UNIVERSITÀ POLITECNICA DELLE MARCHE

Department of Information Engineering

Master degree in Biomedical Engineering

Liver in a Machine Perfusion: a new scenario for diagnostic imaging techniques

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Ph.D. thesis of

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To Luca,

always close to me.

Thanks for believe in me, in every moment.

To my family,

You have shown me determination, dedication and discipline.

You have given me love, hope and faith.

To me,

For having arrived at the end of this way,

aware of my strength and capacities.

Contents

Abstract

Although transplantation is the only cure of end stage organ failure, this treatment is limited by a severe global donor organ shortage. Explanted organs, even of 'good quality', experience severe injury during only a few hours or days of cold storage or ex-situ perfusion.
On this field, imaging studies are becoming essential in the management of liver transplantation. In fact, they have a very important role in the preoperative evaluation and selection of suitable candidates. At the same time, they are essential in the early detection of postoperative complications.
Because of this reason, the aim of this study, in collaboration with Ospedale Pediatrico Bambin Gesù of Rome, is to evaluate the role of MRI scanning of a liver during its perfusion in Liver Assist machine perfusion.
During this study, a first test was performed using a liver puppet and a very powerful MRI: the Magnetom Vida, a Siemens Healthineers MRI.
The results obtained were great, despite the numerous technical and logistic problems that we have encountered during the test.
After this first test, a support for the container where the liver is stored, was designed and manufactured by a 3D printer, in order to improve the quality of future test.
As soon as possible, we would like to replicate the same test using a real discarded liver: in this way we could obtain and study clear images of a liver during its perfusion.
In future, this could be a very powerful approach, through which we can improve the amount of liver available for transplantation solving, at least partially, the problem of donor organ shortage.

Chapter 1

Introduction

It has been an ongoing and fascinating dream for more than 100 years to keep organs alive outside the human body. However, only relatively small steps have been made in order to achieve this prestigious goal, despite numerous advances in the field of organ perfusion [1]. Although transplantation is the only cure of end stage organ failure, this treatment is limited by a severe global donor organ shortage. Surprisingly, the key reason leading to the limited supply of donor organs is not the number of donations; rather, it is time [2]. In fact, while the process of organ donation, cold storage and transport appears completely reversible in healthy livers upon implantation in vivo, with full function for many years, explanted organs, even of 'good quality', experience severe injury during only a few hours or days of cold storage or ex-situ perfusion [3]. The current clinical standard for organ preservation is the static cold storage (SCS) in an ice box, which limits storage to few hours for vascular and metabolically active tissues such as the livers [2]. Therefore, SCS only allows transplantation of the highest quality organs within a matter of hours after procurement. Because of this reason, a new approach is used during these years: machine perfusion (MP). Contrary to metabolic suppression during SCS, MP provides organ support through an extra corporeal artificial circulation [2].
Through a collaboration with Ospedale Pediatrico Bambin Gesù of Rome, I had the possibility to work with prof. Marco Spada, who is the director of the unit of epato-bilio-pancreatic surgery and abdominal transplantation and with all his equipe. Together we decided to start this study, whose aim is to evaluate the role of non-invasive imaging techniques such as Computed Tomography (CT) or Magnetic resonance imaging (MRI) in the evaluation of liver recipients and potential liver donors, and in the detection of potential complications arising from liver transplantation. Actually, in liver transplantation (LT) candidates, the goal of imaging is to evaluate the intra- and extra-hepatic anatomy, identify conditions that can complicate LT and stage of neoplastic disease [4]. Our aim is, instead, to use these imaging techniques during the perfusion of the liver, so when the organ is within the machine perfusion. During our research, we found only few studies that talk about this and nobody did a real test until now, so we decided to move in this way because we think that in future this could be a very powerful approach in order to have a general overview of the liver itself before the transplantation.

Chapter 2

Materials and methods

2.1 Anatomy of the Liver

The liver is the largest solid organ in the body and anatomically is divided into four lobes:

- Right lobe: the largest lobe
- Left lobe: lies to the left of the falciform ligament
- Quadrate lobe: lies between the gallbladder and round ligament of the liver
- Caudate lobe: lies between the inferior vena cava (IVC), ligamentum venosum and porta hepatis.

Surgically, the liver is divided into right and left anatomical lobes and it is further subdivided into eight functional segments, based largely on the intrahepatic distribution of vessels and bile ducts; the divisions are useful for partial hepatectomy and/or the excision of metastatic nodules or specific segments. The external demarcation of the two physiological liver halves runs in an imaginary sagittal plane passing through the gallbladder and IVC [5] (Fig.1).
The liver is important and it receives the venous drainage form the GI tract, its accessory organs, and the spleen via the portal vein.
The liver serves mainly the following important functions:

- Storage of energy sources (glycogen, fat, protein and vitamin)
- Production of cellular fuels (glucose, fatty acids and ketoacids)
- Production of plasma proteins and clotting factors and lymph production
- Metabolism of toxins and drugs
- Modification of many hormones
- Production of bile acids
- Excretion of substances (bilirubin)
- Storage of iron and many vitamins
- Phagocytosis of foreign materials that enter the portal circulation from the bowel.

Its right and left hepatic arteries arise from the proper hepatic artery, a branch of the common hepatic artery from the celiac trunk. Variations in the arterial supply to the liver are common, and surgeons operating in this region must remain cognizant of this variability. The proper hepatic artery lies in the hepatoduodenal ligament with the common bile duct and portal vein [5] (Fig.1).

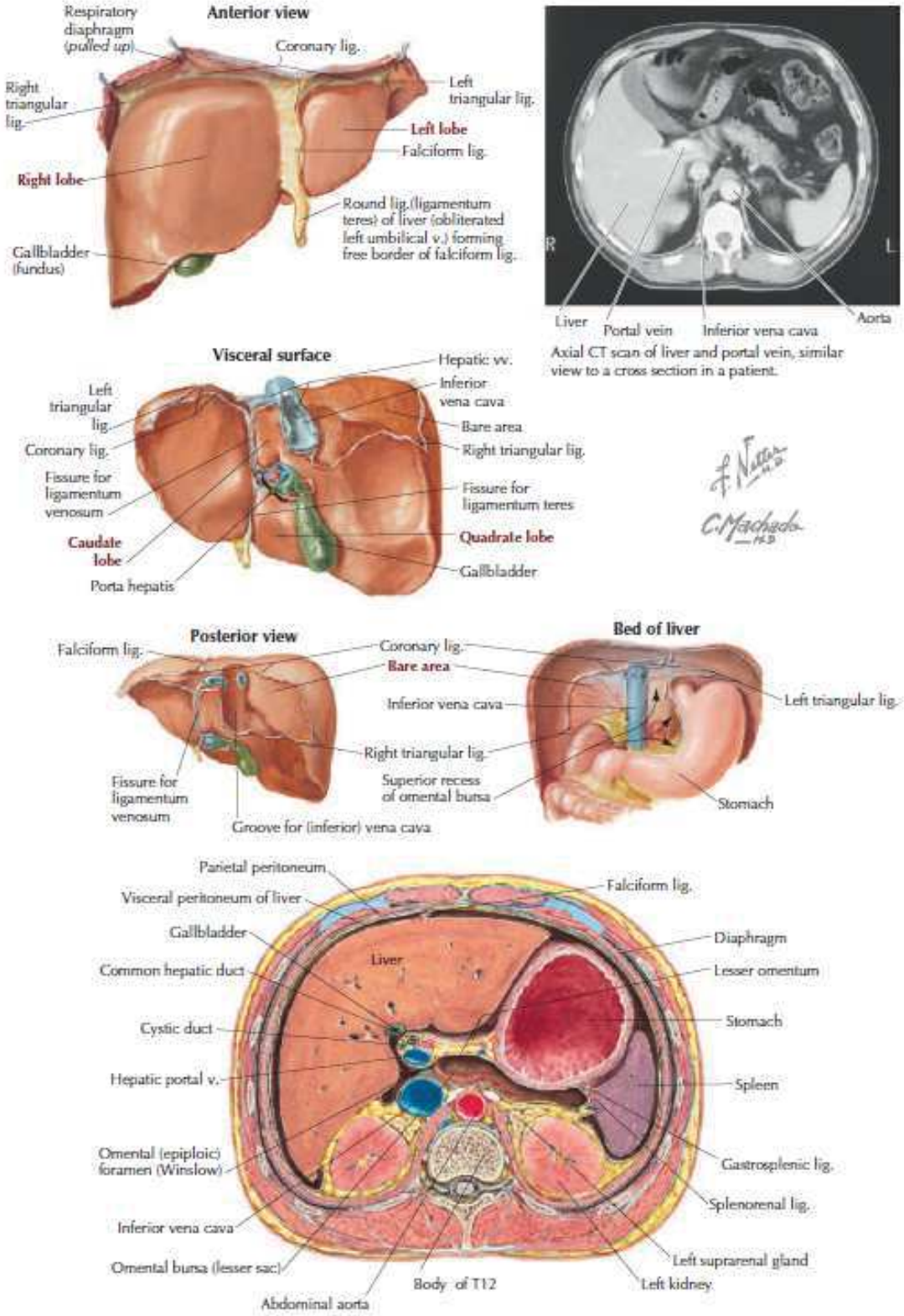


Figure 1: Various views of the Liver and Bed of the Liver

2.2 Machine Perfusion

The imbalance between grafts available for transplantation and demands has moved the focus of many investigators on the search for novel strategies to rescue organs, previously considered to be unsuitable for transplantation. In this setting, machine perfusion is recognised as one of the most significant improvements in the field of transplantation over the past 20 years [6]. Besides potentially improving organ shortage by repairing putative irreversible injuries, dynamic preservation strategies may offer the opportunity to test organ quality before implantation or to manipulate some functions [6] (Fig. 2).

Different machine perfusion modalities have emerged, mainly differing in perfusion temperature and thus the metabolic rate: normothermic (NMP), subnormothermic (SNMP) and hypothermic (HNM) [2]. Whereas HNM focus on slowing down metabolic rates and preserving ATP concentrations, NMP try to generate an ex vivo environment mimicking physiologic in vivo conditions (Fig.3). With this approach, organs are metabolically active and, in contrast to hypothermic models, functional markers of donor organs can be assessed (bile production, urine output, oxygenation). Both NMP and HMP approaches allow measurement of biomarkers which could give additional information on organ quality. Although it becomes more complex to meet the organ's metabolic demand at higher perfusion temperatures, higher metabolic rates enable detailed ex vivo viability assessment and therapeutic intervention. Most often the perfusate is oxygenated, however this can be omitted due to the low metabolic rate during HMP. Perfusate oxygenation is dependent on perfusion temperature and arguably the most complex component of MP. Many different base solution, oxygen carriers and numerous more additives have been reported [2].

Another important distinction in MP modalities can be made with respect to the timing of application during the preservation process. MP can be employed before or after a significant period of SCS but it can also be continuous from procurement until transplantation [2].

During the process of Static Cold Storage (SCS) the liver is flushed and cooled with specialist preservation fluid, then stored in an icebox. Low temperatures decrease metabolic rate by factor 1.5-3 per 10° C, so cooling a donor organ to 4° C reduces its metabolic rate to 10-12 % of the baseline. Slowing down metabolic processes reduce oxygen consumption and ATP depletion. After organ recovery, tissues and organs lack blood supply. This leads to oxygen deprivation, anaerobic respiration, metabolic waste accumulation and electrolyte imbalances, all contributing to ischemic injury. Upon reperfusion, the reintroduction of oxygen and subsequent production of reactive oxygen species aggravates this damage (reperfusion injury) [7].

Despite the injurious potential, static cold storage (SCS) of donor organs is currently considered standard of care in organ transplantation. However, it only enables the preservation of organs and tissues for a few hours, depending on type of organ or tissue [7]. Using strategies to decrease tissue ischemic injury, through organ cooling or machine perfusion, is the basis of multiple preservation techniques aimed at increasing organ viability and thus availability [7].

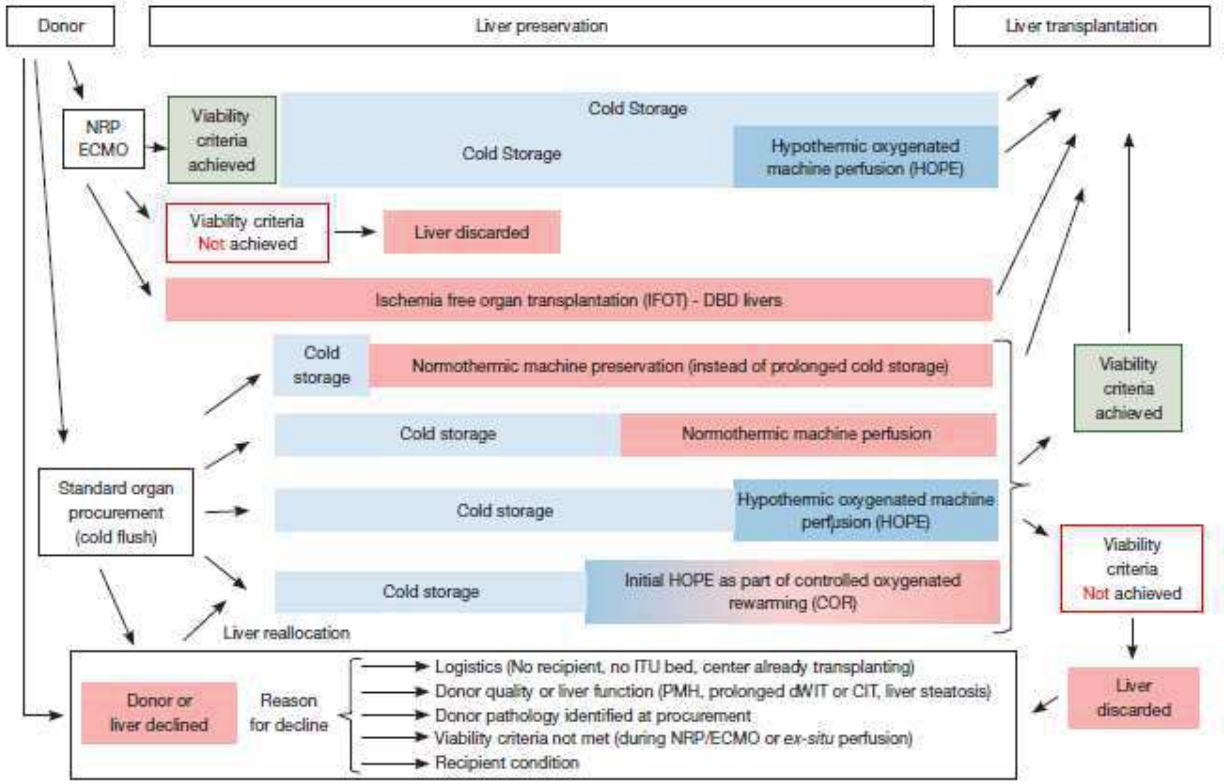
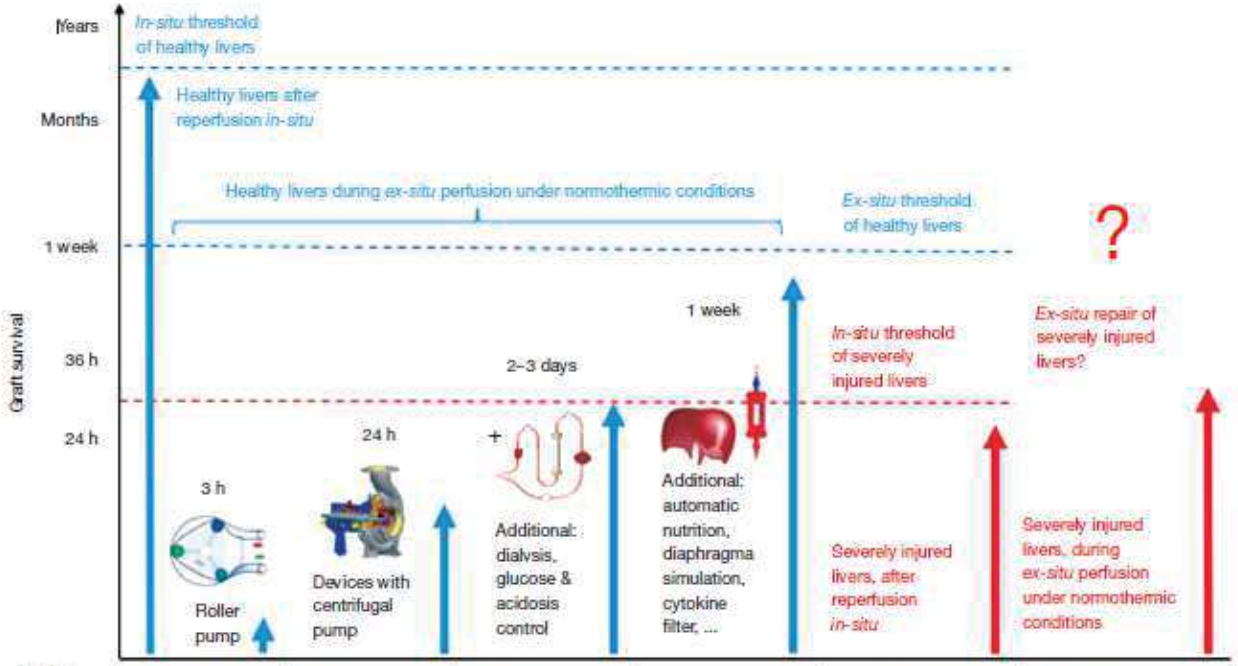


Figure 2: Currently available preservation technology for in-situ and ex-situ machine liver perfusion



Current limitations of perfusion devices:					
Insufficient prevention of reperfusion injury	Insufficient prevention of reperfusion injury	Insufficient prevention and clearance of reperfusion injury	Insufficient prevention and long-term clearance of reperfusion injury in high risk liver grafts	Insufficient prevention of reperfusion injury and subsequent impaired liver function	Insufficient prevention and long-term clearance of reperfusion injury in high risk liver grafts
Occlusion variability affecting flow rate and blood trauma	Prolonged recirculation of perfusate triggering further inflammation	Prolonged recirculation of perfusate triggering further inflammation	Prolonged recirculation of perfusate triggering further inflammation	Recipient condition and disease severity	Prolonged recirculation of perfusate triggering further inflammation
Micro emboli		Inferior simulation of *in-vivo* conditions	Inferior simulation of *in-vivo* conditions		Inferior simulation of *in-vivo* conditions

Figure 3: Survival of liver grafts after transplantation in-situ and ex-situ under the best possible normothermic conditions on different devices.

2.2.1 Normothermic machine perfusion

During NMP, the liver is perfused with oxygenated blood, medications and nutrients at normal body temperature to maintain a physiological milieu. The mechanism underlying these improved outcomes is at least partly related to the metabolic resuscitation of the organ that occurs with preservation under physiological conditions [8]. This has been demonstrated through the replenishment of ATP levels, which in turn contributes to a reduction in the severity of the ischemia-reperfusion injury that is experienced after transplant [8]. There is increasing interest in the clinical application of NMP, with several cases reported in the recent literature [8].
The reason of this interest are the numerous advantages that this technology has. Here are listed some of the most important advantages that this approach has reported.

- *Viability assessment*: one of the most important advantages of normothermic perfusion over SCS preservation is the ability to access viability. Because the organ is functioning during the period of preservation, it is possible to measure key parameters before transplantation to access function before committing the recipient to transplantation. Several parameters (bile output, base excess, aspartate aminotransferase/alanine aminotransferase, hyaluronic acid, portal pressure and portal venous resistance) reliably predicted those livers that would fail after transplantation and did so within 4 hours of the start of perfusion. This would not only decrease the risk of primary nonfunction after transplantation after transplantation nut also minimize the number of viable extended criteria organs that are discarded, thereby expending the potential donor pool. [9].
- *Extended preservation times*: Current cold storage with a preservation solution facilitates the storage and transportation of a liver for up to 16 h before transplantation. NMP devices have the benefit of extending the preservation period for up to 24 h. NMP has also been used in combination with SCS in the context of organ reconditioning. A report by Watson et al. described the successful transplantation of a liver which had been preserved for a total of 26 h of which 8.5 h were normothermic, using the Liver Assist device. Extended preservation times allow a more organized and structured approach to transplantation by improving utilisation of the operating room and elective lists. It could also provide a critical time period which may be required for organ enhancement through liver-directed therapeutic interventions. [10].
- *Drug intervention*: NMP provides a potential platform to treat the liver ex vivo during the preservation period. This is unique to NMP as opposed to other machine perfusion techniques, as active metabolism permits graft intervention and modification during preservation. This could be applied in the context of defatting steatotic livers. It has been hypothesized that NMP may benefits with respect to the transplantation of steatotic liver in a number of ways: the avoidance of cooling, in fact the compliance of steatotic liver tissue alters considerably as the temperature falls and it is possible that this is one mechanism to explain the poor immediate postoperative recovery of such organs; reduction of ischaemia-reperfusion; mobilization of fat and reduction of intracellular fat during NMP and pharmacological strategies to increase fat metabolism.
  Another exciting potential is that of immunomodulation to induce tolerance of the

liver; it has also been suggested that NMP could be used to deliver gene therapies [10].

- *Gene delivery*: the benefit of NMP preservation in terms of organ-specific targeted drug intervention might be more crucial in gene therapy approaches. Potential targets for gene therapy in transplantation are manifold. During preservation, whole organs are uniquely accessible for modification. The advantage normothermic organ preservation offers over hypothermic preservation is again the physiologic environment, which is much more likely to promote both gene transfection and function. [9].
- *Steatotic livers*: in efforts to increase the donor pool, more 'marginal' donor livers are being transplanted. These include livers with substantial intra-cellular fat deposition (steatosis). Steatosis results from altered metabolism of fatty acids with hepatocytes and is characterized by intracytoplasmic accumulation of triacylglycerol (TG) in the form of lipid droplets (LDs). Livers with evidence of steatosis are much more susceptible to ischemia-reperfusion injury (IRI) during SCS: the consequent organ injury is attributed to impaired microcirculation, reduced mitochondrial function and excessive inflammatory response and so it is associated with subsequent poor post-transplant outcomes. [11].
  Steatotic livers constitute the largest individual cohort of organs which might benefit from active intervention during NMP. Pre-clinical models demonstrate that ex-situ liver function can be enhanced by NMP and hepatic triglyceride content can be reduced by enhancing intracellular lipid metabolism and potentiating the reversal of steatosis [11].
- *Liver splitting*: there is an increasing interest in NMP as a means to resuscitate suboptimal donor organs and minimize the effects of preservation. Experimental transplant studies have shown superiority compared with conventional SCS as well as enabling reliable prediction of viability. NMP provides the possibility of carrying out an ex situ liver split in an organ that is still perfused, thereby combining the advantages of the in situ and ex situ techniques [12].

Currently, two devices are available allowing normothermic MP in the context of liver transplantation: OrganOx Metra and Liver Assist.

2.2.1.1 OrganOx Metra

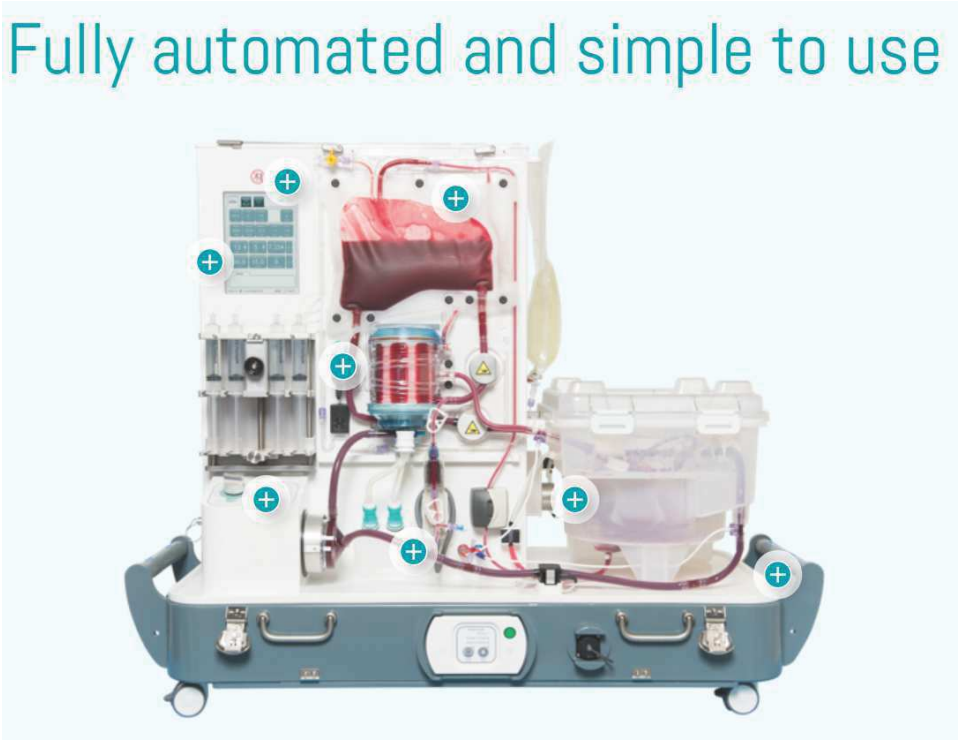


Figure 4: OrganOx Metra

The OrganOx Metra (Fig.4) enables the assessment of donor liver function by allowing surgeons to monitor markers such as perfusate lactate clearance, pH, transaminase levels, glucose metabolism and bile pH during preservation.
The risks of graft injury during static cold storage are well established in marginal donor organs such as DCD (donation after circulatory death) or elderly donors.
Compared to static cold storage, the Metra has been associated with a 50% reduction in graft injury, as measured by a 50% reduction in peak AST during the 7 days after liver transplantation. This was despite a 54% increase in preservation time.
The Metra can be used in two ways: transport mode (continuous NMP) or “back to base” mode (NMP following static cold storage) offering transplant centres maximum flexibility of use.
In a randomised trial, compared to static cold storage, the Metra reduced early allograft dysfunction by 74%, while peak serum AST levels, a predictive biomarker for graft and patient survival, were 49% and 73% lower during the first 7 days after transplantation for all transplanted livers and transplanted DCD (donation after circulatory death) livers, respectively.

How it works:

During normothermic machine perfusion with the OrganOx Metra, the donor liver is continuously perfused with oxygenated blood, medications and nutrients at normal body temperature and near physiological pressures and flows (Fig.5).
This means the liver is functional throughout preservation, enabling functional assessment and evidence-based decisions on whether to transplant a donor organ.

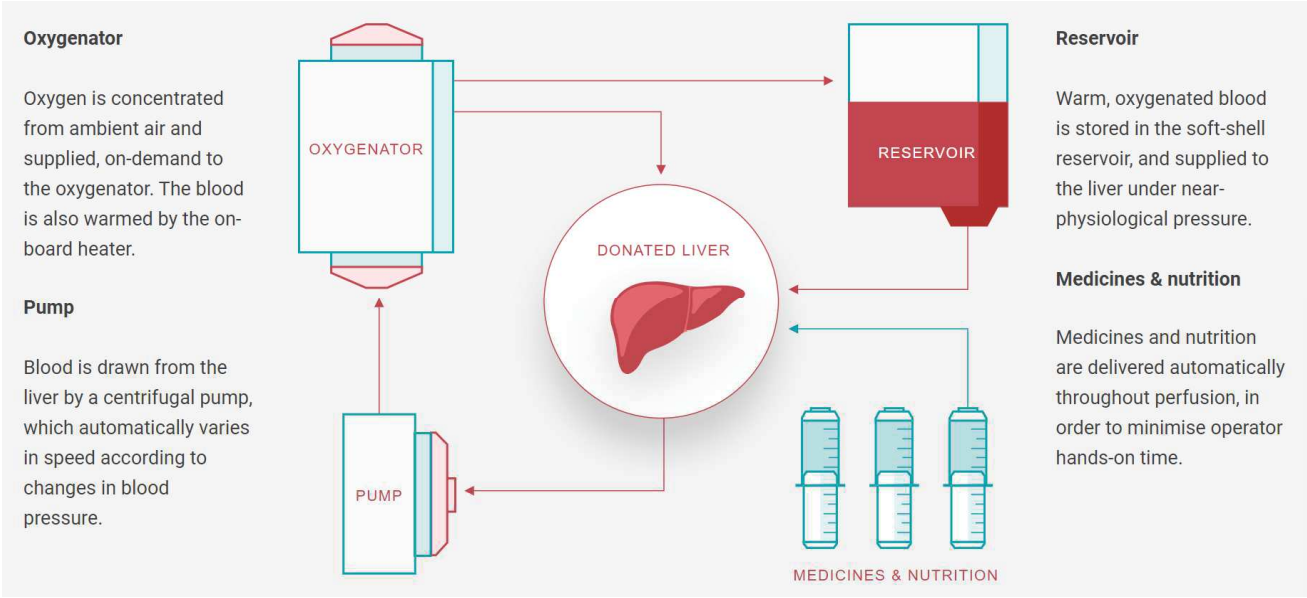


Figure 5: Map of OrganOx Metra

Conventional cold preservation involves storage of the liver at 4°C and aims to minimise liver degradation. The Metra, however, recreates a near physiological environment by continuously perfusing the liver at near physiological pressures and flows with oxygen-carrying red blood cells at 37°C. The liver remains functional during preservation, producing bile, metabolising glucose and maintaining pH, allowing the objective assessment of organ performance prior to transplant.
Via onboard blood gas analysis, the Metra automatically measures and controls blood gases in the perfusate without user intervention.

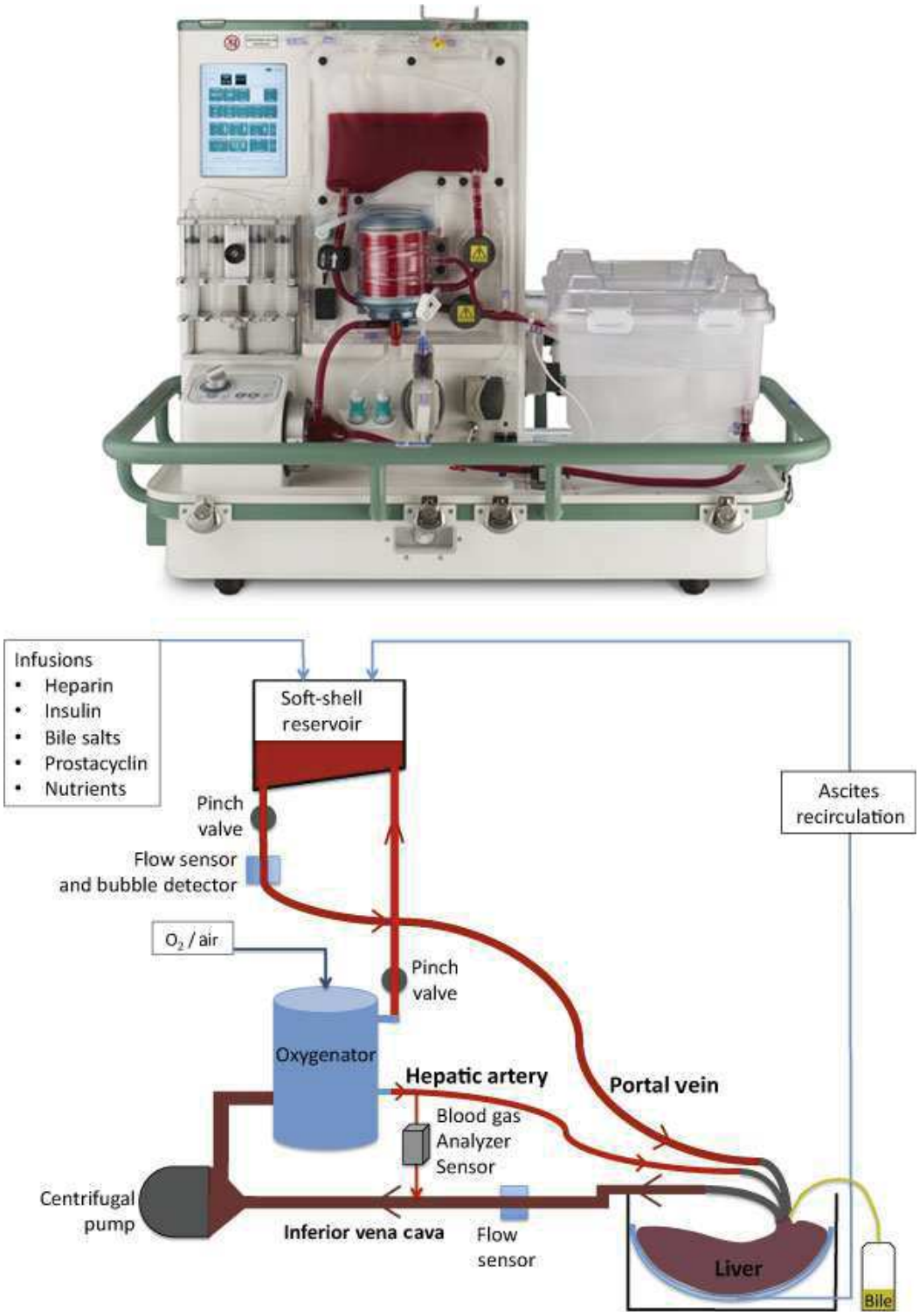


Figure 6: Block scheme OrganOx Metra

In Fig.6 we can appreciate a block scheme of OrganOx Metra useful in order to know all its components. This perfusion machine provides continuous flow through the hepatic artery and portal vein, using a single pump delivering blood to the artery directly and to the portal vein via a reservoir. This technology uses a normothermic suspension of red cells in a colloid to perfuse the liver in a fully cannulated system. Briefly, the perfusate is pumped out of the inferior vena cava using a centrifugal pump, heated and oxygenated. It is then diverted either to the hepatic artery through a high-pressure, low-flow system or to the soft-shell reservoir which feeds the portal vein via a high-flow, low pressure system. Constant blood gas analysis enables monitoring and control of pO2 and pCO2 levels, facilitating maintenance acid-base homeostasis. Continuous infusions ensure sufficient vasodilation, protection against coagulation and the provision of an environment that enables near physiological metabolic and synthetic liver function. The system is designated to perfuse liver for up to 24 h [13].

2.2.1.2 Liver Assist

The Liver Assist (Fig.7) is a dedicated device for ex vivo perfusion of donor livers. Two different pump units provide a pulsatile perfusion of the hepatic artery and continuous flow to the portal vein. The oxygenated perfusion is pressure controlled. Despite the OrganOx Metra, with this MP the temperature can be set from hypothermic to normothermic conditions thanks to an integrated heater/cooler [13]. Here are reported some features which characterize this machine:

- Isolated dual perfusion of donor livers
- Pulsatile perfusion of Hepatic artery (60 bpm)
- Continuous flow pattern to Portal vein
- Pressure controlled perfusion
- Oxygenation of the perfusion solution/blood
- Dedicated perfusion pressure and flow settings
- Easy priming procedure
- Automatic de-airing
- Mobile device
- Easy controllable by two press-dial buttons
- Unique design liver holder which supports liver
- Specially developed cannulas
- Temperature adjustable from 10 to 38 degrees Celcius
- Dedicated flow and pressure settings

Figure 7: Liver Assist

How it works:

The Liver Assist (Fig.8) is a CE-marked pressure-controlled device that provides pulsatile flow through the hepatic artery and continuous flow through the portal vein. For this, it uses a dual system of rotary pumps and hollow fibre membrane oxygenators. Temperature settings are not fixed and can be manually altered by the user. These are displayed on the screen in real time. The liver is cannulated via the coeliac artery or aorta and portal vein. The inferior vena cava is left open and drains into the reservoir. The cystic duct is ligated and the common bile duct is catheterized. As the device is not transportable, livers are cold preserved from the retrieval centre to the transplant centre before being placed on the device. The Liver Assist can be used in hypothermic, subnormothermic or normothermic modes. This enables slow rewarming after cold storage, which has been shown to be beneficial [13].

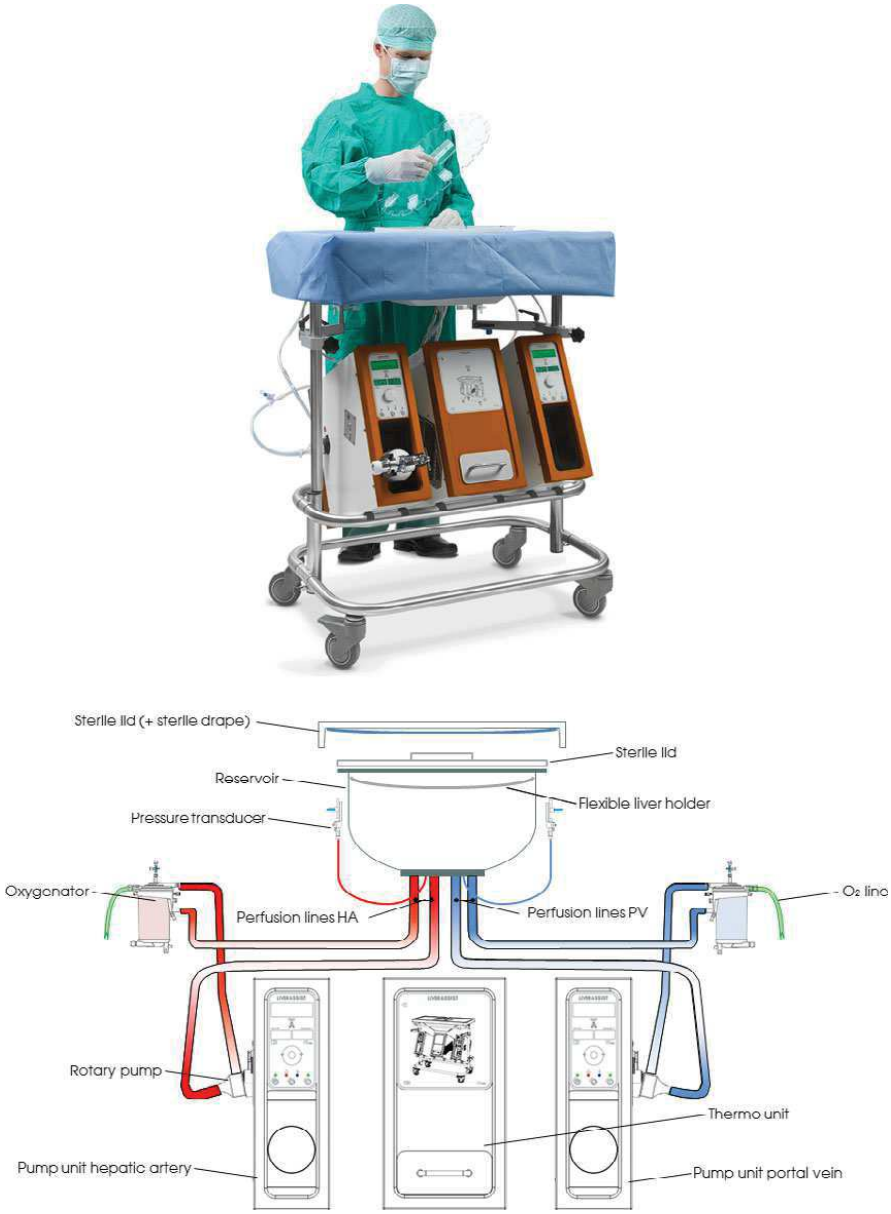


Figure 8: Block scheme Liver Assist

2.2.2 Hypothermic Machine Perfusion

Currently it is avaiable only one device that is able to perfusate the Liver in a hypothermic situation: Liver Assist machine.
As said before, with this machine we are able to choose the desired temperature to be used. Despite to NMP that can be applied both in situ (eg, in donors before procurement) or ex situ, during or after organ transport to the recipient center, HMP is currently performed after cold storage, that is, just before implantation in a recipient. Here are reported some advantages of this approach:

- Significant reduction in peak alanine-aminotrasferase levels
- Reduction in intrahepatic cholangiopathy
- Reduction in biliary complications
- Improved 1 year graft survival when compared to SCS (static cold storage) with donation after cardiac death

However, the advantages or disadvantages of this approach with respect to NMP remain until now unclear.

2.3 Imaging techniques

The liver is affected by various pathologies that maybe characterized as diffused or focal as well as benign or malignant, each requiring different management. Although detection and characterization are the two primary objectives of liver imaging, the new expectations from imaging has increased due to a better understanding of disease processes as well as the availability of more refined and individualized treatment options [14]. Imaging modality current being used to detect and characterized liver lesions, include computed tomography (CT) and magnetic resonance imaging (MRI) [14].
In liver transplantation candidates, the goal of imaging techniques is to evaluate the pre and postoperative assessment of the patients. Preoperative assessment of potential living liver donors requires the evaluation of liver parenchyma to identify steatosis or lesions; the accurate evaluation of intra and extrahepatic biliary and vascular anatomy to identify congenital variants and to prevent accidental removal at surgery; and an accurate estimation of the volume of both liver lobes to exclude complications related to graft volume [4]. In the post-transplant period, the goal of imaging is to identify vascular and biliary complications. The long-term follow-up also allows clinicians to identify recurrence of the primary disease and/or detect disease related to long-term immunosuppression [4]. In particular, in the pediatric recipient a wide spectrum of diffuse and focal diseases are indications for LT.

2.3.1 Computed tomography

The term “computed tomography”, or CT, refers to a computerized x-ray imaging procedure in which a narrow beam of x-rays is aimed at a patient and quickly rotated around the body, producing signals that are processed by the machine’s computer to generate cross-sectional images, or “slices”, of the body. These slices are called tomographic images and contain more detailed information than conventional x-rays. In particular, dense tissues, such as bones, appear white in the pictures produced by a CT scan: less dense tissues, such as brain tissue or muscles, appear in shades of gray and air-filled spaces, such as in the bowel or lungs, appear black.
Once a number of successive slices are collected by the machine’s computer, they can be digitally “stacked” together to form a three-dimensional image of the patient that allows for easier identification and location of basic structures as well as possible tumors or abnormalities [15].
Unlike a conventional x-ray, which uses a fixed x-ray tube, a CT scanner uses a motorized x-ray source that rotates around the circular opening of a donut-shaped structure called a gantry. During a CT scan, the patient lies on a bed that slowly moves through the gantry while the x-ray tube rotates around the patient, shooting narrow beams of x-rays through the body (Fig. 9).

Figure 9: Computed tomography

The process can be divided into different phases:

1. Scanning of the patient
2. Data acquisition
3. Image reconstruction
4. Image display
5. Image archival

At the end, image slices can either be displayed individually or stacked together by the computer to generate a 3D image of the patient that shows the skeleton, organs, and tissues as well as any abnormalities the physician is trying to identify. This method has many advantages including the ability to rotate the 3D image in space or to view slices in succession, making it easier to find the exact place where a problem may be located (Fig.10-11).

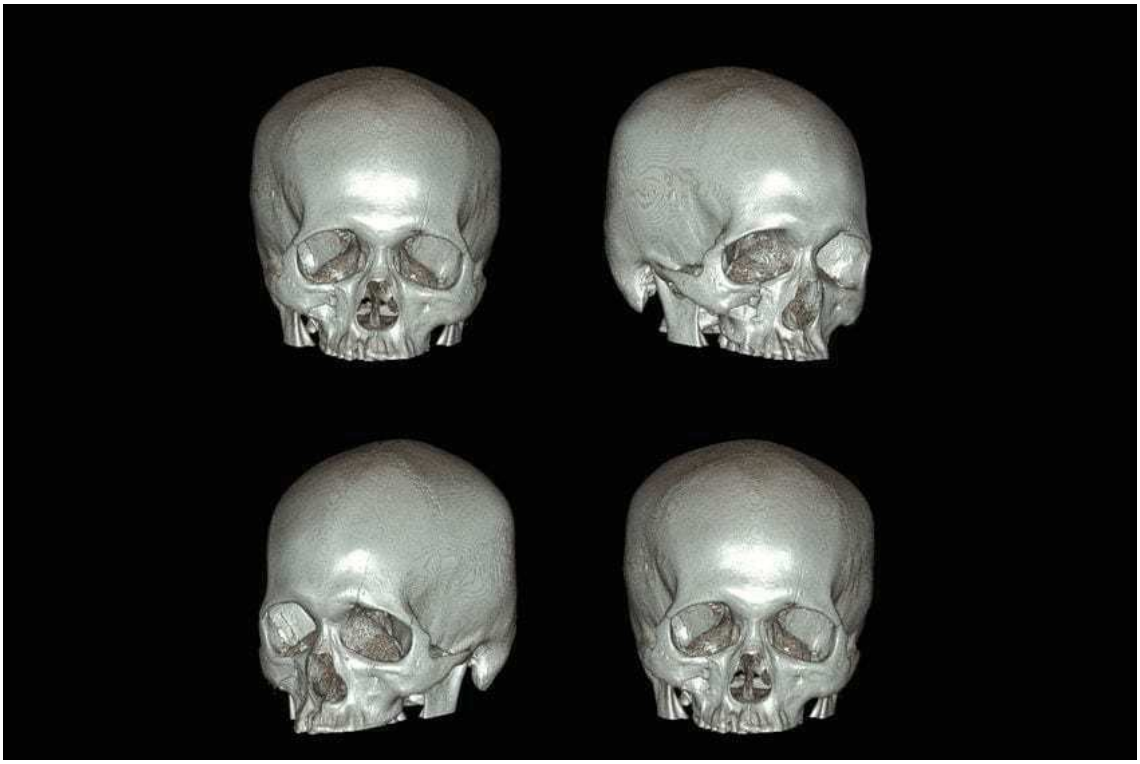


Figure 10: 3D Computed tomography

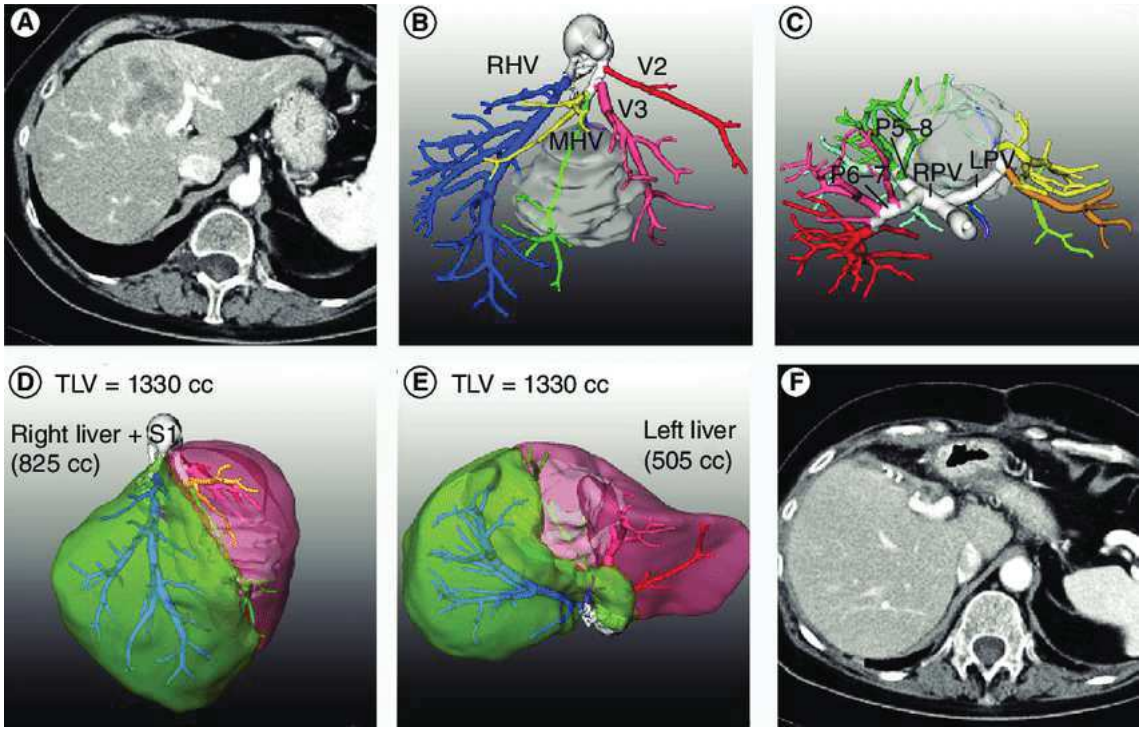


Figure 11: 3D Liver Computed tomography

2.3.1.1 Applications

Dynamic contrast-enhanced perfusion Computed Tomography (CT) measures the temporal changes in tissue enhancement after injection of contrast medium; it has emerged as a powerful tool for the functional evaluation of tissue perfusion in a variety of clinical and research applications, particularly in the assessment of vascular and oncologic diseases [16].
Perfusion imaging has been studied and validated clinically most extensively in the evaluation of cerebral blood flow in acute ischemic stroke owing to the lack of motion within the brain.
In recent years, perfusion CT has found further applications outside of the brain in numerous tissues for the diagnosis, grading, staging, prognostic evaluation and therapeutic planning and monitoring of various diseases [16].
Although perfusion CT has been studied for nearly 3 decades, several technological and practical limitations have restricted its clinical use, such as: limited scan range, temporal resolution and radiation dose. Furthermore, specific applications such as body perfusion are also hampered by respiratory motion artefacts and organ deformability. However, recent advances in multidetector CT systems seem to address some of the aforementioned limitations, due to the current availability of scanners with faster rotation times and larger scanning ranges [16].
As said before, in liver transplantation (LT) candidates, the goal of imaging is to evaluate the intra- and extra-hepatic anatomy, identify conditions that can complicate LT, and stage the neoplastic disease [4]. In order to reach this goal, Multi detector row CT can provide important information about liver morphology (normal or cirrhotic), intrahepatic and extrahepatic malignancy, venous benign and/or malignant thrombosis, patency of main portal vein, portosystemic collateral due to portal hypertension (spleno-renal spontaneous shunt, gastroesophageal and/or paracaval varices, and paraumbilical and caput medusae), celiac stenosis, splenic artery aneurysm, congenital arterial variants, patency, and anomalies of the inferior vena cava [4].
These findings may influence the decision to transplant.

2.3.2 Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) is a non-invasive imaging technology that produces three dimensional detailed anatomical images. It is often used for disease detection, diagnosis, and treatment monitoring [17].
MRIs employ powerful magnets which produce a strong magnetic field that forces protons in the body to align with that field.
When a radiofrequency current is then pulsed through the patient, the protons are stimulated, and spin out of equilibrium, straining against the pull of the magnetic field. When the radiofrequency field is turned off, the MRI sensors are able to detect the energy released as the protons realign with the magnetic field. The time it takes for the protons to realign with the magnetic field, as well as the amount of energy released, changes depending on the environment and the chemical nature of the molecules. Physicians are able to tell the difference between various types of tissues based on these magnetic properties [17].
To obtain an MRI image, a patient is placed inside a large magnet and must remain very still during the imaging process in order not to blur the image (Fig. 12). Contrast agents (often containing the element Gadolinium) may be given to a patient intravenously before or during the MRI to increase the speed at which protons realign with the magnetic field [17]. The faster the protons realign, the brighter the image.

Figure 12: Magnetic Resonance Imaging

Although MRI does not emit the ionizing radiation that is found in X-ray and CT imaging, it does employ a strong magnetic field. The magnetic field extends beyond the machine and exerts very powerful forces on objects of iron, some steels, and other magnetizable objects [17].
Because of this reason, when having an MRI scan some things should be taken into consideration:

- People with implants, particularly those containing iron such as pacemakers, vagus nerve stimulators, implantable cardioverter, defibrillators.
- Noise: loud noise commonly referred to as clicking and beeping in certain MR scanners, may require special ear protection
- Nerve Stimulation: a twitching sensation sometimes results from the rapidly switched fields in the MRI
- Contrast agents: patients with severe renal failure who require dialysis may risk a rare but serious illness called nephrogenic systemic fibrosis that may be linked to the use of certain gadolinium-containing agents, such as gadodiamide and others
- Claustrophobia: people with even mild claustrophobia may find it difficult to tolerate long scan times inside the machine.

Despite these, MRI is potentially one of the best imaging modalities since unlike CT, it does not have any ionizing radiation that could potentially be harmful. However, one of the most difficult challenges that MRI technicians face is obtaining a clear image, especially when the patient is a child or has some kind of ailment that prevents them from staying still for extended periods of time [17].
MRI scanners are particularly well suited to image the non-bony parts or soft tissues of the body (Fig.13). As said before, they differ from computed tomography (CT), in that they do not use the damaging ionizing radiation of x-rays. The brain, spinal cord and nerves, as well as muscles, ligaments, and tendons are seen much more clearly with MRI than with regular x-rays and CT; for this reason, MRI is often used to image knee and shoulder injuries [17].

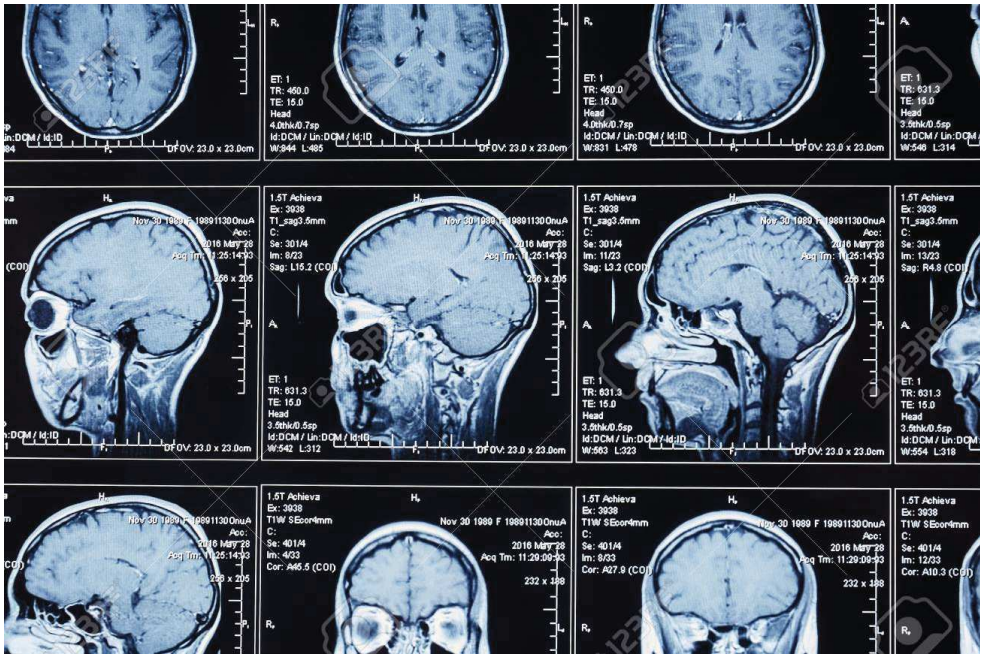


Figure 13: MRI brain scan

A still open discussion regarding the MRI, is the use of a 3 Tesla MRI instead of a 1.5 Tesla MRI. Tesla (T) is the unit of measurement used to quantify the strength of a magnetic field in an MRI machine.
Most MRI scanners operate at a strength of 1.5 Tesla. A 3 Tesla MRI, however, operates at twice the normal strength, providing a greater signal-to-noise ratio, which is a major determinant in generating the highest quality image.
Despite this, moving from the standard of 1.5 T to higher field strength implies a number of potential advantage and drawbacks, requiring careful optimization of imaging protocols or implementation of novel hardware components [18].
1.5 Tesla systems still represent the technical standard for abdominal MRI. Nonetheless, the use of ultra-high field strength is a major focus in liver imaging, given the ever-increasing number of new 3.0 T magnets installed worldwide for research and clinical practice [18]. In theory, 3.0 T magnets have the capability to provide better image quality as the base for improved diagnostic performance.
This is because doubling the field strength (almost) doubles signal-to-noise ratio, that is the quantity of signal made available from the patient in order to build MRI images. Exceeding signal can be converted into better image detail (higher spatial resolution) and/or faster acquisition (higher temporal resolution), as well as more efficient fat suppression and better lesion conspicuity because of improved lesion-to-liver contrast after gadolinium administration. Both conventional imaging and functional techniques such as diffusion-weighted imaging (DWI), dynamic contrast-enhanced MRI and spectroscopy may benefit from the above changes [18].
On the other hand there are some drawbacks: the invisible force associate with these systems can be extremely dangerous if proper precautions and facility planning is not taken. These hazards may be attributed to one or more combinations of the three components that make up the MRI environment. Strong static magnetic fields including its associated spatial gradient, pulsed gradient magnetic fields and pulsed radio frequency (RF) fields are potential hazards when patients and medical devices are placed within the MRI environment [19]. In addition, the gradient magnetic field is responsible for the ambient noise associated with MRI: the noise associated with the gradient fields increase with field strength.
So in conclusion, the potential benefits of MRI are abundant, however there are dangers in the environment that must be acknowledged and respected.

2.3.2.1 Applications

As discussed before, MRI is a non-invasive and sensitive technique that is devoid of ionizing radiation. For this reason, MRI is the preferred modality in the assessment of pediatric recipients. If necessary, contrast medium is injected: using Gadobenato dimeglumina it is possible to obtain information about perfusion of the liver, changes in the parenchyma and the vasculature related to cirrhosis and portal hypertension, and to detect vascular congenital anomalies [4].
Regarding the radiological assessment of potential liver donors, MRI is currently considered the primary imaging tool for biliary anatomy evaluation in potential living liver donors. Although the congenital variants of biliary anatomy do not represent a

contraindication to liver donation, they must be identified before surgery to prevent ligation of major branches of the right lobe in the recipient and/or of the liver lobe in the donor [4]. Multiple biliary anastomoses during the implantation of the right lobe into the recipient can be required to avoid atrophy due to biliary obstruction.
In conclusion, donor imaging is important, and correct identification of donor anatomy is essential to determine whether the donor is a suitable candidate, liver disease is present, and hepatectomy may be safely performed with no risk to the donor [20]. This goal can be reached by using MRI, in order to have necessary information.

Chapter 3

Results

3.1 Test using a liver puppet

In order to achieve the goal of this project, and so to know if it is possible and useful using some imaging techniques for studying a liver during a perfusion, a first test was done. We focused on using a Magnetic Resonance Imaging for our research because of different reasons.
In general, before the transplantation, we can face with two different situations: most of the time the liver is an adult liver, which will be then subjected to a split procedure in order to have two partial livers that can be then transplanted in pediatric and adult recipients. Another situation is to have a liver which comes from a liver transplantation (LT) recipient with metabolic disease.
As said before, regarding the radiological assessment of potential liver donors, MRI is currently considered the primary imaging tools for biliary anatomy evaluation of potential living liver donors. In addition, through MRI we can have more information about the volume of the liver (the entire liver and all its parts). In this way we can create 3D anatomical models of the liver that can be very useful for the simulation of a split procedure or of a transplant. Sometimes we can face also with a situation where the liver comes from a deceased donor, so because it has not been subjected to previous evaluation, it is fundamental doing an evaluation of the organ through MRI for example.
The test was performed on Ospedale Pediatrico Bambin Gesù of Rome - San Paolo, which is one of the three sites of the hospital, because in this location there iù
a very powerful Magnetic Resonance Imaging of 3 T, so one of the most powerful available in the market for a clinical use.
During this first test, we didn't have a real liver available, so we decided to use a liver puppet in order to reproduce the presence of a real organ within the machine perfusion.
First of all, there were numerous technical problems connected with this experiment, so this was a first real approach to the situation, in order to know if it was really possible doing all the things that we studied and planned before.
The first big problem was correlated with the fact that we wanted a liver which was, in the same time, perfused within the Liver Assist machine, which is the perfusion machine used on Ospedale Pediatrico Bambin Gesù, and scanned by a Magnetic Resonance Imaging. As we can imagine this was a very big problem, because this means that two incompatible machines had to work together; I said incompatible because the machine perfusion Liver Assist is made of plastic and metal material, and as we know, we cannot put a metal material in a MRI. In order to solve this problem there were two different possible approaches: to put the entire Liver Assist device within the MRI, trying to screen somehow the metal materials which were present, or to put only

the liver inside the MRI, but in the meanwhile it must be connected with all the pipes to the machine perfusion in order to be perfused.
The second approach was the best one so we decided to move in this way: first of all we measured the distance between the centre of the MRI and the location (outside the room of the MRI) where the Liver Assist device had to be put. Following our approach, we needed that all the pipes that connected the liver to the Liver Assist device were as long as we need in order to go from the inside to the outside of the room where the MRI was placed. This distance was more or less 6 metres, so before doing the experiment we modified all the Liver Assist pipes in order to be as longer as we wanted. Obviously, as I will explain after, this modification was connected to a series of problems regarding the pressure and the flow, because having pipes so long means also loss of flow and so of pression. As said before, we did not use a real liver for this first test but a liver puppet; however, the machine perfusion worked during the experiment, as if the organ was really perfused. The only difference is that both arterial and portal cannula, that are connected to the liver in a real situation, were disconnected; in order to recreate a kind of resistance, we used two clamps to compress these two cannula as if they were connected to an organ.
The Liver Assist device was installed, with all his pipes and sensors and was connected to the puppet liver. After all the checks in order to know if the machine perfusion was working well, we positioned the liver puppet on the bed of the MRI, while the Liver Assist was outside the room where the MRI was placed.
As I anticipated before, there were some problems connected with this configuration: first of all the fact that, because we moved the liver from the perfusion machine to another location, we had to make sure that the height of the actual position was the same of that of Liver Assist, this because of problem of pressure. Another problem was connected with the pipes: in fact, in order to keep the entire Liver Assist outside the room we had to stretch all the pipes to more or less 6 metres; this means that, obviously, there was a loss of flow and pressure between the machine perfusion and the real position of the organ. As a consequence, the values of flow and pressure that we saw on the screen of Liver Assist, that were measured through sensors, were not totally real.
The MRI that we used during this experiment is an extremely powerful machine: it is the Magnetom Vida, a Siemens Healthineers MRI.
Here, I report the images done during the experiment (Fig.14-15).
These images represent the moment where the experiment was done: as we can see in the first image (Fig 14) the technician was positioning the box with the puppet liver inside the MRI; instead, in the second image (Fig.15) we can see the acquisition of the images during the experiment. In both images we can see all the pipes that, coming from the box where the puppet liver is stored, goes outside the MRI. From these images we can also see a coil that must be positioned above the box in order to do the acquisition of the images.

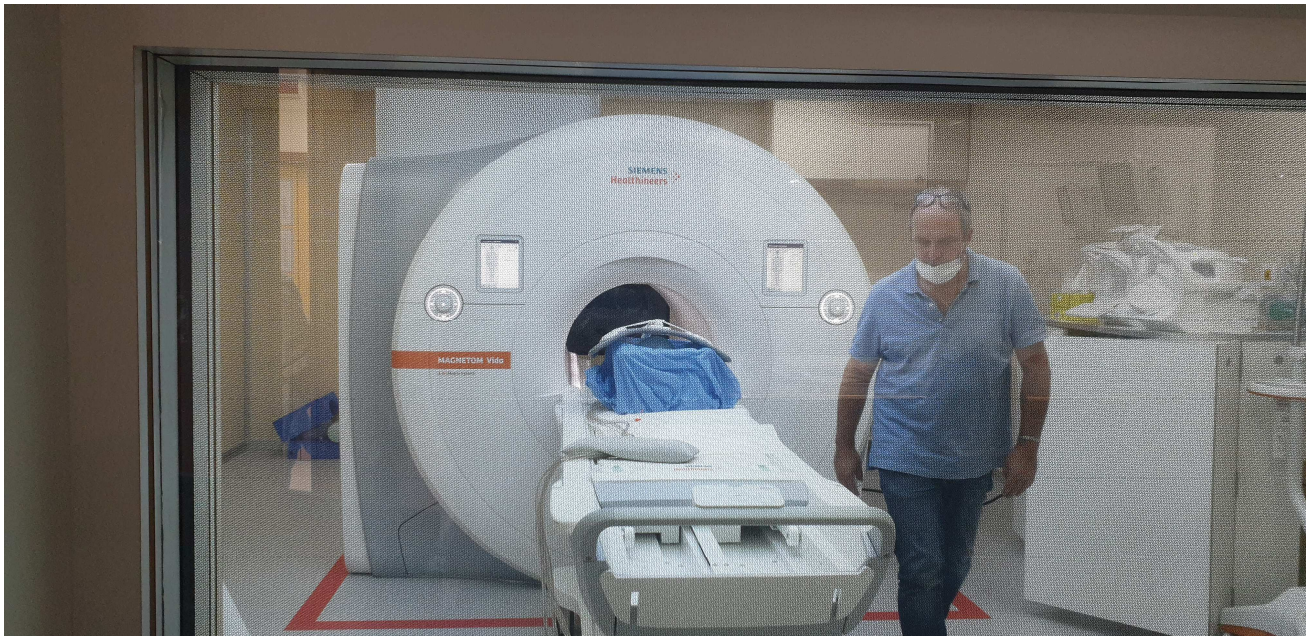


Figure 14: MRI used during the experiment

Figure 15: MRI used during the experiment

The results that we obtained from this first test were good.
In fact, we expected some artefacts coming, first of all, by the fact that the door of the room must remain a bit opened in order to pass the pipes out the room. Another thing that we expected were artefacts coming from the movement of the pipes during the perfusion: in fact, because the perfusion machine works through some pumps normally there is a slight movement of the pipes during the perfusion of the organ. Despite these movements, we didn't recognize artefacts that can affect the quality of the images.
As a first test, we did different images acquisition, here I reported some of these.

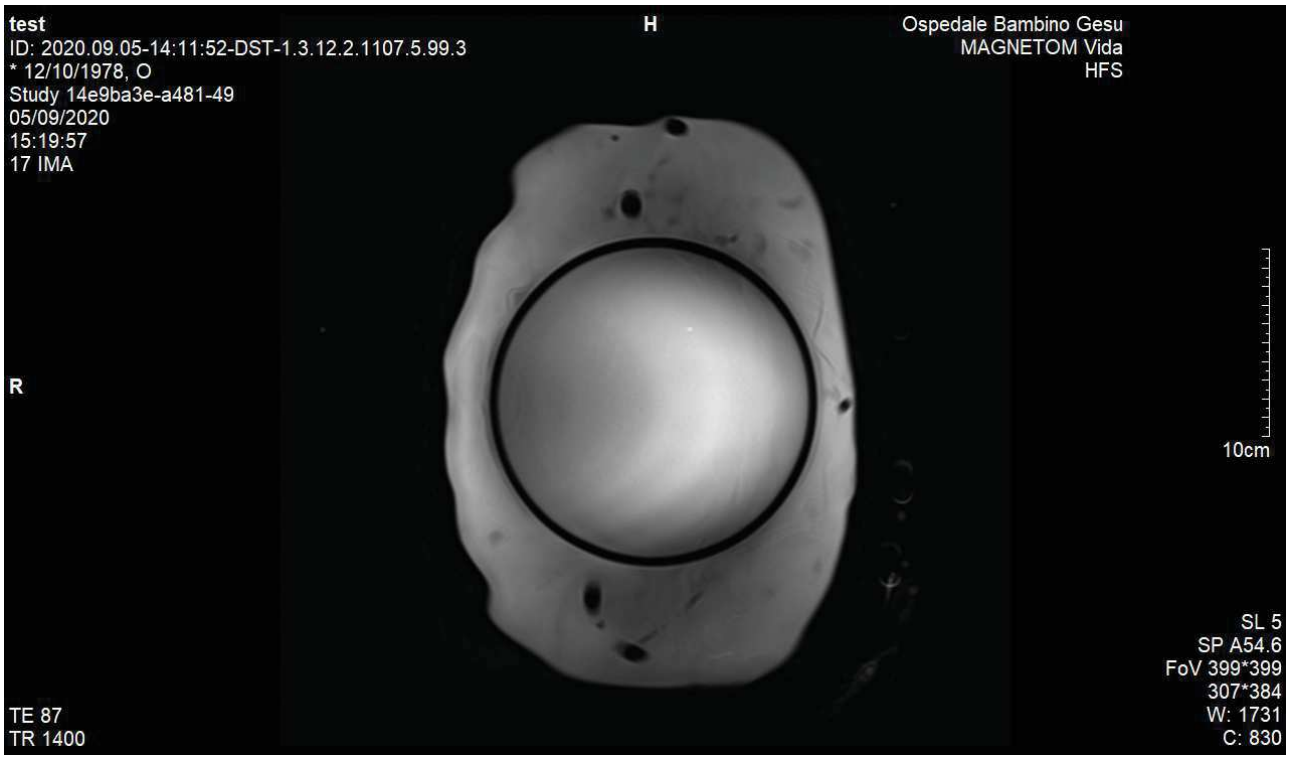


Figure 16: MRI scanned image

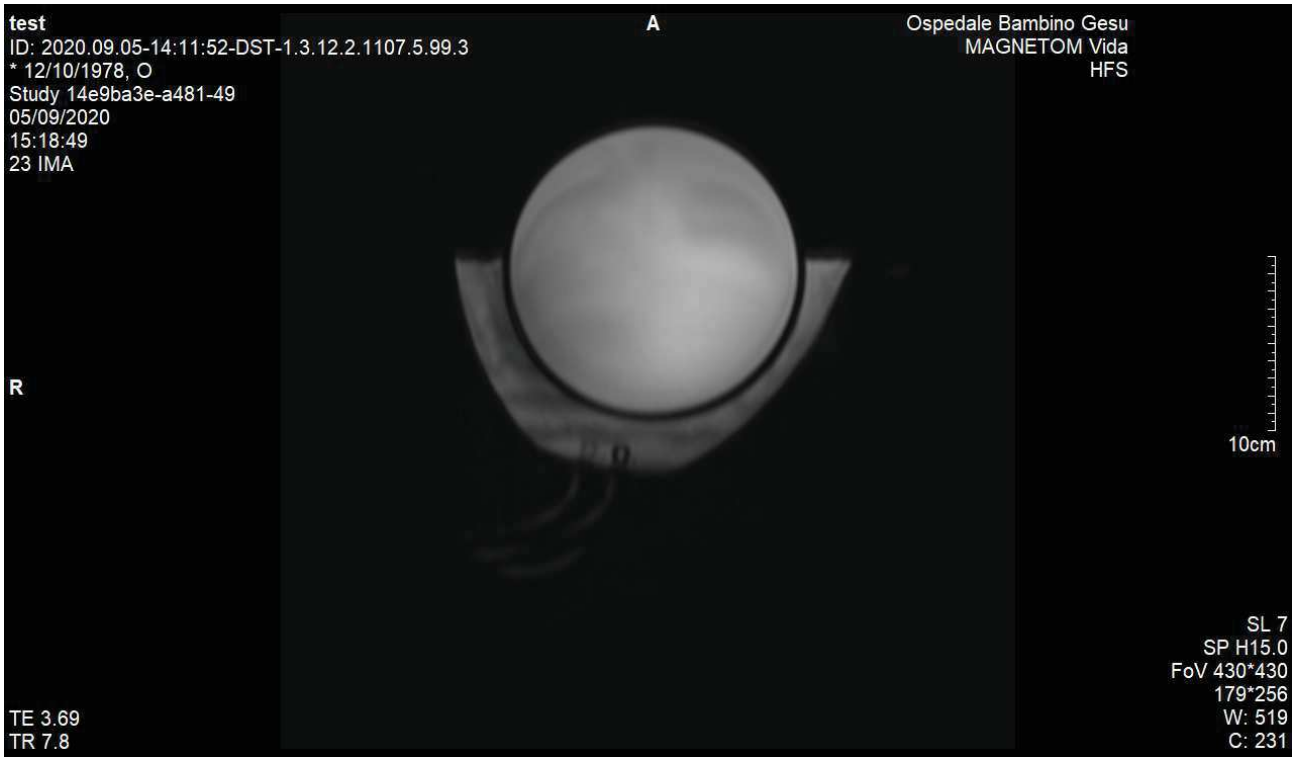


Figure 17: MRI image scanned

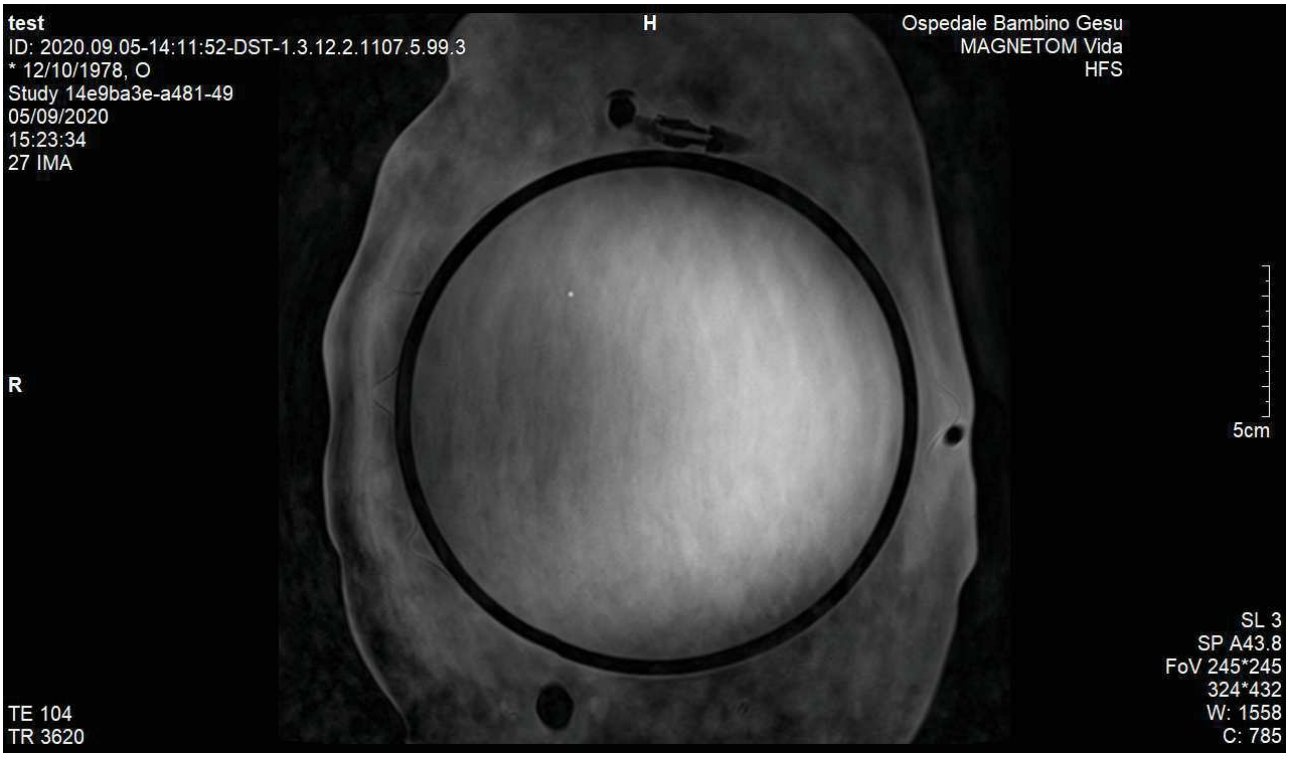


Figure 18: MRI scanned image

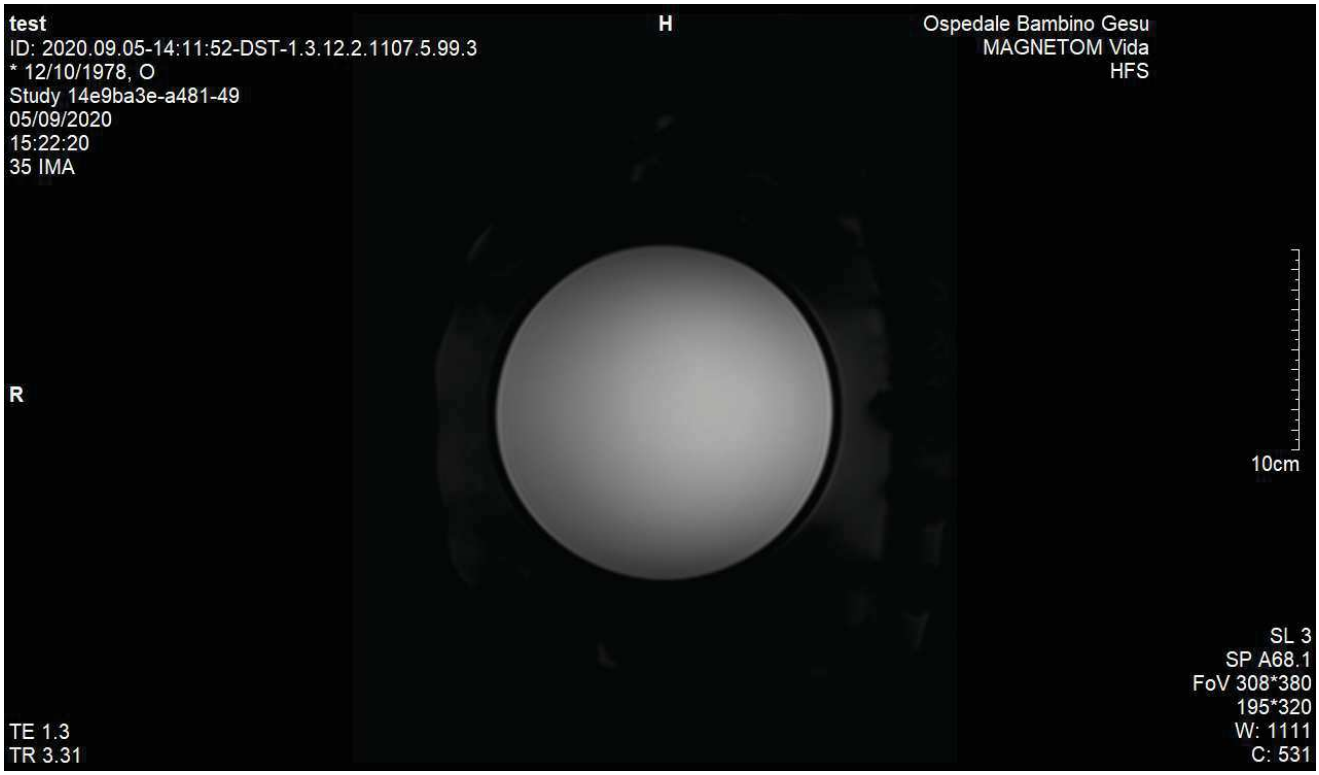


Figure 19: MRI scanned image

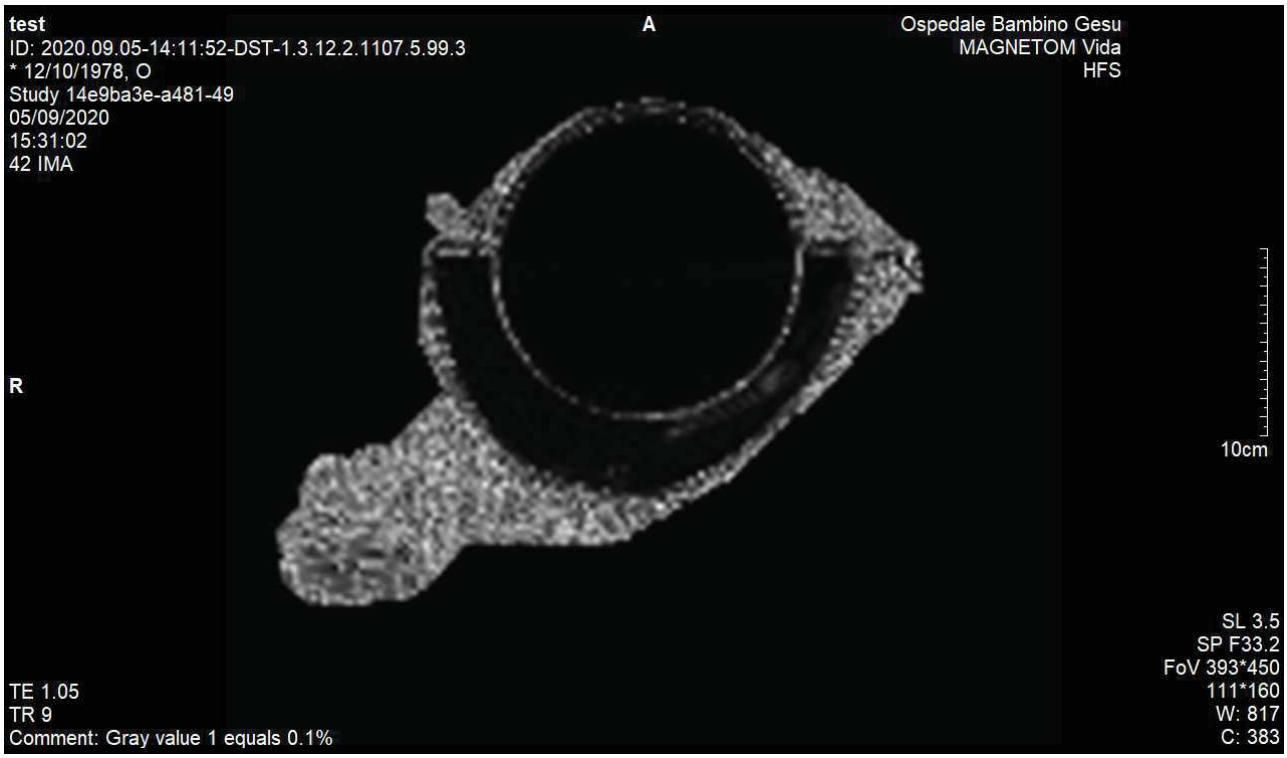


Figure 20: MRI scanned image

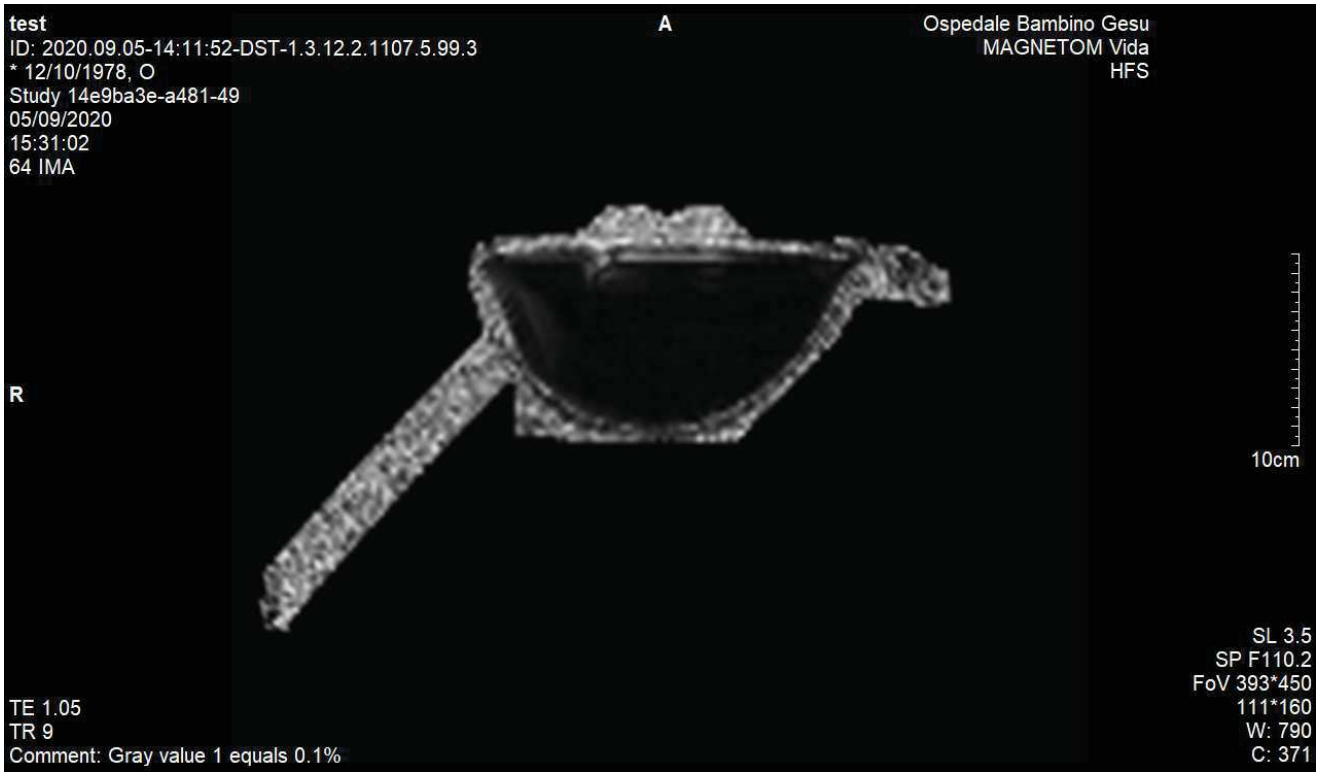


Figure 21: MRI scanned image

As we can appreciate from these images, the results are good.
On Fig.16 we can clearly see the puppet liver, which is like a ball, and a structure around this. The structure is the box where the liver puppet is stored; this is a sort of basin, made of two layers of soft plastic material: the first layer is perforated because it is the basin where the liver is stored and through these holes all the liquid that comes from the organ can pass and go down on the second layer; this last one is closed, a sort of storage. This structure belongs to Liver Assist device and so, in a normal configuration, must be placed above the perfusion machine. Here I report two images of this box (Fig.22-23).

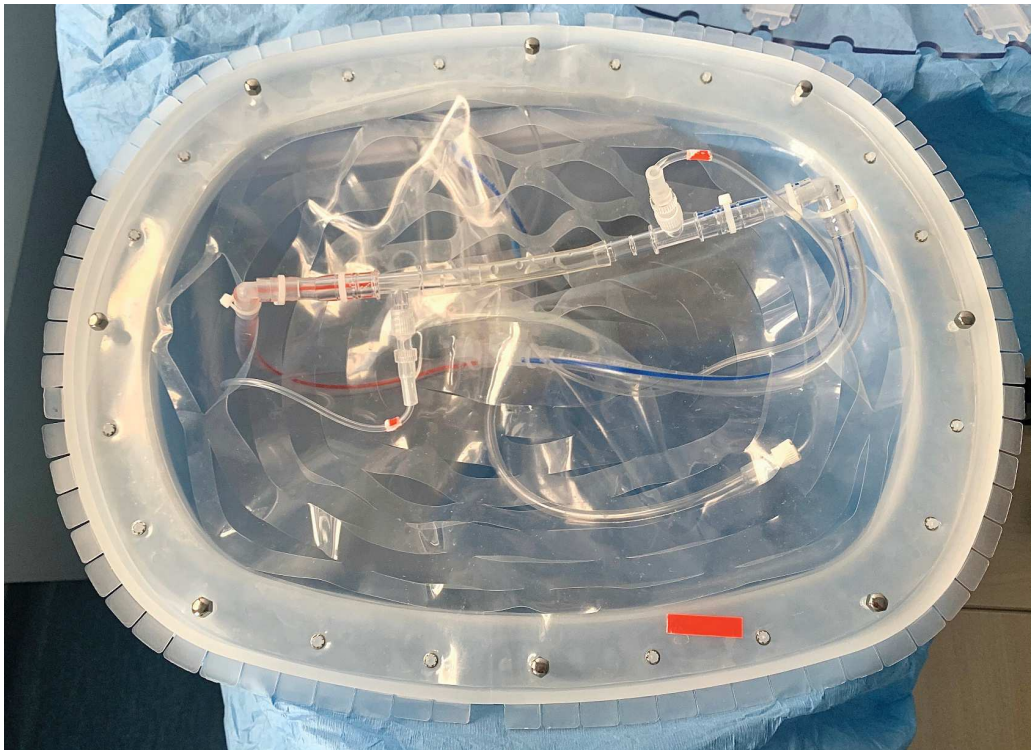


Figure 22: Box for liver during perfusion

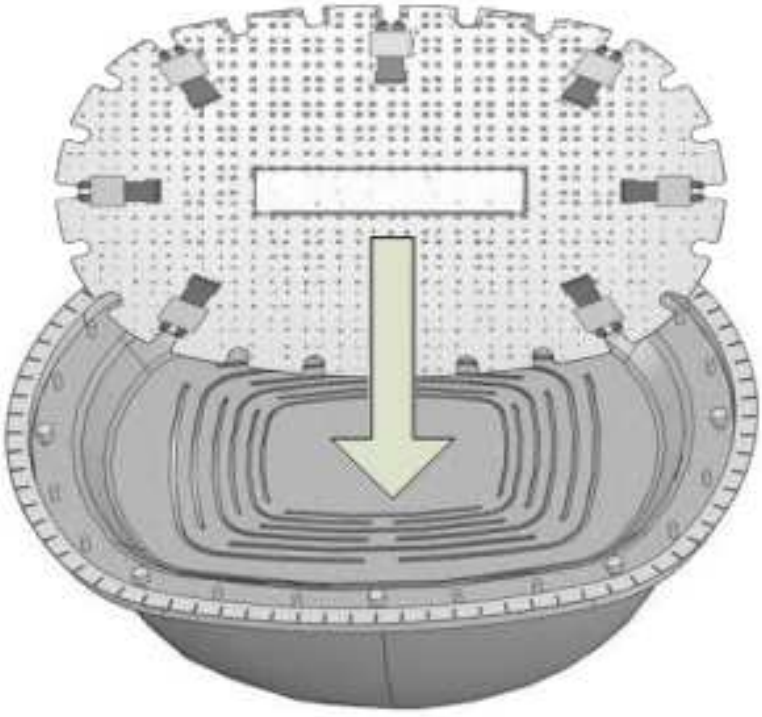


Figure 23: Structure of the box

Obviously, we can appreciate the presence of this structure on the images acquired on MRI. In particular, on the second image (Fig.17) we can also see the pipes that come out from the box. Fig.18 is fundamental, because it is a particular image that we decide to acquire as a test. In this image we can see that there are some artefacts because of the slight movement of the water where the liver is stored. In fact, we have to remind that the liver, because is perfused, is partially immersed into a liquid, and so in some particular acquisitions this fact could affect the quality of the image. On Fig. 18 we can also see some pipes that are present on the box. Regarding the presence of water (or, in general, of a liquid) Fig.20-21 represent a sort of post processing that this new technology of MRI is able to do: in fact, this MRI automatically recognises the presence of a liquid that can affect the quality of the image and deletes it. In this way, a sort of post processing of the image is automatically done by the machine itself.
So, finally, the results are clear and defined images as we can particularly appreciate on Fig.19.

Chapter 4

Discussion

Despite numerous technical problems that were present during this first test, very good results were obtained from the acquisition of the images. Obviously, some little adjustments are needed in order to perform a better test next time, that will be done using a real liver.
The most important thing that came out from this first test was the need of a kind of support for the box containing the liver. In fact, as we said before, the box that contains the liver is made of soft plastic material and it is full of water (or in general liquid), so it is not possible to put directly this box above the bed of MRI, because it is not a rigid body. So, it is fundamental to design a support that must be rigid, stable and made of a material which is compatible with MRI. Starting from the shape of the external ring of the box (Fig.24), which will be put on this support, I design a support using the software Rhynoceros.

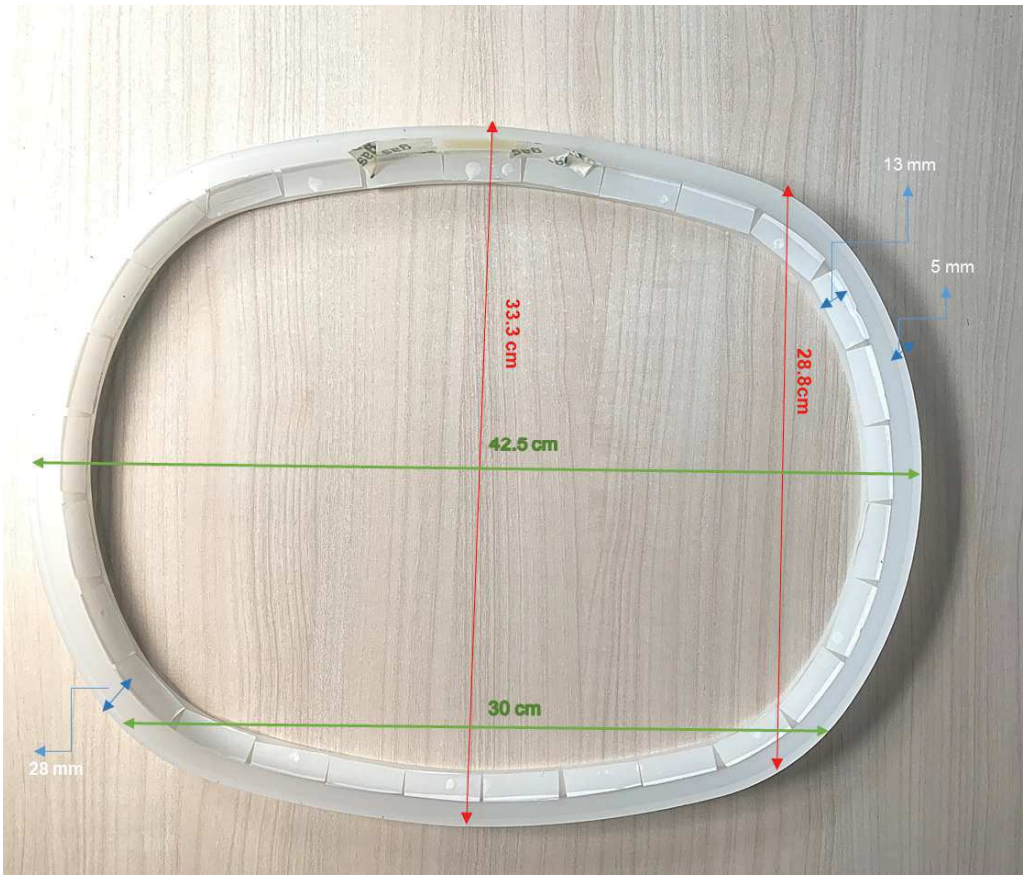


Figure 24: External ring of the box containing the liver

As we can see on this figure, the shape of the external ring is not a regular oval, as a consequence was very difficult to create a support of the same shape. However, I decided to project a support with a shape made in a way that the external ring can lean as much as possible on this (Fig. 25-26-27).

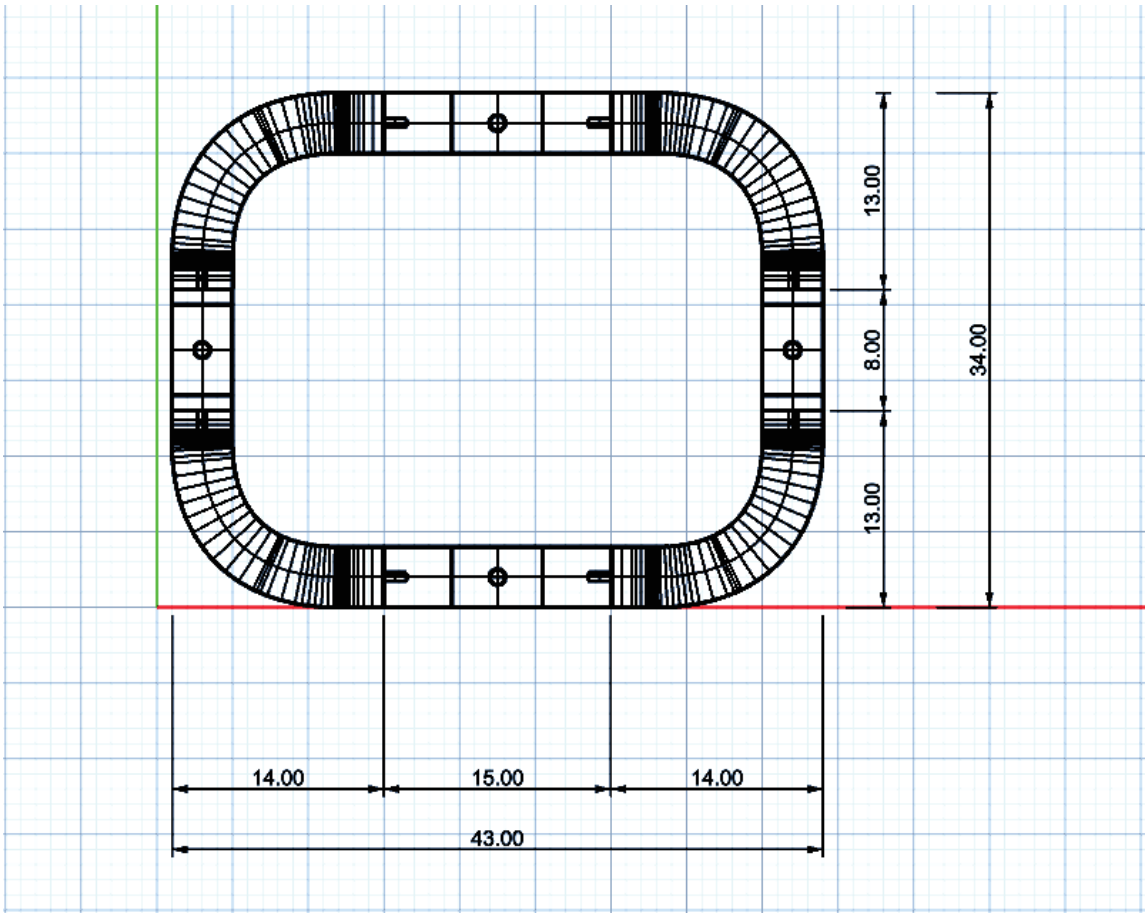


Figure 25: Top view of the support

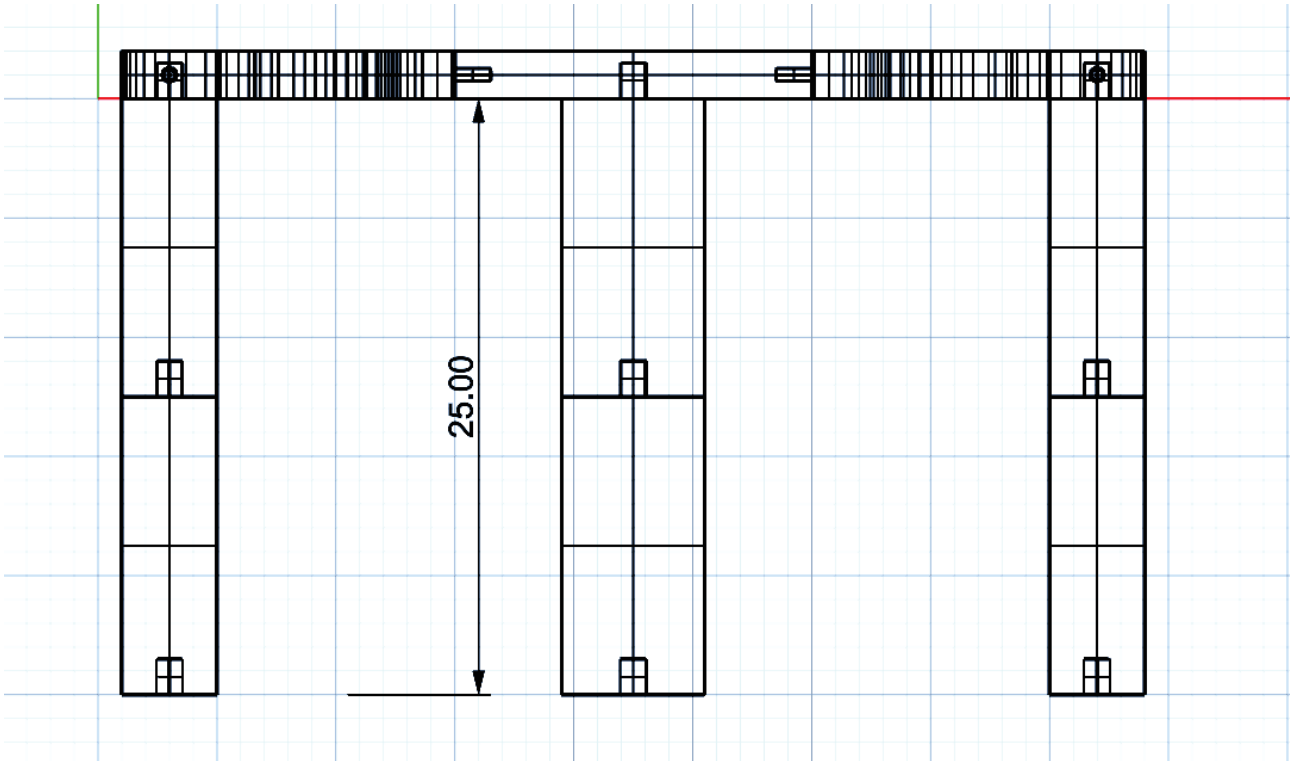


Figure 26: Lateral view of the support

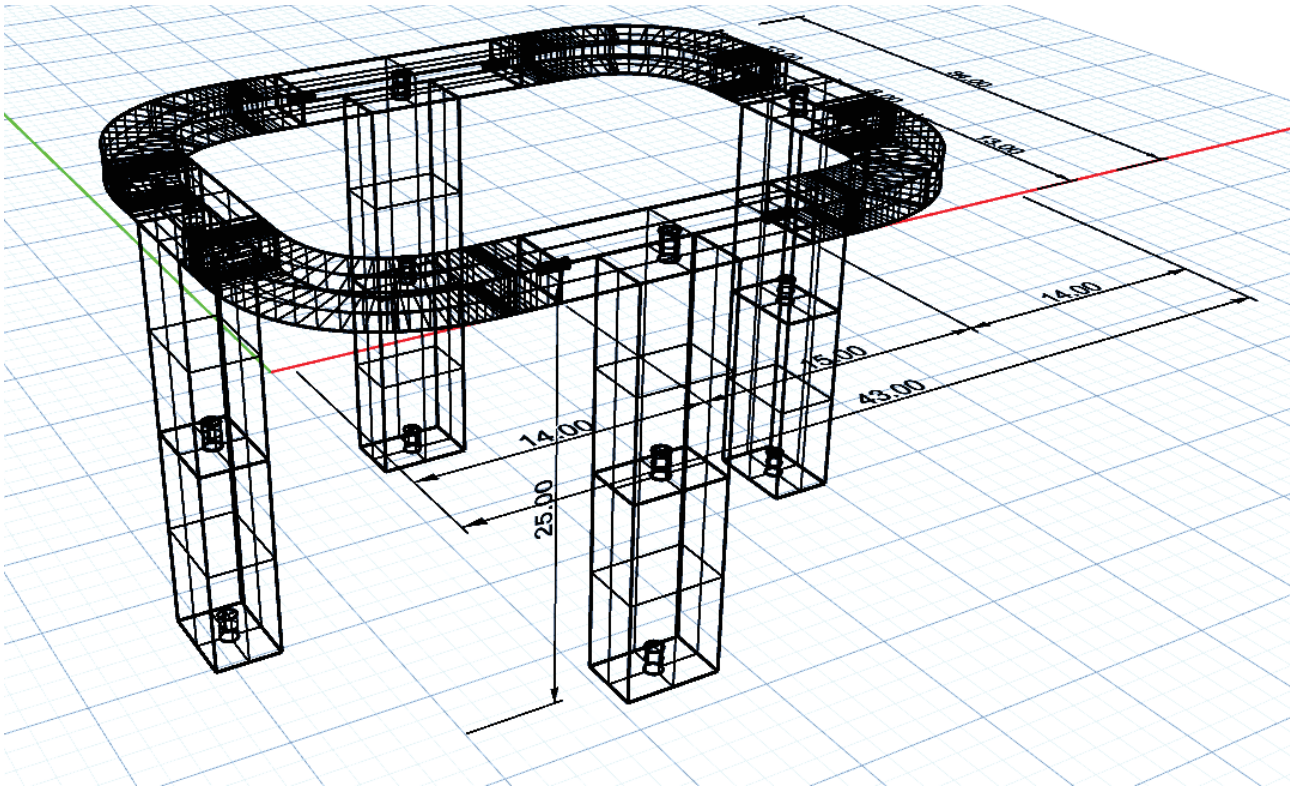


Figure 27: Perspective view of the support

As we can see on these figures, I designed the support in a modular way; this because, in order to manufacture this object, we used a 3D printer which is present in one of the laboratories of Università Politecnica delle Marche. The maximum dimensions that this printer is able to reach are 200 mm x 200 mm x 200 mm, so it was not possible to create a support made of only one piece, but I had to divide them in different pieces. In order to have a final stable structure, I created some joints between the pieces, as we can see on Fig.28.

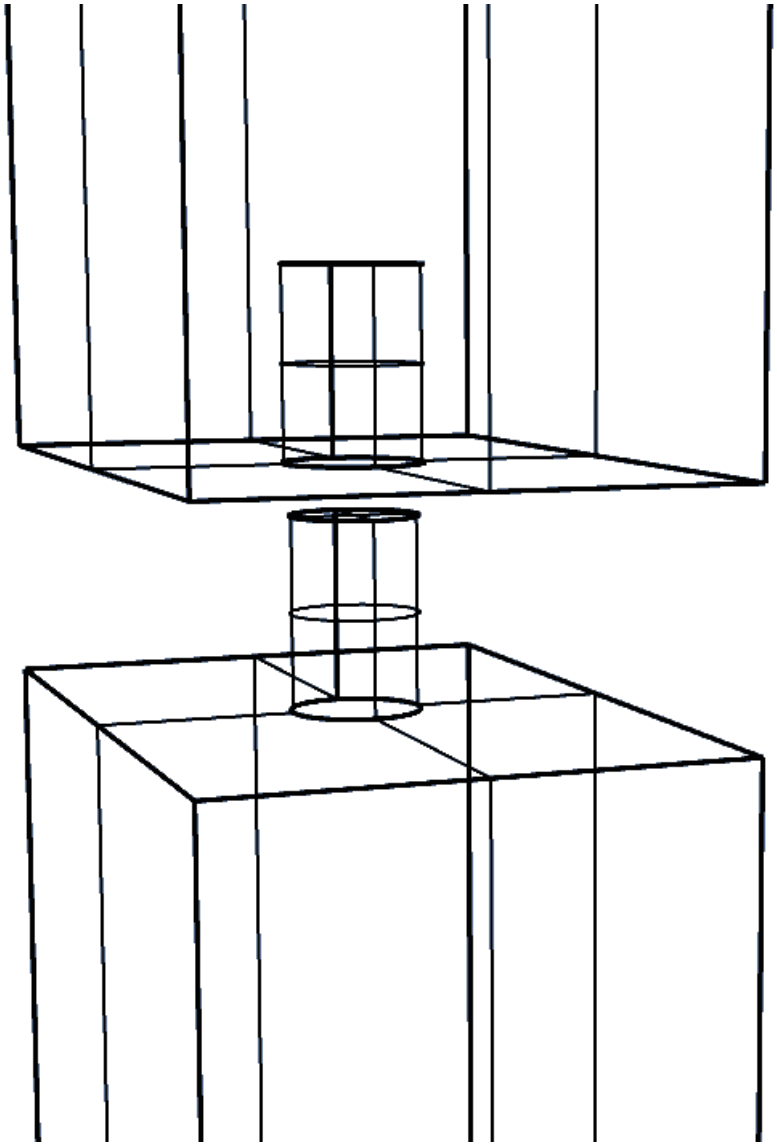


Figure 28: Joint between two pieces

As said before, in order to realize this object I used a 3D printer; the material used during this procedure is PLA (polylactic acid): in this way the support is stable and compatible with MRI and at the same time it is a quite cheap material. Here are reported some pictures of the real pieces during and after the printing (Fig. 29-30).

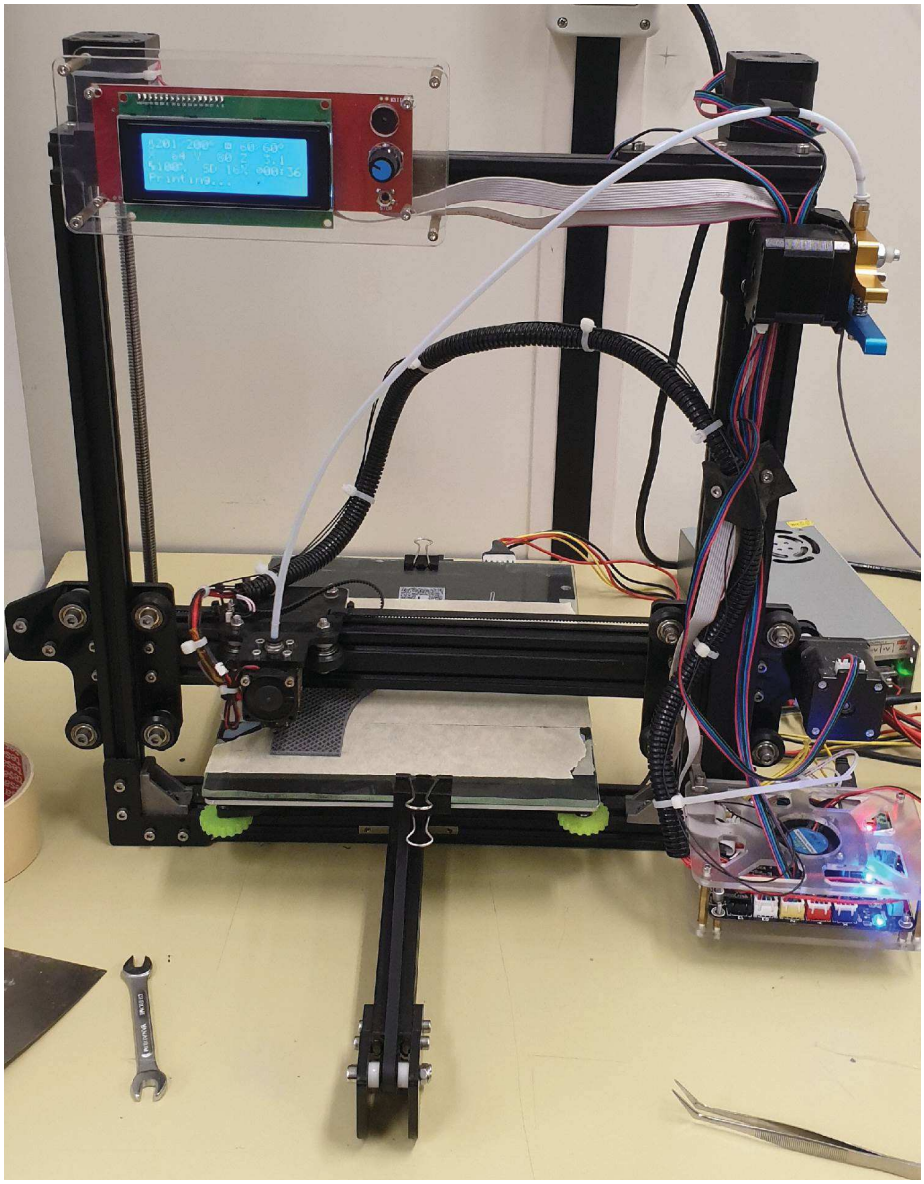


Figure 29: 3D printer used for creating the support

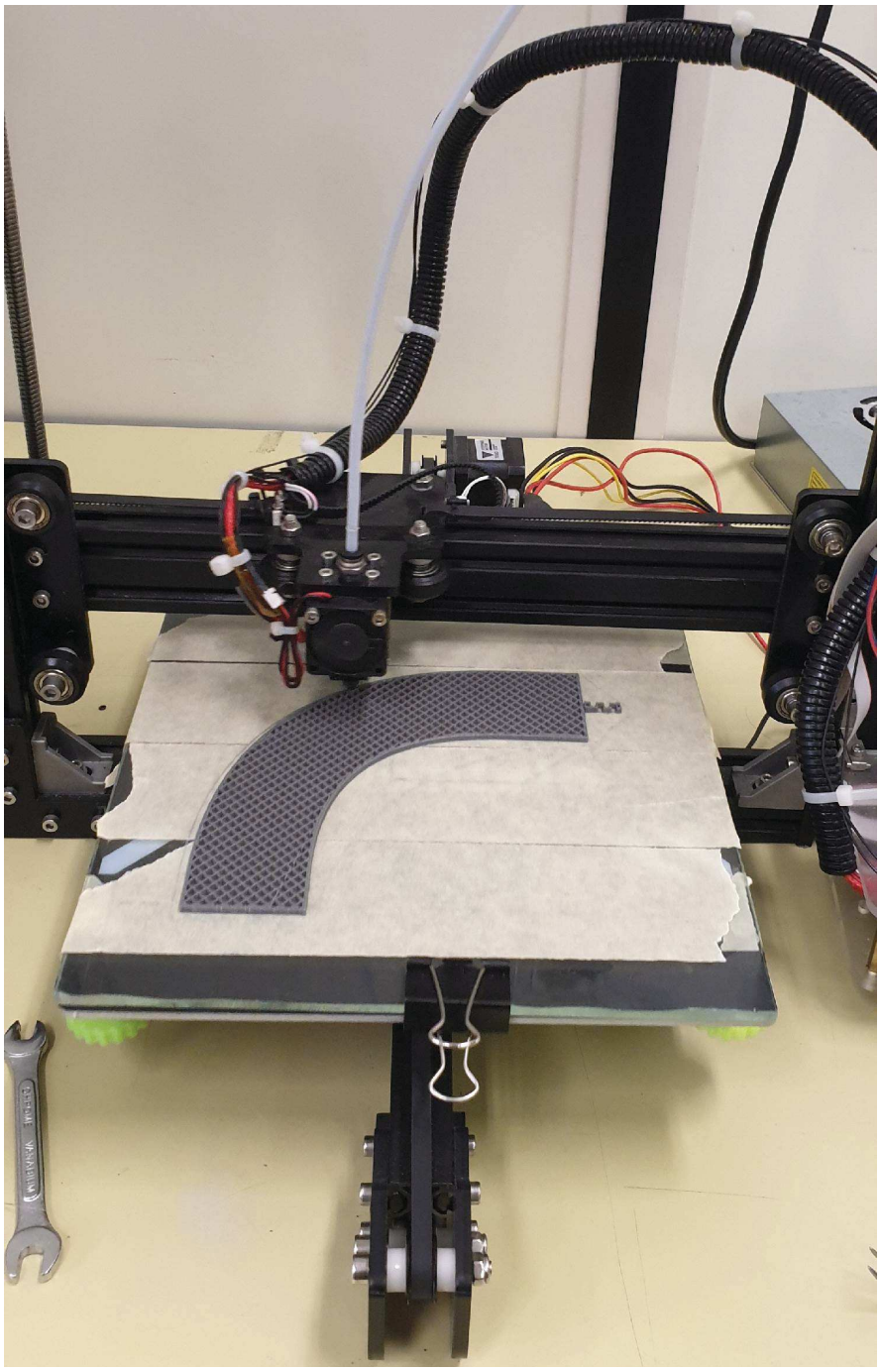


Figure 30: Printing of one piece

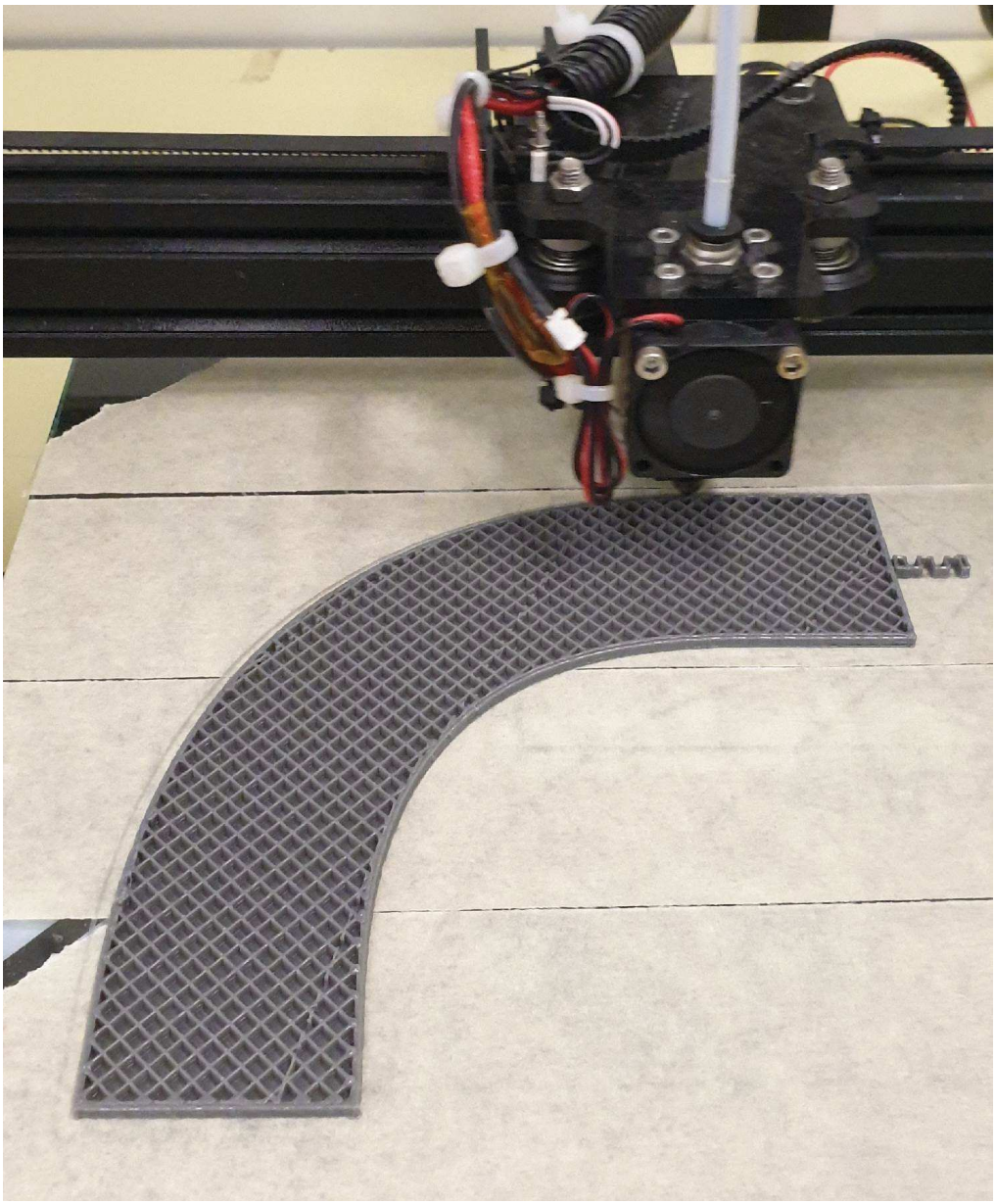


Figure 31: Printing of one piece

Chapter 5

Conclusion

During this study, we started from one simple idea, that nobody has performed previously: studying a liver through an imaging technique such as MRI, during the perfusion within a machine-like Liver Assist.
This apparent simple idea has proven to be, in reality, quite difficult.
During the pathway of this study we found so many problems, first of all technical and logistic problems. As I said before, we started without a reference point, because there were not previous studies about this in literature, and this was not in our favour.
Despite all these problems, we were able to do a first test with a liver puppet, during which we obtained great results.
Until now we are not able to do a test with a real liver, because it is estimated that we could have, more or less, 15 discarded livers in one year, in Lazio region, where Ospedale Pediatrico Bambin Gesù is located.
However, as we saw before, in the meantime we designed a support in order to guarantee a better stability for the container of the liver during the scanning in the MRI.
Through this support, made by a 3D printer, we could obtain better results in future studies, considering that it was designed and projected using the precise dimensions of the original Liver Assist container for the liver.
Despite the great results that we obtained during the first test using a liver puppet, our aim is to improve the quality of this study, first of all trying to replicate the same test with a real discarded liver.
The final goal of this study is to prove that this process could be fundamental in the evaluation of potential diseases and complications that could arise from a transplantation.
With the hope that, in future, this could become a common protocol, applied during each transplant.

List of Figures

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