Diagnosis and management of fetal CMV infection

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Infectious diseases in pregnant women, fetuses and newborns
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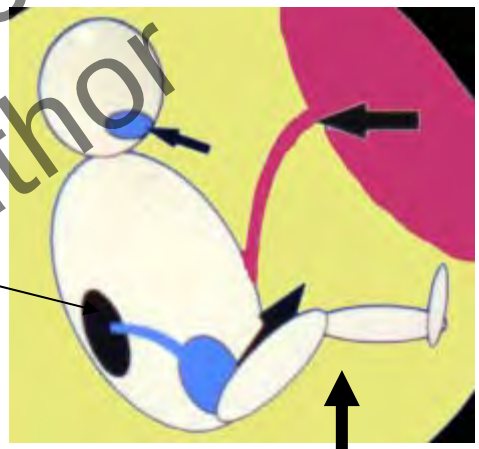
- Diagnosis of fetal infection
- Prognosis of fetal infection
- Treatment of fetal infection

Fetal CMV infection

1. Maternal infection (maternal viremia)

2.Placenta infection

3. Fetal infection



Systemic infection fetal viremia and replication in all fetal organs

Replication In the kidney

Virus eliminated in fetal urine ≥ 16 weeks gestation

Virus accumulated in amniotic fluid over time

CMV DNA is detectable in AF from 7 weeks after maternal infection

The gold standard for the diagnosis of fetal infection is to perform CMV PCR in amniotic fluid after amniocentesis

When should we perform an amniocentesis for CMV prenatal diagnosis?

Compatible fetal ultrasound abnormalities
in the 2sd/3dt trimesters

Amniocentesis should be recommended
for ethical and legal raisons

Known maternal primary infection without ultrasound symptoms

The benefit/risk of invasive procedure should be discussed with the patient

Patients with the lowest risk (periconceptionnal infection…) are more divided

Patients with the highest risk of fetal transmission (cases with certain primary maternal infection) usually choose to do the amniocentesis

This strategy decreases the patient anxiety and avoid unnecessary abortions

Should the presence of CMV DNAemia in maternal blood be checked before amniocentesis?

Performing a CMV PCR in maternal blood prior to amniocentesis had been recommended

Potential iatrogenic risk of performing this invasive procedure when the mother is viremic

However: no clear recommendation when CMV PCR in maternal blood is positive:
cancel?
postpone?
or do it nevertheless if the viral load is “low”?

Moreover there are no data in the literature supporting this strategy

- No iatrogenic risk has been reported in the literature :
  - In 2 studies the risk to deliver an infected baby was not increased if CMV PCR was positive in maternal blood at the time of amniocentesis [1,2]

- In a recent study, a CMV positive PCR in maternal blood at the time of amniocentesis was associated with a 3 X risk of fetal infection (positive CMV PCR in amniotic fluid)

  suggesting that CMV DNA found in maternal blood at the time of amniocentesis could be of fetal origin and therefore rather be an indication for amniocentesis than the reverse

[1] Liesnard C et al, 2000, [2] Revello et al, JID, 2008, [3] Simonazzi G et al, ECCI Conference, 2016

The best timing for prenatal diagnosis

At least 7 weeks after the onset of maternal primary infection

After 20 weeks of pregnancy

The performance of CMV prenatal diagnosis is good

- If the timing of the amniocentesis is appropriate
  - Positive Predictive Value ≈ 100%
  - Negative Predictive Value is ≈ 90 -95% [1,2]
- The are 5 to 10% false negative cases of prenatal diagnosis with a negative PCR in AF but positive PCR in urine or saliva at birth
  - late transplacental passage of virus

[1]Revello et al, J Infect dis, 2008, 197: 595-96, 2 Bilavsky E et al, Clin Infect Dis, 2016

False negative cases have a good prognosis

N= 138 infected neonates	Controls (positive at prenatal diagnosis) N=92	Cases (negative at prenatal diagnosis) N=46
Symptomatic at birth	23 (25%)	2 (4.3%)
Sequelae at 2 years old	13 (14%)	0

Bilavsky E et al, Clin Infect Dis, 2016

After the diagnosis of a fetal infection,
the prognosis must be established

The aim of prenatal management is to ensure livebirth
of asymptomatic fetuses

Fetal CMV disease mirors what is seen at birth

10 to 30% show symptoms of systemic fetal infection may be transient : Hepetatis enterocolitis

10 to 15% show affection of the brain which can been delayed

Most cases are asymptomatic

Severe affection of the brain is due to a high level of CMV multiplication in neural cells in a context of immune exhaustion

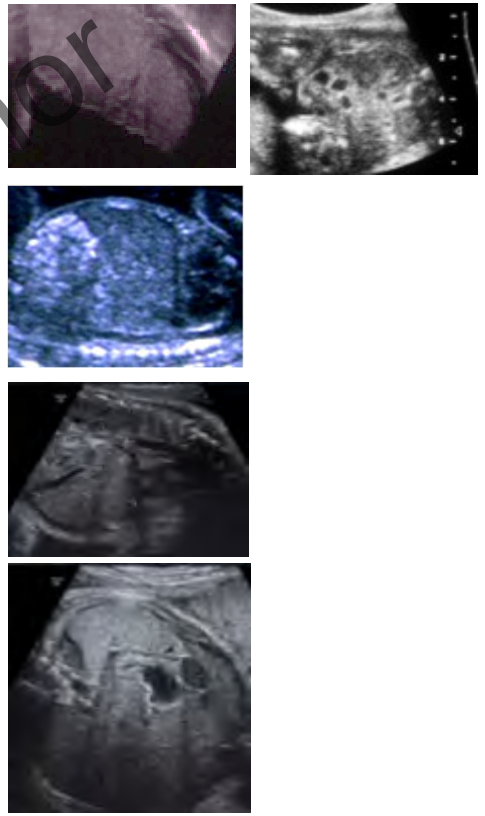
- In severely affected brains: significantly higher concentration of T cells, NK cells and virus are detected
  - But 90% of brain T cells and 70% of brain NK cells are exhausted: express PD1
  - Pro-inflammatory IL (IL6, IL4, IL2) and TNFα and INFγ are not increased when anti-inflammatory protein IL1 RA expression is significantly increased

Ultrasound must look for extra-cerebral features of fetal CMV infection

Presented in the theoretical chronological order of their development

Placentitis
Oligohydramnios
Polyhydramnios
Ileus
Meconial peritonitis /Ascites
Liver & Spleen enlargement
Ubiquitous Calcifications
Pericardial / Pleural Effusion
Dilated Myocarditis
Heart Calcifications
Hydrops

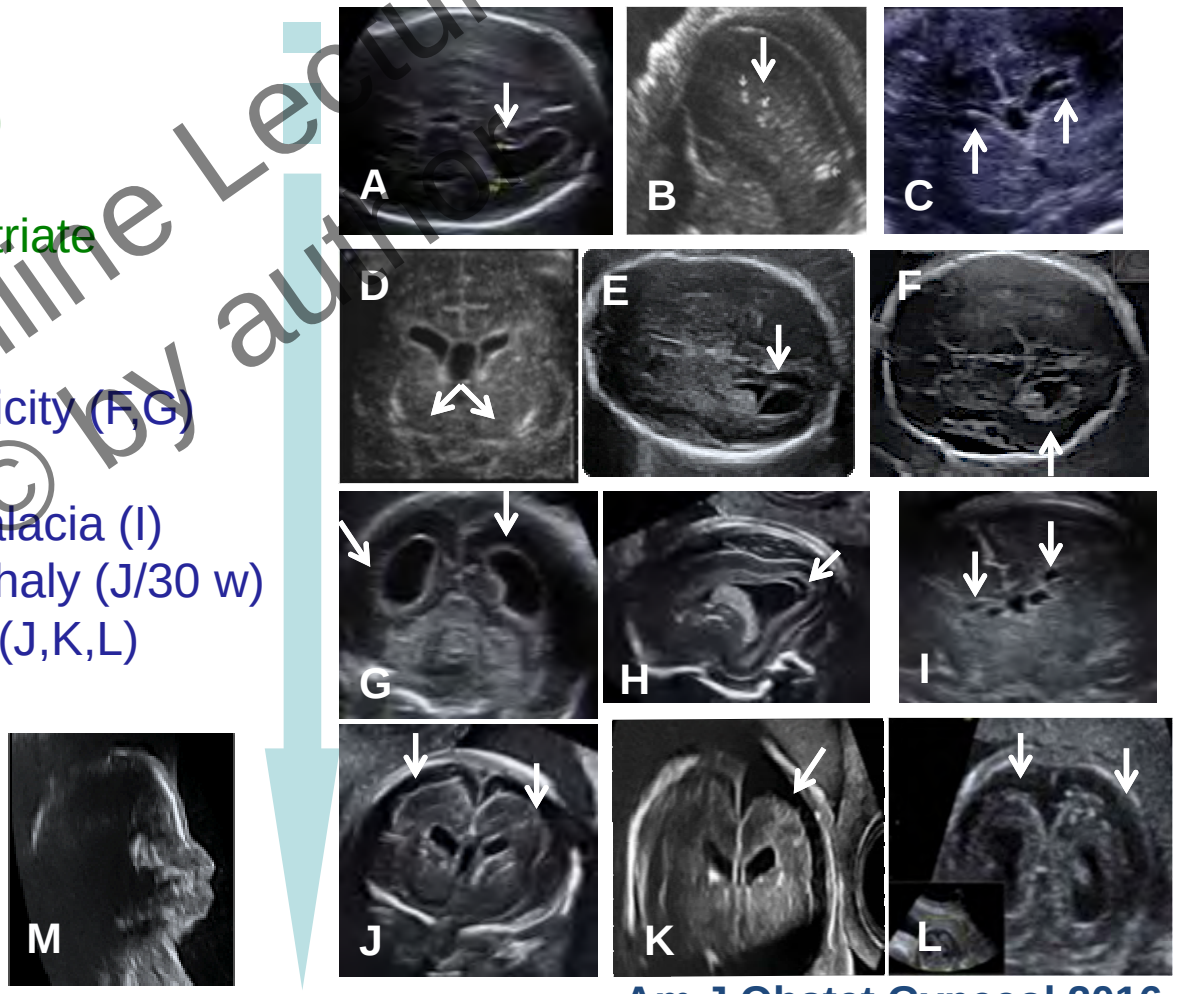
Growth Restriction / Small for GA



And for cerebral ultrasound features of fetal CMV infection

Presented in order of increasing severity

- Ventriculomegaly (A)
- Parenchymal calcifications (B)
- Sub-ependymal Cysts (C)
- Calcifications of the lenticulostriate
- vessels (D)
- Intraventricular septation (E)
- Periventricular Hyperechogenicity (F,G)
- Periventricular Cysts (G
- Cystic Periventricular leukomalacia (I)
- Abnormal Gyration / Lisencephaly (J/30 w)
- Enlarged pericerebral spaces (J,K,L)
- Polymicrogyria (K)
- Microencephaly (L)
- Microcephaly (M)



Am J Obstet Gynecol 2016

Ultrasound features and prognosis of infected fetuses

- The presence of severe cerebral ultrasound features increases the risk to be symptomatic at birth by 40 [1]

  The prognosis of an infected fetus with severe cerebral ultrasound features is poor leading to TOP

- The positive predictive value of isolated extra-cerebral ultrasound features for being symptomatic at birth is ≈50% [1]

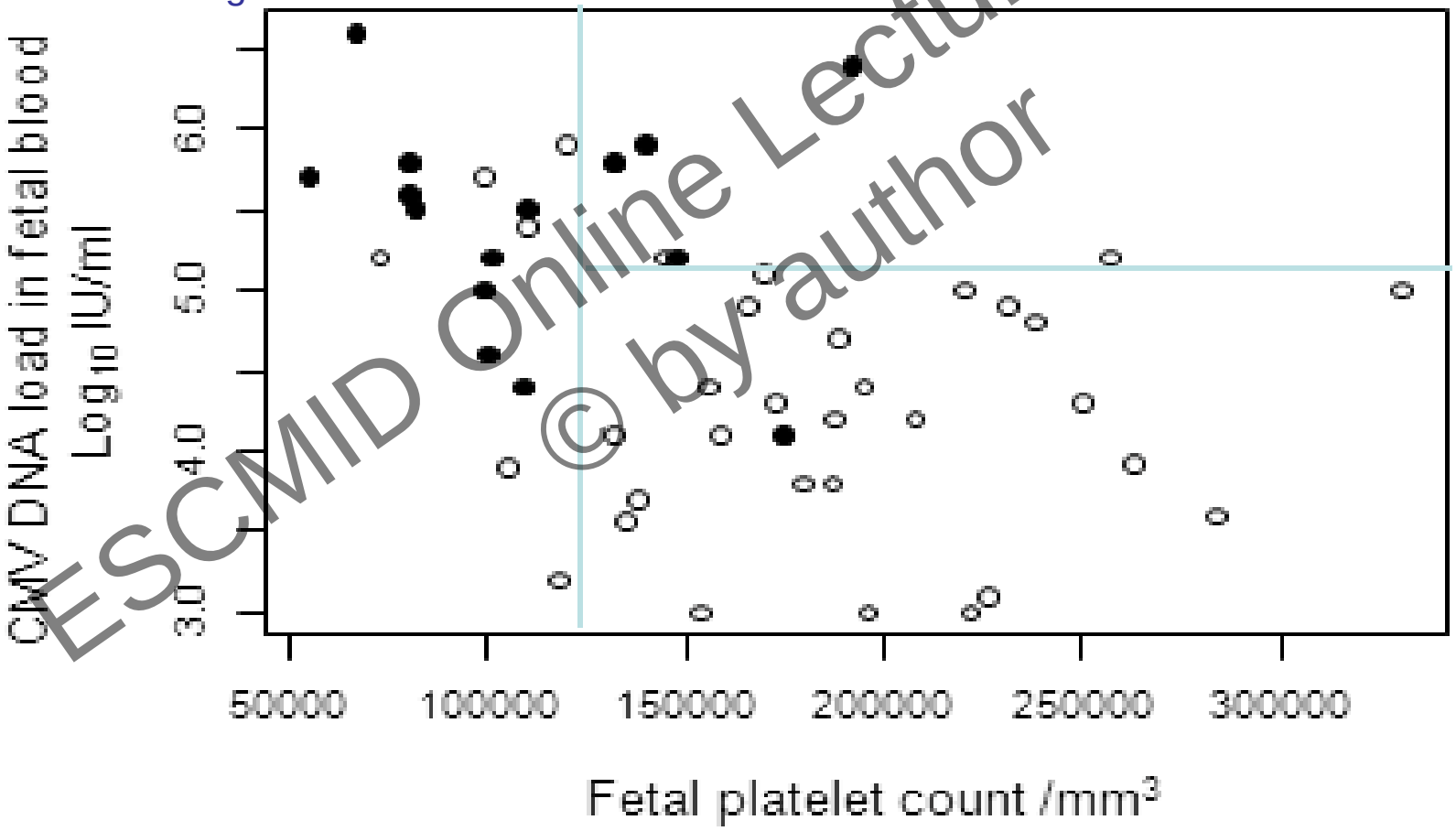
- The negative predictive value of a normal imaging was found 85-~90% in different studies [2-5]

- Need for improvement of Positive and Negative Predictive Value of ultrasound alone
  - ✓ Early reassurance
  - ✓ Early access to TOP
  - ✓ Opportunity for treatment

[1]Benoist G et al , 2008, British J Obstet Gynaecol; 2 Benoist G Ultrasound Obstet Gynecol. 2008 ; [3] Picone O, Prenat Diagn. 2008. 4 Lipitz S, Ultrasound Obstet Gynecol. 2013. ; 5 Farkas N, Prenat Diagn. 2011

Fetal laboratory blood parameters can help to establish the prognosis

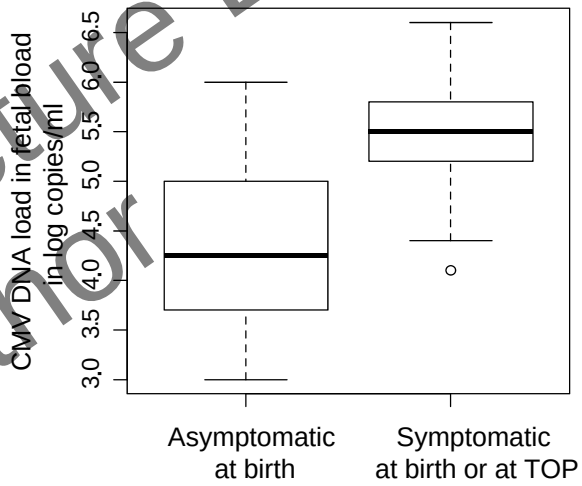
In a serie of 53 fetuses with either no US symptoms or only extra cerebral US symptoms: the cases symptomatic at birth or TOP (black dots) were those with the lower fetal platelet count and the higher level of CMV DNA in fetal bload

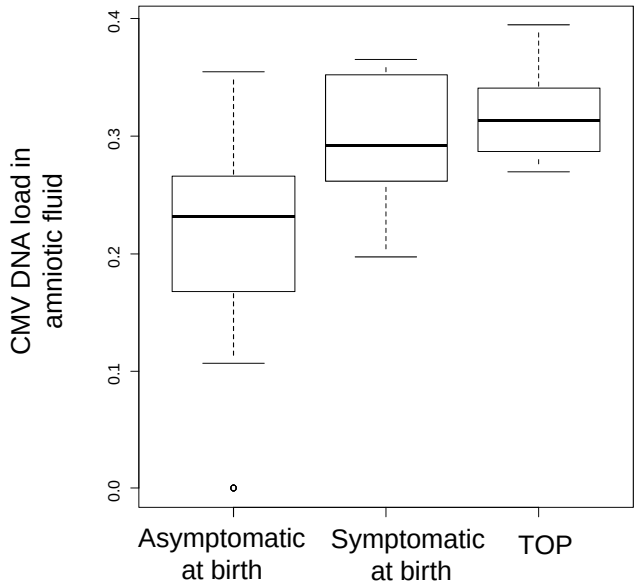


Scatter plot. Empty dots are cases asymptomatic at birth; full dots are cases symptomatic at birth or at TOP (TOP: termination of pregnancy)
Leruez-Ville et al, AJOG, 2016

Viral loads in fetal blood and in amniotic fluid at the time of diagnosis (23 weeks) are predictive of symptomatic or asymptomatic status at birth

	Logistic regression		
	OR	IC95%	**p. value**
CMV DNA in fetal blood (for each 1 log of IU/ml increase) N=54	5.77	2.02-16.53	0.001
CMV DNA in amniotic fluid adjusted to the delay between primary infection and time of amniocentesis (for each 1 log of IU/ml increase) N=48	2.31	1.15-4.64	0.018





M Leruez-Ville et al, Am J Obstet Gynecol, 2016

The Positive Predictive value of non-severe ultrasound features at 23 weeks rose from 60% to 79% when combined with fetal laboratory parameters

The negative Predicitive value of a normal ultrasound examination at 23 weeks rose from 91% to 100% when combined with fetal laboratory parameters

	PPV	NPV
Non-severe US features alone N=63	60 %	93%
Non-severe US features AND adjusted CMV DNA in amniotic fluid > 1MoM N=58	78%	90%
Non-severe US features OR adjusted CMV DNA in amniotic fluid > 1MoM N=58	44%	95%
Non-severe US features AND abnormal fetal blood results * N=53	79%	91%
Non-severe US features OR abnormal fetal blood results N=53	50%	100%

PPV: positive predictive value, NPV: Negative predictive value

* CMV DNA in fetal blood > 4,95 $\log_{10}$ UI/ml and/or fetal platelet count < 114 000/ mm^3

Leruez-Ville M, Am J Obstet Gynecol, 2016

Which options are available for the treatment of infected fetuses?

The efficacy of hyperimmune globulin for the prevention and treatment of materno-fetal is still debated

- One exploratory study: risk reduction of fetal infection of 24% [1]
- One randomized, double-blinded, placebo controlled study:
  - Non significant risk reduction of fetal infection [2]
  - Side effect? Increased risk of prematurity
- The results of an ongoing trial in the US might help to conclude this matter https://clinicaltrials.gov/ct2/show/NCT01376778

[1] Nigro G, N Engl J of Medicine, 2005, 353: 1350-1362,
[2] Revello MG, N Engl J of Medicine, 2014, 370: 1316-26

Antiviral treatment for infected fetuses is another option

- Antiviral treatment with ganciclovir or valganciglovir is a validated option for infected infants with symptomatic CMV infection
- However, although this treatment is beneficial with stabilization or improvement of hearing it is only moderately efficient because severe neonatal cerebral lesions are irreversible
- Therefore there is a need to validate a prenatal antiviral treatment that could be given to infected fetuses to avoid constitution of irreversible cerebral lesions

Which anti CMV drugs to treat infected fetuses?

- Antiviral drugs licensed to prevent and/or to treat CMV infections and diseases in immunosuppressed patients :
  - Ganciclovir (GCV), valganciclovir (ValGCV): first line drugs
  - Foscarnet and cidofovir : second line drugs
  - Valaciclovir (ValACV): prevention of CMV disease

- Which of these antiviral drugs could be and have been used during pregnancy?
  - Foscarnet & cidofovir : NO (no oral formulation, high renal toxicity)
  - GCV & ValGCV: very little data, concerns on teratogenicity, but still a potential candidate?
  - ValACV: the only trial testing the efficiency of antiviral treatment in infected fetuses was based on the use of ValACV

Why valaciclovir?

- Although in vitro
  - ValACV is not the most efficient drug against CMV
  - High IC50 values (10-67 µM) / GCV (2.5-5.5 µM) (Tyms et al, AAC 1981)
- In clinical setting: high valACV dosage (2gx4/day) has proved efficient to prevent CMV disease in transplanted patients (Lowance et al,N Engl J Med 1999).
- ValACV has the best safety profile among anti CMV drugs
  - No cell transformation, no increase risk of neoplasia in vitro / GCV extremely genotoxic
  - Reassuring safety data : no association with an increase risk of birth defects in thousand of women exposed in pregnancy (Stone et al, 2004; Pasternak, JAMA, 2010)
  - Good tolerance

CYMEVAL 2 trial *(NCT01651585)* design

- Phase II, open labeled, one arm: all included women were treated with ValACV 8 g/day up to delivery
- Population: 43 pregnant women carrying a moderately symptomatic infected fetus (showing only extra cerebral ultrasound feature(s) or mild cerebral ultrasound feature(s))
- Primary end point: being asymptomatic at birth
- Efficiency criteria: birth of more than 31 asymptomatic neonates (Simon's methodology)

Leruez-Ville M, Am J of Obst Gynecol, 2016

Cymeval II trial results

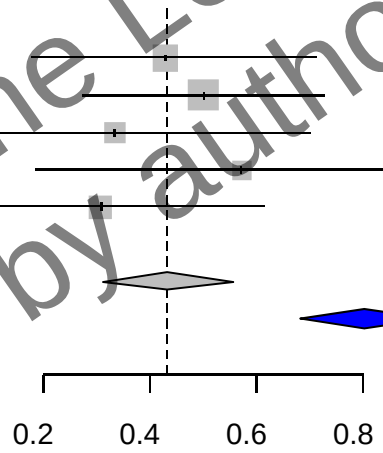
- High dosage ValACV treatment was very well tolerated both by the mother and the fetus
- ValACV treatment was efficient to increase the number of asymptomatic neonates: this could be explained
  - a direct antiviral effect of the drug: treatment induced significant increase of fetal platelets count and significant decrease of fetal viral load
  - a placebo effect of the drug that could decrease parental anxiety and avoid TOP

	Fetal blood baseline	Neonatal cord blood	Differences*	P
CMV DNA (log_{10} IU/ml)				
Median (IQ)	4.0 (3.55–4.6)	3.05 (2.57–3.92)	-0.5 (-2.075; -0.075)	0.01
Platelet count / mm^3				
Median (IQ)	173 000 (141, 500–201, 500)	245 000 (193, 000–274, 000)	101 000 (47, 500–122, 000)	<0.001

Leruez-Ville M, Am J of Obst Gynecol, 2016

Valaciclovir effect compared to historical control group

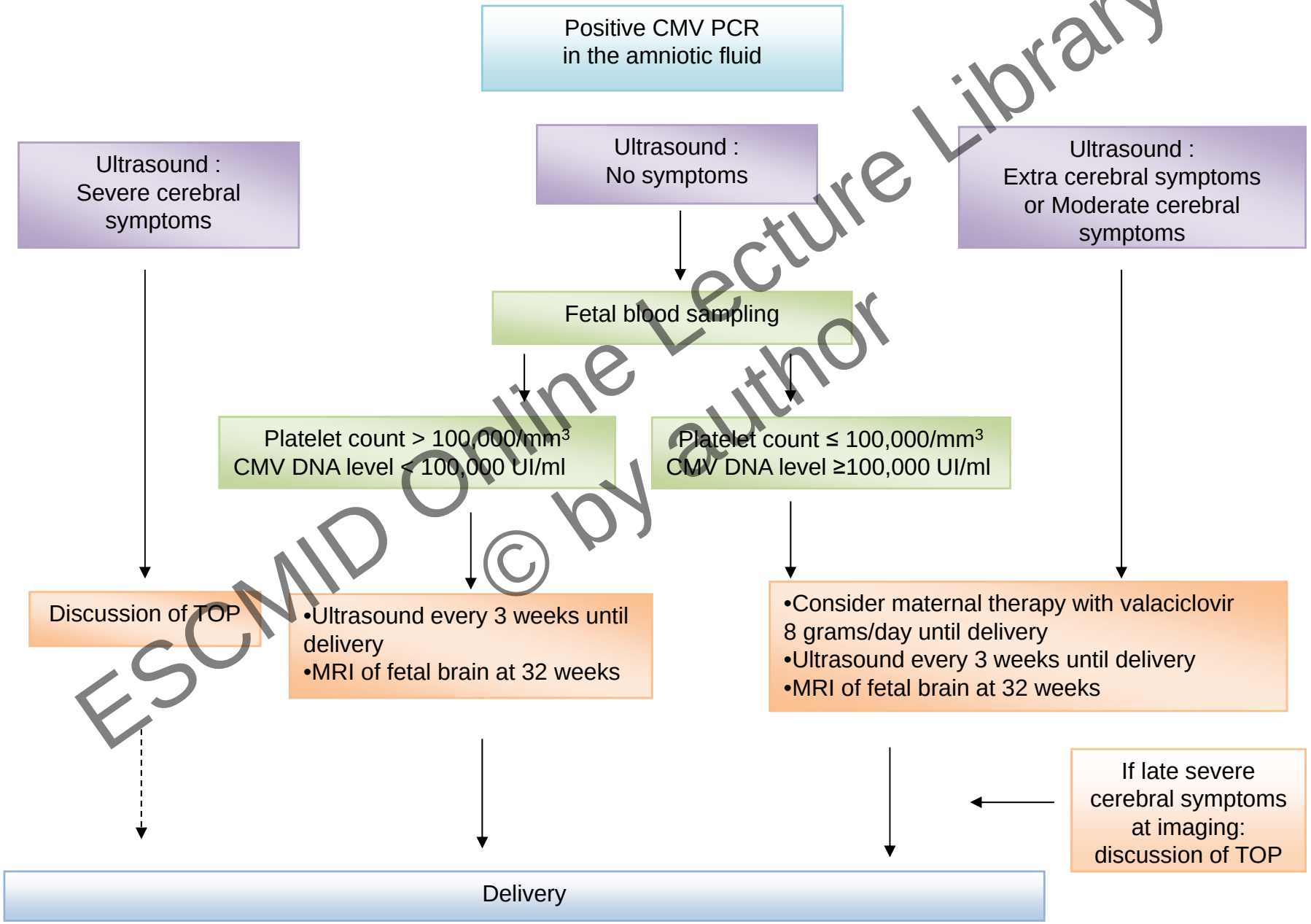
Study	Success	Total	Proportion of asymptomatic neonates	95%-confidence interval	W(random)
Guerra et al, 2008 (16)	6	14	0.43	[0.18; 0.71]	23.0%
Jacquemard et al, 2007 (11)	10	20	0.50	[0.27; 0.73]	33.5%
Liesnard et al, 2000 (14)	3	9	0.33	[0.07; 0.70]	13.4%
Lipitz et al, 2010 (17)	4	7	0.57	[0.18; 0.90]	11.5%
Picone et al, 2008 (15)	4	13	0.31	[0.09; 0.61]	18.6%
Random effect model	**27**	**63**	**0.43**	**[0.31; 0.56]**	**100%**
Valacyclovir effect *	**34**	**43**	**0.82**	**[0.67; 0.88]**	**--**



Comparison of the trial results with those of a historical group indicates that valaciclovir significantly increased the proportion of asymptomatic neonates, from 43% without treatment to 82% with treatment

Leruez-ville M et al, Am J Obstet Gynecol, 2016

Proposed algorithm for prenatal management of infected fetuses



Conclusions

- The diagnosis of fetal infection has a good performance
  - The development of a non invasive test to diagnose fetal infection could be of great value
- Progress have been made in the last decade in establishing the prognosis of an infected fetus
  - But there is room for development of new markers to improve the PPV of mild ultrasound symptoms
- A first step towards antiviral treatment of infected fetuses:
  - RCT testing ValACV efficacy against other anti viral drug (valGCV, letermovir…) should be promoted

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