# PhD in Bioengineering and Medical-Surgical Sciences

## Research Title:
**Normothermic Machine Perfusion for Reconditioning of Steatotic Liver Grafts for Transplantation**

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### Context of the research activity

Liver transplantation is the ultimate treatment for end-stage liver disease, hepatocellular cancer, metabolic disorders and other liver diseases. However, the success and applicability of liver transplantation is utterly dependent on organ donors availability. This is a key point, as there are a great number of potential recipients of a liver transplant who are denied this opportunity due to scarcity of organ donors. In recent years, in the hope to expand donor pool, the transplant community has gained increased experience with the use of so-called “extended criteria donors”. Although a widely accepted definition is currently lacking, the concept of extended criteria normally refers to older age (> 65 years), fatty infiltration or steatosis (> 30%) or donation after cardiac death. Age seems to be no longer a barrier to organ donation, as several groups have employed liver allografts from elderly donors with success. Donation after cardiac death has been implemented as a way to expand donor pool in several countries like Spain, USA, UK, Belgium, The Netherlands and others. Donation after cardiac death differs from donation after brain death as organ cooling and perfusion with preservation solution begins after a period of warm ischemia that damages the organ. Three-year graft survival of livers from this donor subset is reduced compared to that of organs from brain dead donors, mainly because of the onset of ischemic cholangiopathy. Italian law on the matter prescribes a 20-minutes hands-off period after cardiac arrest that has actually halted exploiting donation after cardiac death in our Country. Although there are currently several liver transplant programs that are starting using these donors with several forms of reconditioning (extra-corporeal membrane oxygenation, machine perfusion, etc.) experience in the field is in its outset and these donors are unlikely to constitute a major part of our everyday practice for at least one decade. On the other hand, whether to accept a steatotic liver for transplantation is a situation most transplant surgeons in developed
Countries are very familiar with. Metabolic syndrome and non-alcoholic fatty liver disease are becoming epidemic in Western world with an impact also on organ donors. Our group has pioneered the use of “fatty” liver grafts in Italy, showing how their use is safe and feasible, providing that a correct allocation algorithm is followed\(^3\). Surprisingly, despite a considerable body of literature on the subject, evidence as to whether steatotic liver grafts are suitable for transplantation is far from being conclusive\(^2\). One problem is huge variability in inter-observer assessment of steatosis based on optical microscopy and the lack of a reproducible tool to objectively quantify in the urgent setting of a liver transplant. Another problem is that several papers do not distinguish between macrovesicular and microvesicular steatosis, which have a different impact on immediate liver function after transplantation. Furthermore, several articles have shown an association between steatosis and poor early outcomes after liver transplantation in terms of primary non-function and initial poor function rate but unfortunately definitions of both primary non-function and initial poor function are hugely variable, contributing to further confusion. All this above probably explains – at least in part – while findings have been so conflicting\(^3,4\). However, it is generally accepted that use of grafts with moderate to severe steatosis is associated with an increased rate of primary non-function and initial poor function (whatever the definition) and reduced graft survival. As a matter of fact, there are very few series with > 10 patients transplanted using grafts with > 60% (severe) steatosis\(^2\).

The increased susceptibility of steatotic grafts to ischemia-reperfusion injury has been thoroughly studied. A common finding is that in steatotic liver oxidative stress at reperfusion is increased and the prominent cell death pattern is necrosis rather than apoptosis. Steatotic hepatocytes exhibit a baseline impaired oxidative phosphorylation due to overexpression of mitochondrial uncoupling protein 2, reducing ATP production after a period of ischemia. As apoptosis is an energy-dependent process, cell death pattern is shifted towards necrosis. Furthermore, fatty hepatocytes are in a permanently pro-inflammatory state, caused by endoplasmic reticule instability, reduced expression of peroxisome proliferator-activated receptor and increased expression of toll-like receptor 4. This implies an enhanced release of pro-inflammatory cytokines and leukocyte infiltration after reperfusion\(^5,6\). As they seem to be particularly vulnerable against ATP depletion during cold ischemia, an attractive way to obviate to ATP depletion would be providing hepatocytes with oxygen and other metabolites “to keep them alive” during preservation. This is why normothermic machine perfusion appears to be a logic strategy to reduce ischemia-reperfusion injury, especially for steatotic livers. In normothermic machine perfusion a perfusate
containing an oxygen carrier (red blood cells), glucose, aminoacids, bile salts, insulin and other compounds is pumped through the liver at 37 °C at physiological pressure and flows. During normothermic perfusion hepatocytes are metabolically active, as demonstrated by bile production, lactate clearance and glucose consumption. Normothermic machine perfusion has already been introduced in the clinical setting. A first feasibility and safety study has demonstrated the safety of the technique and a decreased aspartate amino transferase peak after machine preservation. We are awaiting the results of the COPE (Consortium for Organ Preservation in Europe) trial that will hopefully provide further insight into the advantages of normothermic perfusion. There is also some experimental evidence that steatotic grafts could benefit from machine perfusion. In a rodent model of choline-methionine deficient diet-induced steatosis and isolated-perfused liver, 24-hours hypothermic machine perfusion resulted in reduced damage and improved ATP content compared to cold storage.

The aim of this project is to assess normothermic machine perfusion as a way to reduce ischemia-reperfusion injury in steatotic liver grafts and ultimately to improve transplant outcomes using this kind of grafts in the clinical setting. We intend to use a model of isolated-reperfused steatotic rat liver. Steatotic livers will be obtained from Wistar rats fed with a metionine-choline deficient diet or from leptin-deficient Zucker rats, naturally developing hepatic steatosis. Static cold storage will be compared with normothermic perfusion. To mimic clinical setting, after a period of initial cold ischemia, control livers will be preserved with static cold storage during 12 hours then reperfused at 37 °C. During normothermic reperfusion, liver function will be assessed by bile production, enzymes release into the perfusate and histology. Study livers, after a first short 1-hour period of cold ischemia (that in the “real world” would be the time necessary to retrieve the organ and prepare it to be plugged to the machine) livers will be perfused for 11 hours. Then, after a further 30-minutes period of cold ischemia (ideally, the time necessary to disconnect the liver from the machine and flush it while vascular anastomoses are performed), livers will be reperfused normothermically for 4 hours. In this second normothermic perfusion phase liver function will be assessed by bile production, enzymes release into the perfusate and histology. Experiments will be carried out both in normal and steatotic livers. Study groups will be as follows:
- Normal rat liver 30’ cold ischemia + 12 hours static cold storage (total ischemia time 12 h 30’) + 4 hours normothermic reperfusion;
- Normal rat liver 1 hour cold ischemia + 11 hours normothermic perfusion + 30’ cold ischemia (total ischemia time 12 h 30’) + 4 hours normothermic reperfusion;
- Steatotic rat liver 30' cold ischemia + 12 hours static cold storage (total ischemia time 12 h 30’) + 4 hours normothermic reperfusion;
- Steatotic rat liver 1 hour cold ischemia + 11 hours normothermic perfusion + 30’ cold ischemia (total ischemia time 12 h 30’) + 4 hours normothermic reperfusion.

Besides improving preservation and reducing ischemia-reperfusion injury, normothermic machine perfusion offers the invaluable opportunity of reconditioning organs, trying not only to reduce damage but also to enhance organ function through preservation. This opens a window onto two possible ancillary projects with minimal variations to the initial protocol. Several groups have attempted liver defatting during machine perfusion as a way to enable the use of steatotic liver grafts in the clinical setting. Adding defatting agents during normothermic liver perfusion has been shown to increase lipid oxidation and export, as well as upregulate the expression of enzymes involved in fatty acid oxidation and triglyceride clearance⁹, ¹⁰. Is not clear whether active defatting would translate into better function after transplantation. This model would be ideal to test liver function during the second phase of normothermic perfusion after defatting during the first phase.

Another possibility of active reconditioning during normothermic perfusion would be adding biological agents, like stem cells or stem cells-derived microvesicles, the latter being the true message carrier and effectors explaining the paracrine effect of stem cells. Several types of stem cells have been employed both in the experimental and clinical setting to improve regeneration of damaged tissues. In particular, mesenchymal stem cells and human liver stem cells have been shown to reduce ischemia-reperfusion injury in experimental models thanks to their ability to influence cell cycle progression and apoptosis of resident cells¹¹-¹³. These biological agents could be added to the perfusate during first phase of normothermic perfusion to evaluate the impact on liver function assessed during second perfusion.

The successful candidate will have interest in liver transplant surgery and ischemia-reperfusion injury. Training in liver surgery and previous publication on the subject are welcome. She/he will have to show interest or have some background in experimental surgery, as well as being able to set up and actively working with an isolated perfused rat liver model. As this is a difficult model potentially presenting many surgical and technical issues, the candidate will have to be very motivated, with a pronounced attitude to problem solving. She/he will have to show attitude to teamwork as well as being able to productively interact with her/his supervisor, regularly reporting progresses of the project.
References