Author's response to reviews

Title: Molecular evidence of Ureaplasma urealyticum and Ureaplasma parvum colonization in preterm infants during respiratory distress syndrome.

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 ANSWERS TO THE REVIEWERS' COMMENTS

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Molecular evidence of Ureaplasma urealyticum and Ureaplasma parvum colonization in preterm infants during respiratory distress syndrome.

Rosario Cultrera, Silva Seraceni, Rossella Germani, Carlo Contini.

General comments

1. Authors specified in the manuscript text that the research was in compliance with the Helsinki Declaration and successive amendments.

2. Authors ensured that our revised manuscript conforms to the Journal style.

Reviewer 1 (Prof. Robert Schelonka)

ANSWERS

1. As requested by the other Reviewer, Mycoplasma Duo test has been replaced by “culture identification assay”.

2. We agree with the Reviewer that the interpretation of our results was complicated by the lack of a true “gold standard” technique to which compare our PCR results. The Microbiology Laboratory of our Hospital employed a culture identification technique as screening assay (Mycoplasma Duo test kit, Bio-Rad Laboratories, Italy). Considering that most studies have been reported PCR for the detection of Ureaplasma spp. in clinical samples was better than culture method for its high
sensitivity and the short time required to obtain the results (Blanchard A, et al. 1993; Colaizy TT, et al. 2003; Waites KB, Katz B, Schelonka RL, 2005). We employed PCR methods to detect *Ureaplasma* spp. in the respiratory secretions, because of its high sensitivity and short time consuming compared with culture methods. We also focused our attention on the use of species-specific primer toward urease and 16S rRNA genes that detected *Ureaplasma* spp. only, without any possible cross-reactivity with other mollicutes or bacteria. A second gene target combined with sequencing of the amplicons for serovar identification was also employed in order to exclude any false-positive reaction due to contamination.

3. The aim of our study was to investigate the role of PCR, employed directly on clinical specimens, to evidence *Ureaplasma* spp. colonization of the respiratory tract in premature newborns with RDS and to evaluate a possible association of *Ureaplasma* spp. infections with RDS. Our findings indicated that 62.5% of RDS patients resulted PCR positive for *Ureaplasma* spp., evidencing a high prevalence of *U. parvum* in tracheal aspirates of newborns with RDS. In agreement with other Authors, molecular techniques were confirmed more sensitive and time-saving than culture techniques or other direct identification assays for *Ureaplasma* spp., other than to allow a direct species identification.

4. We also focused our attention to detect ureaplasmal infection of the lower respiratory tract in premature newborns with RDS, rather than to relate this pulmonary infection with CLD or BPD. Although not statistically significant, we found only three cases of CLD at 28 days, two of which in RDS group. However, previous studies (Cassel GH. et al., 1988; Jonsson B. et al., 1994; Wang EEL. et al., 1995; Ollikainen J. et al., 2001; Kotecha S. et al., 2004; Kafetzis DA. et al., 2004), based on more consistent
number of patients, were conducted in order to evaluate the presence of *Ureaplasma* spp. in the respiratory secretions, suggesting a possible their role in the pathogenesis of CLD. About the long term follow-up, we were not authorized by local ethic Committee to obtain respiratory secretions during the follow-up until 36th week after the discharge. The short term respiratory outcome was favourable in all cases except one newborn that died at 8 days old. Some newborns were lost during the follow-up, thus it was not possible to define the development of CLD or BPD.

5. The treatment of infected infants with clarithromycin was uncontrolled because we were authorized by ethic Committee to employ this drug in all newborns which resulted PCR positive for ureaplasmal respiratory infection. The employment of clarithromycin in babies with *Ureaplasma* spp. infection was useful to improve the clinical picture as supported by molecular and microbiological findings for *Ureaplasma* spp.

6. A lot of *in vivo* and *in vitro* studies suggested that *Ureaplasma urealyticum* induces the production of proinflammatory cytokines (IL-1β, IL-8, TNF-α). In a previous unpublished study we found that viable *Ureaplasma urealyticum*, serovar VIII, more than its extracted lipoprotein pool, was able to induce the production of IL-1β and TNF-α from THP-1 limphomonocytic cell line. These results were in agreement with other published studies.

7. The manuscript was revised for the spelling and grammar mistakes and errors.
Reviewer 2 (Prof. Dimitris A. Kafetzis)

ANSWERS

1. The manuscript was revised in all sections for the spelling and grammar mistakes and errors.

2. As requested by the Reviewer, Mycoplasma Duo test has been replaced by “culture identification assay”.

3. Abstract: sequencing analyses were put either in “Methods” section or in the “Results” section.

4. As the Reviewer suggested, we avoided paragraphs – one sentence.

5. We revised the disproportion in the length between the “Background” and “Patients and methods” sections as possible. Authors thought that the different parts of “Patient and Methods” section are required to explain point to point the step of the research and its development. Thus, the recruitment of the patients and the laboratory methods needed to be described into details.

6. As required by the Reviewer, we added an additional photo (figure 1) with the PCR results visualized by an agarose gel showing samples of group 1, group 2 and control group patients.