

Diagnostic and prognostic value of molecular and serological investigation of human parvovirus B19 infection during pregnancy

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SUMMARY

To define diagnostic and prognostic markers of parvovirus B19 (B19V) fetal infection, two groups were investigated:

- 1) pregnant women with specific symptoms or contacts with symptomatic households (n=37);
- 2) mothers with pathological ultrasound findings and the relevant fetus at the time of prenatal diagnosis (n=16).

In the first group, diagnosis of B19V infection was achieved using IgM detection in 29/37 (78.3%) of patients, while B19V DNA was detected in 36/37 (97.3%) of infected women. In the second group, intrauterine infection was investigated by amniocentesis (n=5), cordocentesis (n=3) or both (n=5). Median B19V DNA load in amniotic fluid was 8.2x10⁷ copies/ml and in fetal blood was 2x10⁹ copies/ml. Maternal blood was positive for B19V DNA (median 3.8x10⁴ copies/ml) in 14/16 (87.5%) women examined. At time of fetal US investigation, all mothers were B19V IgG positive and B19V IgM were detected in 10/16 (62.5%), while fetal B19V IgG and IgM were detected in 1/8 (12.5%) and 5/8 (62.5%), respectively. Phylogenetic analysis revealed that all B19V maternal and fetal strains belonged to genotype 1A. Diagnosis of maternal, fetal and neonatal B19V infection should be based on both IgM and DNA detection. Prognostic markers of congenital B19V infection need to be defined.

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INTRODUCTION

Human parvovirus B19 (B19V) is a common pathogen. In immunocompetent individuals, infection can be completely asymptomatic or can cause mild and self-limiting clinical manifestations such as erythema infectiosum or fifth disease during childhood and arthralgias and arthritis in adults, particularly in women (White *et al.*, 1985). During pregnancy, B19V is mainly transmitted through respiratory secretions, but the infection may also be transmitted by blood and blood-derived products and can be transmitted vertically from mother to fetus (Norbeck *et al.*, 2002). Fetal infection is unlikely to occur if the mother is immune at the time of exposure. Vertical transmission accounts for asymptomatic congenital infections (33-51% of cases) (Kock *et al.*, 1998) or adverse fetal effects (3-12%) such as fetal anemia, cardiomegaly and pericardial effusion, non-immune fetal hydrops (NIHF), hydropic or nonhydropic intrauterine fetal death (IUFD) and stillbirth

(Enders *et al.*, 2004, 2008), intrauterine growth retardation, thrombocytopenia (Segata *et al.*, 2007), meconium peritonitis (Zerbini *et al.*, 1998), hepatic calcifications and abnormal long-term neurodevelopment (Nagel *et al.*, 2007). Nowadays, fetal anemia is diagnosed by Doppler measurements of the middle cerebral artery peak systolic velocity (MCA-PSV) (Cosmi *et al.*, 2002). However, the accuracy of laboratory tests should be evaluated for a specific diagnosis of B19V fetal infection. Furthermore, prognostic parameters of intrauterine infection remain unsettled.

B19V diagnosis can be performed by serologic and molecular investigation in the mother, fetus and neonate (Enders *et al.*, 2006; de Haan *et al.*, 2007; Bonvicini *et al.*, 2009; Weiffenbach *et al.*, 2012). In the absence of clinical or anamnestic data, negative detection of IgM in maternal serum samples can be misleading since fetal infection can occur even 8-12 weeks later, when maternal infection has already resolved. In fetal blood, IgG represent passively acquired maternal immunity, with passive transfer of immunoglobulins increasing in the second trimester through term, and IgM may be absent due to immaturity of the fetal immune system, as for other viral congenital infections (Simister, 2003). The same diagnostic considerations apply to B19V infection at birth. Therefore, molecular tests have been combined with serological investigation to ameliorate the diagnostic accuracy of B19V infection.

Sequence analysis of B19V isolates showed a genetic di-

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versity with organization into three genotypes (Servant *et al.*, 2002). The prevalence of each genotype varies with geographic origin, population, and sample type but clinical spectrum and pathogenic properties of each genotype remain an intriguing issue (Gallinella *et al.*, 2003).

The present study retrospectively analyzed serological and molecular results from pregnant women to establish the most appropriate diagnostic procedure to detect and manage parvovirus B19V infection during pregnancy. Out of these, fetuses were investigated for a suspected B19V infection to assess the value of serological and molecular assays in the diagnosis of fetal infection.

MATERIALS AND METHODS

Patients and clinical specimens

Between January 2003 and December 2010, 53 pregnant women underwent serological and virological investigations for B19V. Two groups were identified:

- 1) 37 (69.8%) pregnant women with specific symptoms or contacts with symptomatic households;
- 2) 16 (30.2%) mothers with pathological ultrasound (US) findings suggestive for B19V fetal infection. Diagnosis of maternal acute B19V infection was based on B19V-specific IgG and IgM detection and/or viral DNA in serum samples.

At first referral, the median age of pregnant women was 34 years (range 24-42). The median gestational age (GA) was 13 weeks (range 5-33) in the first group, and 21 weeks (range 20-29) in the second group. Maternal serum samples were analyzed at study entry and, when possible, at follow-up during pregnancy (n=24).

Fetal samples were investigated for B19V DNA detection in amniotic fluid (n=14) and for B19V-specific antibodies and B19V DNA in fetal blood (n=8). No multiple pregnancies were reported. Fetal karyotypes were normal, when assessed. Neonatal serum samples were collected within 7 days from delivery (n=9) and tested for B19V-specific IgM and DNA.

Serological and molecular investigations

B19V-specific IgG and IgM (EIA BIOTRIN, Dublin, Ireland) were determined in maternal serum samples according to the manufacturer's instructions. In fetal and neonatal blood samples, B19 IgM were detected by diluting serum samples 1:20 instead of 1:100, as for maternal specimens.

B19V-DNA was quantified in amniotic fluid and blood from mothers, fetuses, and neonates, if available. Briefly, viral DNA was extracted from 0.5 and 1 ml of plasma and amniotic fluid samples, respectively, using the automatic Easy Mag extractor (Biomerieux, Lyon, France). Screening of clinical samples for B19V DNA was performed using real-time PCR. Sequencing of B19V DNA was carried out to investigate the molecular correlation of circulating B19V strains and phylogenetic analyses was performed to investigate the genetic characteristics of virus variants associated with intrauterine infection. Briefly, real-time PCR products from samples that were positive were amplified by using a PCR spanning the NS1-VP1 junction region (Corcoran *et al.*, 2010) and sequenced on a ABI 3130 genetic analyzer with a fluorescent dye terminator kit in accordance with the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). The sequenc-

es were aligned using ClustalW program integrated within the MEGA version 5.0 package (Tamura *et al.*, 2011). The phylogenetic tree was constructed with the Kimura 2-parameter neighbor-joining method with 1.000 bootstrap resamplings. The phylogenetic tree was constructed using references sequences for comparison.

Ultrasound determinations

Pathological findings at US examination, such as polydramnios, pleural effusion, ascites, hydrops, cardiomegaly, and liver calcifications were recorded. A MCA-PSV >1.5 multiples of the mean, according to existing reference values adjusted for gestational age, was interpreted as a clear sign of fetal anemia.

Statistical analysis

For most variables, descriptive statistics, such as median with range, and proportion (%), were calculated. The Mann-Whitney U-test and 2-tests (or Fisher's exact test when applicable) were used for univariate analysis, as appropriate.

RESULTS

Maternal B19V infection

In the first group, 37 pregnant women had a diagnosis of B19V acute/recent infection in pregnancy. In particular, 20/37 (54.0%) were investigated because of the onset of B19V-related symptoms, while 17/37 (46.0%) were tested following exposure to subjects affected by erythema infectiosum. Diagnosis was achieved by IgM detection in 29/37 (78.3%) patients, while B19V DNA was detected in 36/37 (97.3%) of infected women, showing a better sensitivity than IgM. Quantitation of maternal DNAemia in symptomatic and asymptomatic patients was significantly different (median 1.1×10^6 vs 3.2×10^4 copies/ml, $p < 0.001$). Twenty-four patients underwent follow-up. IgM were positive up to a maximum of 196 days post-infection (first negative result at 59 days post-infection), while DNA could be detected up to one-year at the onset of infection (first negative result at 15 days post-infection).

In the second group, 16 pregnancies were investigated because the women presented with fetal pathological findings at US monitoring. Maternal blood collected at time of fetal investigation was positive for B19V DNA (median 3.8×10^4 copies/ml, range $880-1.0 \times 10^5$) in 14/16 (87.5%) women examined. All mothers were B19V IgG positive. B19V IgM were detected in 10/16 (62.5%).

Comparison of DNA load in maternal blood at diagnosis of acute/recent infection (first group) and at discovery of US pathological findings (second group) showed a significant decrease (1.3×10^5 versus 4.1×10^4 copies DNA/ml) ($p = 0.0057$).

Fetal B19V infection and outcome

In the first group, none of the fetuses evaluated showed pathological findings at US examination up to 30 weeks' gestation. Four women decided to undergo amniocentesis (GA, range 16-22) for the risk of congenital infection, and viral transmission was ruled out.

In the second group, of the 16 fetuses presenting with US anomalies, 9 (56%) showed NIHF, 5 (31%) had mild to moderate ascites and cardiac hypertrophy, 1 (6%) hepatic calcifications, and 1 (6%) intrauterine growth retardation.

Table 1 - Characteristics of 16 fetuses complicated by symptomatic B19V infection.

Mother	WOP	Ultrasound findings	MCA-PSV (cm/s)	IUT	Fetal/neonatal outcome	DNA in amniotic fluid (copies/ml)	DNA in fetal blood (copies/ml)	IgM in fetal blood (Index)
#1	20	Ascites	NA	ND	Term, Asymptomatic CI	ND	ND	ND
#2	24	Ascites	NA	ND	Term, Asymptomatic CI	ND	ND	ND
#3	34	IUGR	NA	No	Term, Symptomatic CI	ND	ND	ND
#4	20	Ascites	NA	ND	NA	ND	POS	NEG
#5	21	Hepatic calcifications	NA	No	NA	POS	ND	ND
#6	21	Ascites	NA	ND	NA	7.2x10 ⁴	ND	ND
#7	29	NIHF	70	Yes	Term, NA	3.1x10 ⁷	ND	ND
#8	22	NIHF	58	Yes	Fetal death	ND	6.3x10 ⁹	1.1
#9	26	NIHF	75	Yes	Fetal death	8.2x10 ⁷	POS	5.4
#10	24	NIHF	48	Yes	Term, NA	3.0x10 ⁷	ND	ND
#11	20	NIHF	56	Yes	NA	ND	POS	1.5
#12	21	NIHF	63	Yes	NA	1.0x10 ⁸	2.0x10 ⁹	NEG
#13	21	NIHF	54	Yes	Term, NA	2.4x10 ⁸	1.9x10 ⁹	NEG
#14	22	Ascites	NA	ND	NA	1.0x10 ⁷	ND	ND
#15	21	NHIF	50	Yes	Fetal death	2.5x10 ⁸	7.3x10 ⁸	1.3
#16	22	NIHF	52	Yes	NA	6.2x10 ⁸	1.9x10 ¹⁰	1.1

WOP, weeks of pregnancy; IUT, intrauterine erythrocyte transfusion; NIHF, non-immune fetal hydrops; NA, not available; CI, congenital infection; ND, not done.

Intrauterine infection was investigated by amniocentesis (n=5), by cordocentesis (n=3) or both (n=5) (GA, range 20-29), while 3 fetuses were not submitted to prenatal diagnosis. Quantitation of DNA in AF and FB is reported in *Table 1*. The median B19V DNA load in FB (2x10⁹ copies/ml) was significantly higher than that observed in AF (8.2x10⁷ copies/ml; p<0.01) (*Table 1*). Fetal B19V IgG and IgM were detected in 1/8 (12.5%, #11) and 5/8 (62.5%, #8, 9, 11, 15, 16) DNA-positive cord blood, respectively. Levels of maternal and fetal B19V IgG and IgM as well as B19V DNAemia at prenatal investigation were compared. The mean fetal viral load was log 9.0 and the mean maternal viral load was log 4.0.

Doppler measurements of MCA-PSV allowed detection of 9 anemic (hydropic) fetuses that required intrauterine transfusions (IUTs) of red packed cells. At IUT, 7/9 fetuses underwent also cordocentesis for B19V infection. Four fetuses died in utero (3 shortly after IUTs, and 1 late in gestation). Of the five remaining transfused fetuses, 2 recovered and went to term and 3 were lost at follow-up.

In the remaining 7 fetuses with US pathological findings, MCA-PSV values were normal and anemia due to B19V infection did not occur. Only in one case was cordocentesis performed to assess fetal B19V infection. In 4 fetuses, clinical outcome was not available. The remaining 3 pregnancies went to term (1/3 symptomatic at birth).

Congenital B19 infection

Out of 53 pregnancies complicated by B19V infection, only 9 newborns were virologically investigated at birth

(6/37 newborns in the first group and 3/16 newborns in the second group of pregnancies). Among the first group, 3 babies were not infected, and 3 were congenitally infected. Transient jaundice was reported in one newborn, while the remaining 2 babies were asymptomatic at birth. In the second group, 2 were asymptomatic congenital infections at birth and one had jaundice, hepatosplenomegaly and thrombocytopenia that required exchange transfusions. An intrauterine growth retardation was documented at 34th week of gestation. All newborns were lost at follow-up. Diagnosis of B19V congenital infection was based on DNA detection in blood, while B19V IgM at birth were positive in 2/6 (33.3%) congenitally infected newborns.

In summary, B19V vertical transmission was documented in 19/53 pregnancies (35.8%). Of these, 3 (15.8%) occurred when the mother had B19V-related symptoms or household contacts, and 16 (84.2%) abnormal US scan.

Sequence analysis

Sequence analysis of 15 maternal blood samples collected at time of B19V infection was performed. In 3 cases, the relevant fetal blood could be investigated in parallel. Phylogenetic analysis revealed that all B19V maternal and fetal strains belonged to genotype 1 A (data not shown).

DISCUSSION

The present study reviewed serological and virological data of 53 pregnancies complicated by B19V infection. Two groups were identified:

- 1) mothers with specific clinical symptoms or contacts with symptomatic households, representative of an acute/recent infection;
- 2) mothers with pathological findings at US monitoring, representative of a non-recent misdiagnosed infection in pregnancy. Mother-fetus pairs were analyzed and the clinical outcome of gestation was evaluated to establish the accuracy of serological and virological tests in the diagnosis of B19V infection during pregnancy. In acute/recent infection IgM detected 78.3% of B19V infections, while DNA showed a sensitivity of 97.3%. This means that a negative result of IgM detection cannot rule out a B19V infection, and DNA testing should always be included to avoid incorrect diagnosis. At follow-up, DNAemia persisted longer than IgM, but DNA might disappear earlier than IgM. When B19V infection in pregnancy remained silent and a suspicion arose in the presence of US pathological findings, IgM were positive in 62.5% and DNA in 87.5% of pregnant women. Considering that fetal manifestations can appear 8 to 15 weeks after maternal infection, a negative result of either serological or virological tests in the mother can be misleading. Furthermore, clinical manifestations in the fetus were not influenced by defense of the immunologic system in terms of IgM production or clearance of DNA load in maternal blood.

DNA detection in AF and FB correctly diagnosed B19V transmission from mother to fetus. Therefore, prenatal diagnosis of B19V fetal infection should be performed by amniocentesis in case of US pathological findings, and cordocentesis might be restricted to anemic fetuses requiring IUTs. In our series of fetuses, we observed consistently high B19 copy numbers in FB. For this reason, in the absence of B19V DNA load in AF, detection of low levels of B19V DNA in FB should be regarded with caution due to possible contamination with maternal blood. On the contrary, IgM tested positive in 62.5% of infected fetuses examined, showing a lower sensitivity than DNA. As for IgG, only 12.5% of fetuses showed passively transferred antibodies from the maternal bloodstream. This is not surprising, since at 17-22 weeks of gestation, fetal IgG reaches about 10% of the maternal concentration (Simister *et al.*, 2003). In our study, cordocentesis was performed at median 21 weeks GA (range 20-26). Moreover, the placental transport of maternal B19V specific IgG to the fetus has been advocated to have a protective effect on fetal damage (Weiffenbach *et al.*, 2012), but this aspect could not be confirmed in our small series of fetuses.

Quantitative measurement of B19V viral load was performed to compare fetal and maternal viral load. A consistently high gradient between fetal and maternal viral load was observed. In the hypothesis that the fetus is the source of maternal viremia (de Haan *et al.*, 2007), the amount of DNAemia at diagnosis of acute maternal B19V infection (first group) and at US fetal pathological findings (second group) was compared. A significant decrease was observed (1.3×10^5 versus 4.1×10^4 copies DNA/ml) but it might be interpreted as an ongoing clearance of virus in maternal blood instead of a retrograde transplacental viral transport. Furthermore, despite of the small number of fetuses examined.

As reported by several authors (Bonvicini *et al.*, 2011; Puccetti *et al.*, 2012), the rate of B19V congenital infections was 35.8%. However, this rate could be underestimated

since we examined only fetuses/neonates with a suspected B19V infection and not all newborns whose mothers had a diagnosis of B19V infection in pregnancy. As a matter of fact, prenatal diagnosis of B19V infection should be performed only in the presence of pathological findings at US scan and/or fetal anemia. Indeed, symptomatic women or contacts with infected people enrolled in the present study did not show fetal signs of vertical transmission during pregnancy. However, following a diagnosis of maternal B19V infection in pregnancy, confirmation or exclusion of vertical transmission in utero should be mandatory at birth, even in asymptomatic newborns. In parallel, an enhanced surveillance in public health would be important to establish the risk to pregnant women following in-house/occupational exposure and the rate of fetal loss due to B19V.

From a diagnostic viewpoint, DNA detection in neonatal blood was the most sensitive method to disclose congenital infections at birth since IgM were positive in 2/6 (33.3%) congenitally infected newborns. Both the immaturity of the fetal and neonatal immune systems and B19V antigenicity can contribute to the absence of IgM (de Haan *et al.*, 2007). However, sensitivity of the Biotrin IgM was enhanced using modified cut-off values (data not showed).

In summary, molecular investigation should always aim to correctly diagnose B19V infection in the mother, fetus, and neonate. Serological screening might switch the gap in public health knowledge on infections in pregnancy.

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