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# Tissue inhibitor of metalloproteinase-2 predicts early allograft dysfunction after liver transplantation

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## Abstract

**Background** The lack of objective criteria to assess graft integrity represents a major impediment to the acceptance of livers of limited quality as a way to increase the donor pool in liver transplantation. Extracorporeal machine perfusion prior to transplantation may serve as a valuable platform to second to the surgeon's decision on acceptance or discard of the organ, but, to date, the availability of valuable perfusate biomarkers is rather poor.

Tissue inhibitor of metalloproteinase-2 (TIMP2) is a protein actively secreted upon epithelial cell stress and involved in the suppression of endothelial cell proliferation.

**Methods** A total of 20 livers grafts from extended criteria donors, which were shipped to the implantation clinic by conventional cold storage, were subjected to 90 min of *ex vivo* machine perfusion after arrival. At the end of liver perfusion, perfusate concentrations of TIMP2 were measured and related to the occurrence of early allograft dysfunction after transplantation.

**Results** ROC analysis disclosed a significant ($p = 0.0046$; CI 0.9–0.1) predictive potential of TIMP2 with a sensitivity to predict EAD of 1.0 (CI 0.51–1.0) and a specificity of 0.94 (CI 0.72–1.0) at a cut-off value of 2106 pg/ml.

**Conclusions** Determination of TIMP2, which is possible to be performed as point-of-care measure, may thus become a useful adjunct on the way to better evaluate extended criteria donor livers.

**Keywords** Graft evaluation, Machine perfusion, TIMP2

## Background

The demand for donor organs suitable for transplantation largely exceeds the available number fulfilling current standard donor criteria [1]. Therefore, there is a persisting high medical need to utilize more already available organs, i.e., those with extended donor criteria.

However, those livers may be inflicted with an increased risk of primary nonfunction or early allograft dysfunction (EAD) after ischemic preservation [2]. Thus, pushing the limits of acceptance criteria for donor organs requires tools to support the surgeon with appropriate estimation of the quality of organ grafts.

An increasingly accepted method to better maintain organ viability is provided by oxygenated machine perfusion for ex vivo organ conditioning.

In addition, information on the functional integrity of the organ might be obtained during organ perfusion [3]. For example, the behavior of vascular flow resistance or the determination of perfusate parameters could provide valuable information about graft viability.

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The establishment of further parameters, instantly available during liver perfusion, and defining graft viability still represent an area under investigation of utmost importance [4].

Tissue inhibitor of metalloproteinases 2 (TIMP2) is a novel biomarker, which had recently been shown a sensitive readout of epithelial damage in acute kidney injury. Early upregulated during epithelial cell stress, TIMP2 is operative in preventing cells with a damaged DNA to perform mitosis by inducing G1 cell cycle arrest [5].

We hence sought to evaluate, if machine perfusate concentrations of TIMP2 could be used to predict early allograft dysfunction after transplantation and thus help to decide upon transplantability of extended criteria donor livers.

## Patients and methods

Machine perfusate samples and other conventional parameters were analyzed as part of a randomized-controlled trial on the benefits of a short-term, mid-thermic machine perfusion prior to liver transplantation in clinical routine (ISRCTN 94691167) [6].

### Study approval

The CORNET trial (ISRCTN 94691167) was approved by the ethics committee of the University Duisburg-Essen. All participants provided written informed consent.

### Experimental design

Data from the CORNET trial (ISRCTN 94691167), a single-center prospective randomized-controlled trial that included 40 patients who received a liver graft from extended criteria donors, were analyzed for this study. Details of trial design, inclusion and exclusion criteria participants, treatment, and follow-up have been published [6].

Extended criteria donor livers [7] fulfilling one of the following criteria: donor age > 65 years, intensive therapy including assisted ventilation > 7 days, obesity of donor (BMI > 30), serum sodium > 165 Mol/l, AST or ALT > 3 × of normal, serum-bilirubin > 3 mg/dl, or histologically proven liver steatosis > 40% were included in the study.

All patients listed for a liver transplant at the University Hospital Essen with a minimum age of 18 years and residency in Germany were eligible to participate in the study. Written informed consent had been obtained from all participants.

The impact of 90 min of *ex vivo* machine perfusion immediately prior to transplantation with gentle and protracted elevation of the perfusate temperature from 10° to 20 °C as adjunct to simple cold storage was investigated and compared to the standard procedure without machine perfusion. The trial was enrolled between April 2019 and April 2021 at the University Hospital of Essen, Germany.

Here, we focus on the 20 livers in the machine perfusion arm of the trial aiming to delineate the prognostic value of perfusate concentrations of tissue inhibitor of metalloproteinases 2 (TIMP2) during machine perfusion for the prediction of early allograft dysfunction (EAD) after liver transplantation. EAD was defined according to Olthoff et al. as: bilirubin ≥ 10 mg/dL on postoperative day seven and/or INR ≥ 1.6 on postoperative day seven and/or AST or ALT > 2000 IU/L within the first seven days [8]. Each case was classified as "EAD" or "no-EAD".

### Biomarker measurement

Perfusate samples were drawn after 90 min of rewarming machine perfusion, when steady-state conditions at 20 °C were obtained. Samples for the detection of tissue inhibitor of metalloproteinase-2 (TIMP-2) were stored at − 80 °C and analyzed after conclusion of the study using a commercial ELISA kit (MyBioSource, Brussels, Belgium) according to the instructions of the manufacturer.

All samples were analyzed without any retrospective sample selection.

Lactate was measured on site using a pH-blood gas analyzer (ABL 800flex acid–base laboratory, Radiometer, Copenhagen). Aspartate aminotransferase (AST) was assessed on a biochemical immunoassay analyzer (RC3X, Viernheim, Germany).

### Statistics

Sample size was calculated based on the hypothesis, that the determination of TIMP2 in the machine perfusate would allow to predict the occurrence of early allograft dysfunction after liver transplantation should be tested by establishing a respective receiver-operating curves (ROC). Thereby, an area under the curve of more than 0.9 should be considered as a significant benefit for future use and further investigation of the parameter. The rate of type 2 error has been defined as $\beta = 0.2$. The level of significance for the type 1 error is set to $p < 0.05$. From these assumptions, it follows that the detection of a discriminative significance compared to the null hypothesis (area under the ROC curve = 0.5) requires a sample size of $n = 20$ (MedCalc® Statistical Software version 20.009 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021).

Values are depicted as median ± 95% confidence interval (CI). Differences between groups were tested by non-parametric comparison using the Mann–Whitney test. Statistical significance was set at $p < 0.05$. Data for receiver-operator characteristics curves were calculated

by the method of Wilson/Brown using Prism 9.01 (GraphPad software Inc., San Diego, CA, USA).

## Results and discussion

Our data showed that the concentrations of TIMP2, accumulated during pre-transplant machine perfusion, turned out to be very predictive of later EAD as defined by Olthoff [8] (cf. Figure 1).

ROC analysis disclosed an area under the ROC (AUR) of 0.97 and a level of significance given by $p=0.0046$, thus very much approaching the characteristics of a reliable predictive test. Using a cut-off value of 2106 pg/ml, the concentration of TIMP2 in the machine perfusate showed a sensitivity to predict EAD of 1.0 at a specificity of 0.94 (95% confidence interval: 0.72–1.0).

Other parameters, analyzed in parallel and usually considered helpful in evaluating pre-transplant organ graft integrity, fell short of producing comparable results (Fig. 2).

Median values of lactic acid accumulation during machine perfusion did not differ significantly between livers, that resumed function after transplantation (2.65 mmol/l; CI 2.157–3.18) or that developed EAD (3.75 mmol/l; CI 1.274–6.776; $p=0.1544$).

Likewise, machine perfusate activities of aspartate aminotransferase (non EAD: 915 (753–1714) U/l vs EAD: 1547 U/l (237–2794); $p=0.4279$) as well as total vascular flow during machine perfusion (non EAD: 457 (368–588) ml/min vs EAD: 344 (135–601) ml/min; $p=4372$) were conflicted by a significant overlap of the individual levels between the two collectives and did not allow for predicting early allograft dysfunction after transplantation.

EAD has been chosen as primary estimate for liver graft recovery as it represents one of the best validated, clinically relevant assessment tools early after transplantation [9].

However, even with 1-year graft survival as prognostic goal, TIMP2 values were found to result in an area under the ROC curve of 0.89 (Fig. 3), although the data did not quite reach significance ($p=0.078$), due to the low number of negative cases.

According to local regulations, all donor livers were revisited by an experienced transplant surgeon upon arrival at the hospital and only those grafts primarily accepted for transplantation were included in the study. Up to 20% of all organ offers were declined prior to be included in the trail. Obviously, poor liver grafts were thus not included in our investigation. This might explain the relatively little differences observed in the

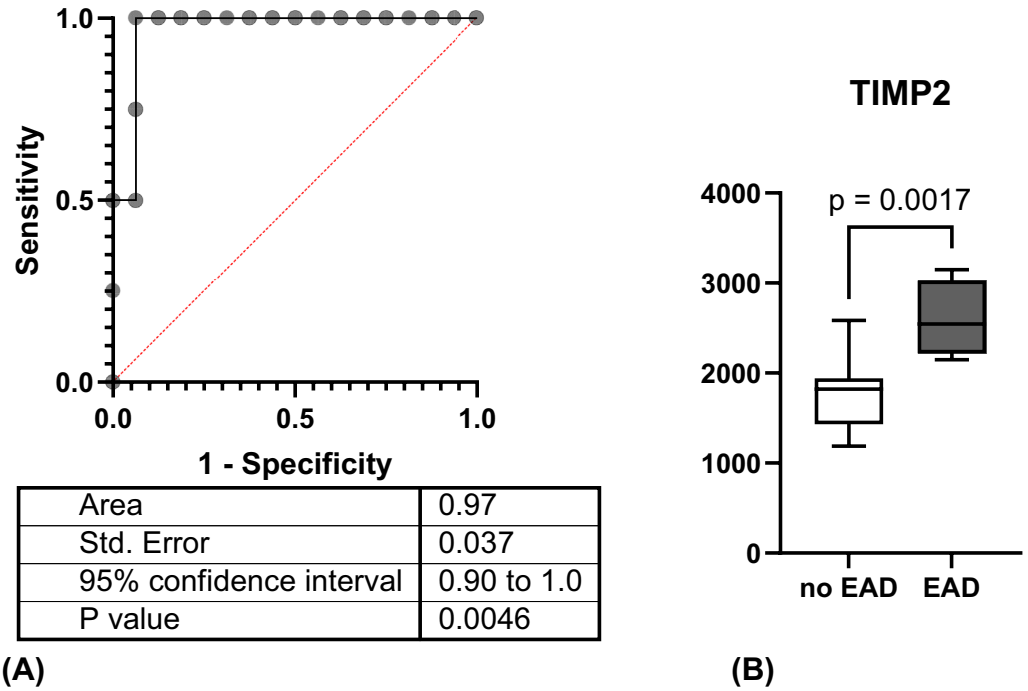
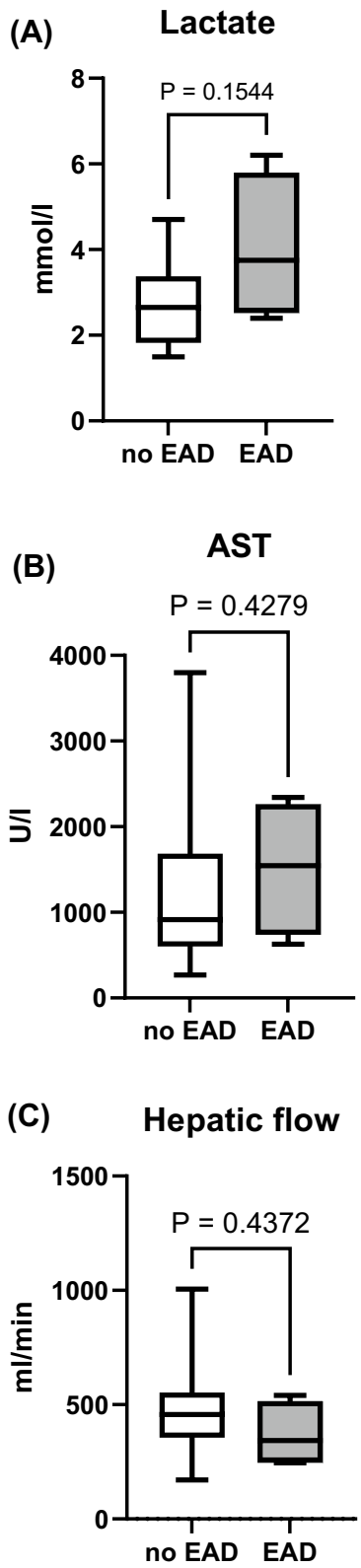



**Fig. 1** Relation between TIMP concentration during machine perfusion and early allograft dysfunction (EAD) after liver transplantation. **a** Receiver-operating characteristics curve illustrating the accuracy for TIMP2 perfusate concentration during pre-transplant ex vivo machine perfusion to allow for discrimination of livers that later underwent early allograft dysfunction after transplantation. Calculated using Prism 9.01 (GraphPad software Inc., San Diego, CA, USA). **b** Box plot (showing median, minimal and maximal values) of TIMP2 levels during machine perfusion of livers that developed or did not develop early allograft dysfunction after transplantation (Mann–Whitney *U* test)



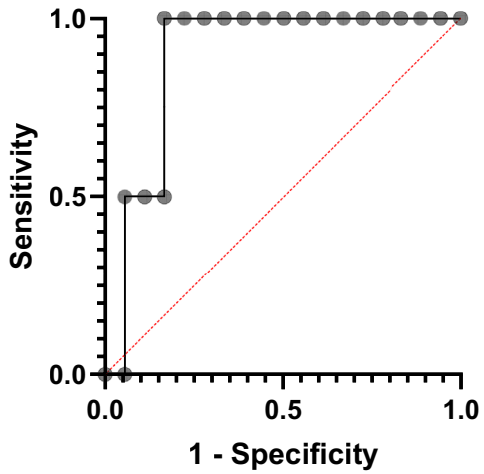

◀ **Fig. 2** Influence of graft quality on functional markers upon machine perfusion. Box-plots showing median, minimal and maximal values of Lactic acid (**A**), aspartate aminotransferase-AST (**B**), and total hepatic flow (**C**) during machine perfusion of livers that developed or did not develop early allograft dysfunction after transplantation (Mann–Whitney *U* test)

conventional parameters during machine perfusion, because most negative outliners were excluded from machine perfusion analysis. On the other hand, this might happen to stress the discriminative value of TIMP2 as a biomarker even in a collective that is positively preselected by experienced transplant surgeons.

A limitation of our study must be seen in the relatively small number of patients included. In contrast to a most recently published large validation trial on another biomarker of graft loss, the release of flavin mononucleotide (FMN) upon hypothermic machine perfusion, disclosing an area under the ROC curve of 0.77 [10], our investigation only constitutes a first pilot trial, evaluating the potential of a new marker. To this purpose, TIMP2 was only measured once at the end of a brief perfusion period to evaluate liver integrity after cold storage.

In the future, serial measurements seem to be warranted to appreciate interventional success also after extended perfusions for therapeutic purposes. Being a stress responsive, actively upregulated parameter, TIMP2 might be particularly suitable to follow up graft regeneration.

If allograft valuation by machine perfusion will be implemented into the decision-making process, measurement of TIMP2, which is possible to be performed as point-of-care measure (e.g., Simple Plex, human TIMP-2 Cartridge, ProteinSimple-www.bio-techne.com), might become a useful adjunct on the way to better evaluate extended criteria donor livers.




| | |
|---|---|
| Area | 0.89 |
| Std. Error | 0.079 |
| 95% confidence interval | 0.73 to 1.0 |
| P value | 0.0778 |

**Fig. 3** Relation between TIMP concentration during machine perfusion and 1-year graft survival after liver transplantation. Receiver-operating characteristics curve illustrating the accuracy for TIMP2 perfusate concentration during pre-transplant ex vivo machine perfusion to allow for prediction of 1-year graft survival after transplantation. Calculated using Prism 9.01 by the method of Wilson/Brown. (GraphPad software Inc., San Diego, CA, USA)

**Abbreviations**

| | |
|---|---|
| AUC | Area under the curve |
| AST | Aspartate aminotransferase |
| EAD | Early allograft dysfunction |
| ECD | Extended criteria donor |
| FMN | Flavin mononucleotide |
| ROC | Receiver-operating characteristics |
| TIMP-2 | Tissue inhibitor of metalloproteinases 2 |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40001-025-03410-9.

Supplementary material 1

**Author contributions**

CvH and TM conceptualized and designed this study, acquired data and wrote and revised the manuscript. HZ contributed to the investigation, acquired data and revised the final manuscript. LM acquired and analyzed data and revised the manuscript.

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**Data availability**

The datasets supporting the conclusions of this article are included within the article and its additional files.

## Declarations

**Ethics approval and consent to participate**

The study was approved by the ethics committee of the University Duisburg-Essen. All participants provided written informed consent.

**Consent for publication**

All authors approved the final version of the manuscript and concur with the submission.

**Competing interests**

The authors declare no competing interests.

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