Multicity Italian Study of Congenital Cytomegalovirus Infection

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Background: Cytomegalovirus (CMV) infection is the most frequent congenital infection in humans. Its prevalence and the frequency of disabling sequelae must be assessed in different populations to permit the formulation or assessment of preventive measures.

Objectives: To check the prevalence of congenital infection and seroprevalence in Italy; to verify the rate of sensorineural hearing loss (SNHL) in infected infants; and to assess the proportion of children with SNHL attributable to congenital CMV infection.

Methods: Diagnosis of congenital CMV infection was sought in 9032 children born between March 2002 and February 2003 by testing for viral DNA [CMV dried blood spot (DBS) test] in each newborn's Guthrie card and confirmation by isolation of CMV from urine collected in the first 3 weeks of life; CMV IgG testing in 1200 women of childbearing age; clinical and audiologic tests in the first 24 months for infected children; CMV DBS tests on the Guthrie cards collected from screening centers for 77 children (3 months–5 years) presenting SNHL of 40 dB or more.

Results: CMV infection was diagnosed in 14 asymptomatic and 2 symptomatic newborns (0.18%). CMV seroprevalence was 80%. In 2 infected infants, transient, unilateral SNHL was found. Nineteen of the 71 children with SNHL >70 dB were congenitally infected.

Conclusions: The prevalence of congenital CMV infection is low in Italy. Population characteristics limiting the circulation of CMV strains in adult women might explain this. The fact that CMV contributes to significant SNHL highlights the need for preventive measures.

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According to epidemiologic and seroprevalence studies, human cytomegalovirus (CMV) is present in all populations, even the most isolated.[1] The time curve for acquisition of the infection differs from one population to another because of the different conditions governing the prevalent modes of transmission.

Immaturity of the immune system, together with immunosuppression of any origin, facilitates the expression of the pathogenic potential of the virus. CMV can induce infection in the fetus and do harm in the intrauterine period, becoming evident at birth or in infancy. The frequency of congenital CMV infection varies in different populations or even within the same population but is usually between 0.2 and 2.2% of live births. The infection is asymptomatic in ~90% of cases, but permanent damage is detected during postnatal development in about one-fifth of infected infants. The most frequent and disabling sequelae are sensorineural hearing loss (SNHL) and psychomotor delay.[2]

These aftermaths require care, corrective measures and rehabilitation. Knowledge of the prevalence of the infection and its consequences as a cause of disability in a population is needed for decisions on preventive measures. These might take an immunologic approach when vaccines become available. At present, the aim is to limit damage through early identification of infected infants.

The few Italian studies on the prevalence of congenital infection have covered only 2 regions of the country, Lombardy[3,4] and Veneto.[5] To obtain more complete and up-to-date figures on the burden of congenital CMV in Italy, we organized a multicenter survey in 4 Italian regions: Lombardy (Milan) in the northwest of Italy; Emilia-Romagna (Parma) in the northeast; Puglia (Bari) in the south; and the island of Sardinia (Sassari). The study comprised 2 parts, in 2 populations: one aimed at assessing the prevalence of the infection in babies born in the 4 cities during a 12-month period and the incidence of sequelae in the infected children; the other aim was to estimate the prevalence of congenital CMV cases among children diagnosed with SNHL.

Both these aims were pursued by testing for CMV DNA in neonatal blood samples dried on cards (dried blood spot; DBS) collected routinely at birth to screen for metabolic and genetic diseases. The test (CMV DBS test) was selected in the light of its accurate results (100% sensitivity and 99% specificity[6]) and its practical advantages: samples do not require special storage conditions, the test is relatively simple and convenient and preliminary examination of samples in pools allows savings.[7]

The prevalence of congenital infection was compared with the seroprevalence assessed in women of childbearing age in the 4 regions.

MATERIALS AND METHODS

Children and Women. To establish the prevalence of congenital infection (study A), we examined 9032 consecutive infants born between March 2002 and February 2003 in the university hospital used as reference for each study region. Criteria for inclusion were (1) signed informed consent from parents and (2) availability of a neonatal blood sample (2 drops) taken in the first week, before any blood transfusions, and dried on a Guthrie card (DBS). Using a questionnaire, we collected clinical and personal data for each infant's mother (age, nationality, education) and the infants themselves (sex, gestational age, birth weight, clinical signs attributable to congenital CMV infection).

We assessed the role of congenital CMV infection in the etiology of SNHL (study B) in 77 children between 3 months and 5 years of age, with hearing loss ≥40 dB in the best ear, attending 3 university hearing centers, in Milan, Bari and Sassari, for diagnosis and/or speech therapy. Hearing loss was bilateral in 69 cases, severe-profound (>70 dB hearing loss) in 64 and medium-severity (40–70 dB hearing loss) in 5. The other 8 cases had unilateral hearing loss, severe-profound in 7. In 48 cases, the cause was not known. The risk factors found in the remaining 29 children were: various syndromes (8); family history (10); neonatal mechanical ventilation (4); hyperbilirubinemia (1); pharmacologic treatment (1); agenesis of the 8th nerve (1); preterm birth (1); and CMV infection (5).

Anti-CMV seroprevalence was assessed on a random, anonymous sample of 1200 women (300 in each center) 14–47 years of age.

The study was approved by the ethics committees of the authors' universities and hospitals. Screening and confirmatory tests were performed in the laboratories of each regional center.

CMV DBS Test. The test was done as previously described,[6] with some modifications. In brief, DNA was extracted from Guthrie cards by thermal shock, and the nested amplification reaction was done in "monotest tubes" (CMV Early Oligo Mix; Amplimedical Bioline, Torino, Italy), by adding the sample DNA and *Taq* polymerase (Dynazyme II DNA polymerase; Finnzymes, Espoo, Finland) to the reaction mixtures. After the amplification cycles and electrophoretic separation, we could detect the 110-bp amplified product. The lower limit of detection of this assay is 400 DNA copies/mL.[8]

For DBS in study A, we did this test on a pool of cases, as recently reported.[8] Each pool comprised 1 card (a 3-mm-diameter disc) for each of 3 newborns. We ran each pool in triplicate, and if at least 1 of the 3 tests was positive, we reexamined the cards for the neonates in the pool, again in triplicate. If the DBS result was positive in at least 1 of the 3 tests, the child concerned was called in for confirmation of the specificity of the finding by testing for the virus or viral DNA in a urine sample. If the urine sample was positive, the child entered the clinical and audiologic follow-up schedule.

The DBS of deaf children (study B) were tested individually, always in triplicate, and cases that gave the expected amplification in 2 of the 3 tests were considered positive.

Viral Isolation. We tested the urine samples by rapid isolation in mink lung fibroblasts in shell vials. The virus was identified by immunofluorescence detection of the immediate-early CMV p72 protein (Anti-Cytomegalovirus IEA; Argène Biosoft, France) 16–24 hours after inoculation.

Follow-up. Clinical and audiologic follow-up was scheduled at 3, 6, 12, 18 and 24 months of age. Clinical examination comprised objective examination, auxiometry, neurologic examination, ultrasound brain scan, ophthalmologic examination and dental check from age 12 months.

The audiologic examination involved standard recording of evoked auditory potentials. The infants were examined while sleeping in a sound-proof room. The stimuli were clicks presented at a rate of 11.1/s, rising in 10-dB steps until the V wave merged with baseline. On average, 2000 clicks were administered. At least 2 tests were done for each recording condition.

Tests for Antibodies. CMV-specific IgG were tested with a microplate ELISA (CMV IgG ELISA PKS; Medac, Hamburg, Germany), following the manufacturer's instructions.

Statistical Analysis. Data were analyzed with the STATA statistical package version 7. P values were calculated by χ^2, z and Fisher's exact tests where indicated because of small numbers. $P < 0.05$ was considered significant.

RESULTS

Population Studied. Table 1 sets out the main details of the newborns and mothers. The sample of neonates amounted to 5.13% (range, 4–10%) of the infants born in the 4 regions we studied. Mean gestational age, sex distribution and birth weight were close to the overall figures for Italian newborns.[9] The mothers' mean age at delivery (31.7 ± 5.1 years) was also comparable with figures for the corresponding Italian population.[9] Their educational levels were higher than average for the Italian women 15–49 years old; 69% versus 58% had a high school diploma.[9] The proportion of foreign versus native mothers in the study centers corresponded to that of the immigrants in the respective regions.[9]

Prevalence of Congenital Infection. The DBS test gave a positive result, according to the 2-stage design, in 41 cases. According to the protocol, we classified only the 16 children (16 of 9032; 0.18%; 95% confidence interval, 0.09–0.26%) with positive results for urine isolation as having congenital CMV infection. Nine cases were in Milan (9 of 4147, 0.22%), 7 in Parma (7 of 2127, 0.33%); no cases of congenital

TABLE 1. Main Details of the Population Studied

Children	Total	CMV-Positive	CMV-Negative
Sex			
M	4752	5	4747
F	4280	11	4269
Gestational age			
<37 wk	801	0	801
≥37 wk	8106	16	8090
Not indicated	125	0	125
Clinical findings*			
Small for gestational age	88	0	88
Jaundice	43	0	43
Direct serum bilirubin ≥3 mg/dL	6	2	4
ALT ≥100 units/L	1	1	0
Cerebral calcification	1	1	0
Others†	4	0	4
None	7593	14	7579

Mothers	Total	Child CMV-Positive	Child CMV-Negative
Age (yr)			
≤24	787	4	783‡
>24	8099	12	8087‡
Not indicated	146	0	146
Nationality			
Italian	7740	14	7726
Foreign	1292	2	1290
Maximum education			
Elementary school	226	1	225
Middle school	2362	5	2357
High school	4054	7	4047
University	2130	3	2127
Unknown	260	0	260

*Suggesting congenital CMV infection; some children had more than one clinical sign; data available for 7731 cases.
†Thrombocytopenia, petechial rash, hearing loss.
‡$P = 0.046$.

infection were found in Bari (0 of 1481) or Sassari (0 of 1277). The differences in the prevalence of congenital infection in the 4 cities were not significant ($P > 0.05$, z test).

The infected babies were born at term and had an appropriate weight for gestational age. The pregnancy had been uneventful, and routine ultrasound scans had not detected anomalies. Children born to women older than 24 years had a significantly lower frequency of congenital infection than those born to younger mothers (0.15% versus 0.51%; $P < 0.05$, Fisher's exact test). The nationality of the mothers did not influence the rate of congenital infection (0.18% versus 0.15%, Italian versus non-Italian; $P > 0.05$, Fisher's exact test).

Two infected children (12.5%) had signs at birth attributable to CMV infection, direct bilirubin 8.1 mg/dL in one, cerebral calcifications, direct bilirubin 6mg/dL and alanine aminotransferase 107 units/L in the second; none had hearing deficits at birth. Clinical and audiologic follow-up until the age of 24 months found no permanent damage in any of the children. However, the first symptomatic child and 1 asymptomatic infant, at 6 and 3 months of age, respectively, had moderate unilateral auditory deficit, with no intercurrent middle ear disease. This regressed at subsequent checks.

Seroprevalence. IgG anti-CMV tests on samples from 1200 women showed that seroprevalence was 79.9% (95% confidence interval, 77.6–82.1%) with the values uniformly distributed between the study centers ($P > 0.05$, χ^2 test for homogeneity). Statistical comparison of the seroprevalence figures for different age brackets (90.6%, 15–20 years; 76.1%, 21–25 years; 85.3%, 26–30 years; 81.3%, 31–35 years; 88.1%, 36–40 years; 93.5%, 41–45 years) showed no significant differences ($P > 0.05$; χ^2 test).

Congenital CMV Infection and SNHL. The DBS test on the neonatal Guthrie cards retrieved from the regional screening centers identified congenital CMV infection in 19 of 77 cases, 12 with no risk factors for deafness and 1 with agenesis of the 8th nerve. For the other 6, the DBS test confirmed the diagnosis of congenital CMV infection made at birth. All positive cases had severe-profound hearing loss. The prevalence of CMV infection was therefore 24.67% (19 of 77) of all the children with hearing loss and 25% (12/48) if we limit our analysis to the children with no evident cause of deafness or risk factors.

DISCUSSION

The first part of this study confirms that the prevalence of congenital CMV infection in Italy is among the lowest reported in the literature.[2] This proportion is lower than the previous figures for Milan (0.5–1%)[3] and Lombardy as a whole (0.47%)[4] on smaller populations of partly selected neonates, or for Padua (0.43%).[5] The results are comparable with the findings of other studies that used viral isolation as the analyzing tool, as the algorithm established for defining positive results in the DBS test[6,7] indicated that sensitivity was reliable: 100% in comparison with cell culture isolation. Urine tests on all cases with a positive result in the molecular test confirmed specificity only on a certain proportion, as expected from the 99% calculated specificity for the test[6] and the low prevalence of the condition in this population.

The older age of mothers at delivery and the spread of prenatal serologic screening in the last 20 years in Italy might have helped lower the prevalence of congenital CMV infection even though the seroprevalence of women of reproductive age has not changed. Mean age at delivery has risen from 25.2 years in 1981 to 30.3 in 2000.[10] Fowler et al,[11] reporting on a population with seroprevalence similar to our finding, indicated that maternal age over 25 years was associated with a lower risk of congenital infection. We report a similar finding.

Prenatal serologic screening is now done on average in 45% of pregnancies,[10] normally in the first trimester. For seronegative women, instructions on how to avoid at risk behavior can lower the number of cases of primary infection in pregnancy. A positive IgM finding can lead to a decision to terminate the pregnancy should further diagnostic investigations show the infant is at high risk of symptomatic infection.[12,13]

We could not compare the history and clinical findings for infected children and their mothers and those of the noninfected population because the infected group was too small. Seroprevalence turned out to be high, with already high rates in the youngest women. This is similar to reports from earlier Italian studies,[4,5,14,15] 2 of which also found that 50% of infants acquire CMV infection in the first year of life. The high seroprevalence was accompanied by a low preva-

lence of congenital infection in our series, like in earlier studies from Italy[4,5] and in other populations.[16,17]

As regards the clinical effects of the congenital CMV infection, the frequency of symptomatic cases at birth was as expected, but the number of infected babies is too small, and the follow-up still too short to draw any firm conclusions on the consequences. It will be interesting to see whether the regression of the hearing loss detected at follow-up visits in 2 children is permanent or whether the deafness fluctuates.[18]

The frequency of congenital CMV infection in the children with hearing loss confirms that this is an important cause of deafness, in terms of both frequency and severity.[19,20] The agenesis of the acoustic nerve in 1 case suggests this might be one of the manifestations of CMV damage to the fetal central nervous system.

The present results will help evaluate strategies for preventing the burden of congenital CMV infection in the Italian population. Right now, because we have no vaccines with proven efficacy in preventing vertical transmission,[21] preventing disability caused by the infection through virologic screening for all newborns and clinical follow-up for infected children might be a more constructive strategy. The screening model we used, modified as necessary for routine use and coupled with the procedures and structures for universal neonatal hearing screening now being widely set up in various countries including Italy, brings closer the possibility of preventing deafness.

In conclusion, this study provides an up-to-date picture of the prevalence of congenital CMV infection in Italy. Widespread and early positivity for CMV antibodies in childbearing age, together with a high mean age at delivery and a widespread good level of education, result in a very low frequency of vertical transmission of the virus. The relatively high proportion of cases with hearing loss caused by congenital infection confirms the importance of this virus as a cause of sensorineural deafness and the heavy impact of congenital CMV infection on the population and on the public health system. Planning public health preventive measures calls for thorough analysis of the local situation.

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