Author's response to reviews

Title: Impact of Cytokine Release on Ventricular Function after Hepatic Reperfusion: A Prospective Observational Echocardiographic Study with Tissue Doppler Imaging

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Author's response to reviews: see over
Reviewer 1:

Reviewer's report

Title: Impact of Cytokine Release on Ventricular Function after Hepatic Reperfusion: A Prospective Observational Echocardiographic Study with Tissue Doppler Imaging

Version: 4 Date: 18 May 2015 Reviewer: Paolo Feltracco Reviewer's report:

Comment for Authors

The authors correlated cardiac performance measured by TEE and TDI with cytokines release at different points during liver transplantation; any hemodynamic instability after the reperfusion stage was also evaluated. They concluded that cytokine release might not be the major cause of circulatory derangement at reperfusion.

The methods are quite appropriate, the results well reported, the discussion understandable, but sometimes slightly supported by data obtained.

The content of the paper is of scientific interest, even though the main message (no link between cytokines release and PRS) may not be valid for the whole range of cirrhotic patients undergoing OLT.

Some issues and concerns with this paper:

Main message: No observed impairment of LV function, no changes in MAP as a consequence of an increase in pro-inflammatory mediators after reperfusion.

Issue with definition of baseline cardiac performance and MELD score of candidates

Authors only mention at the end of paper a rather low MELD, and report a mean value of 15 on tab 1.

They should emphasize the low MELD, the fact that none of recipients at baseline displayed a LV-EF <50% (average 72%), and the extremely low cardiovascular risk factor of their case series. That 11 patients tolerated a complete cross-clamping of IVC without veno-venous bypass indirectly suggests that they underwent surgery with a “well-maintained” cardiovascular function at baseline. This may be one important factor for not observing PRS.


Thank you very much for your careful review of our manuscript. We agree with you, that most of our patients either have a low cardiovascular risk at baseline or that a coronary artery disease has been treated prior to the transplant. We included this information and extended the discussion on the rather low MELD scores into the discussion section (p.17, l. 10-18).
The non-occurrence of hemodynamic impairment cannot be true for the population of candidates with high MELD, and represents a limitation that deserves mentioning.

We did now extend the discussion of low MELD and include more information about cardiac remodeling and diastolic and systolic function in patients with higher MELD scores (p.17, l. 10-18). In addition we added a paragraph of the study limitations and strengths (p. 17, l. 14- p. 18, l. 6).

The development of a clinically relevant PRS (manifested not only with >30% reduction in MAP) could be facilitated by a reduced cardiac reserve. A reduced myocardial performance (systolic and diastolic dysfunction) is almost always present in the late cirrhosis, where, despite a normal to increased cardiac output and contractility at rest, an abnormal and blunted response to pathologic stress may occur.

We agree with the reviewer that in patients with low MELD scores there seems to be a normal cardiac response to stress [1]. We also agree that diastolic dysfunction progresses along with the severity of the disease [2]. But systolic dysfunction does only develop in a subset of patients [3]. The fact, that systolic function is preserved in most patients, however does not provide much information because of the low peripheral resistance in end stage liver disease.

The assertion that significant cytokines liberation is not coincident with hemodynamic instability and impaired cardiac function might be therefore true only for these low-risk, not advanced liver recipients (conclusion not applicable to all end-stage cirrhotic patients currently included in the modern liver transplant programs).

This comment is well taken and is now included in the new paragraph about study limitations (p. 18, l. 5-6).

Amount of Vasoactive drug infused

Another issue to be clarified is the rate of infusion of noradrenaline. High dose noradrenaline, a sudden consistent increase in venous return (liberation of total clamp of IVC), and an optimal myocardial reserve may have accounted for the non-reduction of MAP after reperfusion, despite cytokines liberation. Based on how noradrenaline is reported (mcg/min) it is very difficult to appreciate the exact dose/per patient/ per stage.

Thank you very much for this important comment. We tried to clarify the catecholamine doses. Usually our patients receive a continuous infusion of norepinephrine in order to keep a MAP of >60-65 mmHg. In addition small boluses of 5-10ug noradrenaline are administered for immediate correction of a drop in blood pressure, for example after declamping. We have now added the exact amount of patients requiring a catecholamine bolus in the manuscript. (p. 12, l. 9-12).

Was the hemodynamic status immediately before reperfusion prophylactically "assisted" "reinforced" by an increase in catecholamine administration,
vasoconstrictors, or fluid infusion? It does not emerge... It is difficult to assert “no myocardial depression” if an incremental dosage of amines had been given.

The 2nd statement of discussion underlines that echocardiographic parameters reflected the hemodynamic changes due to administration of catecholamines, and volemia changes.

With the extended information about catecholamine doses before and after unclamping and especially about catecholamine boluses, a relevant pharmacological reinforcement of the heart (p. 12, l. 9-12) can be excluded. Only in 6 of the patients bolus catecholamine (noradrenaline, neosynephrine or adrenaline) was applied after unclamping. Only a single patient received adrenaline with a strong inotropic effect. We are unfortunately not able to answer your question about the fluids. We recorded the total amount of fluids administered, also the start and the end of the infusion bag, but we are unable to extract the exact volume in a certain time unit out of the patient history.

In addition we did not observe changes in systolic or diastolic function in our patient population throughout surgery (p. 12, l. 23-p.13, l. 1).

A desirable, common goal of anesthesia conduction during clamping in OLT is to maintain MAP with vasoconstrictors or amines (or fluids). How can we differentiate the primary spontaneous variations of myocardial fibers function from those influenced by catecholamines?

This is indeed an interesting question. In our study all patients received a continuous infusion of noradrenaline (the mean dose before cava clamping was 9.4 ± 8.6 ug/min and 2-5 min after reperfusion 11.0 ± 7.0 ug/min, p. 12, l. 9-12). None of the patients received an infusion of adrenaline or another inotrope (e.g. dobutamine). Noradrenaline acts mainly as a vasoconstrictor and myocardial fiber function should thus not be influenced too much. We are aware however, that it has some inotropic effects and therefore we cannot completely exclude its effect on myocardial contractility.

Importance of the quality of the grafts

Also the quality of implanted graft may be of importance in determining PRS, both as regard the amount of cytokines released and vasoregulation of tone of systemic circulation and pulmonary artey. The fact that IL6 and IL8 increased significantly both during clamping and after reperfusion does not necessarily mean that the measured increase be clinically relevant. TNF alpha was below the detection level. Marginal graft and high-risk patients would potentially show far greater levels than those observed.

Statistically significant does not always mean clinically significant.

We agree with the reviewer. The fact, that the increased cytokine levels may still be too low to impair cardiac function is now discussed in the section about study limitations (p. 18, l. 5-6).
Good grafts, adequate filling pressure in PA after reperfusion (sudden massive increase in venous return), and optimal basal cardiac reserve (plus amines) may act synergically to mask PRS manifestations. The reduction in SVR after reperfusion may be not only due to cytokines but also to the “doubling” of CO (mathematical result).

We agree with the reviewer that we observed an increase in CO and a reduction in SVR after unclamping of the liver and that therefore a kind of masked PRS may be present. Despite these facts our patients do not meet the criteria for PRS defined as a drop in mean arterial pressure of >30% for at least one minute (during the first 5 minutes after reperfusion).

Methods of evaluation of cardiac performance, TEE and TDI

Where there difficulties in recording clear images in the transgastric short-axis while the stomach was pushed away during liver dissection and grafting?

You are completely right. There were some difficulties in obtaining the transgastric views, particularly during liver grafting. That’s why we used different ways to assess systolic function, including Simpson’s rules obtained in mid-oesophageal views. The following comment has been added in methods: “Different measurement of systolic function were used, as far as the transgastric views were not always available, particularly during liver grafting” (p. 9, l. 4-6)

Some “minor” doubts in interpreting the TEE measurement of some parameters: FAC in transgastric short-axis may not well define systolic function of LV, depending on momentary LV filling. Even FAC and EF% may consistently reflect the changes in volume loading and/or the administration of catecholamines, and not necessarily the variations of fiber function (i.e. dp/dt). A reduction in CO is reported after clamping, with a “compensatory” increase in SVR....however, LV-EF% increased at this stage.....

Unfortunately, there is no true echocardiographic measurement of inotropy or of myocardial fiber function. The FAC or EF are all very load-dependent and more closely reflect an adaptation of the heart to hypovolemia during clamping than a true reflection of inotropy. The acceleration time of s in TDI might have been a better, less load dependent, monitoring of the systolic function. Unfortunately, we did not measure it at this time. This has been added in the discussion. (p. 15, l. 1-6)

- How was INRT easily measured with transmitral Doppler?

The isovolumetric relaxation time (IVRT) was obtained after a slight modification of the interrogation window into the left ventricular outflow tract. This has been added the methods. (p. 9, l. 14-16)

- Line 23 on page 9 ..... mitral annulus on the lateral position... better than on the medial aspects
We used both medial and septal aspects of the mitral annulus, although we agree with your comment that the lateral aspects is generally more reliable.

- Line 9-10 page 10 E/A ratio >1.5 ???: likely 1?

Thank you very much for this comment. It should be E/A 1 to 2. But since this paragraph is complicated to read and the measurement of diastolic function has been published before by Garcia MJ et al (1998) and by Oh JK et al (2006) we allowed ourselves to reference this section to the published works. We hope this meets the reviewer’s approval. (p. 9, l. 20-21)

Minor Errors or imprecisions

Abstract: Line 17 2-5 minutes after…? lines

This has now been corrected. (p. 2, l. 16)

19-20 MPAP CVP and CO unchanged…when??

This has been specified. (p. 2, l. 19)

Line 16 on page 9 indice?

This has been corrected to indicative. (p. 9, l. 10)

Line 23 on page 7 inversion better…to respect chronological sequence

This sentence has been rephrased.

Lines 5-6 on page 16…pertinent statement, citation and reference?

This paragraph has been revised to allow a distinction between our opinions and published factors impacting on hemodynamic stability. (p. 16, l. 4-15).

Line 16 on page 16 fortified?

This sentence has been rephrased. (p. 16, l. 23)

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

No
Reviewer 2:

Reviewer's report

Title: Impact of Cytokine Release on Ventricular Function after Hepatic Reperfusion: A Prospective Observational Echocardiographic Study with Tissue Doppler Imaging

Version:4 Date: 24 May 2015

Reviewer: Maged Salah

Reviewer's report:

Thank you very much for reviewing our study and for your inputs. We are happy to answer and complement our manuscript upon your suggestions.

I find it acceptable study written in acceptable language. Study question is important and clearly answered.

figures and tables are clear and informative abstract is acceptable methodology is well written results are clear

discussion is acceptable

no major compulsory revisions

discretionary revisions:

-small number of patients in study

We agree with the reviewer, that the number of the patients in the study is rather small. This lies in the fact that the ethical approval was only valid for a certain time period and that only our most experienced cardiac anaesthetist (Dr. Dominique Bettex) performed the echoes – she had to be available and not be occupied during the entire case of liver transplantation, which can be scheduled to a desired time-point only on rare occasions. We therefore feel, that this study still may be important and exclude investigator variability to the minimum despite the low number.

- no comment on bleeding caused by TEE probe

We observed no bleeding caused by the TEE probe, most likely because varicose veins were an exclusion criteria (p. 7, l. 12-15). The fact, that we did not observe a bleeding in our patients is now given on (p. 12, l. 15-16).

- Did the researchers use an ordinary TEE probes or small size probes
The probe was an ordinary TEE probe and not a small size probe. The exact details of the echo machine and the probe can be found in the method's section (p. 8, l. 19-22).

- did manipulations of stomach and liver affected the quality of transgastric views

You are completely right, there were some difficulties in obtaining the transgastric views, particularly during liver grafting. That's why we used several ways to assess systolic function, including Simpson’s rules obtained in mid-oesophageal views. The following comment has been added in methods: “Different measurement of systolic function were used, as far as the transgastric views were not always available, particularly during liver grafting” (p. 9, l. 4-7.)

- authors mentioned FAC,FS and EF for systolic function assessment but tables only show EF, is this related to difficulty in obtaining transgastric views

This is correct. Therefore the above mentioned statement was added: “Different measurement of systolic function were used, as far as the transgastric views were not always available, particularly during liver grafting” (p. 9, l. 4-7.)

- TEE findings sound reasonable

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests
Reviewer 3

Reviewer's report

Title: Impact of Cytokine Release on Ventricular Function after Hepatic Reperfusion: A Prospective Observational Echocardiographic Study with Tissue Doppler Imaging

Version: 4 Date: 22 May 2015 Reviewer: Hossam El Ashmawi Reviewer's report: Major Compulsory Revisions:

- The article is well written and has no major revisions.

  Thank you very much for revising our manuscript and this statement.

Minor Essential Revisions:

Methodology

1- It is customary to write the statistics at the end of the methods.

  The statistics section has been moved to the end of the methods section

2- Last line in Anesthesia and Monitoring: the authors state that echocardiographic measurements were done by an "experienced cardiac anaesthetist (DB), who is an expert in TEE". The abbreviation DB understandably refers to Professor Dominique Bettex, but I fear that this will not be readily comprehensible by the readers.

  This has been clarified and is now written out.

3- TEE Measurements and Techniques: The authors measured EF using either Simpson's method OR Teicholz method; I wonder if the two methods should be used interchangeably, as this might lead to differences in measurements.

  You are right, these methods should not be used interchangeably. EF reported in the table were obtained with Simpson’s rules, the most reliable measurement of EF. We did however use various ways to assess EF throughout the procedure (such as Teichholz or eye-balling). A notice has been added for comprehension in table 5.

Results:

1- In the tables, the mean and standard deviation are reported in two different formats; e.g., Mean (SD) in table 1, and Mean +/- SD in table 2.

  Thank you very much for this comment. The style of table 2 has now been adapted to the rest of the tables.

Discussion:

1- Please mention study limitations and future recommendations.
A section about study strengths and limitations has now been included (p. 17, l. 11-22) as well as recommendations for future studies (p. 17, l. 23 – p. 18, l. 2).

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests
References

