The use of CMV qPCR in the blood as an indicator for treatment for Congenital CMV infection

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CMV
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Background

- There role of blood quantitative CMV PCR (qPCR) in the initial management (at baseline diagnosis) and follow-up of infants with cCMV infection is not well defined.

- At diagnosis, a small number of studies have shown
  - The absence of viremia at baseline to be associated with better long term outcomes (Ross et al, 2009; Forner et al, 2014)
  - A difference in baseline viral load among those infants with, and without SNHL (Bradford et al, 2004; Boppana et al.2005)

- In follow-up, the role and duration of viral load monitoring for infants is not clear (possible benefits including ensuring absence of drug resistance, therapeutic drug effect)

Background

- In 2007, blood qPCR for CMV was introduced at the CHU Sainte-Justine laboratory, and its use in the management of CMV infection was at individual physician discretion.
- cCMV managed by pediatric infectious diseases → viral load testing routine
- Previously reported on outcomes/PCR guided management while on antiviral therapy (Leduc et al, ID Week, 2015)
- **What is the role of PCR at baseline (<3 weeks of age, at the time of diagnosis?)**

PCR → Treatment in cCMV infection

- Treatment is only recommended in moderate-severely symptomatic cases → considerable time and cost necessary to determine level of symptoms after a diagnosis of cCMV is made.

- Treatment initiation is recommended early (within the first month of life), however the cascade of care from time of diagnosis to completion of all investigations necessary to determine level of symptoms can be long, resulting in delays in treatment initiation.
  - Some failed newborn hearing assessments subsequently normal after weeks of follow-up
  - Imaging studies may require considerable time to initiate and may be difficult to interpret in non-tertiary care centers

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Investigations for children with confirmed diagnosis

Investigation	Turnaround time
Labs: CBC, LFTs, Bilirubin, urea, creatinine	Same day
qPCR blood	**2-3 days**
Head ultrasound (pediatric radiologist)	Same week*
MRI (some)	1-3 months
Ophthalmology	Same week*
Hearing confirmation	2 weeks – 6 months*

Best case scenario (tested at birth), with a work intensive, coordinated clinical/administrative/effort, all investigations can be completed within the first month of life

Could the baseline PCR value be used to determine the need for antiviral therapy?

Objectives

- To understand the role of PCR in the diagnostic workup of cCMV infection
- To determine whether the PCR value can reliably predict which infants will require treatment
- To review the impact of this predictive value of PCR on screening program

Methods

- Retrospective cohort study of children diagnosed with cCMV infection at CHU Sainte-Justine between 2008 and 2016.

- Cases of cCMV infection were identified via the microbiology laboratory clinical database, based on positive testing by culture or shell vial assay on urine or saliva, or qPCR in blood, urine, saliva or CSF, within the first 21 days of life.
  - If initial test was qPCR in saliva, confirmatory urine PCR was done.

- **Inclusion criteria**: Complete baseline information available on all clinical findings, laboratory results, specialized investigations and neuroimaging (HUS, CT or MRI) and qPCR at baseline (<21 days of age)

Standard newborn assessments in all cases cCMV

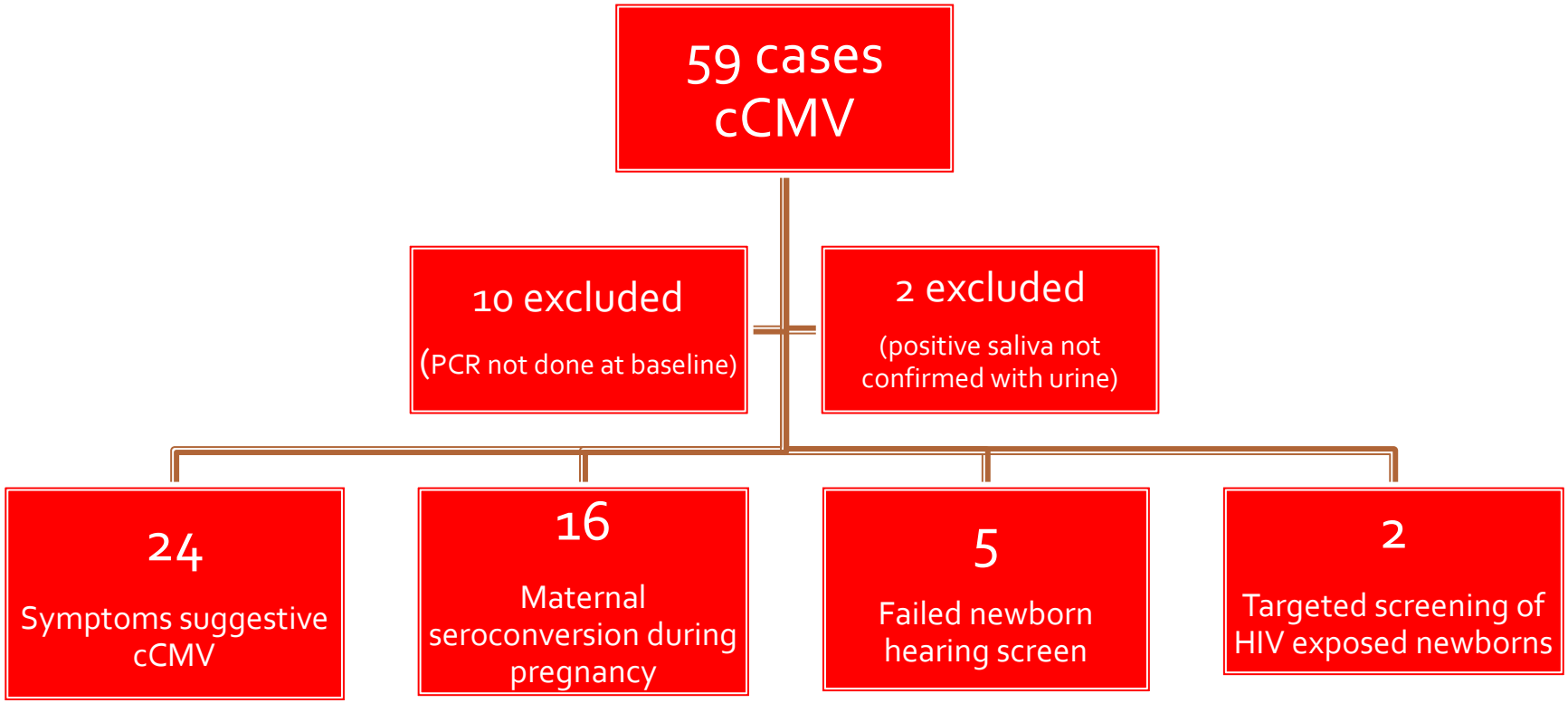
ALL cases

- Bloodwork: CBC, ALT, Bilirubin (total/direct), electrolytes/urea/creatinine
- Imaging: Head ultrasound, if abnormal → MRI
- Ophthalmology
- Audiology: A-ABR → Not passed, full audiology evaluation
- Physical exam: Pediatrician, or neonatologist

Physician discretion:

qPCR, lumbar puncture, HUS and MRI, other studies

Results: Cases identified 2008-2016



Classification of cases (symptoms*)

Moderate-severe	Multiple manifestations attributable to congenital cytomegalovirus infection: Thrombocytopenia, petechiae, hepatomegaly, splenomegaly, intrauterine growth restriction, hepatitis (raised transaminases or bilirubin), or central nervous system involvement such as microcephaly, radiographic abnormalities consistent with cytomegalovirus central nervous system disease (ventriculomegaly, intracerebral calcifications, periventricular echogenicity, cortical or cerebellar malformations), abnormal cerebrospinal fluid indices for age, chorioretinitis, sensorineural hearing loss, or the detection of cytomegalovirus DNA in cerebrospinal fluid.	31 (66%)
Mild	Up to 2 isolated manifestations of congenital cytomegalovirus infection that were mild and transient	0
Asymptomatic	if they had no clinical or laboratory or imaging abnormalities to suggest congenital cytomegalovirus disease, but sensorineural hearing loss	4 (8.5%)
Asymptomatic with isolated SNHL	no abnormalities to suggest congenital cytomegalovirus disease, only SNHL	12 (25.8%)

**Lancet Classification*

Common clinical findings among newborns

- 75% of patients had at least one symptom suggestive of cCMV infection (n=32).
- The most common clinical findings were:
  - Evidence of CNS involvement (microcephaly, seizures and brain abnormalities on neuroimaging) (n=24, 52.1%)
  - SNHL (n=18, 38.3%)
    - IUGR (n=18, 38.3%)
- Other common clinical findings included
  - Thrombocytopenia (n=17, 36.3%)
  - Neonatal jaundice (n=10, 21.3%)
  - Petechiae or purpura (n=10, 21.3%)
  - Hepatitis (n=9, 19.1%)
  - Hepatomegaly or splenomegaly (n=6, 12.8%)
  - Lethargy, hypotonia or poor feeding (n=5, 10.6%).
  - *Pneumonitis and eye abnormalities were the most uncommon of clinical findings found in only 2 patients (4.3%) one patient (2.1%), respectively*

Baseline viral load according to symptom group

	Moderate to severely symptomatic	Mildly symptomatic	Asymptomatic with isolated SNHL	Asymptomatic
n	31	0	4	12
Median VL (copies/ml)	13 736	NA	28 392	1496
IQR	9917- 187 999	NA	3890-83951	835-2811

p= 0.004

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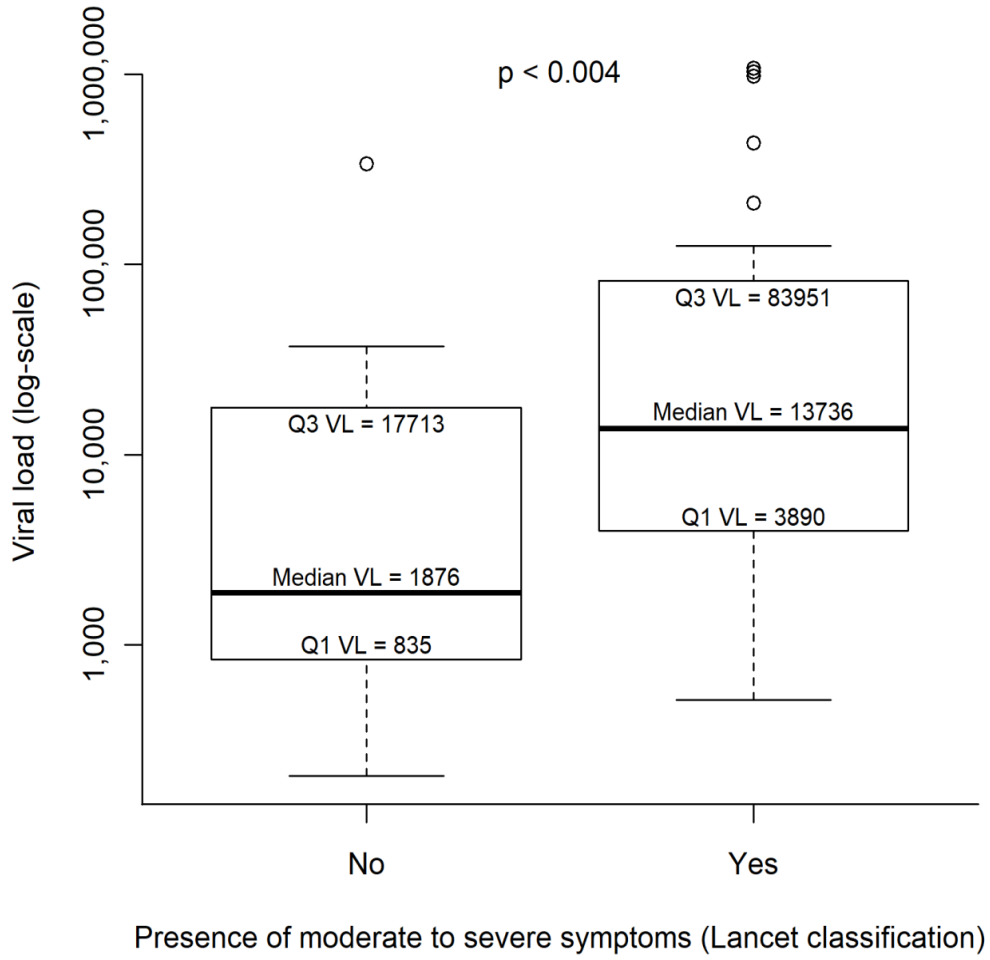
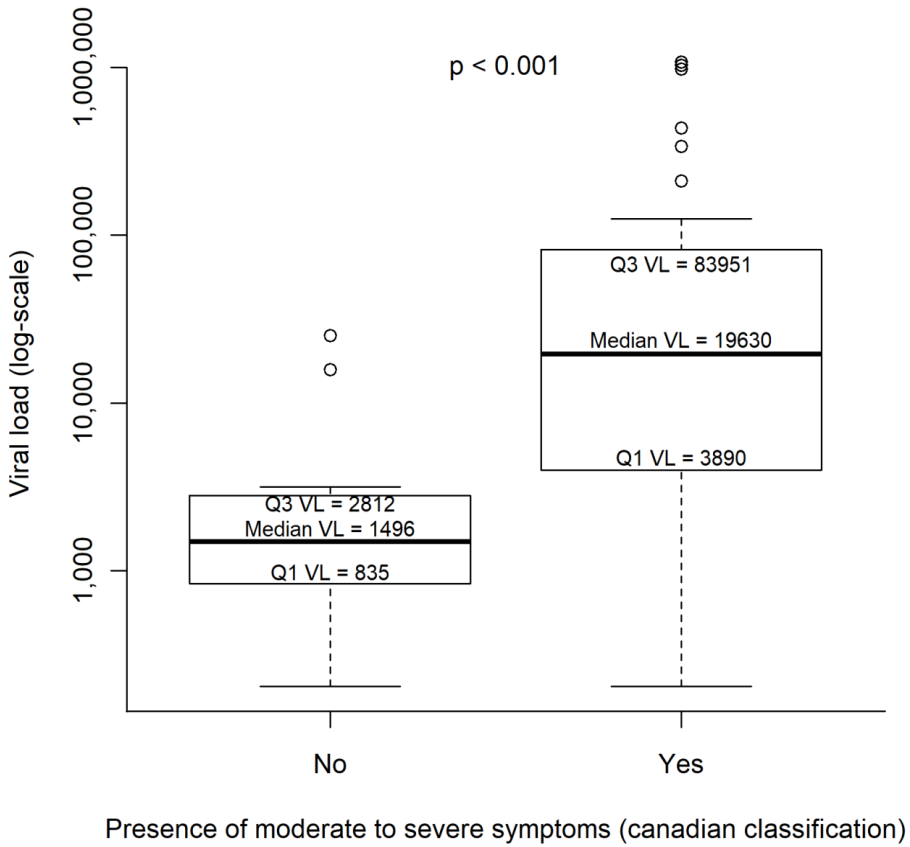
Baseline viral load according to treatment criteria

Lancet classification	Treatment	No treatment	Treatment (Canada)*	No treatment (Canada)
	Moderate to severe	Mild, asymptomatic, asymptomatic isolated SNHL	Moderate to severe, asymptomatic isolated SNHL	Mild, asymptomatic
n	31	16	35	12
Medial VL (copies/ml)	13 736	1876	19 630	1496
IQR	9917- 187 999	835-17713	3890-83951	835-2812
p	0.004		<0.001	

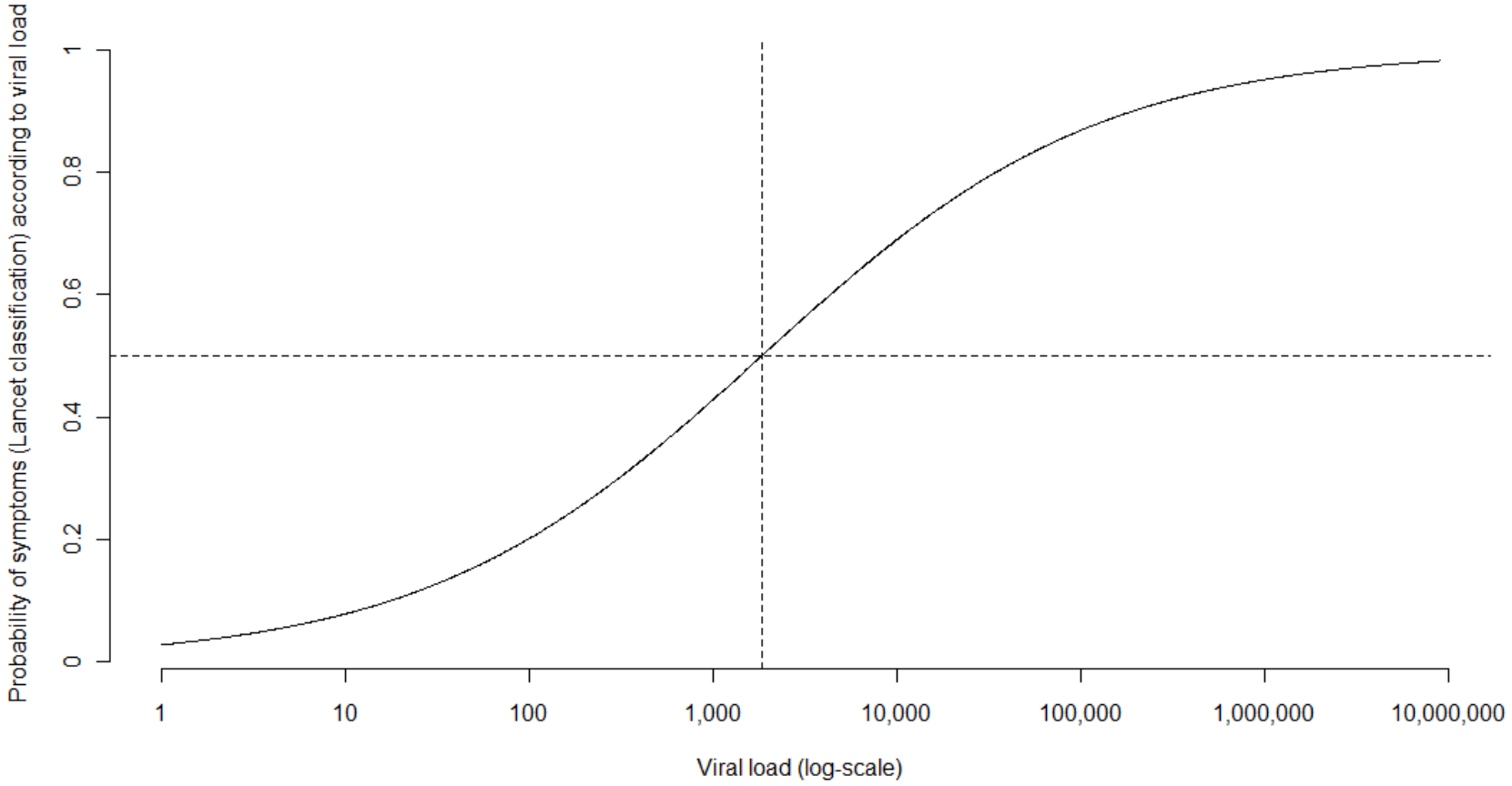
Baseline viral load according to presence of symptoms (Lancet classification)



Baseline viral load according to presence of symptoms (Canadian classification)



Probability of requiring treatment (Lancet classification) according to viral load (log scale)



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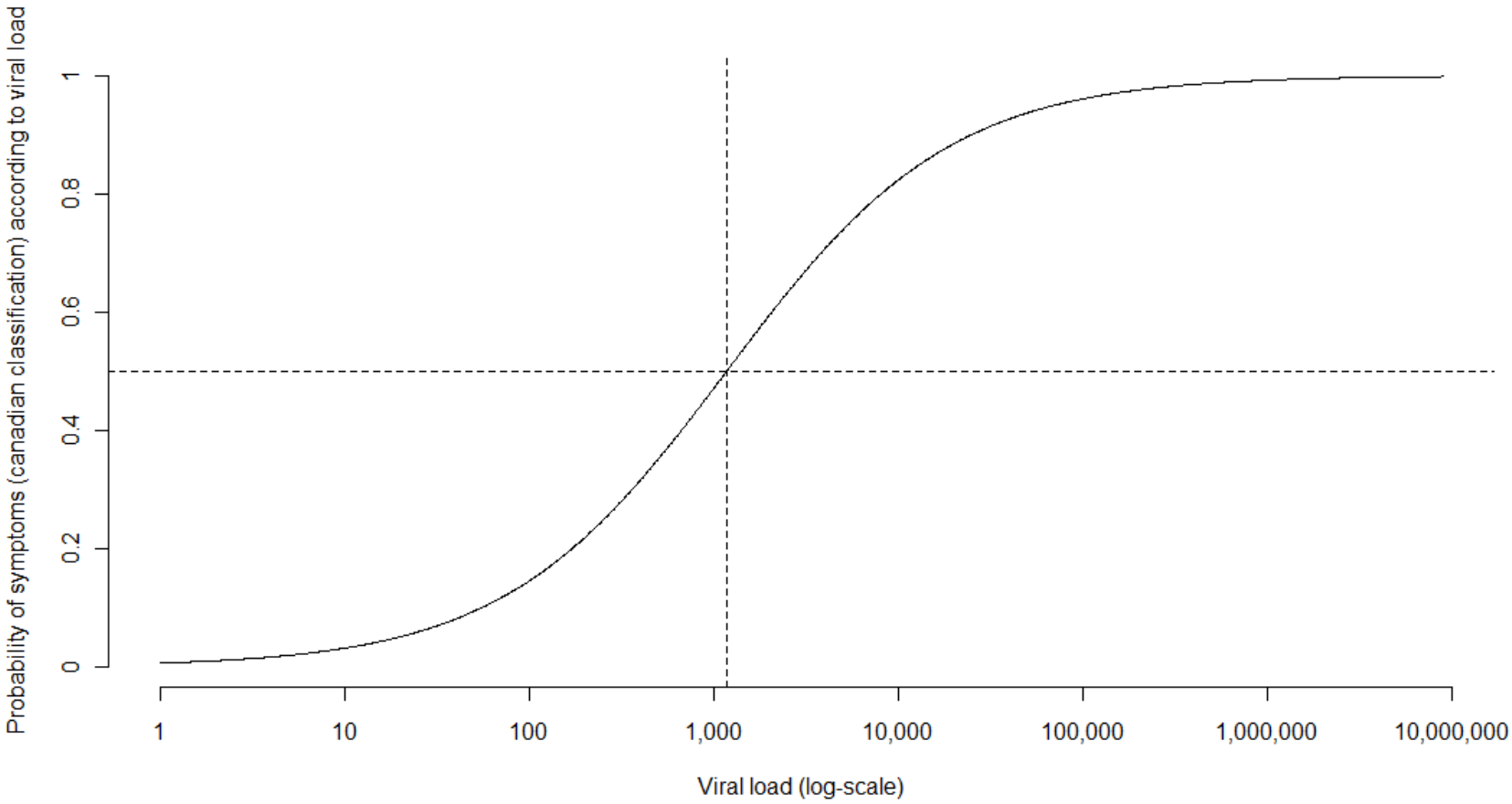


Summary

- Significant difference of at least 1 log in baseline qPCR among those meeting treatment criteria compared to those who do not.
- Clear set point varies depending on treatment criteria (weather including those with isolated SNHL), in both cases the probability approaches 100% at a level of 100 000 copies/ml (or 10 000 copies/ml according to local practice).
- Between 1000 and 10 000 copies/ml, the probability of treatment increases in a linear fashion such that there is no clear cutoff.

Implications

- Baseline qPCR could be a contributing factor in determining the indication for antiviral therapy, pending the full diagnostic work-up
- Rapid turnaround time for qPCR results could allow for rapid initiation of treatment prior to full diagnostic evaluation (confirmatory hearing or neuroimaging), shortening delays in treatment initiation
- If findings replicated at a larger scale, PCR could potentially be used as a independent indicator for treatment where resources are limited

Limitations

Lack of a standardized assay for qPCR across laboratories

- These identified thresholds of 10 000 and 100 000 copies/ml may not be applicable across laboratories and centers.

How do our results compare to previous studies?

Branford et al, 2004.

Difference in viremia (present/absent) and presence of SNHL, and level and specific symptoms

BSER	Baseline CMV viremia		*P*
	Present	Absent	
At baseline			
Best ear	(*n* = 32)	(*n* = 10)	.045
Normal	14 (44)	8 (80)	
Abnormal	18 (56)	2 (20)	
Total ear	(*n* = 64)	(*n* = 20)	.001
Normal	19 (30)	14 (70)	
Abnormal	45 (70)	6 (30)	

Virus load at baseline, ge/mL	BSER		*P*
	Normal	Abnormal	
	At baseline		
	(*n* = 22)	(*n* = 20)	.13
<200	8 (80)	2 (20)	
200–5400	10 (45)	12 (55)	
>5400	4 (40)	6 (60)	

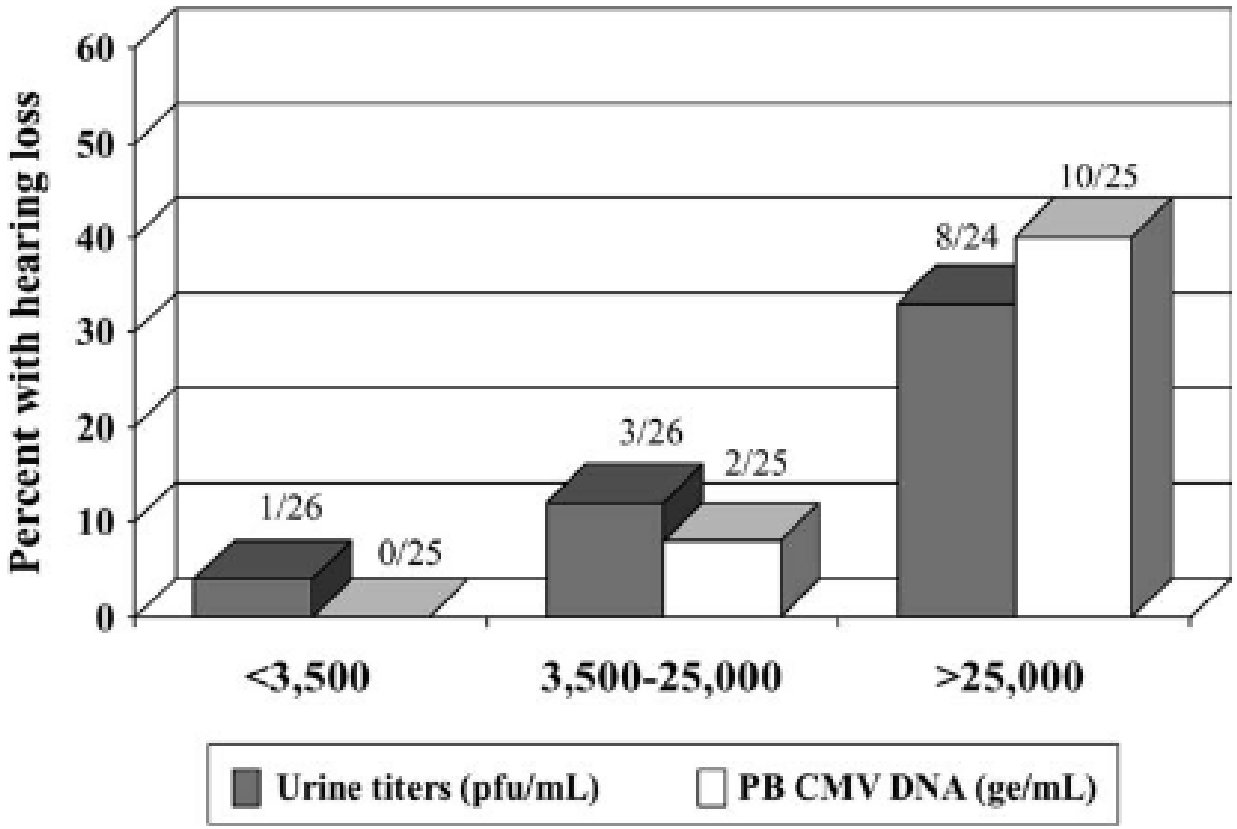
Table 5. Clinical features and laboratory evaluations of infants with symptomatic congenital cytomegalovirus infection, stratified on the basis of virus load expressed in terms of genomic equivalents per milliliter (ge/mL).

Virus load at baseline, ge/mL	Characteristic		*P*
	Alanine transaminase		
	Normal (n = 20)	Abnormal (n = 29)	.003
<200	9 (75)	3 (25)	
200–5400	10 (42)	14 (58)	
>5400	1 (8)	12 (92)	
	Petechial rash		
	Absent (n = 27)	Present (n = 20)	.01
<200	10 (91)	1 (9)	
200–5400	13 (57)	10 (43)	
>5400	4 (31)	9 (69)	
	Splenomegaly		
	Absent (n = 18)	Present (n = 31)	.03
<200	8 (67)	4 (33)	
200–5400	8 (35)	16 (65)	
>5400	2 (15)	11 (85)	
	Thrombocytopenia		
	Absent (n = 24)	Present (n = 5)	.05
<200	10 (91)	1 (9)	
200–5400	18 (75)	6 (25)	
>5400	6 (46)	7 (54)	

NOTE. Data are no. (%) of infants, unless indicated otherwise.

Boppana et al, 2004.

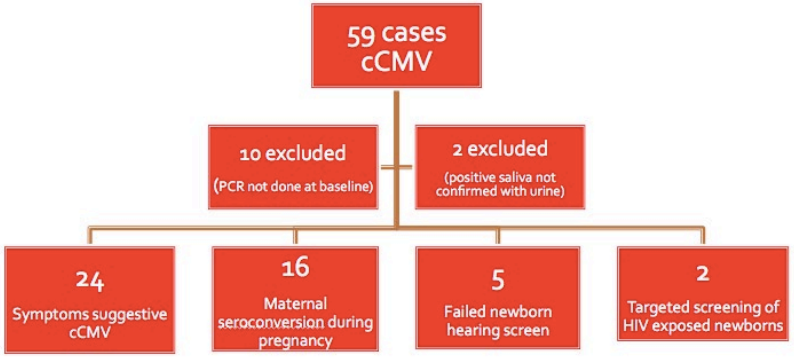
Difference in baseline VL and presence of hearing loss (urine)



Limitations

2. CIME cohort comprises mainly symptomatic infants and children at risk of infection → indications for testing at our center (those with maternal seroconversion or symptomatic infants).

- Not representative of the general population



Implications for screening programs

- While the sensitivity of the DBS for cCMV infection is not clear (ranging from 30-80%), the sensitivity increases with increasing viral load.
- Level of viremia at birth could help determine how quickly infants are triaged for treatment should DBS be used in large scale screening programs.
- If DBS sensitivity is determined at specific VL thresholds – could then DBS only be used to identify those who would likely benefit from treatment?

Conclusions

- Higher baseline viral load at birth was present in moderate to severely symptomatic infants with cCMV infection, which are the main criteria supporting treatment.
- This may have useful clinical implications in screening programs where large numbers of cases of cCMV may be identified.
- The role of viral burden in cCMV-infected infants should be further investigated in prospective studies to better understand viral pathogenicity/host response, predictors of long-term outcome and response to therapy.

Acknowledgements

CIME clinic team

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