Author's response to reviews

Title: AuTop trial: Screen-and-treat program by Point-of-care of Atopobium vaginae and Gardnerella vaginalis in preventing preterm birth: protocol study for a randomized controlled trial

Authors:

Florence Bretelle (florence.bretelle@ap-hm.fr)
Florence Fenollar (florence.fenollar@univ-amu.fr)
Karine Baumstarck (karine.baumstarck@ap-hm.fr)
Cécile Fortanier (cecile.fortanier@ap-hm.fr)
Jean-François Cocallemen (jean-francois.cocallemen@ap-hm.fr)
Valérie Serazin (vserazin@chi-poissy-st-germain.fr)
Didier Raoult (didier.raoult@univ-amu.fr)
Pascal Auquier (pascal.auquier@univ-amu.fr)
Sandrine Loubière (sandrine.loubiere@univ-amu.fr)

Version: 2 Date: 9 September 2015

Author’s response to reviews: see over
Dear Prof. Altman,

Please find enclosed the revised manuscript of our study protocol entitled: "AuTop trial: Screen-and-treat program by Point-of-care of Atopobium vaginae and Gardnerella vaginalis in preventing preterm birth: study protocol for a randomized controlled trial", which I and my colleagues are submitting for the exclusive consideration of publication as an article in Trials.

We would like to thanks the referees for his/her appreciations of our work and for all his/her very interesting comments on the paper and the study protocol. Replying to the referees comments has helped us improving our paper.

Please find below in bold our answers to the referee 1 and referee 2.

1 – Answers to referee 1

1 to 3: No question

4. Reference list could be shortened.
   We have deleted some of the references. The reference list is now made of 44 citations.

5. a. I foresee criticism about allocation concealment when results will be published. Why did not the investigators use sequentially numbered, opaque, sealed envelopes rather than open-label? Describe justification for open-label choice.

   The referee raised a significant point that has been discussed at length within the study team. We agree that the implementation of allocation concealment techniques would prevent selection bias; however, under some circumstances an open-label design may be unavoidable. In our study, achieving a non-analyzed vaginal swap may raise both ethical and regulatory issues (legal/penal risk for the institution and risk of refusal from the Ethical bodies). For example, in the case of premature delivery in a patient for which the result would have been positive, the issue of sponsor’ liability is raised. Thus, these final arguments were that we have chosen the open-label scheme for our RCT.

5.b. I suggest start recruiting after first trimester scan if this is not the norm. This will avoid recruiting before early miscarriages and will confirm viability, wish of termination of pregnancy, gross fetal anomalies and singleton pregnancies. If it is the norm, I suggest to clarify this in the protocol.

   There is not down age gestational limits for inclusion. In our study, patients are recruited around 10-14 weeks of gestation after the first US screening. In addition,
several studies have indicated that the link between bacterial vaginosis and preterm delivery is stronger when the diagnosis of vaginosis was fixed early in the course of pregnancy (1-3). Andrews et al. estimated that the contamination occurs early in pregnancy even in pre-conceptional step. Other studies showed an association between concentrations of vaginal cytokines and an excess risk of premature birth or the occurrence of chorioamnionitis in early pregnancy (before 20 SA) (4-5). Based on these results, we have considered that screening after the first trimester was probably too late to prevent pregnancy complications. Therefore, we have chosen to start recruiting during the first trimester.


5.c. In exclusion criteria I would add “...known diabetes or hypertension or fetal/uterine malformation...”. Would be increased nuchal fold be considered “fetal malformation”?

We confirm that in our study, we have considered increased nuchal fold as “fetal malformation”.

Would be increased risk of preeclampsia (if uterine Doppler are tested at 1st trimester” be considered “hypertension”?

Uterine Doppler testing is not currently performed routinely in French practices.

May I suggest to re-write it as: Known conditions at the time of recruitment that have either increased risk of spontaneous preterm birth (previous preterm birth, uterine malformation, multiple pregnancies…) or that may need preterm delivery due to medical indication: hypertension, diabetes, fetal malformation, increased risk for preeclampsia (or other conditions that the investigators may consider).

We agree with the referee and we have re-written the “exclusion criteria” paragraph as proposed.

5.d. In cases of positive PCR, needs to be clarify if follow-ups are going to be in a predefined weeks or if they will be after pre-defined time after treatment. I wonder if D0, D18, D48 and D78 applies for pre-specified days of rescreening. If so, a call to the figure has to be introduced in the text.

PCR results are expected within 48 hours after inclusion. Following the PCR result, an appropriate treatment will be provided for positive women. Successive vaginal swab at home for monitoring will be performed 15 days after the treatment intake. Consequently D18, D48 and D78 applied for pre-specified days of screening controls (with a small ‘window’ of +2/3 days). We have introduced a call to the figure 1 in the section “Experimental group: screen-and-treat strategy”.
5.e. No question

5.f. Consider secondary outcome delivery <34 weeks as the “late preterm” issue is a hot topic. WHO defines “Preterm” as babies born alive before 37 weeks of pregnancy are completed; sub-categories of preterm birth are defined as follows: extremely preterm (<28 weeks), very preterm (28 to <32 weeks), moderate to late preterm (32 to <37 weeks). In our study, we used the WHO’s definition of moderate to late preterm sub-category as an endpoint. However, we agree with referee 1’s comment, as “late preterm” infants are born at a gestational age between 34 weeks, and 36 weeks and 6 days. We have therefore added in the paragraph “Secondary endpoint” this additional threshold.

5.g. Consider also spontaneous delivery at each gestational age (besides overall preterm birth rate) as a secondary outcome as bacterial vaginosis has been related to this. If so, define how will you consider induction of labor because of preterm rupture of membranes. Will that be a spontaneous preterm birth or indicated preterm birth? This is a very difficult question to answer. In our study, preterm rupture of membrane will be considered as a preterm event. If induction of labor should be decided thereafter, it will be defined as indicated preterm birth.

5.h. Define “spontaneous abortion” Spontaneous abortion or miscarriage is the spontaneous loss of the fetus before the 22 weeks of amenorrhea according to WHO definition. In our analysis, we will distinguish spontaneous abortion between 14-22 weeks of amenorrhea versus late abortion between 22-24 weeks of amenorrhea.

5.i. I suggest including late miscarriage (15-24w?? both included) as a specific secondary outcome as it has been related to presence of bacterial vaginosis. We agree with the referee’s suggestion. We have now defined spontaneous abortion and added late miscarriage as a specific secondary outcome in the paragraph “Secondary endpoint”.

5.j. Define better secondary outcome:” woman’s hospitalization”. It means hospitalization for preterm labor or for any condition? “Duration of the woman’s hospitalization” in the section “Obstetrical outcomes:” includes hospitalization for delivery or preterm labor and potential previous hospitalizations due to gynecologic complications during pregnancy. Our analyses will have a specific focus on the duration of hospitalization for preterm labor. Future hospitalizations after delivery will be analyzed and collected in a separate section (“Other health care utilization”). We have clarified the definition of woman’s hospitalization in the text.

5.k. Define also policy when preterm rupture of membranes (induction of labor at 32 or 34 36 weeks?) In case of preterm rupture of membranes, a “wait and see” attitude is the rule until 36 weeks.
5.1. Findings of short cervix if routine cervical length is carried out should be collected. If routine screening is not the rule, treatment with pessary or progesterone during pregnancy should be collected too.

**Routine cervix screening is not performed routinely in France. However, treatment with pessary or progesterone during pregnancy will be collected. This precision was added in the section “Data collection and follow up”**.

If there are different centers with different policies, this should be taken into account in the eCRF.

We agree with the referee’s comment. The different policies implemented at different centers are taken into account in the eCRF (particularly in regards to the concomitant treatments).

5.m. Why did the authors not plan an interim analysis? If clear benefit arises after the first 3400 women, efforts may be shortened. If no differences, study may be followed as planned. It is for the investigators to decide how clear should be this benefit as a stopping rule.

**For our study we have decided to define the incremental cost-effectiveness ratio (ICER) as the primary endpoint. This means that the difference in effectiveness should be calculated together with the difference in costs between the two groups. The expected difference in costs is based on 6-month follow-up after delivery. The sample size was calculated to obtain a statistically significant difference between the two groups in terms of ICER ratio. Therefore, we are not confident with an interim analysis, particularly because of: - attending completion of the trial will increase precision, reduce errors, increase statistical power as reported previously, increase ability to look at different thresholds or subgroups, and gather information on secondary endpoints.**

5.n. Regarding treatment: azithromycin sure has appropriate spectrum and might increase adherence rate. Clindamycin seems better second choice to me considering spectrum and in order the results to be compared to previous literature. Clarify call to the Figure for antibiotic strategy in recurrences.

**We have added a figure on the treatment algorithm (Figure 2); this provides a better understanding of the complex treatment schema as suggested by the referee.**

5.o. Decreasing from 4.3% to 3.0% is a huge difference as it is decreasing a 30% the prematurity.

The expected difference in effectiveness is based on the results from a previous RCT conducted by Kiss et al. in 2004 (6). Their design is quite similar to our protocol study with the exception that they used Nugent score testing instead of PCR point of care screening; and they recruited women in their second trimester of pregnancy. Nevertheless, they found a decrease of 45% in the prematurity rate between the two groups (difference 2.4%, CI [1.3 – 3.6] between screened and treated women versus non-screened women). We have decided to use the low figure of the confidence interval.

(6) **Kiss H, Petricevic L, Husslein P: Prospective randomised controlled trial of an infection screening programme to reduce the rate of preterm delivery. Bmj 2004, 329(7462):371.**

5.p. Prevalence of BV in the general population is around 10%. However, as this is a low risk population and as BV is more prevalent in high risk population which is excluded (i.e previous preterm birth), 10% might be overestimated. Include in limitations.

**We have some arguments which may comfort our expected prevalence of BV.**
Firstly, in Kiss et al. (6), the percentage of bacterial vaginosis in their study population was 7.3%. Their exclusion criteria were quite similar (women with subjective complaints, i.e. contractions, vaginal bleeding, or symptoms suggestive of vaginal infection, or women with multiple pregnancies). In addition, both women with high-risk and low-risk factors of preterm birth or late miscarriage were included. However, the percentage of women having an obstetric history including preterm delivery or a history of late miscarriage did not differ significantly between the two groups at inclusion (less than 2.3%).

Secondly, it is well known that the ethnic origin influences the prevalence of bacterial vaginosis in pregnancy (7-9). In Kiss’s RCT, the population was 98% white ethnic origin. In our study population, we will expected less than 65% for the white ethnic group, based on a previous RCT conducted in the same French centers. Therefore, we think that a prevalence of BV of 10% in a population with or without clinical symptoms of BV and well balanced in terms of ethnic origins is not so optimistic. Nevertheless, we will be particularly attentive to this specific point. We have added these discussion points in the “Discussion”.

6. Define D0, D18 D48 and D78 in the Figure: are this the days of re-screening?

The referee is correct; D0, D18 D48 and D78 are the days of re-screening. We have provided the definition of the different times of the trial in the revised Figure 1.

7 to 8: No question

2 – Answers to referee 2

Major revisions:
The study is interesting and well structured. It opens new horizons regarding the screening for vaginal infection in pregnancy and the related preterm delivery risk.

However, the aim of the study and the potential cost savings cannot be fully achieved by searching just *Atopobium vaginae* and *Gardnerella vaginalis* without a complete analysis of the vaginal ecosystem (*Enterococcus*, *Streptococcus*…). In particular it does not consider the amount of lactobacilli in view of the wellness/disease status of the vaginal flora. It could be significant to add lactobacillus colonization status for further studies. We agree with the referee’s comment. Besides, several bacterial species, including *A. vaginae*, *G. vaginalis*, *Eggerthella*, *Prevotella*, Clostridiales BVAB2, and *Megasphaera* type 1, or even *Lactobacilli* depletion have been reported to be highly predictive for bacterial vaginosis. In addition, lactobacillus quantification could be another clue. However, for economic reasons we had to limit the number of bacterial species included in the study. Why did we choose *A. vaginae* and *G vaginalis*? Firstly, it has been clearly shown in several studies performed by different teams that the diagnostic accuracy of
this polybacterial dysbiosis is best for *A. vaginae* (1-4). More recently, our previous study, performed in 813 pregnancies, targeting the relations between the concentration of several bacterial species (*A. vaginae*, *G. vaginalis*, *Lactobacilli* and *Mycoplasma hominis*) and the risk of preterm birth, showed that *A. vaginae* and *G. vaginalis* had a stronger association with preterm birth than other bacterial species (5). Secondly, treatments against *G. vaginalis* and possibly against *A. vaginae* exist and may decrease PB, whereas we have no information to date on the impact of additional lactobacilli on PB.


Minor revisions:
Could the authors be more exhaustive regards the usual care management in the control group?
Usual pregnancy care includes 6-8 obstetrical consultations, no systematic vaginal swab, 3 ultrasound scans, 1rst trimester Down syndrome screening and blood sampling for toxoplasmosis, syphilis, rubella, and complete Blood group testing. This precision was added in the section “Control group: usual care management”.

Why the authors have chosen the oral use of azitromicin or amoxicillin as the unique appropriate therapy for the treatment of vaginal infection and other topical therapies are not considered. Authors could have used topical nifuratel which is effective against *Atopobium vaginae* and *Gardnerella vaginalis* without affecting the lactobacillaris flora. Which criteria do the authors used to choose the specific antibiotic? Why do they not used topical therapy with lactobacillaris strains highly lactic acid producers that lead to a significant reduction of vaginal PH determining favorable conditions for endogenous lactobacilli growth? To assess the cost-effectiveness of a systematic screen-and-treat program we ask which advantages this study has compared to the use of fresh vaginal swab and traditional culture followed by a treatment with vaginal lactobacilli and nifuratel that has a real effectiveness against *Atopobium* even if it is not identified with traditional molecular technique. This could lead to a reduction of recurrence.

Amoxicillin and azithromycin could both be used safely in pregnant women. Besides, it has been already shown that *A. vaginae* and *G. vaginalis* were highly susceptible to azithromycin and amoxicillin (1,2).

Recently *in vitro* studies also suggested that nifuratel, a nitrofuran derivative, has a better spectrum of activity in comparison to metronidazole and clindamycin, being highly active against *G. vaginalis* and *A. vaginae* without affecting lactobacilli (3). However, to our knowledge, large randomized studies evaluating the efficiency of
nifuratel on bacterial vaginosis in the real life have not been performed yet. In addition, atopic reaction has been already reported with nifuratel (4). Thus, it would be highly delicate to use this compound for pregnant women. Finally, conflicting data have been reported about the efficiency of the use of vaginal Lactobacilli probiotic in bacterial vaginosis. Indeed, it has been clearly shown that combining the first line therapies of oral metronidazole and vaginal clindamycin, or oral metronidazole with an extended-course of a commercially available vaginal-L. acidophilus probiotic, does not reduce bacterial vaginosis recurrence (5).

(1) De Backer et al. Antibiotic susceptibility of Atopobium vaginae. BMC Infectious Diseases 2006, 6:51

Thank you for your consideration of our work.

Please address all correspondence concerning this manuscript to me at the address below and feel free to correspond with me by e-mail.

Sincerely,

Sandrine Loubière

Correspondence to: Sandrine Loubiere, Laboratoire Santé Publique, Faculté de Médecine, 27 bd Jean Moulin, 13005 Marseille Cedex 5, France. Email address : sandrine.LOUBIERE@univ-amu.fr

Files attached:
- Revised manuscript (DOCX), with yellow highlighting on the text
- 2 figures’ files (PDF)