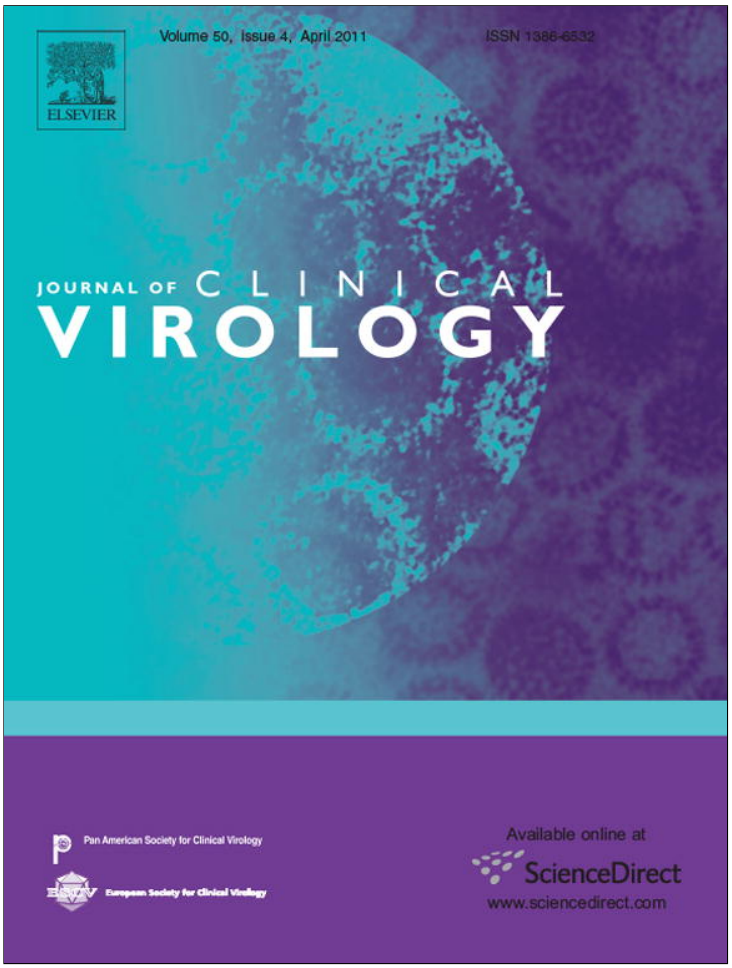

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Role of prenatal diagnosis and counseling in the management of 735 pregnancies complicated by primary human cytomegalovirus infection: A 20-year experience

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ABSTRACT

Background: The burden of congenital human cytomegalovirus (HCMV) infection is well recognized. However, screening for maternal infection remains controversial in view of diagnostic challenges, counseling difficulties, and absence of medical treatment.
Objective: To assess the role of prenatal diagnosis and counseling in the management of pregnancy complicated by primary HCMV infection.
Study design: Retrospective study aimed at investigating diagnostic features, options, and pregnancy outcome in 735 women with primary HCMV infection over a period of 20 years (1990–2009).
Results: Overall, 25.6% women were found to be seronegative before the actual pregnancy. However, none were informed about HCMV infection and potential prevention strategies. Diagnosis of primary HCMV infection was achieved by seroconversion in 44.4% cases and by different combinations of virus-specific IgM, low IgG avidity, and DNAemia in 43.9% cases. Non-specific symptoms and/or haematological/biochemical alterations were recalled by 73.5% women. The onset of infection could be established, and counseling adjusted accordingly in >90% cases. The overall rate of vertical transmission was 37.1%, ranging from 5.6% for preconceptional infections to 64.1% for third trimester infections. Amniocentesis was chosen by 43.1% women, whereas pregnancy termination was requested by 15.6%.
Conclusions: Reference virology centers and ad hoc trained and experienced physicians are required for accurate diagnosis of primary infection in pregnancy and ensuing counseling. Prenatal diagnosis has a central role in the management of pregnancies complicated by primary HCMV infection. HCMV-seronegative women should receive adequate information.

1. Background

Human cytomegalovirus (HCMV) is the most common cause of congenital viral infection leading to sensorineural hearing loss and neurodevelopmental delay. In a meta-analysis study, the birth prevalence of congenital HCMV infection was reported to be 0.64% (95% CI 0.60–0.69), with a rate of symptomatic infections at birth of 0.07%,[1] i.e. about 11% of live-born infants with congenital infection are symptomatic. *In utero* HCMV transmission may follow either primary or recurrent infection.[2–7] However, the rate of transmission to infants born to mothers with primary or recurrent infection during pregnancy was 32% and 1.4%, respectively.[1] In a parallel study on estimates of the prevalence of permanent sequelae based on universal screening, it was found that the percentage of symptomatic children with permanent sequelae was estimated to be 40–58%.[7] In addition, the percentage of newborns without symptoms at birth who developed permanent sequelae was estimated to be 13.5%.

The urgent need for interventions aimed at reducing the burden of this overlooked disease has been recognized by the US Institute of Medicine (2000),[8] which has given the highest priority to development of an HCMV vaccine. However, at this time, testing for HCMV antibody in women of child-bearing age is neither recommended

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nor performed on a routine basis, as well as screening of newborns for HCMV congenital infection.[9,10]

Nevertheless, in some countries, HCMV testing is randomly performed during pregnancy. In Italy, in particular, HCMV testing was included in the TORCH panel and was performed free of charge from 1995 to 1998. Presently, although no precise data are available, it is estimated that about 40% of pregnant Italian women are routinely tested for HCMV-specific antibody.

2. Objectives

In this paper, we report the experience of our center covering 20 years and including 735 pregnant women diagnosed with primary HCMV infection, with special emphasis on the role of prenatal diagnosis and counseling on the outcome of pregnancy.

3. Study design

3.1. Patients

This retrospective cohort study was conducted on 735 pregnant women with primary HCMV infection acquired either before or during pregnancy in the period January 1990–December 2009. This population was identified among 3426 women who, over the same period, were referred to our institution in Pavia for confirmation/interpretation of an HCMV IgM-positive result obtained elsewhere.

Institutional Review Board approval was not requested for this retrospective study. Starting in 2000, written informed consent was obtained from each woman undergoing invasive procedures.

3.2. Diagnosis of maternal and congenital HCMV infection

Primary HCMV infection in the mother was diagnosed by one or more of the following criteria: seroconversion (i.e. *de novo* appearance of specific antibodies in a previously seronegative individual), kinetics of HCMV-specific IgM and IgG antibody, low IgG avidity index,[11,12] and detection of HCMV and HCMV products in blood.[13–16] Non-specific maternal symptoms (fever, headache, asthenia, and upper respiratory symptoms) as well as biochemical and haematological alterations (i.e. abnormal values of liver enzymes and/or lymphocytosis), were also investigated.[9] Prenatal diagnosis was performed by virus isolation from and viral DNA detection by PCR in amniotic fluid, as previously reported.[17] Post-natal diagnosis of congenital infection was achieved by virus recovery from an urine sample taken within two weeks after delivery.[15]

3.3. Management of pregnancy and counseling

Fig. 1 summarizes the protocol followed at our center, whenever a pregnant woman is referred because of suspected primary infection. Each woman is interviewed by a medical virologist who manages the test performance and interpretation of laboratory results. The same medical virologist also provides the woman with the most appropriate virologic counseling according to confirmed, excluded or uncertain diagnosis of primary infection. Since 2002, at the Policlinico San Matteo residents in Obstetrics and Gynecology are routinely involved in this process in order to gain specific experience, and discuss obstetrical issues as necessary. Counseling is individually tailored by time of gestation as well as, in case of primary infection, by time of gestation at onset of infection (when established). Advantages and limitations of invasive diagnostic procedures (amniocentesis, funiculocentesis) for prenatal diagnosis

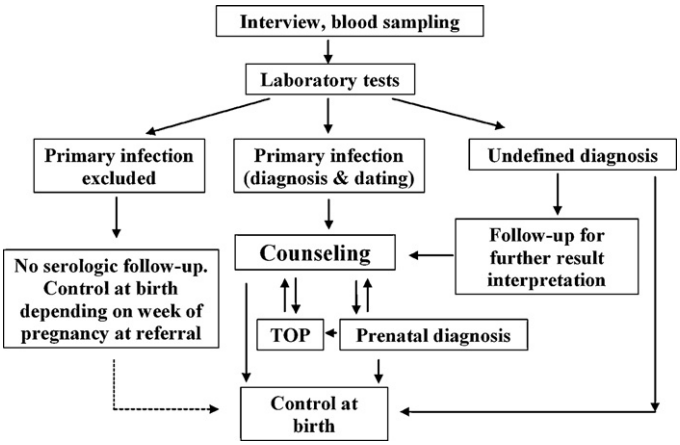

Fig. 1. Flow diagram of pregnant women with suspected primary HCMV infection referred to our institution.

(if appropriate) as well as ultrasound monitoring are discussed in detail.

In case of HCMV detection in amniotic fluid, the possibility of gaining additional information on the fetal condition by funiculocentesis is further discussed. Eventually, all available data are reviewed by the same medical virologist together with the obstetrician performing the invasive procedures and ultrasound examination, and a final counseling session is held. The woman/couple then decides whether to continue the pregnancy or request a therapeutic abortion (up to 22 weeks' gestation).

Each woman is given a written report with diagnostic conclusions and detailed instructions for newborn testing if necessary. In uncomplicated cases, i.e. cases not requiring a prospective follow-up for confirmation/exclusion of primary HCMV infection, laboratory results and counseling are routinely provided to the woman within 72 h from the referral. Amniocentesis/funiculocentesis results are routinely available within 24 h after the procedure.

3.4. Statistical analysis

Differences in the transmission rate between the preconceptional period and the three trimesters of pregnancy were evaluated by means of the χ^2 test.

4. Results

4.1. Characteristics of the study population

On the whole, 735 pregnant women (mean age 31, range 18–42 years) were diagnosed with primary HCMV infection at our institution in Pavia between January 1990 and December 2009. In parallel, 2691 women (mean age 33, range 21–42 years) were found not to be affected by a primary HCMV infection (ctrls). The majority were Italian (722/735, 98.2%; ctrls 94.0%), and came from Northern Italy (586/649, 90.3%; ctrls 95.0%). Most women were professionals (357/405, 88.1%; ctrls 90.0%) and married (506/619, 81.7%; ctrls 71.0%). As for parity, 254/623 (40.8%; ctrls 61.0%) women were primiparae, and 369/623 (59.2%; ctrls 39.0%) already had one child or more. It is noteworthy that 21/254 (8.2%) primiparae and 138/366 (37.7%) multigravidae had been tested for HCMV-specific antibodies and found to be seronegative before the actual pregnancy.

Table 1
HCMV-specific and non-specific laboratory and clinical findings and their combinations used for diagnosis of primary HCMV infection in 735 pregnant women.

	No. positive/No. examined (%)
(A) Finding	
Seroconversion	324/729 (44.4)
IgM antibody	637/725 (87.8)
Low AI[a]	437/724 (60.3)
DNAemia	370/654 (56.6)
Antigenemia	87/381 (22.8)
Viremia	18/310 (5.8)
Symptoms and/or biochemical haematological alterations	530/721 (73.5)
(B) Combination	
IgM + low AI	144/724 (19.9)
IgM + low AI + DNAemia	157/654 (24.0)
IgM + low AI + DNAemia + antigenemia	28/381 (7.3)

[a] AI, avidity index.

4.2. Diagnosis and dating of primary infection and virologic outcome of pregnancy

Serologic and virologic findings in the study population are listed in Table 1. In this series, a major contribution to diagnosis was attributed to seroconversion, (324/729 women, 44.4%). As a rule, in the absence of seroconversion, primary HCMV infection was diagnosed when at least two viral assays were simultaneously positive. However, a fair proportion of additional cases was diagnosed based on both the association of IgM and low AI (144/724, 19.9% women), and the same association combined with DNAemia (157/654, 24.0% women) (Table 1). The remaining cases were diagnosed based on multiple assay combinations.

Non-specific symptoms (fever, headache, asthenia, and upper respiratory symptoms) and/or alterations of biochemical/haematological parameters were found in 530/721 (73.5%) pregnant women. By combining serologic, virologic, and anamnestic (i.e. previous symptoms and/or signs) data it was possible to date the onset of primary infection in 695/735 (94.6%) women.

The transmission rate (Table 2) was 5.7% in the preconceptional period, between 42% and 44% during the first and second trimester, and significantly higher (64.1%) in the third trimester ($p = 0.035$, χ^2 test). The overall transmission rate was 37.1% (206/555).

4.3. Options selected by pregnant women and outcome of pregnancy

Overall, amniocentesis was chosen by 302/700 (43.1%) women and declined by 398/700 (56.9%). Termination of pregnancy was requested by 67/284 (23.6%) women who underwent amniocentesis and by 34/363 (9.4%%) women who had declined amniocentesis. Altogether, pregnancy was terminated in 101/647 (15.6%) women.

As far as termination is concerned, it must be considered that voluntary abortion is allowed by the Italian law within the first 90 days of pregnancy at the woman's request. After 90 days, therapeutic abortion can be performed only when: (i) continuation of the pregnancy poses a risk to the woman's life or (ii) fetal anomalies cause serious danger to the mental or physical health of the woman. After 23 weeks' gestation, pregnancy termination is permitted only when continuation of the pregnancy poses a risk to the woman's life and every effort is made to save the fetus' life. Since the chance of fetal survival has progressively increased over the years, the maximum gestational time for therapeutic abortion has consequently decreased from the original 25 to the actual 22 weeks of gestation.

We therefore examined women's decisions according to the time of gestation at diagnosis/counseling (Table 3). Moreover, in order to investigate variations occurring over time among different options, the study period was divided into two decades: 1990–1999 and 2000–2009. Onset of the infection and the woman's decision were available in 647/735 (88.0%) cases. Overall, of the 334 women who knew they were infected before 12 weeks' gestation, as many as 205 (61.4%) decided to undergo amniocentesis, while 71 (21.1%) decided to continue their pregnancy with ultrasound monitoring only, and 58 (17.4%) requested termination. By contrast, about two-thirds of women with preconceptional or 2nd trimester infection chose ultrasound monitoring and one-third prenatal diagnosis. Termination was requested by 8/111 (7.2%) women with preconceptional infection. Within the limited number of women examined, the most important variation observed over 20 years was relevant to the increase of women requesting amniocentesis following diagnosis of preconceptional infection from 6.6% to 31.2% (Table 3). The rate of termination performed within the first 12 weeks of gestation remained substantially unchanged.

We then examined outcome of pregnancy following prenatal diagnosis results. Overall, 302 women (284 with known onset of infection and 18 in whom onset could not be established) underwent amniocentesis. Fetal infection was diagnosed in 110/302 (36.4%) cases and excluded in the remaining 192 (63.6%) cases (data not shown).

Pregnancy outcome was available for 107/110 (97.35) women with positive amniocentesis, and 168/192 (87.5%) women with negative amniocentesis. Results are shown in Fig. 2. Of the 107 pregnancies with a prenatal diagnosis of fetal infection, 68 (63.5%) went to term, 32 (29.9%) were terminated, and in 7 cases (6.5%) miscarriage occurred following invasive procedures (3 after amniocentesis and 4 after cordocentesis). Congenital infection was confirmed at birth or at autopsy in all cases.

Of the 168 women with negative amniocentesis, 163 (97.2%) went to term, two pregnancies were terminated because of trisomy 21 (1 case) and severe fetal abnormalities (1 case), while miscarriage occurred in three cases (Fig. 2). Absence of congenital infection was confirmed at birth in 150/163 (92%) newborns.

Finally, time of gestation was compared in transmitter and non-transmitter women and found not to be significantly different (39, 31–42, weeks *vs* 40, 34–42, weeks). Similarly, no significant difference in time gestation was found between symptomatic (38, 31–41, weeks) and asymptomatic (39, 36–42) congenital infections.

Table 2
Rate of vertical transmission according to gestational age at onset of primary HCMV infection.

Gestational age at onset of infection	No. (%) of women observed	No. of transmitters/no. of women examined (%) throughout the study
Pre-conceptional	118 (16.9)	6/106 (5.7)
1st trimester	371 (53.4)	111/263 (42.2)
2nd trimester	166 (23.9)	64/147 (43.5)
3rd trimester	40 (5.7)	25/39 (64.1)
Total	695	206/555 (37.1)

5. Discussion

Notwithstanding a general awareness of the severe handicaps occurring in congenitally infected newborns and the availability of assays for the diagnosis of primary HCMV infection in the mother, screening of pregnant women remains a controversial issue.[18,19] Common criticisms to HCMV screening are that a number of unnecessary terminations are performed because of incorrect interpretation of serological results and that, in case of primary infection, termination is the only option that can be offered. Results of the present study can be useful for assessing the actual outcome of HCMV screening.

Table 3
Options selected by 647 pregnant women with known onset of infection during the 20-year study period.

Weeks of gestation at onset of infection	Period	No. of women examined	No. of women (%) choosing		
			Prenatal diagnosis	Termination of pregnancy	Ultrasound monitoring
Pre-conception	1990–1999	15	1 (6.6)	1 (6.6)	13 (86.6)
	2000–2009	96	30 (31.2)	7 (7.3)	59 (61.4)
≤12	1990–1999	88	60 (68.1)	15 (17.0)	13 (14.7)
	2000–2009	246	145 (58.9)	43 (17.5)	58 (23.6)
13-22	1990–1999	34	13 (38.2)	0 (0)	21 (61.7)
	2000–2009	103	32 (31.1)	1 (0.9)	70 (67.9)
≥23	1990–1999	16	2 (12.5)	0 (0)	14 (87.5)
	2000–2009	49	1 (2.0)	0 (0)	48 (98.0)
Total		647	284 (43.9)	67 (10.3)	296 (45.7)

Firstly, our study confirms that seronegative women in successive pregnancies are at increased risk of acquiring HCMV infection.[20,21] In our series, about 60% women with primary HCMV infection already had one or more children. Therefore, women in their second or more pregnancy, should be considered a target population for HCMV testing and, when seronegative, for hygiene information.[22]

A second consideration is that all of the women who were found to be seronegative and seroconverted during pregnancy denied receiving any information about HCMV infection and its potential prevention strategies. This sobering observation confirms that awareness and knowledge of congenital HCMV are rather poor among women[23] even in countries, such as Italy, where a fair proportion of pregnant women are routinely tested for HCMV. We believe that limited knowledge of physicians in general and obstetricians in particular is the main reason for missing a great opportunity of reducing the rate of seroconversion after providing adequate information.[24]

A third consideration arising from the present study is that when testing is performed for the first time during pregnancy, positive IgM results must be interpreted as quickly and accurately as possible in order to confirm or exclude maternal primary infection in pregnancy. Given that primary HCMV infection is eventually diagnosed in only about 20–25% IgM-positive women,[25] the correct interpretation of IgM results is crucial for avoiding unnecessary pregnancy termination[26] and for providing the most appropriate counseling. To achieve this result, reference virology laboratories and physicians with specific competence are required. Our experience has been quite unique in that it has been based on a team of medical virologists and obstetricians with a specific clinical background supported by a virology laboratory equipped with a wide array of commercial and in-house developed assays.

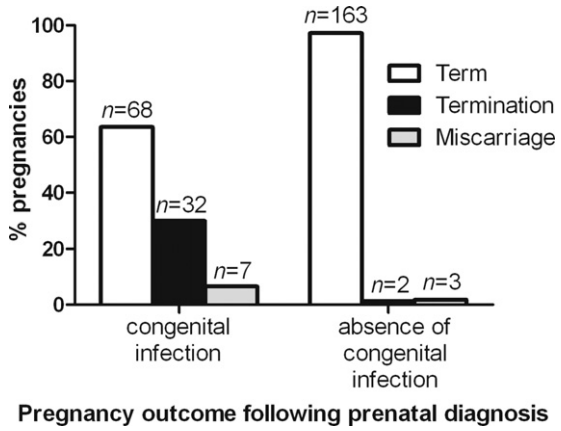

Fig. 2. Frequency of diagnostic options selected by pregnant women after diagnosis of fetal infection. Blank column, pregnancy at term; dark column, termination of pregnancy; grey column, miscarriage.

Results of the present study indicate that the presence of IgM antibody was the most important contributing parameter to diagnosis of primary infection (87.6% patients). However, apart from IgG seroconversion, final diagnosis was based on the association of IgM, low AI, and DNAemia results in multiple combinations. It is worth mentioning that, albeit in a minority of women, IgM antibodies were not detectable and IgG values were already in the medium–high range at referral; hence, the importance of performing multiple/additional assays, as well as storing IgM-positive serum samples for further investigations.

This study confirms that, following a careful medical interview, as many as 75% of pregnant women recalled symptoms that helped in timing the onset of infection.[9] Dating the onset of primary infection is critical as a prognostic factor as well as an indicator of the optimal time for prenatal diagnosis. In addition, our data confirm that virus transmission reached its highest peak during the third trimester of pregnancy.[27,28]

As for options chosen by pregnant women with respect to continuation/termination of pregnancy, two observations are worthwhile mentioning. The first is relevant to the high percentage (43%) of women selecting amniocentesis. Indeed, amniocentesis represented a key option in the management of pregnancies complicated by HCMV infection in that none of the women with a negative amniocentesis terminated their pregnancy because of HCMV infection, and only one third of the women diagnosed with fetal infection requested termination for medical reasons. In the majority of these cases, termination was performed on the basis of abnormal ultrasound/magnetic resonance findings and/or abnormal findings in fetal blood compatible with a poor prognosis.[9,29–31]

The second observation concerns the overall rate of pregnancy termination (15.6%) observed in this study. Our result is comparable with that reported (11.9%) by Guerra et al. in a series of 445 women with primary HCMV infection.[26] Undoubtedly, termination performed without knowing whether the fetus is infected represents one of the most negative aspects of HCMV screening. However, in Italy, the rate of termination due to HCMV infection in pregnancy does not seem to be different from general national data. In fact, the abortion ratio (i.e. number of terminations/1000 live births) in 2008 was 224.3.[32] When the same data are extrapolated from our series, a value of 184.8 is obtained. However, a direct comparison is not feasible.

In conclusion, this study indicates that prenatal diagnosis appears to be a very important option and a beneficial intervention in terms of reduction of voluntary abortions. On the other hand, the option of pregnancy termination, does not seem to be the

moving factor in the decision for antenatal testing, since the majority of women decided to continue their pregnancy despite the knowledge of fetal infection. Correct counseling, however, remains the key factor for the woman/parents in order to make an informed decision.

Conflict of interest

The authors declare no conflict of interest.

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