal counseling based on the compliance of these specific markers can be misleading.

CLINICAL CASE #1

Primary infection in the first trimester of pregnancy
Prenatal diagnosis at 20 weeks of gestation

Amniotic fluid *Virological testings*	*Results*
Virus isolation (shell vial)	Positive
Real Time PCR	Positive 5.100.000 copies/mL

Ultrasound Findings and Cerebral Magnetic resonance	negative

Fetal blood	cut-off	*Results*
ß2- Microglobulin	*(> 11 ng/mL)*	14.6 ng/mL
Platelet count	*(≤ 110x10^3 /µL)*	161x10^3 /µL
CMV DNAemia levels	(> 5.000 copies/mL)	1.470 copies/mL

Autopsy: symptomatic fetus had CMV positive brain with severe histological brain damage and necrosis of cerebral tissue.

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CLINICAL CASE #7

Primary infection in the first trimester of pregnancy
Prenatal diagnosis at 21 weeks of gestation

Amniotic fluid *Virological testings*	*Results*
Virus isolation (shell vial)	Positive
Real Time PCR	Positive 3.072.000 copies/mL ●

Ultrasound Findings and Cerebral Magnetic resonance	negative

Fetal blood	**cut-off**	*Results*
ß2- Microglobulin	*(> 11 ng/mL)*	9.7 ng/mL
Platelet count	*(≤ 110x10^3 /µL)*	126x10^3 /µL
CMV DNAemia levels	(> 5.000 copies/mL)	16.335 copies/mL ●

Autopsy: CMV infected fetus without brain damage

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STUDY LIMITATION

- Limited sample size does not allow firm conclusions.

However these data indicate the need to take further caution when:

1) offering cordocentesis for fetal prognosis of CMV infection;

2) evaluating blood markers for fetal prognosis of CMV infection.

CONCLUSIONS

Further studies in a larger number of symptomatic and asymptomatic cases should be performed to verify the prognostic efficacy of determination of different parameters in fetal blood.

At present, fetal blood sampling at 20-21 weeks gestation cannot be justified for the prognosis of fetal CMV infection due to the high risk of fetal demise.

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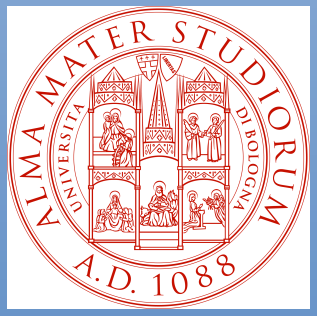

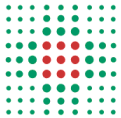

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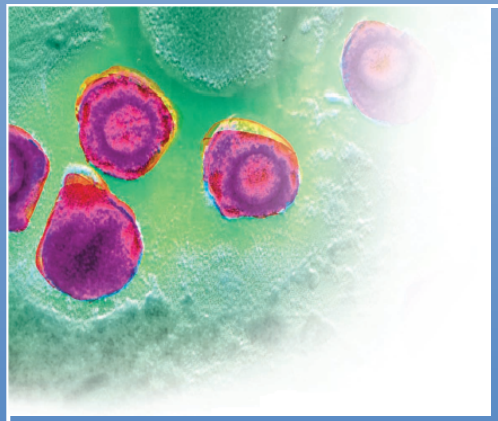

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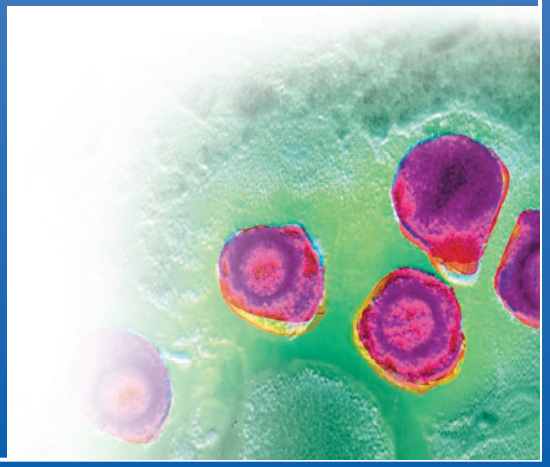

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