**Thesis**

# Novel strategies for non-invasive monitoring of liver function during machine perfusion


submitted by

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*Declaration of Academic Integrity*

*I hereby confirm that the present diploma thesis is the result of my own independent scholarly work. I also confirm that in all cases, where material from the work of others (in books, articles, essays, dissertations, and on the internet) is acknowledged, quotations and paraphrases are clearly indicated. No material other than that cited in the reference list has been used. I have read and understood the Medical University's regulations and procedures concerning plagiarism.*

*Graz, 12.09.2024* *Julian Kühnl m.p.*

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## Abstract in German

In Österreich wurden im Jahr 2020 insgesamt 672 Organe transplantiert. Mit 158 Lebertransplantationen sind Lebern die zweit häufigsten transplantierten Organe in Österreich. Steigender Bedarf und verbesserte Transportstrategien zielen immer mehr auch auf „extended criteria donor“ Transplantate ab und schaffen es, diese Organe erfolgreich vorzubereiten.

Erst kürzlich hat ein Züricher Forschungsteam eine initial nicht transplantierbare Leber 3 Tage lang mit einer Maschine perfundiert und nach Besserung der Organqualität die Leber erfolgreich transplantiert.(Clavien et al., 2022)

Diese und viele weitere Publikationen zeigen die Richtung, in welche sich der Forschungsbereich bewegt. Auch diese Arbeit möchte Ihren Beitrag dazu leisten und stellt zwei neue Methoden vor, die eine nicht-invasive Monitorisierung von Lebertransplantaten während Maschinenperfusion ermöglichen wollen. Die Tests laufen aufgrund von Ressourcenmanagement und Verfügbarkeit mit Organen von gesunden Minischweinen direkt am Campus der Medizinischen Universität Graz ab.

Die bewährte Methode der LiMAx® Syntheseleistung findet bereits seit Jahren in der klinischen Überprüfung der Leber von Patient*innen statt. Dabei stellt das verabreichte Methacetin eine gute Möglichkeit dar, Rückschlüsse auf die Metabolisierungsleistung des Organs zu machen, da diese Substanz spezifisch in der Leber vom Cytochromenzym 1A2 verarbeitet wird. Mit diesem Testsetup wurden insgesamt drei Lebern überprüft und es konnte gezeigt werden, dass Methacetin signifikant abgebaut werden kann.

Das andere System arbeitet über ein Absorptionsspektrum des Indocyaningrüns (ICG), welches mit einem Laser angeregt und in Echtzeit mit einer hochauflösenden Infrarotkamera ausgewertet werden kann. Aufgrund der Verteilung der Perfusionsflüssigkeit und des darin gemischten ICGs im Organ, kann die Absorption direkte Rückschlüsse auf die Perfusion an individueller Stelle geben. Hier wurden insgesamt vier Leberorgane verwendet, um Zusammenhänge zu erforschen und eine Möglichkeit zu finden, dieses System im klinischen Alltag einzusetzen.

Obwohl die Größe der Stichprobenanzahl sehr gering ist, und statistische Aussagen dadurch erschwert sind, konnten vielversprechende Ergebnisse gezeigt werden.

Eine größere Follow-up Studie mit geschädigten Leberorganen könnte noch bessere Vergleichbarkeit liefern und stellt den nächsten Schritt dar.

## Abstract in English

In Austria a total of 672 organs were transplanted in 2020. 158 liver transplants were carried out making livers the second most transplanted organs in Austria.
Recently, a Zurich research team perfused an initially non-transplantable liver with a machine for three days and successfully transplanted the liver after organ quality improved.(Clavien et al., 2022) These and many other publications indicate the direction in which this field of research is moving. This work would like to make its contribution and present two new methods which aim to make a non-invasive monitorization of liver transplants possible during machine perfusion. Due to resource management and availability, the tests are running with organs from healthy minipig directly at the Medical University of Graz.

LiMAx® is a proved method for synthesis analysis and has already been used for years in clinical testing of liver performance in patients. The administered methacetin represents a good opportunity to draw conclusions on the metabolization performance of the organ, since this substance is specifically metabolized in the liver by the cytochrome enzyme 1A2. A total of three livers were tested with this test setup, and it was shown that methacetin can be significantly reduced, leading to good metabolization function of the test livers.
The other system uses an absorption spectrum of the indocyanine green (ICG), which is stimulated with a laser and can be evaluated in real time with a high-resolution infrared camera. Based on distribution of perfusion fluid and ICG in the organ, the absorption gives direct conclusions about the perfusion for an individual tissue spot or the whole organ. A total of four liver organs were used to investigate correlations and to find ways to use this system in clinical practice.

Although the sample size is very small, making statistical conclusions difficult, promising results could be shown. A larger follow-up study with healthy livers as control and damaged liver organs as test group could provide even better comparability and is the next step.

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# Abbreviations and Definitions

ALT ..... alanin-aminotransferase
AST ..... aspartat-aminotransferase
ATP ..... adenosine triphosphate
BE ..... base excess
$CO_2$ ..... carbon dioxide
CSS ..... cold static storage
HCO3- ..... hydrogen carbonate
HMP ..... hypothermic machine perfusion
ICG ..... indocyanine green
IR ..... InfraRed
LDH ..... lactate dehydrogenase
LTX ..... liver transplantation
MELD ..... Model for End-stage Liver Disease
miRNA ..... micro ribonucleic acid
NMP ..... normothermic machine perfusion
$O_2$ ..... oxygen dioxide
PAS ..... periodic acid Schiff
pCO2 ..... carbon dioxide partial pressure
pH ..... potential of hydrogen
pO2 ..... oxygen partial pressure
RNA ..... ribonucleic acid
SNMP ..... subnormothermic machine perfusion

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# 1 Introduction

In 1967 Thomas Starzl performed the first successful liver transplantation (LTX).(Fricker, 2017) From this point on, surgical techniques improved and immunosuppressive regimens evolved, and todayorgan transplantation is a routine procedure. The process consists of different stages: finding the right donor-receiver match, preparations, explantation of the donor organs, transport, qualifying organ qualitybefore implantation, the surgical procedure and post-operative care(Clavien et al., 2022),.

After explantation the graft usually needs to be transported to the recipient, with the duration depending on geographical location and method of transport.(Sibulesky et al., 2016)

The aim during transport is to keep the organ viable and to slow metabolism, inflammation and destabilizing processes down, this is depending on time and on the transportation method. When cooling down the transplant with cold static storage CSS, these damaging processes can be slowed down significantly. (Sibulesky et al., 2016)

In recent years, machine perfusion gained interest and showed better outcome for extended criteria donors than static cold storage during transportation time. (Jia et al., 2020) Many different machine perfusion methods have been developed, such as hypothermic machine perfusion (HMP), normothermic machine perfusion (NMP) and subnormothermic machine perfusion (SNMP). These new methods all helped with maintaining transplant health and will be explained further in the next chapter.
Before implantation there needs to be verification of organ health, which is a key factor in a successful transplantation.(Resch et al., 2020, Watson et al., 2018) There are many methods to examine the health of an organ, but only fast and highly precise methods can be used. For liver organ health verification there are, in Europe, parameters like: pH, bile bicarbonate, bile pH, histology, ultrasound, perfusate lactate clearance, transaminases, vascular resistance and glucose levels.(Watson et al., 2018, Mergental et al., 2016)

This comprehensive review endeavors to find state-of-the-art liver transplant health indicators and compare those to each other. The following chapter will give an overview of existing methods and parameters used during the transplantation process.

## *1.1 Transplant storage/transportation methods*

After explantation of a viable transplant the physiological circulation stops, and organs degrade due to enzymes, infections and missing protection. Transportation methods endeavour to hold against this degradation process and keep the organ in a good physiological condition for implantation.

Cold static storage (CSS) was one of the first methods to be used and still shows great results. Especially with high quality transplants, this simple and easy method shows its advantage over the complex and expensive machine perfusion. (Jiang et al., 2018) Machine perfusion delivers oxygen, nutrition and near physiological conditions to the organs, which results in better outcome with extended criteria donors.(Tchilikidi, 2019)

### 1.1.1 Cold Static Storage

CSS or hypothermic cooling of transplants aims to reduce the cellular metabolization rate and oxygen demand with longer preservation time of organs. (Eghtesad et al., 2006) Organs are placed in bags filled with special solutions, rich in nutrients and biochemic substances such as Wisconsin solution, Histidine-tryptophane-ketoglutarate (Custodiol®) solution, Celsior®, Institute Georges Lopez solution and Tissue and Organ conservation solution surrounded with ice for cooling.(Tchilikidi, 2019)

### 1.1.2 Machine perfusion

Machine perfusion allows the organ to be in a circuit of arterial and venous vessel flow, with a machine pumping blood or a special perfusion liquid through the organ. This enables ex vivo tissue perfusion and helps to avoid organ damage due to mimicking physiological conditions.(Panconesi et al., 2021) Flow per minute, flow pressure and temperature can be set for different strategies, allowing adjustable machine perfusion for each transplant's needs. Perfusion can be set pulsatile or continuous and for hepatic artery alone (single perfusion) or also for portal vein (dual). Perfusion machines log resistance parameters during perfusion, which allow an indirect idea of the vessel situation of an organ. Machine perfusion liquids aim to further slow degradation down and help to improve the organ health, but this has only been proved in small animal studies or simulated in vitro reperfusion thus the superior clinical evidence over CSS remains to be proven. (Jiang et al., 2018)

NMP provides dynamic organ transportation at around 37°C to mimic physiological temperature conditions. Comparison of NMP against CSS showed that, NMP has a significantly prolonged preservation time with less ischemic bile duct lesions.(Fodor et al., 2021) Normothermia shows the setup for a physiological environment, enabling the liver to better establish homeostasis and recovery processes. (Xu et al., 2012) In most NMP studies Gelofusine, a gelatin-based solution, and Steen solution have been used. (Nasralla et al., 2018, Selzner et al., 2016)

The temperature at HMP is set at 4°C. This temperature level decreases the aerobic metabolic needs and allows a reduction in cellular insults during reperfusion.(Bejaoui et al., 2015) Though promising results with extended criteria donors such as organs from patients after brain death, further investigation is needed in proving the superiority over CSS. (Tchilikidi, 2019) Belzer, one of the authors of the University of Wisconsin solution established machine perfusion in 1960. This first machine perfusion used the Belzer-MP® solution, a slightly changed formula from the standard Wisconsin solution that switched lactobionate to gluconate. (Bejaoui et al., 2015) Polysol proved better preservation of liver grafts than Belzer-MP®, which concluded to more bile production and lower enzyme release. (Bessems et al., 2005) A newer version of the Belzer-MP® solution is the

Vasosol with promising antioxidant results, due to vasodilators, metabolic substances and antioxidants.(Bae et al., 2014)

The latest strategy for machine perfusion aims to combine the functional metabolism at NMP and reduced metabolism rate at HMP. This in-between strategy is called SNMP and the temperature is set for 20-25°C and different perfusion solutions can be used, such as University of Wisconsin solution. (Bruinsma et al., 2014) Another promising study by Shonaka et al. showed that a human-derived haemoglobin-based oxygen vesicles (HbV) perfusate gave promising results with SNMP, with increased oxygen consumption of the liver graft. (Shonaka et al., 2018) The team around Leber et al. used Custodiol® and Belzer MPS® perfusion liquids for non-allocable human liver grafts, which showed promising results.(Leber et al., 2022)

### 1.1.3 Cold static storage vs machine perfusion

SNMP shows an improvement in various parameters important for transplants. In a study by Bruinsma comparing CSS to SNMP, vascular resistance and ATP increased significantly over the control group and showed that SNMP prolonged the transplant viability positively.(Bruinsma et al., 2013)
Another advantage of machine perfusion over the CSS is the ability to extract metabolic and hemodynamic parameters during the perfusion which may predict the viability of transplants in the future.(Black et al., 2014) Important parameters for such a prediction might be pH stability, bicarbonate corrections, lactate clearance, bile production, portal vein and hepatic artery flow and pressure.(Banan et al., 2016)

## *1.2 Overview of methods to monitor liver viability*

Rising demand in transplants is pushing the possibility of preserving these valuable organs better and thus estimating their viability to the best standards.(Laing et al., 2016)
Normothermic machine perfusion makes a viability check for the organ at nearly in vivo conditions possible, thereby stimulating the organ to normal body temperature,

metabolization and perfusion.(Watson et al., 2018) This allows to check for health parameters at nearly physiological conditions, enabling a better overview of the organ's function than compared to the cold ischemia storage method.(Mergental et al., 2016)

Although the importance of an objective transplant viability check before implantation is clear, there are still no worldwide agreed parameters allowing that.(Cardini et al., 2020) This makes it difficult to identify true liver health and is often ultimately decided by the gut feeling by an experienced doctor.(Muller et al., 2019)

Finding such objective parameters would allow for a reliable liver health assessment without damaging the liver and has been the aim of many studies for years. In the following section, some of the important parameters of the latest research will be introduced.

### 1.2.1 Perfusion liquid pH and gas analysis

pH, the short form of potential of Hydrogenii from Latin, is defined as the negative decadic logarithm of protons or hydrogen ions in a liquid.

For machine perfusion, there are many different perfusion liquids available, depending on the perfusion strategy, research team and liver status. pH levels can differentiate and show varying behaviour, due to whichever perfusion liquid being used during perfusion. Many research teams try to hold the often-acidic livers, a result of ischemia time and liver injury, at a nearly physiological pH range of 7.3 to 7.45, which can be achieved with bicarbonate administration.(Mergental et al., 2016, de Vries et al., 2019, Zhang et al., 2020, Matton et al., 2019)

A blood gas analysing device can determine pH and electrolytes, which allows for easy and fast results. The perfusion liquid sample can be taken during machine perfusion and the results usually contain pH, pCO2, pO2, BE, HCO3-, glucose and lactate.

The research team of R. Panconesi et al. suggested that liver viability should only be checked with multiple parameters, like bicarbonate depending on urea production, acid balance and other cellular parameters.(Panconesi et al., 2021)

### 1.2.2 Bile production and bile parameters

Bile is produced in the liver during metabolization processes. This liquid, consisting mostly of water, bile salts, bilirubin, electrolytes, and lipids, enables the liver to get rid of harmful substances like toxins, and at the same time helps the human body to better assimilate food, especially fat. (Behrends et al., 2016)

In physiological conditions the pH of the common bile stays in a range of 7.50 -8.05 pH, being higher than when the bile liquid is stored in the gall bladder.

During machine perfusion, the transplant is connected to three tubes. One for the hepatic artery, one for the portal vein, both pumping liquid in the liver and one at the exit of the common bile duct. The cannulated bile duct makes gathering and analysing bile liquid possible. The research team of A. Matton et al. points out that storing the liquid in mineral oil is very important, otherwise exchange of $CO_2$ with the air would be possible and this would change the pH and bicarbonate parameters.(Matton et al., 2019)

The study in May 2020, “Transplanting Marginal Organs in the Era of Modern Machine Perfusion and Advanced Organ Monitoring” from Resch et al. shows different methods for monitoring during machine perfusion. In one section, the focus was placed on bile and bile parameters. (Resch et al., 2020) The research team was able to show that a biliary bicarbonate concentration higher than 18mmol/L is connected to a histological bile duct injury. (Matton et al., 2019)

Another research team could identify some other parameters for good biliary health. The parameters include biliary pH higher than 7.48, biliary glucose less than 16mmo/L, bile/perfusate glucose concentration ratio less than 0.67 and biliary lactate dehydrogenase (LDH) concentration less than 3,689 U/L. (Watson et al., 2018, Resch et al., 2020)

The paper of Cardini et al. “Clinical Implementation of Prolonged Liver Preservation and Monitoring Through Normothermic Machine Perfusion in Liver Transplantation” describes bile pH as a representative value for bile duct viability and function. (Cardini et al., 2020)

Bile analysis shows an easy and fast method to check the transplant’s health. With the information, that bile breaks down metabolites and cleans the body of toxins, having bile liquid within the physiological limits of the transplant, can be representative for a good transplant. (Behrends et al., 2016)

### 1.2.3 Histology

Histological assessment remains the gold standard for assessing liver viability but being very time-consuming(1-2h) per probe and tissue damaging when taking the biopsy, it requires an experienced pathologist. The biopsy is usually placed in 10% formalin coloured with standard procedure like haematoxylin and eosin or periodic acid Schiff (PAS) stain for better evaluation and sometimes also with antibodies, e.g. the von Willebrand factor.(Eshmuminov et al., 2020)

#### 1.2.3.1 Suzuki Criteria

A known evaluation method for a preserved tissues graft is the Suzuki Criteria, depending on different levels of Congestion, Vacuolization and Necrosis.(Ge et al., 2017, Zhang et al., 2020) The tissue samples are fixed in formalin, stained with haematoxylin and eosin and evaluated by an experienced pathologist.(Suzuki et al., 1991)

| Grade | Congestion | Vacuolization | Necrosis |
|---|---|---|---|
| 0 | None | None | None |
| 1 | Minimal(10%) | Minimal(10%) | Single-cell necrosis |
| 2 | Mild(<30%) | Mild(<30%) | Mild(<30%) |
| 3 | Moderate (30-60%) | Moderate (30-60%) | Moderate (30-60%) |
| 4 | Severe(>60%) | Severe(>60%) | Severe(>60%) |

Figure 1: The Suzuki histological Criteria (Suzuki et al., 1991)

### 1.2.4 Macroscopy

Macroscopic alternations are results of bigger damage in organs and therefore are exclusion criteria for transplantation. Macroscopic pathologies include small or large droplet macro vascular steatosis resulting in steatohepatitis, hepatocyte necrosis and glycogen accumulation.(Mergental et al., 2016) Those macroscopic findings

often result in needed transplantation therefore those grafts can't be used as donation organs.(Behrends et al., 2016)

### 1.2.5 Ultrasound

Ultrasound uses high sound wave frequencies and these reflected soundwaves, when hitting different refraction in tissue and liquid transition, generate pictures. These pictures enable safe, fast and non-invasive analysis of liver and helps to recognize liver steatosis.(Schmidt and Becker, 2004) In some countries this is an authorized method for validating donor livers. During the explantation process also the visible quality of the liver can be assessed, which refers to the shape, size, colour and main vessel structure of an organ.(Panconesi et al., 2021, Resch et al., 2020)

### 1.2.6 Lactate clearance

Lactate is the end product of anaerobic glycolysis and is mainly processed in the liver.(Behrends et al., 2016) Liver injury can be associated with slower lactate metabolization and therefore accumulation.(Cardini et al., 2020) Lactate is produced from pyruvate by lactate dehydrogenase when tissue hypoxia occurs. (Behrends et al., 2016)

Mergental et al. show in their studies that lactate clearance and bile production with consistent flow rates of hepatic artery and portal vein are sensitive parameters for liver transplant viability check. They used lactate levels under 3mmol/L after 3h as a time stamp and concluded that it could be used as a viability parameter for transplantation. (Mergental et al., 2016)

The research team of D. Nasralla et al. described the lactate clearance as a useful parameter for organ quality during normothermic machine perfusion. In their study, the organs usually cleared 9.99 ± 3.13 mmol/L at 15 min and 0.93 ± 0.63 mmol/L at 4 hours. The ability to reduce lactate over time is seen as a positive prediction marker, but is usually combined with other parameters like pH, hepatic artery flow and bile production. (Nasralla et al., 2018)

The study team of R. Panconesi et al. examined publications regarding the predictive value of lactate for liver transplant health. They concluded that lactate clearance usually follows phases during normothermic machine perfusion, due to

variations in liver metabolism. Recent findings suggest, that consistent high levels of lactate are connected to pan lobular injury and liver dysfunction. Initial phase shows rapid clearance and maintenance phase with slower clearance of about 2 to 4 mmol/L lactate remaining.(Panconesi et al., 2021)

### 1.2.7 Transaminases

Aminotransferase, or transaminase are enzymes that catalyse the exchange of amino groups to amino acids and alpha-cetoacids. Aspartat-aminotransferase (AST) and alanin-aminotransferase (ALT) are the main enzymes and have high intracellular liver concentrations. The De-Ritis-Quotient, a clinically used quotient of AST/ALT, can usually indicate a low or high liver injury.(Behrends et al., 2016)
Liver transaminases are released during cell damage and can be measured from blood or during machine perfusion from the perfusion liquid. This would enable easy and non-invasive sampling, thus the problem remains, that the analysis of AST and ALT takes time. High values of AST are often seen as non-viable and therefore labelled as non-transplantable.(Cardini et al., 2020)
Studies couldn't prove a predictive value in transaminase levels for liver resection or transplantation alone, but maybe a combination of weight normalized data with other parameters could show more significance.(Panconesi et al., 2021)

### 1.2.8 Perfusate glucose level

During ischemia when anaerobe metabolization occurs, cells tend to have higher glycogenolysis which results in rising glucose levels. This hyperglycaemia can be measured during machine perfusion and can be counter measured with insulin dosage, which enables viable livers to metabolize glucose.(Panconesi et al., 2021)
The glucose levels can be measured with the blood gas analysing devise, making fast, multiple and easy monitoring possible.
The research team around A. P. M. Matton et al. suggested that a bile to perfusate glucose ratio of less than 0.7 is predictive as a liver viability cut off point. A significant correlation with glucose clearance in bile and cholangiocyte viability was noted.(Matton et al., 2019, Watson et al., 2018)

A research team in Zurich therefore suggests responsive tests, such as the monitoring of glucose levels after insulin administration. These tests could provide better information of the viability status of the liver and could even be combined with other parameters.(Dutkowski et al., 2020, Eshmuminov et al., 2020) Fibroblast Growth Factor 21 FGF21 has a similar effect as insulin regulates the glucose uptake in adipocytes.(Behrends et al., 2016) An elevation 21 times over the normal levels of FGF21, resulted in a scale of Ischemic Reperfusion Injury IRI of liver graft transplants. (Bhogal et al., 2020)

### 1.2.9 Haemodynamic parameters during machine perfusion

Machine perfusion enables the possibility of screening organs continuously and without damaging the tissue. Modern perfusion machines have inbuilt flow and pressure sensors to get resistance parameters. Vascular resistance is dependent on perfusion flow and pressure. (Monbaliu et al., 2012)

Some research teams have shown that high vascular resistance during machine perfusion over time is associated with poorer graft function and can be predictive for graft failure within one year. It should be noted that above-mentioned conclusions derived from kidney studies.(Resch et al., 2020, Impedovo et al., 2012, Nyberg et al., 2005)
Reduced perfusion flow can also be expected in damaged liver grafts, because macro vascular liver steatosis leads to narrower sinusoids and therefore reduced perfusion flow. (Behrends et al., 2016, Fukumori et al., 1997) Ongoing organ dysfunction can be described by reduced flow, which leads to tissue hypoxia and furthermore to ischemic reperfusion injury, cytokine release and inflammation.(Watson et al., 2018)

A research group, which worked with porcine livers in 2012, stated that a drop in arterial flow is connected to graft degradation. They had different warm ischemia time (0, 30, 60 minutes) groups to vary the viable liver health and a significant correlation with the pressure drop rate (pa/p0) could be shown. The team suggested not to only use flow parameters as an indicator of liver health during machine perfusion. However, the team found a link between the minimum normalized

pressure and lactate dehydrogenase LDH level. Showing a connection between low pressure and low LDH levels with WIT0 and high LDH levels with the WIT60 livers. (Obara et al., 2012, Resch et al., 2020)

As many other parameters for assessing liver health during machine perfusion, they heavily depend on their perfusion strategy (NMP, HMP, SNMP). More than 500mL/min for portal vein and >150mL/min for hepatic artery have been set by some studies as good viability criteria.(Mergental et al., 2020, Ravikumar et al., 2016, Zhang et al., 2020)
High resistance levels and obstructed flow can be linked to advanced histologically confirmed liver injuries. This states that perfusion parameters can be linked to late onset liver complications, and not ideally suited for early viability prediction.(de Vries et al., 2019, Laing et al., 2016, Watson et al., 2018)

## *1.3 Conclusion of ex vivo liver health parameters*

The liver health parameters, described above and summarized in Figure 2 are considered as standard for assessing health of liver transplants before implantation. Every parameter alone stands for a specific functionality of the organ, though only the combination of these parameters makes an evaluated decision reasonable. For assessing some parameters, organ tissue has been damaged, with others enabling an objective non-invasive overview. Often these parameters must undergo a time-consuming lab process for gold standard evaluation.

| **Detection Method** | **Material** | **Parameters in Clinical Use** | **Time Needed for Assessment** | **Advantages** | **Disadvantages** |
|---|---|---|---|---|---|
| Macroscopic assessment | Entire liver | Size, perfusion quality, steatosis, fibrosis, vessel | Minutes | Routine, rapid, non-invasive, cheap | No information on function, imprecise, assessor dependent |

| | | | | | |
|---|---|---|---|---|---|
| | | quality, injuries | | | |
| Ultrasound | Entire liver | Size, level of steatosis, lesions | 30 min | Easy, assessment of liver parenchyma, rapid, cheap | Assessor dependent, no information on function |
| Histology | Liver tissue | Level of macro and microsteatosis, fibrosis, inflammation | 1–2 h | Histological evidence of quality provides criteria to exclude organ transplantation (e.g., Fibrosis) | Invasive, variability in interpretation, biopsy covers only small part of organ, no information on function |
| Heamodyn-amics during Perfusion | Entire Liver | HA & PV perfusion flow (pressure) | continuous | Real-Time | Not specific for cell type |
| Blood gas analysis | Perfusate, Effluate, Bile | pO2, pCO2, Lactate, Na, K, pH, Glucose | 5–15 min | Non-invasive, any type of perfusion, multiple parameters, indirect cholangiocyte assessment, different time points | Timing, different parameters, not specific for a certain cell type |
| Biochemical analysis | Perfusate, Effluate, Bile | AST, ALT, LDH, HCO3-, ALP | 5–15 min | Non-invasive, indirect cholangiocyte assessment, different time points | Not specific for a certain cell type, no functional assessment, no reliable prediction of outcomes after transplantation |
| Metabolomics/proteomics/genomics | Liver tisue, perfusate, effluate, bile | Various molecules from all cellular and sub-cellular compounds | Days/Weeks | Multiple parameters, can be performed in any material (tissue, perfusate, bile) | Requires long time, expensive, not specific for a certain cell type |

Figure 2: technologies available to assess the liver quality during the transplantation process.(Panconesi et al., 2021)

When liver failure occurs in patients, e.g. after acute liver failure or cirrhosis, the only option is to switch to a liver transplant. Liver transplants are in high demand and in Europe there is a well-functioning system for the distribution of those and for finding the best donor-receptor-match.(Tchilikidi, 2019) The MELD-Score classifies the urgency of a transplant for patients and is ranging from six to 40, including

parameters like internal normalized ratio, creatinine, bilirubin and serum sodium. Higher numbers are more urgent and usually receive a replacement organ faster.

## *1.4 Transplant graft viability scores*

Right now, in 2023, there is no broadly accepted score for liver viability transplant assessment. Studies suggest that there are two key strategies for making a score like this possible. Either a combination of parameters evaluated by artificial intelligence or one yet to be found simple key parameter might be the suggested solution in finding a viability score. (Panconesi et al., 2021)
A known problem is that almost every study group, surgeon and transplant center use different machine perfusion techniques and therefore also have different parameter thresholds for a viable transplant. As stated by the research group Panconesi et al. a large, normalized sample group with systemic biomarkers, micro ribonucleic acid (RNA) detection and metabolic profiling would be needed to making a broader score analysis possible. (Panconesi et al., 2021) The collection of these parameters is time-consuming and not cheap, making a clinical analysis with current technologies not efficient. (Brüggenwirth et al., 2020)

## *1.5 New methods*

The following two methods represent state-of-the-art possibilities of technology applied in medicine. The systems fulfil medical standards to its highest level, as they have been developed by established medical corporations. The installation process for both methods is easy and fast. They aim to solve an existing problem in analysing the health of liver transplants – making fast, easy and non-invasive assessment of liver viability possible.

### 1.5.1 LiMAx CORLab®

The liver LiMAx system from CORLab® uses the basic metabolization function of the liver. Cytochrome P450 enzymes of the liver have the assignment to get rid of internal and external waste products in the body. The activity of the CYP450 enzymes relates to core hepatocyte function. (Schurink et al., 2021) $^{13}$C-Metheacetin, a substance which is known to be only metabolized with CYP450 1A2 specifically in the liver, is used during the test. The fact that $^{13}$C-Methacetin is only metabolized by CYP450 makes an indirect assessment of liver function possible. (Schurink et al., 2021)

$^{13}$C-Methacetin, N-(4-[($^{13}$C)methyloxy]phenyl)acetamide is metabolized through CYP450 1A2 the Paracetamol and $^{13}$C-Formaldehyd. The $^{13}$C connected formaldehyde is then very rapidly transformed to $^{13}CO_2$. This $^{13}CO_2$ can then be exhaled from a patient.(Musialik et al., 2015)
The LiMAx Test describes the ratio from $^{13}CO_2/^{12}CO_2$ in the (exhaled) gas from a patient.
In this study, it was assumed that the $^{13}CO_2$ traced carbon dioxide is also released from the perfusion liquid and could be found in the waste air of the system.(Modak, 2007)

The study group around Schurink et al. used the LiMAx test in a NMP setup on eleven human extended donor criteria livers. The concept of proof study showed a significant correlation between viable extended donor criteria livers and LiMAx® evaluation. (Schurink et al., 2021)

### 1.5.2 EleVision™ IR-Plattform MedTronics®

The EleVision™ system shows real time high-definition pictures with indocyanine green feedback. It can be used in open and laparoscopic surgeries and displays a direct way of the tissue perfusion. This allows surgeons in time to see anastomoses or to get information about the perfusion. Furthermore, the system can quantify the indocyanine green concentration, thus making feedback of maximum perfusion and the elimination of indocyanine green (ICG) possible.

ICG has been used since 1959 in medical clinics and can be found in various medical applications, e.g. finding anastomosis, evaluation of blood flow in retina vessels and for navigation to sentinel lymph nodes in tumour biopsy.(Cherchi et al., 2021, Serra-Aracil et al., 2022, Gilmore et al., 2013)

The substance has a good stability in blood and plasma due to its inert characteristic, and is usually bound to plasma proteins, naming albumin and alpha1-lipoprotein. (Sakka, 2018)

The fluorescence maximum is reached at approximately 800 nm, and its fluorescent ability is enabled with a near infrared laser, mounted directly on the high-definition camera of the MedTronic® device.(Nishino et al., 2020) This allows for two pictures at the same time and the same camera angle, one with the ICG displaying and the other one with a white light high-definition image for optical navigation.

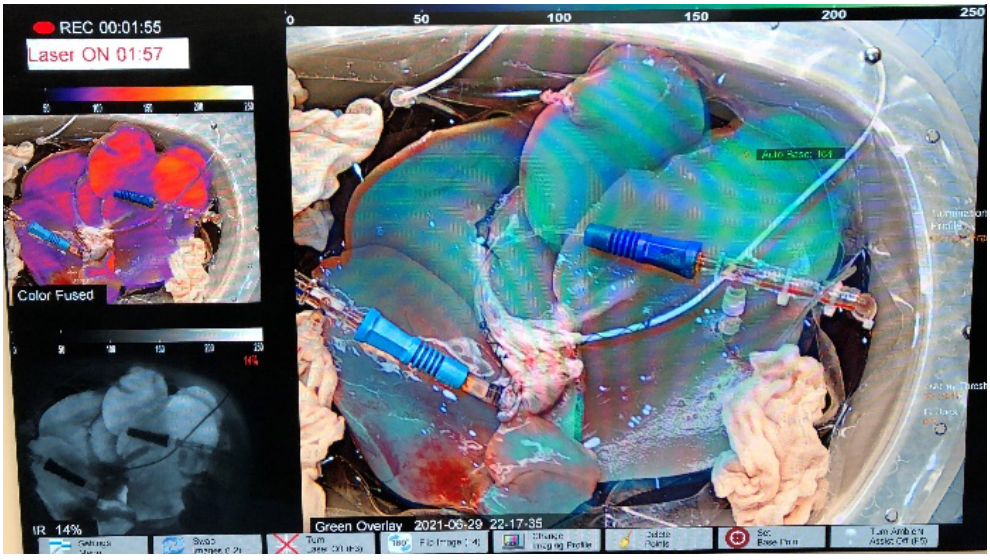


Figure 3: live view of MedTronics® overlay display, with autobase indicating the most absorption point

MedTronics® created a complete medical device with an in-built analysis function at the main computational unit and a program, called VisionSense Player to analyse video sequences on any Windows device.

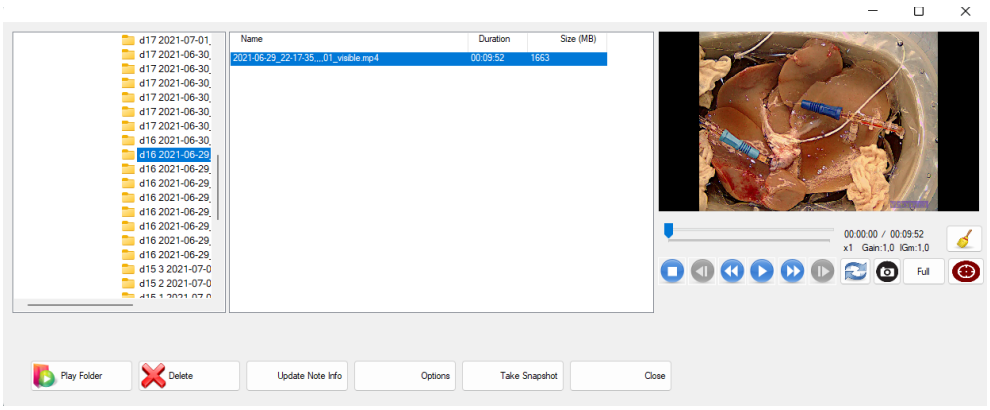


Figure 4: VisionSense Player with file system for analyzing absorption levels of indocyanine green records.

The VisionSense Player shown in Figure 4 allowed easy navigation and overview of all the sequences made with the EleVision™ device.

# 2 Material and methods

The experimental section of this thesis shows the use of two new strategies during liver perfusion. The experiments were held at the clinical surgery department of the medical university in Graz. The department has already a well-equipped infrastructure and is known for its research team specialized for liver perfusion.

For better comparison and resource management, seven healthy minipig livers were used as test organs in the thesis. These animal liver organs show equivalent results to the real human livers, as stated by many studies. From explantation to start of the experiment the organs were transported on cold ice and this transportation time lasted from one to 36 hours.
After each liver arrived at the facility, it was prepared on the back table and connected to the perfusion machine. The perfusion time was set for 24 hours, during this time, samples for biopsy, cryology and information about the perfusion parameters were gathered.

This study aims to demonstrate two novel methods for monitoring liver health during liver graft machine perfusion. One group used liver grafts which were connected to a LiMAx® device from CORLab® to draw conclusions about the liver synthesis capacity. The other group examined liver grafts for perfusion parameters in real time using EleVision™, a MedTronics® optical laser system.

The LiMAx® and the EleVision™ systems were always used separately, and the test started with machine perfusion. The experiments were held for a maximum of 24 hours and had specific time stamps for monitoring liver health. Diagrams were made with Microsoft Excel and statistical analysis were done with IBM SPSS Statistics.

## *2.1 Organs*

Due to high demand in research and human organs, only a number of seven minipig livers were used. Pig organs are often used in studies and show good comparability because of their similarity in cardiovascular and organ specifications. (Swindle et al., 2012)

The minipigs were held at the research facility “Hahnhof” near the medical clinic of Graz and showed very good liver health. After explantation the organs were transported in cold storage directly to the surgery research facility. For better comparability, the organs were stored over different time periods. The range varies from nearly zero, 12 and 24 hours for cold storage time. This results in varying organ quality and could equal real life transport times.

The LiMAx® group used a total of three minipig livers in excellent viability condition. The EleVision™ group used four animal livers with above-described varying starting times for perfusion. The small sample size makes statistical conclusions not significant, but still shows a analysis where the systems could help.

All livers examined showed not only great visual health, e.g. no noticeable cirrhosis, fatty encapsulations or other pathologies, but also their health measurements were well within transplantation checkpoints. This represented the high quality of organs and was a positive consideration for research. All livers showed equal health parameters, though some minor performance and perfusion differences could be noticed.

Some organs showed better performance after a few hours of subnormothermic machine perfusion. With performance being the indicator of less vascular resistance, better perfusion and metabolization rates.

## *2.2 Setup*

The main goal in the transplantation process is to keep organs viable during explantation and implantation, or even improving their performance. In recent years, subnormothermic machine perfusion of transplants could show good outcome compared to the standard cold storage transportation. (Tchilikidi, 2019)

For machine perfusion, the research team used the LiverAssist® from OrganAssist®. The device has three simplified flow circuits, one pulsating mid-flow for the hepatic artery, one high flow for the portal vein and one cooling/heating circuit for the machine. Machine perfusion works with real blood, and for research purposes it often uses special perfusion liquids. In this experiment, Custodial and Becker MPS® were used and showed great performance, even stabilizing and improving the health parameters of livers. The study used subnormothermic perfusion liquid temperatures at 21 degrees Celsius for the experiment.
The organs were prepared by back table cleaning for removing blood, flushing and connecting the tubes to the vessels.
In order to get an overview of the liver parameters, blood gas analysis was performed and if the pH, bicarbonate or glucose levels were out of range, countermeasures were taken.
For gold standard comparability, samples of the perfusate and liver biopsy for histology were taken. The biopsy still remains the gold standard for liver tissue information as inflammatory processes or fibrosis, but require costly preparations to get results. (Musialik et al., 2015)
Due to limitation of time and costs, the histology results remain undiagnosed in this study. The focus was placed on introducing the two new devices to organ perfusion and the improvements they bring.

### 2.2.1 Test setup of LiMAx® System

For oxygenation of the perfusion liquid, 100% $0_2$ was used, which was connected to the membrane oxygenators of the LiverAssist® machine. The $O_2$ came from a gas bottle and was set to put approximately 2 liters/min to the system. The waste tube, where the $^{13}CO_2$ was transported, was connected to the LiMAx CORLabs® device.

Before the experiment started there was a 60-minute time period in order to calibrate and collect baseline parameters. This meant that organs were brought to subnormothermic test temperature and an average circulation of $^{13}CO_2$ was recorded from the measurements.

For good metabolization, the liver was kept in a tight range of gas and resistance parameters. If transplants tend to fall out of range parameters, countermeasures with bicarbonate, insulin or flow change were taken. Methacetin was given in a dosage of 6-8 ml directly in the oxygenators. The dosage was adapted to the liver weight and corresponds with body weight to methacetin ratio used in clinical applications. The system recorded continuously, with over 84,000 data points per liver.

$$Ratio = \frac{R - RPDB}{RPDB} * 1000$$

$$R = \frac{^{13}CO_2}{^{12}CO_2}$$

$$RPDB = 0.0112372$$

Figure 5: LiMAx® ratio calculation formula

Figure 5 shows the basic calculations for the Ratio between traced $^{13}CO_2$ and $^{12}CO_2$, with RPDB being a constant which was set by CORLab® LiMAx® developers.

The LiMAx® group had a total of three minipig livers, liver 1 and 3 starting shortly after explantation and liver 2 for cold static storage being stored on ice for 24 hours. Here again, the small sample size is nowhere near significant, but was nevertheless able to show the direction in which the data was moving.

### 2.2.2 Test setup of EleVision™ System

The EleVison™ system has a computational unit, a monitor and the camera system with a 360° moving arm. The camera was placed approximately half a meter above the organ, which was connected to the liver perfusion machine and laying in the reservoir chamber. This allowed for a good overview of the liver and made the

perfusion of the hepatic artery possible, following the main vessel branches and later the whole tissue. Approximately 0,1-0,2 ml was injected, which corresponds to around 0,5-1 mg of ICG to the oxygenation unit of the hepatic artery system.

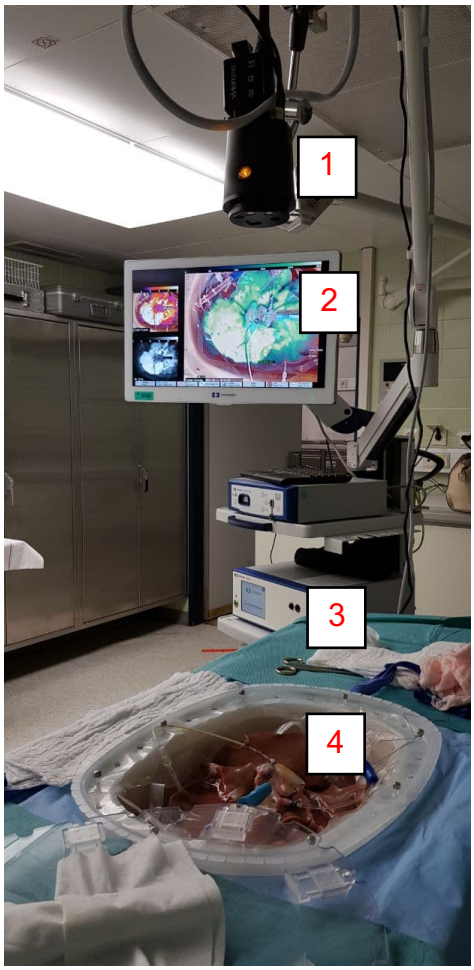


Figure 6: overview of the EleVision™ setup; 1)IR camera 2) touch display 3) computational unit 4) liver organ in perfusion reservoir chamber

Every hour during each experiment, a recording for one minute was made, making a dynamic observation possible. A few seconds after the first ICG was applied, the ICG flooding phase was visible, after that the recordings showed increasing and decreasing ICG levels.

# 3 Results – cross reference with non-invasive methods

## *3.1 Results and diagrams of LiMAx® system*

The LiMAx® test is a well-known test used in patient care for getting an overview of the liver functionality. In vivo, usage usually takes 2mg/kg body weight intravenously to get sufficient results.(Holzhutter et al., 2020)
This study used between 6-8 ml of an 0.4% Methacetin mix, which resulted in all 3 test livers in a visible raise of the ratio.

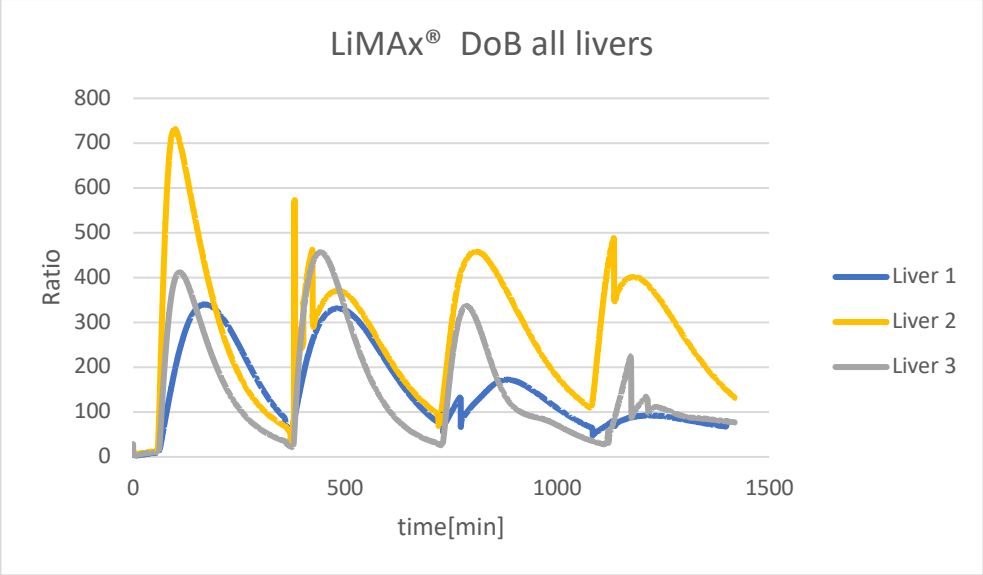


Figure 7: Ratio. Application of methacetin dosage was set for all livers at the same time at 60, 360, 720 and 1080 minutes. Outliers should be treated as artifacts, e.g. due to bent gas lines. Liver 1 and 3 were perfused after explantation, whereas liver 2 was set on ice for 24h

Figure 7 shows merged data points from the individual LiMAx® experiments over 24 hours (=1400 minutes). The calculated ratio gives information about the traced methacetin degradation rate. The graph shows a peak in Ratio after every application of methacetin. The first cycle of methacetin lasted for 5 hours, and the following three cycles lasted for 6 hours.

Every liver was observed for 60 minutes, and during this time the baseline range was set. At 60 minutes into the experiment the first methacetin dosage was administered and shortly after a rise in Ratio was noticeable. This indicates that methacetin was quickly distributed in the circulation and peaked approximately after 65.2 minutes, with an average of 475,6 in ratio change from the first methacetin dosage. Liver 2 and 3 show a fast rise, whereas liver 1 takes longer and doesn't peak that high.

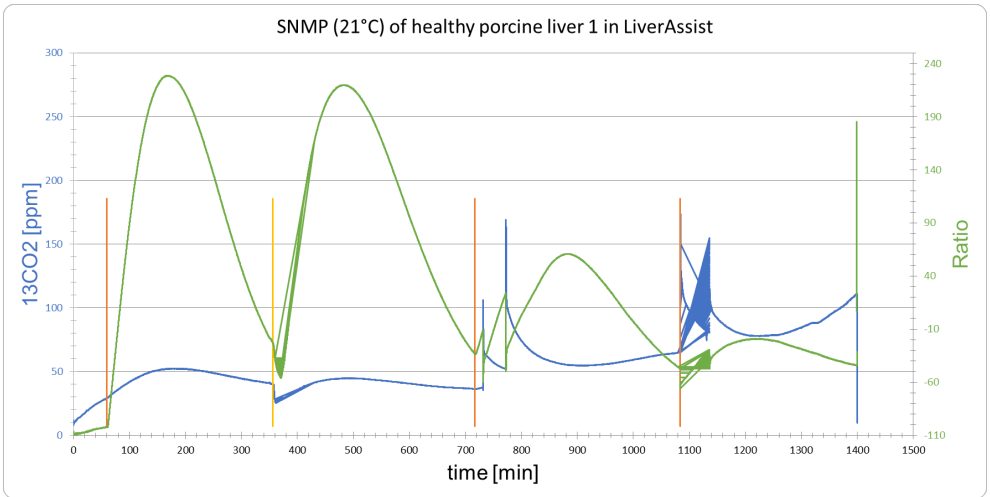


Figure 8: LiMAx® test on liver 1 with 0,736g; the left scale shows in blue $^{13}CO_2$ and the Ratio on the right is for $d^{13}CO_2$ in green; orange displays the time methacetin was added and on the yellow timestamp, perfusate was changed and directly after methacetin was administered, lines appearing as areas are seen as artefacts and the mean value over this distance was assumed

Figure 8 shows a diagram computed from the CORLab® LiMAx® device of liver 1 over the complete experiment time of 24 hours. The diagram usually includes all markers, which were set during the perfusion, but for better visuality only relevant methacetin markers were included. For all tests, there was a 60-minute baseline period without methacetin to evaluate the normal parameters (ratio for figure 5 calculations). The graph shows good correlation between methacetin dosage and rising $d^{13}CO_2$, the green line on the right scaled as Ratio. $d^{13}CO_2$ is showing two high curves in the beginning and a third and fourth declining peaks. This could be due to over saturation of methacetin, therefore decreasing the ability to metabolize methacetin or due to worsening liver

health. With flow and perfusion gas parameters well within limits, this phenomenon could be due to over saturation. There are some outlines visible, especially after approximately 716 minutes and again after 1083 minutes, which could be put down to a bent gas pipe to the LiMAx® device. Over time, the $d^{13}CO_2$ baseline could not be reached again, which would indicate that methacetin was never fully degraded during the four cycles and thus accumulation might have occurred. In order to be sure, medication concentration could have been analysed, but wasn't done due to resource management.

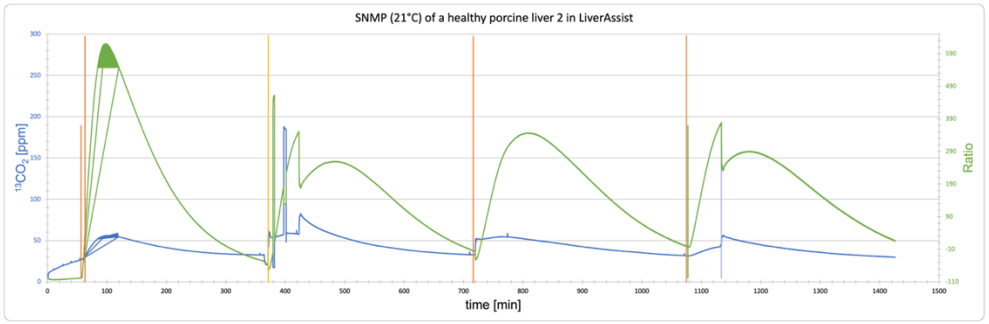


Figure 9: shows 13CO2 in blue over time for liver 2 of 0,954g; orange vertical lines indicate the 3.2mg methacetin dosage and at yellow indicator the perfusate was changed and methacetin injected after

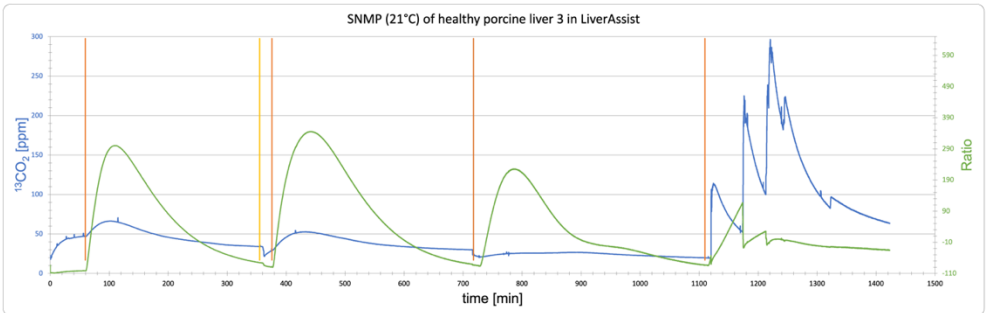


Figure 10: liver 3 of LiMAx® test with orange indicators showing methacetin dosage and yellow perfusate change

Figures 9 and 10 give a more detailed overview of the LiMAx® tests done on liver 2 and 3.

With stabilized perfusion liquid gas parameters and degradation of methacetin in all livers after every dosage, we can accept that livers were in good health conditions.

To quantify, the metabolization starting and ending ratio values were compared. Liver 1 has a total change of 75,1, liver 2 115,1 and liver 3 shows a 47,9 raise in the ratio at the end of the experiment. Though ratio values never reached baseline, neither through nor at the end of the experiment, the metabolization graphs still indicate a good connection between LiMAx® dosage and liver metabolization.

As shown in the research paper of “A proof of concept study on real-time LiMAx® CYP1A2 liver function assessment of donor grafts during normothermic machine perfusion”, LiMAx® values of ex vivo perfusation can correspond to liver health. The research team of I. J. Schurink et al. compared LiMAx® values over time, ALT, AST and lactate clearance.(Schurink et al., 2021) They found significant correlations, which can be compared with this test setup and correspond to test values.

There is a good visible correlation between ex vivo liver perfusation and in vivo LiMAx® tests, with similar dynamic metabolization rates. Though in this LiMAx® ex vivo experiment, only “healthy” pig livers were used and in order to get a meaningful statement, a follow-up study should be done with good and damaged liver tissue in order to show the difference in methacetin separation thus resulting in varying metabolization curves. Also, the methacetin amount could be adapted and be a counterpart of over saturation of the organ, thus resulting in small rising baseline plateaus in this study.

## 3.2 results and diagrams of EleVision™ system

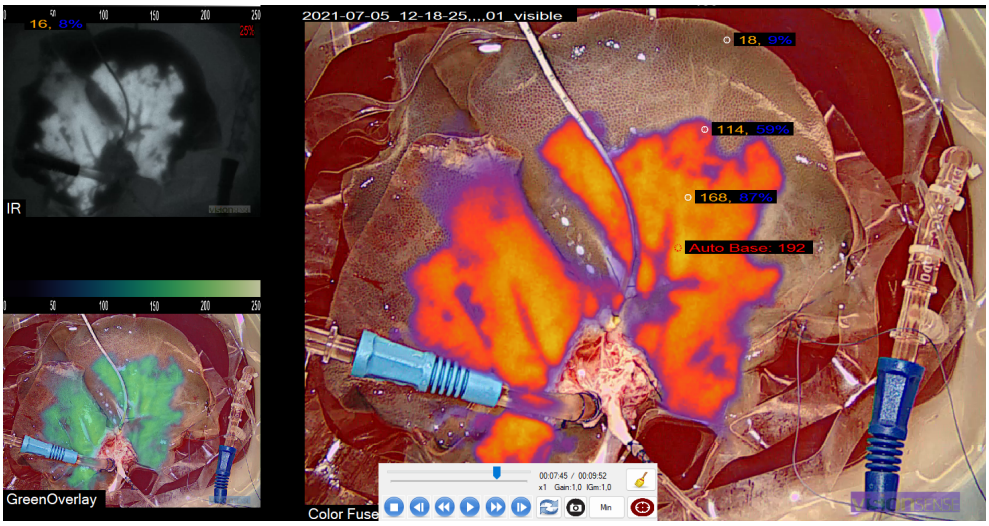


Figure 11: Full-screen of VisionSense Player; auto base was set automatically; individual points were set, with orange as absolute and blue as relative values regarding the auto base

Figure 11 shows the key value of the VisionSense Player, making the video data points analyzable. Auto Base is set automatically and indicates the point with maximum absorption of indocyanine green. This base point can be adjusted, allowing an individual reference point for measuring levels of absorption on the organ. Figure 11 shows three manually set points, to evaluate different perfused areas on the liver.

On the left top side of Figure 11 there is an InfraRed (IR) screen implemented with a red number, indicating the sensitivity of the Infrared camera at this point.

Adjustments in Auto Base were often taken to keep this reference point at the same place over time. The key value of this device would be to make a quantifiable answer to whether the periphery of an organ is well enough perfused. In order to make statements, three individual points with different levels of absorption in the beginning were set for every liver. Figure 11 gives an example of these three points, with point 1 having good absorption, point 2 having less absorption and point 3 with none to less absorption at the beginning.

This and all three points to make references to absorption were set manually with visual mouse clicking. This beholds therefore the mistakes of millimeters in accuracy.

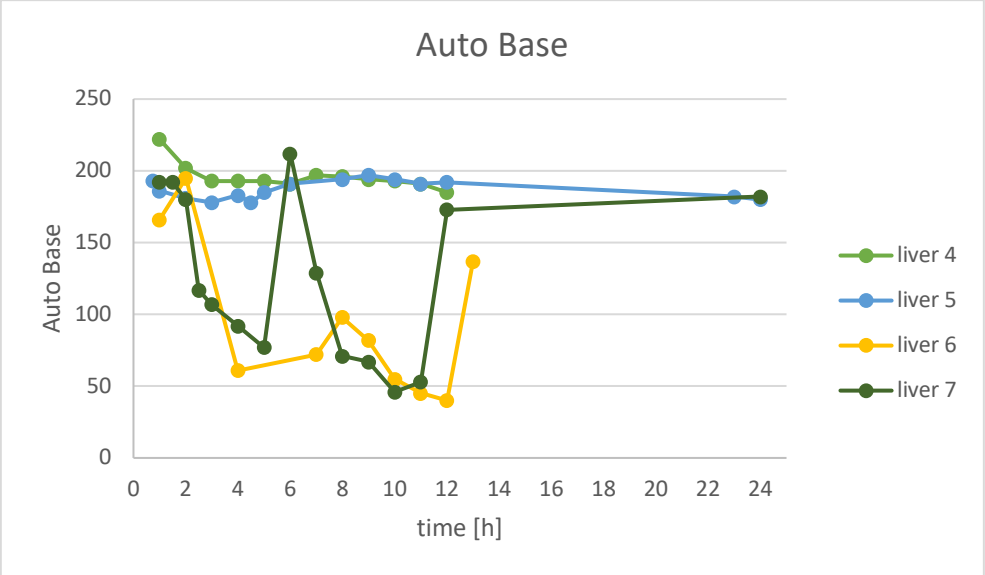


Figure 12: Auto Base of all four EleVision™ livers for each recorded time stamp

Figure 12 shows the Auto Base levels of all four livers used with the Indocyanine green EleVision™ system. Livers 4 and 6 were perfused for 12 and 13 hours, which is nearly half of the 24-hour perfusion time of liver 5 and 7. This difference in perfusion time was due to resource management. Figure 12 also gives an overview on the range of base absorption levels. Liver 4 and 5 have a rather small range, whereas liver 6 and 7 show variability.

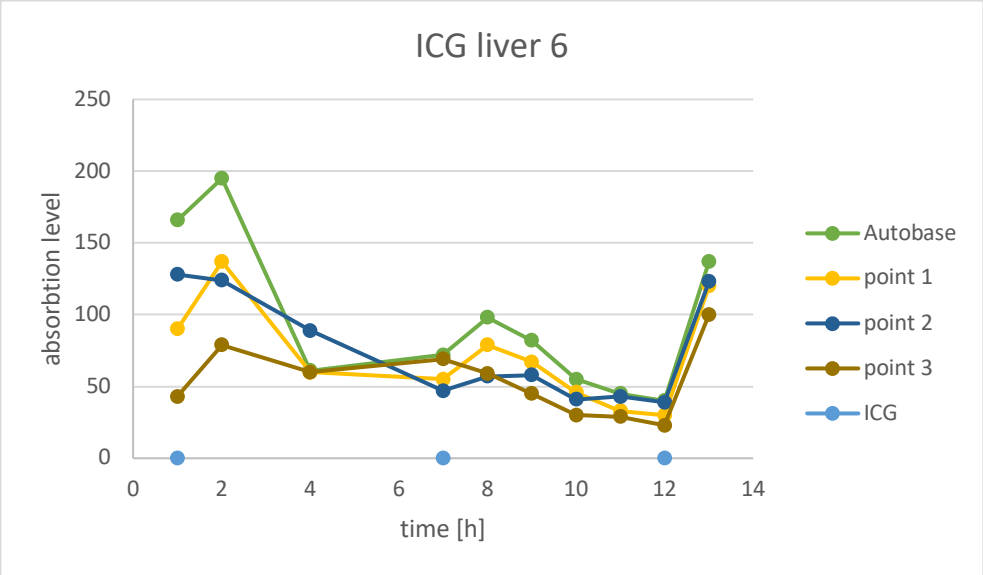


Figure 13: EleVision™ liver 6 with blue ICG dosages at 1, 7 and 12 h and 3 points relative to auto base with individual perfusion/absorption levels at the beginning

Livers 6 and 7 showed good change of absorption levels after ICG dosage, and thus those livers will be looked at more closely. In Figure 13 liver 6 is recorded over 13 hours and four significant absorption points were picked out.

At the beginning of the experiment an average of 106,75 with a standard derivation of 74,82 occurred, whereas in the end absorption levels showed an average of 120 with standard deviation of 20,77. The smaller range of absorption at the end of the experiment would indicate, that the indocyanine green is better distributed. This better distribution could also be seen as better perfusion of the organ during the perfusion time.

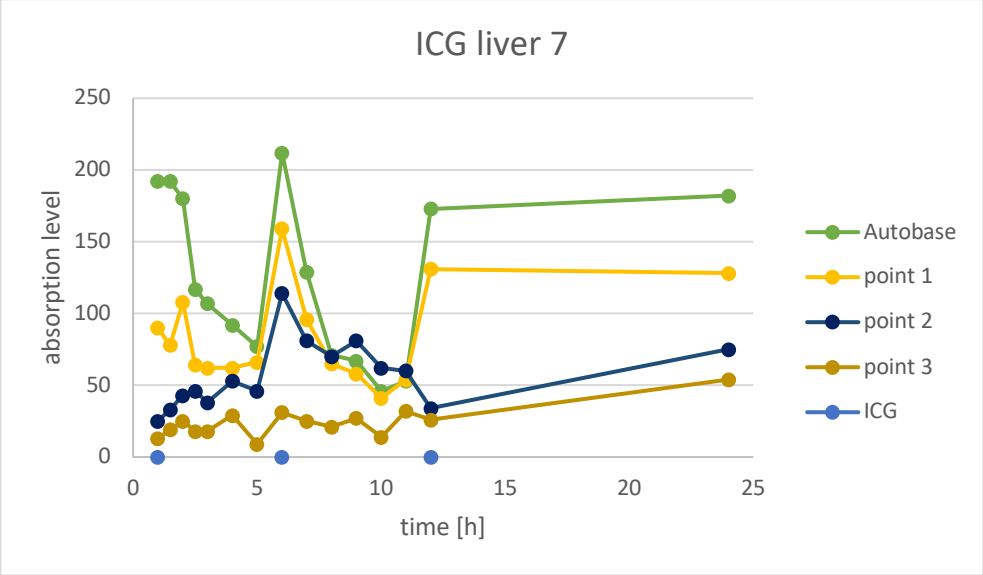


Figure 14: ICG absorption levels of liver 7 for 24 hours; ICG dosage at 1, 6 and 12 hours; each point/color line represents a set absorption point (Figure 11) at the liver over time;

Figure 14 shows the ICG absorption of liver 7, which was machine perfused over 24 hours. After the three ICG shots were administered, a rise in all examined points is noticeable. This indicates the correlation between ICG and the measured absorption. After the first application of ICG at 1 hour into the experiment, the average absorption was 80 with a standard deviation of 131. After 12 hours in the experiment these values changed to an average of 91 with a standard deviation of 113,9 and after 24 hours the average was 109,75 with a standard deviation of 88,3. The significant change of standard deviation indicates the better absorption in points, which had initially far worse absorption levels.

Overall, the EleVision™ indocyanine green system shows great practicability, and is a fast and non-harmful way to assess the liver perfusion state. However, it should be noted that the laser system is limited to surface absorption levels. Deep perfusion malfunctions might be tougher to measure, but should be looked at in a follow-up study with damaged, e.g. cirrhosis livers as a comparable group.

## *3.3 Combined results and cross reference with existing non-invasive methods*

The above methods presented for monitoring non-invasive liver health during machine perfusion show promising results. Although the sample group was small due to resource management, significant changes were noticeable and indicate the value of these new strategies. Follow-up studies should be done with more test transplants and importantly with damaged or slightly damaged organs. This would allow the better differentiation of healthy, transplantable livers to damaged, non-transplantable ones.

# 4 Discussion

Assessing organ/liver health is an ongoing research topic and is still looking for the right approach. Machine perfusion shows promising physiological conditions, to further monitor the organ in ex-vivo but near in-vivo conditions. Even today, there is no suitable solution, meaning a marker making a good prediction for liver health possible.(Brüggenwirth et al., 2020)

Mergental et al. recently released viability parameters for livers considered appropriate for transplantation assessment. The review was set to 3h of perfusion and liver transplants had to have the following characteristics: perfusate lactate less than 2.5mmol/L or bile production. Two of the next three criteria also had to be met: perfusate pH higher than 7.3, constant arterial flow more than 150mL/min and portal venous flow of more than 500mL/min and soft organ parenchymal consistency. The review used declined UK liver transplants and could show that primarily rejected livers could regenerate under normothermic machine perfusion and regain transplantable criteria.(Mergental et al., 2016)

The research paper of R. Panconesi et al., states that there are two solutions: one being to analyse multiple parameters with computational help and probably with artificial intelligence. This would require international collaboration, high similar standards for analysing the parameters and following randomized control trials to verify the conclusions. The other solution would be to find a key parameter to assess the liver viability. With technological improvements, faster and exacter qualification and quantification of micro ribonucleic acid (miRNA), ATP chain reactions, ATP metabolites and other metabolic biomarkers could be possible. (Panconesi et al., 2021)

This study displayed two novel strategies with promising fast, live, non-invasive and indirect results for the organ transplantation field. The LiMAx® system could offer assessment of liver metabolization rates, whereas EleVision™ could show information about the perfusion state of liver tissue. Both systems could thereby deliver important fast live data but have great acquisition costs. To bring those newly

found perfusion and metabolization data into more clinical context, a lager and normalized study for setting range parameters would be needed.
Finally, the basic parameters like pH, blood gas analysis with glucose and lactate make fast, easy and cheap assessment of transplants possible and will probably always be standard parameters. Those basic parameters remained well within limits for the experiments, approving the good viable status of the livers. Histology assessment like the Suzuki-Score remain the gold standard but have high costs and are time-consuming. As suggested by many study groups mentioned above, a combination of these parameters for viability assessment of liver transplants might be the solution for finding an applicable viability score.

The ongoing research and steady development of new technologies pushes the boundaries in every aspect of our lives. With ethics playing a major role in medicine, knowing the boundaries should always be considered in pushing new technologies.

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