ANSWERS TO REVIEWERS

March 7, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 7745-review.doc). Our revised manuscript has been rewritten taking into account all comments and questions pointed out by the reviewers.

Title: Portopulmonary hypertension and hepatopulmonary syndrome.
Challenges for liver transplantation anesthesia

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Specific answers to the Reviewer 1 (*reviewers' comments in italic*):
Abstract:
"*In POPH, there is mainly vascular remodeling and not vasoconstriction*".
This has been corrected according to the reviewer's comment.
"*Medical treatment is disappointing and the only definitive treatment is LT*".
This has been corrected: "Medical treatment is disappointing. Whereas LT results in the disappearance of HPS within six to twelve months, its effect on POPH is highly unpredictable."
Page 4:
"*I don't have access to ref 13, but the value of 50% of acute responders to vasodilators in POPH seems too high*".
According to Krowka, (Hepatology, 1999 september, 641-648), the rate of acute responders (defined as a >20% decrease in mPAP) to Epoprostenol, is 43%. Therefore the text has been changed in that way.
Page 5-6:
"*The chapter on POPH treatment is not sufficient*".
We have completed the chapter with a table summarizing the main treatments. (table 4)
Page 6:
"*The threshold of 400 dyn.s.cm-5 is too high.*"
We disagree with the reviewer but we have modified the text to make it clearer. However, we have kept the PVR value of 400 dyn.s.cm-5, as it is part of the UNOS policy guidelines. Indeed, if a patient with mPAP>35mmHg is responsive to a vasodilator therapy as defined by a decrease in mPAP<35mmHg, he receives MELD exception as long as the PVR is <400 dyn.s.cm-5 and the right ventricular function is preserved (Machicao, Pulmonary complications in chronic liver disease, Hepatology 2013). We fully understand that our text can be confusing as figure 1 uses 250 dyn.s.cm-5 as a cut-off value rather than 400 dyn.s.cm-5. However, this has been the practice in our institution until now. The figure 1 title has been modified to show that this algorithm is used in Geneva only.
Page 10:
"*The cited papers are not sufficient to write that pulse oximetry is a reliable test to detect HPS.*"
We have added articles by Arguedas M (Clin Gastroenterol Hepatol 2007 5:749-54) and Abrams GA (Liver Transplant 2002 8:391-6), to prove that pulse oximetry is a reliable mean of detecting hypoxemia. In the article by Abrams, 200 cirrhotic patients were screened with pulse oximetry. Using a SpO2 cut-off value of 97%, the sensitivity was 96%. Moreover, the accuracy of pulse oximetry was assessed in a meta-analysis of 82 trials. The mean bias was only 1.99% +/-0.23%. Jensen LA (Heart lung

1998;27:387-408). Even though we agree with the reviewer's argument that SpO2 might be insensitive to the detection of hypoxemia in the 60mmHg region because of the flat aspect of the haemoglobin dissociation curve, the literature proves us that on the contrary, pulse oxymetry is very sensitive when using the right cut-off value, i.e. 96-97%.

Specific answers to the Reviewer 2:

This is a review of the diagnosis and management of portopulmonary hypertension and hepatopulmonary syndrome. In light of the fact that evolution of portopulmonary hypertension is not well known, there is little evidence-based medicine upon which to base this discussion.

The strength of this review is to propose an anesthesic peroperative management of patients with PoPH and HPS during liver transplantation.

However, the authors should be more explicit in the pre operative management and in the indication to consider liver transplantation especially in patients with PoPH that can be also diagnosed in patients with mild cirrhosis.

There are many points on PoPH that need to be clarified especially as they are a lot of reviews on this subject published in the literature.

Specific comments appear below:

Portopulmonary hypertension:

1. Page 3: "*Putative harmful mediators are serotonin and endothelin, increased plasma concentrations of these mediators being identified in patients with portal hypertension. - Could you add references?"*

For endothelin [1]: In a study from Benjaminov et al, 62 patients (53 males, 9 females; mean age 54.5 (1.4) years) with biopsy proven cirrhosis and refractory ascites underwent angiographic measurements of pulmonary and splanchnic haemodynamics. Endothelin 1 levels were measured from the pulmonary artery, showing significantly higher endothelin 1 levels in PoPH patients (3.04 (0.40) v 1.98 (0.12) pg/ml; p=0.02).

For Serotonin [2-4]: Hyperplasia of pulmonary artery smooth muscle cells is a hallmark pathological feature of primary pulmonary hypertension. Pulmonary artery smooth muscle cells from patients with primary pulmonary hypertension grow faster than those from controls when stimulated by serotonin or serum and that these effects are due to increased expression of the serotonin transporter, which mediates internalization of indoleamine. However, variation of the serotonin transporter gene appears unlikely to confer significant susceptibility to pulmonary arterial hypertension.

"*In my view, this physiopathological explanation is too simplistic. It has been also suggested that hyperkinetic status could promote shear stress and induce endothelial dysfunction. Moreover, inflammatory condition observed in patients with cirrhosis (endotoxinemia, bacterial translocation) could participate to the pulmonary vascular remodelling.*"

The pathophysiology of POPH remains unclear: it is observed in cirrhotic and non-cirrhotic portal hypertension and is related to neither the etiology of liver disease nor the severity of portal hypertension. However, female sex and autoimmune disease are important risk factors (Kawut SM Hepatology 2008). POPH has the same features of plexogenic arteriopathy of idiopathic pulmonary hypertension involving endothelial and smooth muscle proliferation. It has been also suggested that hyperkinetic status could promote shear stress and induce endothelial dysfunction. Moreover, inflammatory condition observed in patients with cirrhosis (endotoxemia, bacterial translocation) could participate to the pulmonary vascular remodelling. Another widely accepted explanation is that mediators produced in the splanchnic circulation and normally metabolized by the liver reach the pulmonary circulation through portosystemic collaterals with subsequent injury to pulmonary vessels. Putative harmful mediators are serotonin and endothelin, increased plasma concentrations of these mediators being identified in patients with portal hypertension. Besides their vasoconstrictive effect, these mediators also promote cell proliferation. (Michelakis ED, et al. Emerging concepts and translational priorities in pulmonary arterial hypertension. Circulation. 2008 30;118:1486-95). Nevertheless, despite sophisticated testing, the pathogenesis of POPH remains elusive.

"The author must insist in the fact that portal hypertension is sufficient to induce PoPH. More than 10% of patients with PoPH have extrahepatic portal hypertension and patients with mild cirrhosis child Pugh stage A can also develop the disease. This concept is important for the rest of the paper which unfortunately focus only in patients candidates for LT. However, it's important to specify that severity of liver disease is not related to the risk of portopulmonary hypertension."

We have modified the text and stressed the fact that portal hypertension per se is sufficient to induce POPH and that the severity of the liver disease is not related to the development of POPH. Indeed, as mentioned by the reviewer, approximately 10% of patients with portal hypertension without cirrhosis (typically infected with *Schistosomia mansoni*) present this picture. (Zafdar Z, Liver transpl, aug 2012)

Regarding the fact that our paper focuses only on LT candidates, we must say that this is deliberate. Indeed, as the title says, our article deals with the challenges of liver transplantation for the anaesthesiologist. It is beyond the scope of this paper to cover all the aspects of diagnosis and non-surgical management of LT candidates with HPS or POPH.

Page 3: "*POPH is a relatively common condition among LT candidates with a prevalence varying between 6-16%.*" *These % are wrong and are not those mentioned in reference 8. Could you please add other references on prevalence studies and propose a more appropriate prevalence?*

We agree that our numbers are wrong and we have corrected the reference (Colle). In the study by Benjaminov (Benjaminov, F.S., et al., Gut, 2003. 52(9): p. 1355-62) the quoted rate of POPH is 16%. This was found in a subgroup of patients with decompensated cirrhosis or refractory ascites, which is not representative of all LT candidates. We have added one reference concerning the prevalence of POPH among LT candidates. (Krowka, Hepatology 2006 44 1502-1510). In this study of a cohort of 1235 LT candidates, the prevalence of POPH is 5.3%.

Page 3-4: "Their outcome is poor with a one-year survival of 85% and three-year survival of 38%". The reference 9 is not a survival study. These results have been reported by Kawut et al in another small retrospective series (Kawut SM, Taichman DB, Ahya VN, et al. Hemodynamics and survival of patients with portopulmonary hypertension. Liver Transpl. 2005;11(9):1107-1111.) Prognosis of patients with PoPH has been reported more recently by: - Le pavec et al: Portopulmonary hypertension: survival and prognostic factors. Am J Respir Crit Care Med. 2008;178(6):637-643. - Krowka et al: Portopulmonary hypertension: a report from the US-based REVEAL Registry, Chest 2012 There are conflicting results in these studies probably explained in part by dissimilarities concerning the severity of the underlying liver disease.

We agree with the reviewer that reference 9 is not a survival study and we have therefore replaced it by (Kawut SM, Taichman DB, Ahya VN, et al. Hemodynamics and survival of patients with portopulmonary hypertension. Liver Transpl. 2005;11(9):1107-111). Regarding the prognosis of POPH patients, we have added the references by Le Pavec (Portopulmonary hypertension: survival and prognostic factors. Am J Respir Crit Care Med. 2008;178(6):637-643) and Krowka (Portopulmonary hypertension: a report from the US-based REVEAL Registry, Chest 2012), and we have added the 1, 3 and 5-year survival rates, as suggested. We also have explained that the differences between studies can probably be explained by the severity of the underlying liver diseases.

Page 4: "It is now clear from these findings that patients with POPH should be properly diagnosed preoperatively, to initiate the right treatment promptly and select exclusively those with a non-prohibitive level of POPH for LT (table 2 and 3)" Table 2 and 3 don't specify hemodynamic data allowing LT.

We have added figure 1 to illustrate the three step approach to POPH management. First, a proper diagnosis of POPH must be made (table 2). Second, the severity of POPH must be graded (table 3). Finally, depending on the severity of POPH and its response to vasodilators, LT can be considered or is contraindicated (figure 1).

Page 4: "Furthermore, RHC is also used to carry out reversibility tests on the pulmonary vasculature with various vasodilators. However, even though 43% of patients are acute responders, their long-term response to therapy cannot be predicted" This sentence is very confusing and may give a wrong message: - Acute pulmonary

vasodilator testing have an interest only for predicting the long-term effectiveness of treatment with calcium channel blockers (and not specific PAH therapies) - The proportion of responders is very low among patients with PoPH (Montani et al, Long-term response to calcium-channel blockers in non-idiopathic pulmonary arterial hypertension, Eur Heart J 2010). Therefore, treatment with calcium channel blockers has no place in PoPH.
According to the European guidelines for the diagnosis and treatment of pulmonary hypertension (Nazzareno European Heart Journal (2009) 30, 2493–2537), POPH should be diagnosed and treated like pulmonary hypertension of any other cause. Therefore, a reversibility test is carried out first and the response is assessed. In case of acute response, theoretically, a calcium channel blockers therapy should be initiated. However, calcium channel blockers may cause an increase in portal hypertension and edema. They are therefore poorly tolerated in POPH patients and rarely used in this setting (Krowka, Clev clin journ med 75, 2, 2008).
Regarding the rate of acute responders, the results in the literature are conflicting. Responders vary from 1.3% (Montani D, Eur heart Journ, 2010, 31,1898-1907) to 43%(Krowka, hepatology 30:3, 641-648).
In POPH, the reversibility test is useful to assess whether a patient is eligible for LT or not (figure 1). Indeed, in moderate to severe POPH, a post-vasodilator decrease in mPAP <35mmHg makes LT an option, whereas if the mPAP does not decrease under this level, LT is not recommended. Furthermore, in borderline cases, RHC is carried out every three months to reassess treatment response.

About treatment, in my view it will be more appropriate to use the term of "specific PAH therapies" and not "vasodilatators." It is now well established that long term effect of these drugs is due to the antiproliferative effect and not the vasodilatator effect.
Vasodilators have been changed to specific PoPH therapies.The antiproliferative effect of specific PoPH therapies has been added as their main effect.

Page 5: "Nitric oxide (NO) is a selective pulmonary vasculature dilator when used in the inhaled form. It is effective but necessitates an intubated patient."
NO can be used in patients not intubated. However, you have to mention that this treatment have a therapeutic interest only in a context of acute right failure due to PAH worsening.
We have modified the text. NO can also be used in non-intubated patients, in the context of acute right ventricular failure, although, its accurate administration is difficult.

. Chapter on PAH specific therapies is confuse and must be reformulated
- "Epoprostenol, a prostacyclin, is a vasodilator and a platelet inhibitor, which is used parenterally": the main effect is a antiproliferative effect
- "In a retrospective study, it was shown to improve pulmonary circulation hemodynamics and allowed a minority of patients to achieve a sufficiently low mPAP to permit LT"; The reference 11 is not good. Can you give the proportion of patients that achieve sufficiently low mPAP?
- Information you provide on other treatments (ERA, PDE-5, other prostacyclin derivative) are not appropriate. In my view, It seems to be hazardous to give comment on the survival impact of these treatments in PoPH.
You must specify that retropective studies seem to show a significant effect on hemodynamic which justifies using these treatment as bridge-therapy before liver transplantation. These treatments seem to be well tolerated. Nevertheless, the severity of liver disease should be included in the therapeutic choice (risk of portal hypertension worsening, risk of cytolysis with ERA…).
The main effect of Epoprostenol being antiproliferative has been changed accordingly.
Reference 11 indeed is wrong. It has been replaced by reference M. Ashfaq Am Journ Transpl 2007, 7 :1258-64, a study which prospectively included all potential LT candidates for a 8 year period (1997 to 2005). Of 3433 screened patients, PoPH was diagnosed in 40 (10 mild, 8 moderate and 12 severe). Epoprostenol was initiated in 16 of 20 patients with moderate to severe PoPH. 12 of these achieved a drop in mPAP < 35mmHg, which accounts to a reduction rate of 75%. This allowed LT in most patients. In another study by Sussman N, American Journal of Transplantation 2006; 6: 2177–2182, 7 of 8 (88%) consecutive LT candidates with moderate to severe PoPH who had been started on epoprostenol

showed a significant reduction in mPAP.

Regarding POPH treatments, a table (Table 4) summarizing the main effects and adverse effect of these drugs has been added. Also, comments on the context of the treatments' validation (idiopathic pulmonary hypertension) have been made. The weak level of evidence to support their use and the need to choose one or another, on an individual basis has been stressed.
"All these treatments have mainly been validated in patients with idiopathic pulmonary hypertension, which is a different disease from POPH. They have their limitations and must be chosen on an individual basis, taking into account their various adverse affects (Table 4). The various treatment recommendations come from small retrospective trials with inherent limitations. A confirmation by randomized controlled trials is needed. Finally, one must bear in mind that these treatments are mainly useful in lowering mPAP and make LT feasible."

You don't give any recommendation on the management of PoPH in patients with less severe cirrhosis or extrahepatic portal hypertension. Should Liver transplantation be propse whatever the severity of the underlying liver disease (in my view, no)? What about specific treatments for PAH in these patient?...
Our article focuses on the implications of POPH and HPS for LT from an anesthetic point of view. Therefore, it is beyond the scope of this review to give any recommendation on the management of POPH in patients with less severe cirrhosis or extra-hepatic portal hypertension.

The figure 1 must be modified.
- Reversibility tests are not use helpful for the diagnosis
- The use of specific therapies as bridge therapy for patients with more severe PoPH is not mentioned
- They are no recommendation to perform RHC/6 months. However, RHC must be perform to assess efficacy of specific therapies.
- You have to mention in the text the recommendations of the ERS task force
We have modified figure 1.

In case of right ventricular failure, the use of epoprostenol can not induce a immediate improvement of hemodynamic conditions and must be introduce on the long term with gradually increasing doses.
We have already mentioned in the text, that: "It seems that giving a pulmonary vasodilator only intraoperatively is insufficient. It should be started preoperatively to improve outcome as it probably acts on vascular remodeling." We therefore agree with the reviewer's comment, but we believe that no modification needs to be made.

Hepatopulmonary syndrome

"Therefore, HPS must be actively sought in every LT candidate."
HPS must not be actively sought only in LT candidates but also in symptomatic patients whatever the severity of the underlying disease. Unlike PoPH, LT must be considered in sever HPS even if there is no liver indication for transplantation. The same for the figure 3.
We disagree with the reviewer's comment. Indeed, the MELD classification is always the cornerstone of organ allocation in LT. HPS grants MELD exception points to the LT candidate. For instance, the UNOS policy grants 22 points to every patient with severe HPS, whatever the severity of the underlying liver disease.

All references and typesetting were corrected.

Specific references for PoPH physiopathological explanations:
1. Benjaminov, F.S., et al., Portopulmonary hypertension in decompensated cirrhosis with refractory ascites. Gut, 2003. 52(9): p. 1355-62.
2. Roberts, K.E., et al., Serotonin transporter polymorphisms in patients with portopulmonary

hypertension. Chest, 2009. 135(6): p. 1470-5.
3. Machado, R.D., et al., Genetic association of the serotonin transporter in pulmonary arterial hypertension. Am J Respir Crit Care Med, 2006. 173(7): p. 793-7.
4. Eddahibi, S., et al., Serotonin transporter overexpression is responsible for pulmonary artery smooth muscle hyperplasia in primary pulmonary hypertension. J Clin Invest, 2001. 108(8): p. 1141-50.

We hope that we have given satisfaction to all Reviewers' queries and that you will find this original work in its revised version, now valuable and interesting for the readers of the World Journal of Gastroenterology.
Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

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