Author's response to reviews

Title: Monitoring of oxidative and metabolic stress during cardiac surgery by means of breath biomarkers: an observational study

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Version: 2 Date: 19 July 2007

Author's response to reviews: see over
Answers to the reviewers

Reviewers’ comments have been answered in a point to point manner. Reviewers’ suggestions are printed in italics, our answers in standard style. Text from the manuscript that was changed is shown underlined. In the manuscript changes are indicated by standard word format for document control.

Reviewer 1

We thank Prof. Amann for his evaluation of our manuscript.

Reviewer 2

General comments:

To substantiate their project, the authors remark that breath markers could offer - compared with markers of clinical chemistry – the advantage of a non-invasive approach for intra-operative control of the metabolism. In my view, this is only an artificial advantage because stepwise and multiple sampling of blood for clinical chemistry during surgery is no problem and established praxis during surgery.

We agree that clinical chemistry offers a great number of well established tests assessing different aspects of metabolism and tissue damage. Nevertheless, breath tests have the advantage of being non-invasive and not requiring blood withdrawal. The corresponding statement (background 2nd paragraph) was changed according to the reviewer’s suggestions: Early recognition of these pathological conditions is the only way to minimize organ damage. Methods used so far (e.g. Swan-Ganz-Catheter, PICCO, Doppler based cardiac output measurements, laboratory parameters) often are invasive [3], not fast enough and not always available at the bedside.

In my view, it is more important that exhaled substances could be less from hemodilution, infusions and other confounders than blood markers. Furthermore, the authors suppose, that breath testing can be outclass markers of clinical chemistry due to their time and money consuming and non-satisfactory sensitivity and specificity for early recognition of the metabolic stress. Also this argumentation, I think, is artificial. Based on the whole money consumption of heart surgery, cost for
clinical chemistry is marginal. Additionally, modern clinical chemistry with bed-side-testing is an effective strategy to minimize time consuming. In my view, measurement of volatile substances for intra-operative control would then overclass clinical chemistry if devices were constructed enabling measurement of volatile substances continuously or by multiple measurements. very close meshed.

As breath testing can be performed frequently without any burden to the patient, it could be used in the future to recognize metabolic or oxidative stress in a very early phase through continuous analysis of breath biomarkers such as pentane or acetone. As Prof. Schimke points out, this certainly cannot be achieved by offline GC/MS analysis. But seen the rapid development in analytical science towards sensitivity in the ppt range and miniaturization one may suppose that online real time measurement of well defined biomarkers will soon become possible at patients’ bedside.

The authors should point out that realizing such a concept for intra-operative control by volatile markers, first of basic research and especially feasibility studies are necessary to find out which and when breath markers are able to mirror special metabolic stresses intra-operatively.

Paragraph 3 in the background section was changed as follows:

Exhaled concentrations of these compounds can, therefore, be used to detect pathological conditions in the body at an early stage [9]. As breath tests are completely non invasive they can be performed repeatedly without any burden to the patient. Early recognition of pathological conditions, such as oxidative or metabolic stress during surgery, requires continuous online measurements of volatile biomarkers. In order to conceive procedures and devices capable of real time analysis of volatile substances basic research on breath markers and feasibility studies are necessary. Hence, this study was intended to assess the impact of heart surgery with extracorporeal circulation (ECC) onto breath biomarker profiles.

In addition 1st paragraph of discussion was also changed:

Profiles of volatile biomarkers measured during and after cardiac surgery showed correlations with clinical conditions or clinical parameters. Acetone in breath mirrored metabolic stress, exhaled pentane concentrations increased during well defined surgical actions, exhaled isoprene showed a correlation to cardiac output. The profile of acetone exhalation parallels facts known from clinical studies on outcome and serum dextrose control [2]. The results of this study confirm that non invasive
recognition of oxidative and metabolic stress during cardiac surgery is feasible by means of breath analysis. If these data are transferred into appropriate devices continuous surveillance and early recognition of oxidative or metabolic stress should soon become possible.

Specific comments

The word count of the abstract is 347. The guidelines for authors read: “The abstract of the manuscript should not exceed 350 words and must be structured into separate sections”.

Methodological aspects
Description of breath sampling was extended (Methods – Breath sampling):
Although it was known from pre-study experiments that samples in the vials remain stable for at least 6h, preconcentration and analysis was begun immediately after sampling. I.e. samples in the vials can be transported and stored. In order to obtain reliable results time between sampling and preconcentration/analysis should not exceed 6h.
Reproducibility and variation of SPME has been extensively studied by Pawliszyn et al (see references given in the manuscript). Limits of detection and variation of sampling, preconcentration and analysis were investigated in prior studies (see reference below).

Methods - Preconcentration and analysis of volatile substances:
Tables 1 and 2
As proposed by the reviewer tables 1 and 2 were omitted, data are summarized in the text (methods, 1st paragraph).

Correlations between breath markers and data from clinical chemistry:
Clinical chemistry parameters were determined preoperatively, at arrival on ICU and 4h after end of surgery. The reasons for not measuring all these parameters at any time point of the study were:

1. Effects of hemodilution and infusion could considerably have influenced serum parameters during surgery and ECC.
2. Analysis of all serum parameters at any time point would have increased requirements for blood withdrawal for study purposes.
3. Some of the serum parameters are known to need a certain time to show an increase after tissue damage (CK-MB, troponine).
4. During surgery and ECC it is sometimes difficult to differentiate between increase of parameters due to direct surgical trauma (cutting, manipulation on heart tissue) and those due to oxidative/metabolic stress.

Separation of patient groups according to duration of ECC
In table 1 positive correlations between exhaled acetone concentrations and duration of ECC are shown at t4 30 min, t5 60 min after surgery.

Figure legends
Figure legends were revised. All abbreviations used in the figures are explained in detail. We chose to show time of measurements as t1 – t8 rather than complete descriptions (e.g. “150 min after end of surgery”) which would be too bulky and long to be put directly into the figures.