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Neonatal Cytomegalovirus Blood Load and Risk of Sequelae in Symptomatic and Asymptomatic Congenitally Infected Newborns

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ABSTRACT

OBJECTIVE. Human cytomegalovirus (CMV) is a ubiquitous human-specific DNA virus and is the main cause of congenital virus infection in developed countries leading to psychomotor impairment and deafness. Diagnostic techniques for CMV detection have greatly improved during recent years with the advent of sophisticated serological and virological methods. The aim of the present study was to assess the diagnostic and prognostic value of detection and quantification of virus in neonatal blood samples of symptomatic and asymptomatic newborns with CMV congenital infection.

METHODS. Between January 1997 and December 2003, we studied 99 newborns who were born to women with primary, recurrent, and undefined CMV infection during pregnancy. CMV congenital infection was identified by isolation of the virus in urine within the second week of life. Fifty-eight of 99 infants were infected and were assessed clinically for disease in the newborn period and classified as having symptomatic or asymptomatic infection on the basis of physical, instrumental, and laboratory findings. The infants were followed up from birth according to a protocol of the tertiary NICU at the University of Bologna in a prospective study of long-term sequelae of congenital infection. Forty-seven blood samples were obtained from 47 infants in the neonatal period: 34 were examined for pp65 antigenemia test and 44 for qualitative and quantitative polymerase chain reaction (PCR and qPCR). Sequelae at 12 months were evaluated in a group of 50 infants.

RESULTS. Antigenemia was positive in only 10 of 34 samples of infected newborns (29.4% sensitivity). PCR was performed in 44 samples of infected newborns and was positive in all (100% sensitivity). qPCR showed a finding of ≥100 copies per 10^5 of polymorphonuclear leukocytes (PMNLs) in 39 of 44 samples; in the other 5 cases, the number of copies per 10^5 PMNLs was <100. Between symptomatic and asymptomatic newborns, the mean values of viral blood load determined by

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Key Words
cytomegalovirus, DNAemia, pp65 antigenemia, sequelae, sensorineural hearing loss, neurodevelopmental defects, prognostic value

Abbreviations
CMV— cytomegalovirus
PCR—polymerase chain reaction
Ig—immunoglobulin
ALT—alanine aminotransferase
PMNLs—polymorphonuclear leucocytes
qPCR— quantitative PCR
BSER— brainstem evoked response
PPV—positive predictive value
NPV—negative predictive value

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qPCR turned out to be significantly higher in symptomatic newborns. Mean values of neonatal blood viral load were statistically higher in newborns who developed sequelae than in those who did not. Of 20 children with a neonatal viral blood load of <1000 copies per 10^5 PMNLs, 19 did not develop sequelae (negative predictive value: 95%), whereas 2 of 3 with a viral blood load of >10 000 copies did develop sequelae.

CONCLUSIONS. Different viremia value ranges are correlated to a different risk of sequelae: ~70% sequelae were found in newborns with a qPCR higher than 10 000 copies per 10^5 PMNLs. Low neonatal viral blood load detected by pp65 antigenemia test and qPCR was highly predictive of absence of sequelae: DNAemia <1000 copies per 10^5 PMNLs has a negative predictive value of 95%. As an independent predictive factor of outcome, neonatal viremia is another useful element for neonatal counseling and therapeutic choices in symptomatic and asymptomatic newborns.

HUMAN CYTOMEGALOVIRUS (CMV) is a ubiquitous human-specific DNA virus that belongs to the Herpesviridae family. More than 90% of primary infections are asymptomatic in healthy adults and children, but CMV is an important cause of morbidity and mortality in immunocompromised individuals, pregnant women, newborns, and preterm infants.[1]

Diagnostic techniques for CMV detection have greatly improved during recent years with the advent of sophisticated serological and virological methods, but the significance and usefulness of different assays depend on patient categories. Quantitative virological methods (viremia, antigenemia, and DNAemia) are used to predict and monitor CMV disease, institute preemptive therapy, and monitor antiviral treatments in immunocompromised patients such as transplant recipients and those with AIDS.[2] In pregnant women, CMV isolation and/or detection of viral DNA by polymerase chain reaction (PCR) in amniotic fluid is useful in defining the risk for fetal infection.[3–7]

The diagnosis of congenital CMV is best accomplished by viral culture of saliva or urine or by PCR testing of either specimen within the first 2 weeks of life.[8] Congenitally infected infants are asymptomatic at birth in ~85% to 90% of cases.[9] Infection in symptomatic infants ranges from mild to severe disseminated life-threatening disease resulting in up to 20% perinatal mortality.[10] Up to 80% of symptomatic newborns will exhibit sequelae such as mental retardation, cerebral palsy, seizures, visual defects, and sensorineural hearing loss. In addition, 8% to 15% of infants who are asymptomatic at birth will later develop complications, mainly neurodevelopmental defects and deafness.[11,12]

Symptoms at birth therefore have a negative prognostic significance. Studies that have been performed to define the prognostic value of clinical, laboratory, and instrumental findings in symptomatic infected newborns have reported contradictory findings.[10,13–16] Moreover, insufficient data are available on the possibility of an early definition of outcome in the larger group of asymptomatic newborns.

The identification of ≥1 prognostic markers in newborns who have congenital CMV infection and are at increased risk for sequelae could allow careful monitoring to institute prompt rehabilitative and therapeutic strategies to curb subsequent damage. The aim of the present study was to assess the diagnostic and prognostic value of detection and quantification of virus in neonatal blood samples of symptomatic and asymptomatic newborns with congenital CMV infection.

METHODS

Study Population

Between January 1997 and December 2003, we studied 99 newborns who were born to women who were referred to us by gynecologists as affected by primary, recurrent, and undefined CMV infection during pregnancy. Women who had anti-CMV immunoglobulin (Ig) G of low avidity or who seroconverted to CMV IgG positivity were classified as undergoing primary infection. Those with anti-CMV IgG of high avidity and blot-confirmed IgM positivity were classified as having a recurrent (reactivated) infection. Finally, doubtful serological results were classified as undefined.

Congenital CMV infection was identified by isolation of the virus in urine within the second week of life. In the first 2 years of our study, we conducted viruria and viremia determinations at the same time, and the next viremia determination was performed only when viruria was positive.

Fifty-eight of 99 infants were infected and were followed up from birth according to a protocol of the tertiary NICU at the University of Bologna in a prospective study of long-term sequelae of congenital infection. Of the 58 infected newborns, 52 were from mothers who developed a primary CMV infection during pregnancy: 17 were symptomatic and 35 were asymptomatic at birth. Four newborns were delivered from mothers who developed a recurrent CMV infection, and all were found to be symptomatic. The 2 remaining newborns were from mothers with undefined infection in pregnancy: 1 was symptomatic, and 1 asymptomatic.

All children were assessed clinically for disease in the newborn period and were classified as having symptomatic or asymptomatic infection on the basis of physical, instrumental, and laboratory findings. Newborns were considered symptomatic when they showed ≥1 of the following findings: signs and symptoms of systemic involvement such as intrauterine growth retardation, hep-

atosplenomegaly, skin petechiae/purpura, thrombocytopenia (platelet count: $<100\,000/mm^3$), jaundice with direct bilirubin (>3 mg/dL), alanine aminotransferase (ALT) elevation (>80 U/L), pneumonia, neurologic involvement (microcephaly, lethargy/hypotonia, poor sucking, and seizures), and sensorineural defects (chorioretinitis and deafness), and CMV-associated patterns at neuroimaging (abnormal periventricular hyperechogenicity, intracranial calcifications, ventriculomegaly, hyperechogenicity of lenticolo-striatal vessels, etc).[17]

All infants were followed up at birth, at 1, 3, 6, 12, and 18 months of life, and then annually up to school age. Follow-up included clinical evaluation, neurodevelopmental and psychointellectual assessment, cranial ultrasound, cerebral computed tomography (in case of doubts at sonographic examination) and MRI (in case of neurologic symptoms or pathologic ultrasound), funduscopic examination, and audiological assessment. When pathologic features were detected, children were seen more frequently.

Of 58 newborns enrolled, 2 with severe congenital symptomatic CMV infection (1 with aortic arch thrombosis[18] and 1 with severe hydrops) died during the neonatal period and 2 were lost to follow-up. Sequelae at 12 months of life were evaluated in 50 of 58 infants (15 with and 35 without symptoms at birth) because 4 children were younger than 1 year (Fig 1).

Forty-seven blood samples were obtained from 47 infected children in the neonatal period. Of 47 polymorphonuclear leukocyte (PMNL) samples, 34 were examined for pp65 antigenemia test and 44 for qualitative PCR and quantitative PCR (qPCR). Twenty neonatal blood samples were obtained for determination of pp65 antigenemia and PCR tests from 20 infants who were found not to be infected as shown by negative virus isolation from urine. This group was followed up and served as a control group.

Virological Tests

CMV Isolation

Human embryo fibroblasts were grown in Eagle's minimal essential medium with 10% fetal calf serum. For virus isolation, the shell vial procedure was used.[19] The inoculated cells were fixed 24 to 48 hours after inoculation and were stained by an indirect immunofluorescence assay with a monoclonal antibody that reacts with the CMV IE1 and EA gene product (E13 + 2A2; Argene, Varilhes, France).

Antigenemia

Blood samples were collected in EDTA-treated tubes. PMNLs were separated using a standard dextran sedimentation procedure. An aliquot of 2×10^5 PMNLs was spotted onto a slide for antigenemia assay as described originally by van der Bij et al[20] and modified by Revello et al,[21] and indirect immunofluorescence was performed using a pool of 2 monoclonal antibodies specific for CMV pp65 (1C3 and AYM-1; Argene). Results were expressed as the number of positive cells per 2×10^5 PMNLs.

DNAemia

Two aliquots of 10^6 PMNLs were frozen at −80°C before subsequent PCR studies.

PCR

CMV-DNA was extracted from aliquots of 1×10^6 PMNLs by CMV-Ibridoquant Extraction Kit (Bioline, Turin, Italy). Nested PCR was performed for amplification of a highly conserved region of the viral genome (major immediate-early) using 2 primer sets described elsewhere.[22] PCR products were separated by electrophoresis on a 2% Nusieve-Seakem (2:1) gel (FMC Bioproducts, Rockland, ME) and visualized by transillumination. The sample was regarded as positive when a band corresponding to a 110-bp DNA fragment was detected. For excluding false-positive results owing to contamination, 4 samples that contained the reaction mixture but no target DNA were processed for each PCR batch. All samples that contained no target DNA yielded negative results.

qPCR

Quantitative assessment of the CMV DNA load was performed with the COBAS AMPLICOR CMV MONITOR Test (Roche Diagnostics, Branchburg, NJ). DNA was extracted from 10^6 PMNLs that had been resuspended in 200 μL of phosphate-buffered saline. From this material, 50 μL of processed specimen was loaded into the COBAS AMPLICOR instrument. Nucleic acid amplification and detection proceeded in an automated manner. The results of the PCR assay were reported as number of DNA copies per 10^5 PMNLs. One CMV low-positive control (mean value: 3.79 copies per 10^3 PMNLs; SD: 0.62), 1 CMV high-positive control (mean value: 4.56 copies per 10^4 PMNLs; SD: 0.65), and 1 CMV negative control were processed with each batch sample. The limit of detection of this quantitative assay is 120 copies of CMV DNA per 10^6 cells.

The blood samples were tested during the neonatal period of each patient in routine laboratory activity. The laboratory personnel who undertook molecular testing were aware of the derivation and outcome of the infants studied.

Clinical Examinations

Clinical examinations that were conducted at birth, at 1, 3, 6, 12, and 18 months of age, and annually thereafter included measurement of anthropometric parameters (weight, length, and head circumference) and assessment of the relative percentiles.

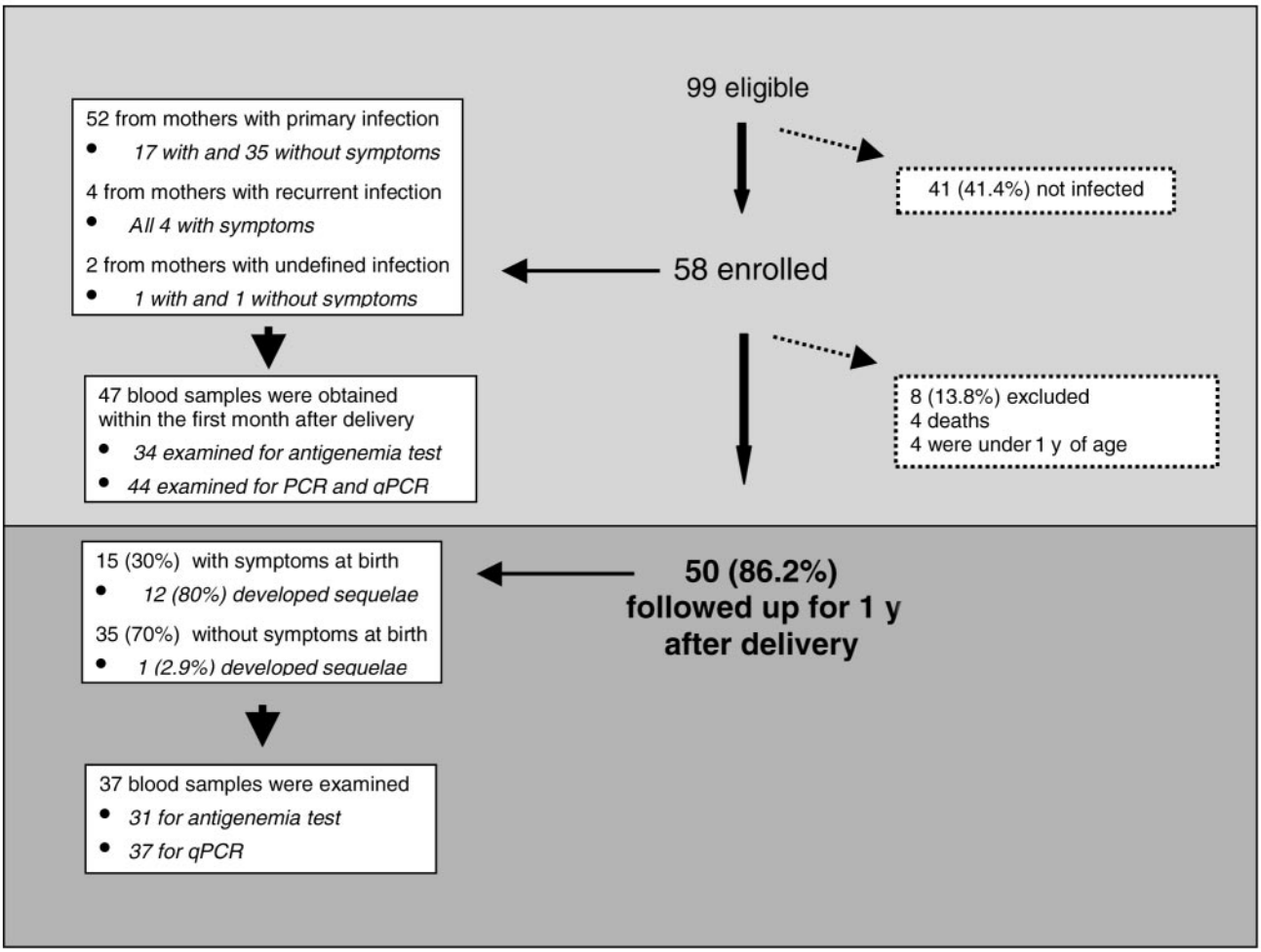

FIGURE 1
Derivation of study population.

Psychomotor and Neurodevelopmental Assessment

Neurologic assessment and the Brunet-Lezine test (which includes 4 subscales for evaluation of posture, coordination, speech, and socialization) for children at 3, 6, 12, 18, and 24 months of age and the Stanford Binet L-M Intelligence Scale (Termann Merril revision) for older children were performed. The tests were always administered by the same investigators.

Audiological Assessment

An audiological evaluation was performed at birth and at 6 and 12 months of age and annually until school age. Brainstem evoked responses (BSERs) were used to assess audiological function during the first year of life. Behavioral audiometry was performed thereafter. Middle-ear disorder was excluded by tympanometry.

Visual Evaluation

Fundus examination was undertaken after topical oculoplegic administration in the neonatal period and at 6 and 12 months of life.

Cranial Ultrasound Examination

Cranial ultrasound examinations were performed at birth and at 1, 3, and 6 months age by the same investigators (Gina Ancora, MD, and Fabrizio Sandri, MD), using an Esaote AU5 (Esaote Biomedica, Genoa, Italy) with a 5- to 7.5-MHz sector probe. Coronal and sagittal views were collected at different times.

Statistical Analysis

The statistical significance of the associations between the diagnostic tests and symptoms and/or sequelae in the newborns was determined using Fisher's exact test or the χ^2 test for trend (when appropriate).[23] The comparison of the mean values of quantitative DNAemia was statistically assessed (after log transformation) by means of Student's t test for independent samples.[24] A 2-tailed $P < .05$ was considered statistically significant. The sensitivity, specificity, and positive (PPV) and negative predictive values (NPV) of the tests were also assessed.[24]

Ethics

The study was conducted in accordance with the ethical rules of St Orsola-Malpighi General Hospital (Bologna, Italy).

RESULTS

The derivation of the final study cohort, consisting of infants who were followed up for up to 12 months, is shown in Fig 1. Fifty-eight children with congenital CMV infection were followed up from the neonatal period: 24 (41.4%) boys and 34 (58.6%) girls. The mortality rate was 3.4% (2 of 58 congenitally infected). Twenty-two (37.9%) of the 58 newborns had symptomatic infection; their clinical, laboratory, and instrumental findings are summarized in Table 1.

Results of virological assays that were performed in the neonatal period are shown in Table 2. Antigenemia was performed in 34 samples from CMV-infected newborns, and positive results were detected in only 10 (29.4% sensitivity). Qualitative PCR was performed in 44 samples and was positive in all (100% sensitivity). All 20 congenitally uninfected newborns were negative for CMV DNA and for pp65 antigenemia test; therefore, these methods showed 100% specificity.

qPCR showed a finding of ≥100 copies per 10^5 PMNLs in 39 of 44 samples (22 asymptomatic and 17 symptomatic). In the other 5 cases, the number of copies per 10^5 PMNLs was <100, 4 of which were without and 1 of which was with symptoms.

Between symptomatic and asymptomatic newborns, the mean values of viral blood load determined by qPCR turned out to be significantly higher in symptomatic newborns ($P = .020$). Considering symptomatic only those newborns with clinical evidence of congenital CMV infection, thereby excluding infants with only isolated transient changes in laboratory or instrumental findings (ALT of >80 U/L, thrombocytopenia, hyperechogenicity of lenticolo-striatal vessels), the differences in viral blood load between newborns with and without symptoms became more evident ($P < .001$; 95% confidence interval of the difference between the 2 means: 0.36–1.12).

The average duration of follow-up was 18 months. The incidence of sequelae was determined in the group of 50 infants who were followed for >12 months, 13 (26%) of whom developed sequelae induced by congenital CMV infection (Fig 1).

Twelve (80%) of 15 symptomatic newborns developed sequelae (Fig 1), with delayed psychomotor development and/or neurologic deficits and sensorineural hearing loss in 6 (50%) infants, 3 with severe bilateral deafness (BSER value ≥71 dB). Two (16.6%) of 12 infants presented only mild monolateral hearing loss (BSER value between 21 and 45 dB), whereas sequelae were confined to delayed psychomotor development and/or neurologic deficits in 4 (33.3%). Of the 35 newborns with asymptomatic CMV infection and a follow-up of at least 12 months (Fig 1), only 1 (2.9%) developed sequelae that consisted of hearing loss and hypertonia of the lower limbs. Of the 20 uninfected newborns in the control group, 16 had a clinical follow-up that lasted up to 12 months, and none developed sequelae.

Of the 50 infants with a follow-up ≥12 months, 31 were tested for pp65 antigenemia and 37 underwent qPCR determination (Table 3). The mean value of neonatal blood viral load determined by qPCR was significantly higher in newborns who developed sequelae ($P < .001$), and the pp65 antigenemia test was more frequently positive in newborns who developed sequelae ($P = .043$; Table 3).

Figure 2 shows the significant association between neonatal blood viral load determined with qPCR and the development of sequelae at 12 months of age (test for trend, $P = .002$). None of the 4 children with CMV viral load <100 copies per 10^5 PMNLs developed sequelae. One of 16 children with viral blood load between 100 and 1000, 7 of 14 with viral load between 1000 and 10 000, and 2 of 3 with viral blood load >10 000 copies developed sequelae.

The prognostic value of virus quantification in the blood of newborns with CMV congenital infection evaluated in the neonatal period is shown in Table 4. At a

TABLE 1 Clinical, Laboratory, and Instrumental Findings in Symptomatic Congenitally CMV-Infected Newborns

Findings	No. Positive/No. Total (%)
Prematurity	8/22 (36)
Intrauterine growth restriction	5/22 (22.7)
Petechiae-purpura	5/22 (22.7)
Hepatosplenomegaly	5/22 (22.7)
Neurologic involvement	
≥1 of the following signs	10/22 (45.4)
Microcephaly	7/22 (31.8)
Seizures, motor or postural abnormalities	2/22 (9)
Chorioretinitis	1/21 (4.7)[a]
Aortic thrombosis	1/22 (4.5)
Neonatal hydrops	1/22 (4.5)
Elevated ALT (>80 U/L)	4/22 (18.2)
Hyperbilirubinemia (direct bilirubin ≥3 mg/dL)	0/22 (0)
Thrombocytopenia	
Platelet count, <100 000/mm^3	9/22 (41)
Platelet count, <50 000/mm^3	4/22 (18.2)
Neuroimaging abnormalities	
≥1 of the following	14/21 (66.6)[a]
Cerebral calcifications	9/21 (42.8)[a]
Ventriculomegaly	8/21 (38)[a]
Other[b]	5/21 (23.8)[a]
Hyperechoic bowel	1/22 (4.5)

[a] One newborn died before the evaluation.

[b] Periventricular cysts (2 cases), hyperechogenicity of lenticolo-striatal vessels (1 case), intraventricular hemorrhage grade 1 in a term newborn (1 case), and periventricular radiolucencies (1 case).

TABLE 2 **pp65 Antigenemia and CMV-DNAemia Performed in Neonatal Blood Samples of Infected Newborns in Relation to the Presence or Absence of Symptoms at Birth**

	pp65 Antigenemia		Qualitative DNAemia	Quantitative DNAemia		
	No. of Positive Samples	No. of Negative Samples	No. of Positive Samples	No. of Samples With Cutoff <100 Copies per 10^5 PMNLs	No. of Samples With Cutoff ≥100 Copies per 10^5 PMNLs	Log Mean (SD)
Symptomatic	5	4	18	1	17	3.24 (0.69)
Asymptomatic	5	20	26	4	22	2.79 (0.56)
Total	10	24	44	5	39	
P		.060[a]			.634[a]	.020[b] (0.07–0.84)[c]

[a] Fisher's exact test.
[b] Student's *t* test.
[c] 95% confidence interval of the difference between the 2 means.

TABLE 3 **pp65 Antigenemia and CMV-DNAemia Performed in Neonatal Blood Samples of Infected Newborns in Relation to the Presence or Absence of Sequelae at 12 Months of Life**

	pp65 Antigenemia		Quantitative DNAemia		
	No. of Positive Samples	No. of Negative Samples	No. of Samples With Cutoff <100 Copies per 10^5 PMNLs	No. of Samples With Cutoff ≥100 Copies per 10^5 PMNLs	Log Mean (SD)
Sequelae	4	2	0	10	3.61 (0.54)
No sequelae	5	20	4	23	2.72 (0.56)
Total	9	22	4	33	
P		.043[a]		.557[a]	<.001[b] (−1.32 to −0.46)[c]

[a] Fisher's exact test.
[b] Student's *t* test.
[c] 95% confidence interval of the difference between the 2 means.

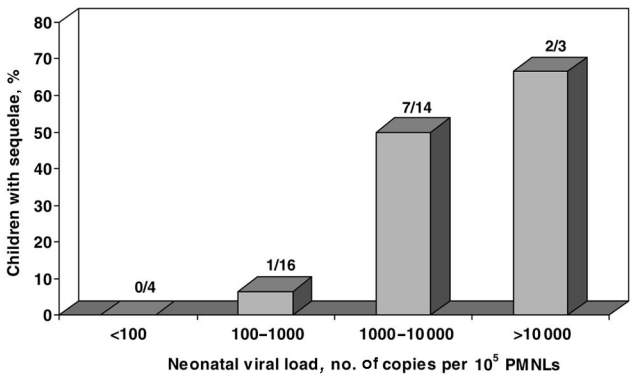

FIGURE 2
The proportion of children who developed sequelae at 12 months according to the neonatal CMV blood load.

cutoff point of at least 1000 copies per 10^5 PMNLs, among the 17 samples with neonatal viral blood load ≥1000, only 9 newborns developed sequelae (PPV: 53%). Only 1 of the 20 children with neonatal viral blood load <1000 copies per 10^5 PMNLs developed sequelae (NPV: 95%). The samples that were collected from this child had a value very near the cutoff point of 1000 (960 copies per 10^5 PMNLs). We obtained similar results with the pp65 antigenemia test because only 2 of 22 newborns with a negative determination developed sequelae (NPV: 91%).

DISCUSSION

Vertical transmission of CMV is the most common cause of congenital viral infection in developed countries (0.3%–2% of all live births, 1.1% in Bologna; unpublished data) and the leading nongenetic cause of sensorineural hearing loss. At birth, it is essential to use appropriate tests for the diagnosis of congenital CMV infection. The gold standard method is virus isolation from urine/saliva in the first 2 weeks of life because subsequent virus excretion may represent neonatal infection acquired in the birth canal or after exposure to breast milk.[8] The determination of DNA in blood by PCR at birth seems to be as sensitive and specific as virus recovery from urine for diagnosis of congenital CMV infection.[25] Negative results by these tests rule out congenital CMV infection, and no additional investigations are required.

CMV-infected newborns must undergo additional clinical, laboratory, and instrumental evaluation to identify those who are symptomatic. The presence of symptoms at birth strongly conditions the treatment and long-term outcome of infected infants. Nevertheless, the prognostic significance of single clinical, laboratory, and

TABLE 4 **Sensitivity, Specificity, PPV, and NPV of Neonatal pp65 Antigenemia and DNAemia (Cutoff Point 10^3 Copies per 10^5 PMNLs) With Regard to the Presence or Absence of CMV Sequelae at 12 Months of Life**

	CMV Sequelae		*P*[a]	Total	Sensitivity, %	Specificity, %	PPV, %	NPV, %
	Yes	No						
pp65 antigenemia (no. of pp65-positive cells per 2×10^5 PMNLs)					66.6	80	44.4	91
Positive	4	5	.043	9				
Negative	2	20		22				
Total	6	25		31				
qPCR (no. of copies per 10^5 PMNLs)					90	70.4	53	95
$\geq 10^3$	9	8	.002	17				
$< 10^3$	1	19		20				
Total	10	27		37				

[a] Fisher's exact test.

instrumental findings observed in the neonatal period remains unsettled.[10,13–16] Prognostic indicators for the larger group of asymptomatic CMV-infected children are lacking, so there are few objective elements to support neonatal counseling, with a high risk for poor family compliance at follow-up.

Our study investigated the diagnostic and prognostic significance of neonatal virological tests (pp65 antigenemia and DNAemia) in relation to psychomotor sequelae and hearing loss in symptomatic and asymptomatic congenitally CMV-infected newborns. In 1995, Nelson et al[26] reported the detection of CMV DNA in the serum of 18 (100%) of 18 infants with symptomatic congenital CMV infection against none of the 32 control subjects. Revello et al[27] investigated the diagnostic and prognostic value of CMV load at birth determined by different assays in the blood of 41 newborns with congenital infection and 34 uninfected newborns. Sensitivities of CMV DNAemia determination were 100%, whereas specificity was 100% for all assays.

We found the same results for qualitative PCR performed on PMNLs, with a positive determination in all 44 samples of infected newborns (100% sensitivity) and negative in 20 of 20 uninfected newborns in the control group (100% specificity). Similar results were found for the pp65 antigenemia test as regards specificity (100%), whereas the statistical value of sensitivity was very low (29.4%), in agreement with other authors.[27]

We found that the neonatal viral blood load in symptomatic newborns (pp65 antigenemia and DNAemia) tended to be higher but did not reach statistical significance. However, we failed to find a correlation between viremia and symptoms from the reticuloendothelial system, including isolated elevated levels of ALT and thrombocytopenia or hyperechogenic lenticolo-striatal vessels deemed a nonspecific sign.[28] Considering only newborns with clinical evidence of CMV infection, the differences in viral blood load between newborns with and without symptoms became statistically significant.

A report by Rivera et al[16] demonstrated that a high viral load in early infancy, expressed by a high amount of virus in urine, is highly predictive of audiological impairment. Finding CMV in blood indicates an active viral replication with ongoing dissemination, but its prognostic role in congenital infection has not been defined.

Our study disclosed that DNAemia performed at birth is significantly higher in newborns who went on to develop sequelae than in infants with a good outcome. Different DNAemia value ranges were correlated to a different risk of sequelae: none for qPCR <100 copies per 10^5 PMNLs values and ~70% sequelae in newborns with qPCR higher than 10 000 copies per 10^5 PMNLs.

Considering a cutoff point of 1000 copies per 10^5 PMNLs in our population, we selected a group of newborns who had DNAemia <1000 and were at very low risk for sequelae (NPV: 95%). Only 1 infant in this group developed sequelae that consisted of neurodevelopmental retardation and late-onset hearing loss, but his DNAemia at birth had been only slightly below the cutoff. In addition, another DNAemia >10 000 copies at 12 months of follow-up did not have any sequelae. This finding is difficult to interpret and may result from late-onset sequelae.

Our study presents 1 of the largest cohorts of CMV-infected children acquired prospectively, but the sample distribution in terms of different intervals of viral blood load reduces the number of infants for each interval and awaits confirmation in larger series. The median value of pp65 antigenemia was significantly higher in the group of newborns who developed sequelae, as shown in Table 4. However, its scant diagnostic utility, the inherent variability resulting from the lack of standardization, the need for immediate processing of samples after collection, and the subjective nature of quantification discourage its use as a diagnostic tool for congenitally infected newborns.[2]

To our knowledge, this is the first study to have assessed the prognostic significance of virological tests on blood samples (pp65 antigenemia and qPCR) from symptomatic and asymptomatic congenitally CMV-infected newborns. As in pregnancy, viral markers are

very useful for neonatal counseling. Predicting neonatal outcome and the subsequent appearance of sequelae has undoubted advantages: (1) it will decrease the family's anxiety if the predictive factors of good outcome are present; and (2) it stratifies patients who are at different risks for sequelae, modulating the follow-up steps and permitting early rehabilitative and/or therapeutic intervention to minimize long-term damage.

In agreement with Bradford et al,[28] treatment decisions currently cannot be based solely on the presence of high CMV blood viral load at birth. However, this finding could identify the population in greatest need of antiviral therapy, whereas a low viral load may save the clinician from administering unnecessary treatments.

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