Author's response to reviews

Title: High frequency of Human Cytomegalovirus DNA in the Liver of Infants with Extrahepatic Neonatal Cholestasis.

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Author's response to reviews: see over
We thank you for the suggestions and we have attempted to answer all the questions. Below we specify comments raised by each question. Answers are in blue font.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Major point is that the authors repeatedly report (in the abstract, both in the results and conclusions, in the results, page 9 line 1, and in the discussion) that serology had a low accuracy, when compared to PCR results (which are considered by them 100% specific, like viral isolation). This consideration, and consequently the sensitivity and specificity of the serology, are based on erroneous calculations of the results.

RESULTS: page 9, line 1: compared to PCR, serology (IgM + IgG or IgG alone) had a specificity of 75% (9/12) but was more sensitive than PCR (65.7% versus 34.3% of the patients).

Active HCMV infection was defined if one or more of the following conditions are present: positive result for HCMV DNA by PCR, a four-fold or greater increase in IgG HCMV antibodies, and a positive test for IgM HCMV antibodies.

Hepatitis or hepatic involvement was defined by findings of elevated bilirubin and/or enzyme levels during liver function testing, absence of any documented cause of hepatitis, and detection of HCMV infection in a liver biopsy specimen.

We didn’t use variations of IgG antibodies to detect active HCMV infection because we didn’t have these results. Then, we didn’t use IgG alone for compare with PCR.

DISCUSSION
- page 9, line 17: seroprevalence is based on the presence of IgG, then the percentage was 65.7% (23/35) ⇒ we thank you for the observation of the right percentage of seroprevalence. Now we correct in the new text.

- page 10, lines 5-10: the presence of IgM is related to a recent infection, while the biliary atresia occurred early in pregnancy: since the mean age of the patients was about 3 months, and maternal CMV antibodies are generally negative after 3 months, the development of atresia was CMV-associated more probably in the 3 infants with only IgG and PCR+ than in the 6 with IgM and PCR+ (in these infants, the presence of CMV DNA in the liver could have been simply due to circulation in the blood, according to the authors, during a perinatal or postnatal infection)
Two major forms of BA are recognized, based on the presumed timing of the obliteration of the lumen of the extrahepatic bile duct: the fetal or embryonic form (occurs in 20-30% of cases, is associated with other congenital anomalies) and the acquired or perinatal form (70-80% of cases, is believed to occur at or following birth with progressive postnatal destruction of a biliary tree that developed normally during embryogenesis).

In our study, only one patient present by sure the embryonic form, because the situs inversus that had. This patient was ELISA negative and PCR positive. In the others patients, the correct diagnosis is difficult based in the clinical presentation. The jaundice during the first few days of life maybe mistakenly believed to represent physiologic jaundice. Dark urine staining the diaper is often seen but not appreciated as abnormal and seldom reported by the mother. Similarly, she may not appreciate that the acholic stools are abnormal, especially if the child is her first.

The presence of IgM and PCR positive results maybe related with the perinatal form of BA. We agree that the detection of HCMV DNA in patients IgM+ maybe caused by the virus circulation since we didn’t use in situ hybridization. However, we believe that the presence of HCMV DNA in liver and porta hepatis samples of the patients IgM-/IgG-indicate that the virus is within the hepatocytes and/or biliary duct cells.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

All the items related with “Minor Essential Revisions” was done and now we think that the paper improved a lot and we hope became suitable for publication.

ABSTRACT: Results: Line 2: To evidence the discrepancy between serology and PCR, it should be included, after Nine liver and seven porta hepatis samples: from 12 infants, 9 of whom had IgG and/or IgM antibodies… Line 5: serology does not detect HCMV but antibodies

BACKGROUND: Line 16: Hepatic involvement (hepatomegaly) is frequent and clinical evidence of hepatitis (persistent jaundice and elevated and persistent aminotransferases) are occasionally found.
Last line: (by ELISA systems)
METHODS: Serological investigations: specify manufacturers of the ELISA systems. ⇒ SORIN BIOMEDICA, Italy.

Ethical Committee approval (last line): all infants’parents.

DISCUSSION: page 11, line 8: 3 PCR+ infants out of 12 ELISA negative are too many, thus it is better to avoid the word only.