Comparison of Ex-vivo Perfused and Non-perfused Porcine Liver Ablations using Uncooled Microwave Applicators

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Abstract: The paper compares thermal ablation on perfused and not perfused porcine liver produced by 17 and 14 Gauge non internally cooled (NIC) microwave (MW) applicators. To the knowledge of the authors this comparison, already made using cooled applicators has not so far been carried out with uncooled applicators that have a very different thermo-kinetic behavior when they operate inside a biological tissue. The purpose of this preliminary study is to explore the possibility of using ex vivo perfused liver in order to define an optimal protocol to allow more reliable translation of the experiments to the clinical practice and compare the obtained results with those of previous studies that used similar energy delivery.

1 INTRODUCTION

A typical MW system consists of a generator and almost one minimally invasive applicator with its cable, however there are no two equal MW systems, in particular as regards the geometry of the applicator.

There are several technological approaches that every doctor should adopt to plan and perform a microwave thermal ablation ensuring that the tumor is completely treated with sufficient ablative margins (> 0.5 cm).

Thanks to continuous advances in microwave technology, manufacturers of MW systems are proposing increasingly advanced minimally invasive technologies (Ruiter 2019, Yung 2017, Meloni 2017) with the valuable support of experienced clinical interventionists.

To minimize costs and the need for multiple insertions, the current tendency of most manufacturers is to use a single high powered (up to 140W) water or gas cooled applicator to create a pseudo-spherical ablation with up to 5 cm diameter in less than 10 minutes (Kodama 2018).

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Moreover, this high-energy approach is not without risks, especially when the treatment involves tumors that are very close to delicate organs, which must be preserved from thermal damage.

A possible alternative to this method is to subdivide the energy needed to destroy the tumor among multiple applicators (Biffi Gentili 2014). In this case the input power of each applicator can be reduced to the level that make the use of cooling unnecessary.

Non Internally Cooled applicators are structurally simpler and robust than the Internally Cooled ones (IC), and also more reliable because they operate at limited power (40 W maximum) and are free from cooling system failures that can cause serious damage to healthy tissues.

Shape and volume of the ablation zone after MW treatment are depending on (De Cobelli 2017): biophysical parameters as thermal conductivity and perfusion rate of the liver parenchyma that can be different in human liver tissue due to fibrosis, cirrhosis or steatosis; effective MW power and hyperthermic treatment duration; structure, gauge and cooling mechanism (if present) of the applicator (MW needle).

Planning for ablation is essentially based on manufacturer algorithms or ablation charts in combination with personal experience of the

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operator. These algorithms and charts, which try to predict the three-dimensional diameter of the ablation zone in relation to the amount of applied energy, are often based on experiments which have serious shortcomings, preventing a reliable translation to daily clinical practice.

These shortcomings are the result of studies performed in non-perfused ex vivo bovine or porcine livers (as opposed to perfused in vivo human livers with variable arterial and portal blood flow).

These differences affect the way in which the applied energy is transferred into heat, resulting in highly unpredictable ablation zone dimensions and volumes.

Despite several individual papers reporting on these shortcomings, a systematic review on this topic is lacking.

A higher reproducibility of the experiments with better predictability of the clinical results could be achieved by defining a standardized procedure to be shared among all manufactures, allowing to extract the treatment parameters through ablation tests on normothermic (body temperature) perfused ex-vivo organs.

2 MATERIALS AND METHOD

Ablation procedures where performed using the TATO thermal ablation system with 14 and 17 gauge NIC applicators.

TATO is a multi-applicator system that has been developed by a small multidisciplinary team at the University of Florence, in the framework of a collaborative academic-industry agreement with Biomedical Srl, Florence.

The experiment was performed in ex vivo fresh porcine liver retrieved from animals in the food chain. In this way important ethical implications can be overcome and the economic burden is lower than more common in vivo studies.

The Experimental platform for hepatic flow simulation developed at the Industrial Engineering Department (DIEF) of the University of Florence (patent pending) was employed to maintain the explanted porcine liver in a normothermic physiological perfusion state.

Freshly taken porcine liver from adult animal with intact in-and outflow vessels where obtained from an abattoir and immediately connected to the perfusion platform. Hepatic inflow was simulated trough flexible plastic tubes sutured to the veins and connected to a perfusion pump system. The hepatic flow was established to emulate the average human cardiac output. To acquire information on the maximum temperature reached by the MW applicators during thermo ablation procedures and the divergences between active perfusion and blocked perfusion, the test process was video recorded with a Thermal-CAM A320 thermal imaging camera (FLIR Systems, Inc., Wilsonville, OR).

3 PERFUSION PLATFORM

The experimental platform for the simulation of the hepatic flow is composed of 3 distinct sections, with the possibility of independent activation / management, which perform the following functions:

1. pre-heating the blood and filtering any clots;
2. perfusion of the venous tree;
3. perfusion of the arterial tree, equipped with a high efficiency oxygenator.

Each section is controlled by a dedicated processor which, following the automatic learning approach, processes the sensor signals (pressure, temperature, flow) placed in strategic areas and performs the actions necessary to maintain the normal physiological conditions of the organ during the extracorporeal perfusion process.

In the literature are described analogous systems which perform similar functions but not in an integrated and adaptive manner such as the current system that was designed and built at the prototype level at the DIEF of UNIFI (R. Ravikumar 2016). and for which a patent application was filed.

4 THE ABLATION PROCEDURE

The ablation procedure was performed in collaboration by a surgeon (FS) and an engineer (MD).

As a first step, the perfusor was replenished by filling the main tank with the perfusion fluid which has the same characteristics as normal blood. In order to evaluate the functioning of the system avoiding the use of blood, a fluid was identified that was able to simulate its behavior. The blood has a dynamic viscosity of about 3 cP. To make a fluid capable of reproducing the rheological properties of blood in terms of viscosity and density, it is possible to use distilled water, glycerol and cornstarch. Since the blood has a physiological temperature of about

37 °C, the percentages of the constituent elements of the fluid must therefore be determined. With a capillary viscometer, it is possible to measure the kinematic viscosity [m2⁄s], from which it is possible to calculate the dynamic viscosity [cP]. It was estimated that at 37 °C the fluid with a viscosity as close as possible to the blood is composed of:

- 70% distilled water; 30% vegetable glycerol. Dynamic viscosity $\mu = 3.52 \pm 0.01$ cP
- 70% distilled water; 30% vegetable glycerol; 1% cornstarch. Dynamic viscosity $\mu = 3.52 \pm 0.15$ cP.

Therefore, since about 3.5 L of fluid is circulated in the system in question, it is possible to simulate blood using 2450 mL of distilled water and 1050 mL of glycerol (and 35 mL of cornstarch).

The main pump was then activated and the heating system was switched on to gradually increase the fluid temperature up to 38 °. Gradual heating of the fluid is necessary to avoid thermal shock and clot formation. The temperature was then monitored through the RP thermocouple and the RA thermocouple and kept constant until the liver cannulation was completed.

The liver was removed immediately after slaughtering the pig. The hepatic hylum was carefully dissected. The main hepatic artery was isolated and cannulated with a flexible 8Fr Nelaton catheter. The choledocic duct was isolated and sutured. The main portal vein was isolated and cannulated with a 16 fr Nelaton catheter. The lower cave vein has been identified and sutured. The upper cave vein was cannulated with a 45fr plastic tube. The suprahepatic accessory vein was isolated, cannulated and connected to the upper cave vein tube with a Y-shaped joint.

Before the connection to the perfusor, the hepatic vessels were washed with a heparinized physiological solution to prevent the formation of clots.

The hepatic artery and the portal vein cannulas were connected to the respective pumps and the upper vena cava tube was connected to the main reservoir. At the end of this procedure the perfusor was turned on and the correct functionality was checked.

Fig. 1 shows the final installation immediately before the thermal ablation tests.

Figure 1: Final set up immediately before the thermal ablation tests.

4.1 Thermal Ablation Tests

For the microwave thermal ablation tests two NIC applicators of 14 and 17 G were used respectively. In order not to influence the results the two needles were inserted in two distant hepatic lobes. The TATO system microwave generator was then set to supply 40 W to the 14 G needle and 30 W to the 17 one. The TATO microwave generator was switched on for 10 minutes immediately after the perfusor reached the normothermia state.

At the end of this first procedure the perfusion was stopped and the needles were repositioned to repeat the same procedure but in the absence of perfusion.

At the end of both procedures the parenchyma was sectioned longitudinally to photograph the obtained ablations and to measure their diameters along the two main axes (Fig. 2, 3).

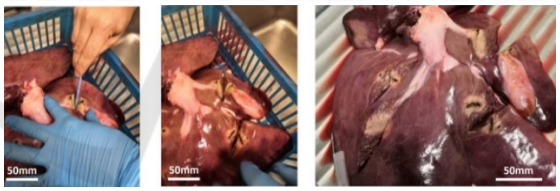

Figure 2: Post ablation sectioning.

5 PRELIMINARY RESULTS

The results of the ablation test are summarized in Table 1.

Table 1: Preliminary results wit perfusion blocked.

Applicator Gauge	Length (cm)	Diameter (cm)	Ablation volume (cm^3)	Aspect ratio
14	4.2	3.3	191	0.66
17	4	2.2	81	0.55

Table 2: Preliminary results with perfusion active.

Applicator Gauge	Length (cm)	Diameter (cm)	Ablation volume (cm^3)	Aspect ratio
14	3.7	2.2	74	0.59
17	3.3	1.5	31	0.45

Without hepatic perfusion, the 14G needle generated a 4.2x3.3 cm ablation (Fig. 3a and 3b) while in perfused mode the ablation was smaller due to the heat sink phenomenon, with dimensions of 3.7x2.2 cm (Fig. 3c and 3d).

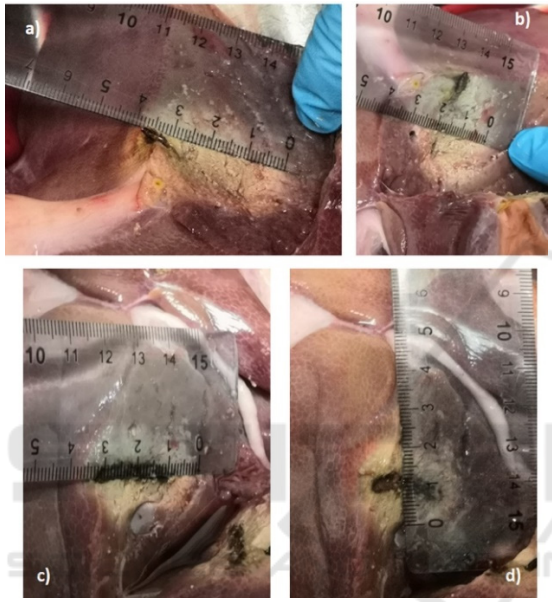

Figure 3: Comparison between 14 Gauge ablation in non-perfused (Fig. a and b) and in perfused (Fig c and d) liver.

The 17G needle produced an ablation of 4x2.2 cm in non-perfused mode (Fig. 4a and 4b) and 3.3x1.5 in perfused mode (Fig. 4c and 4d).

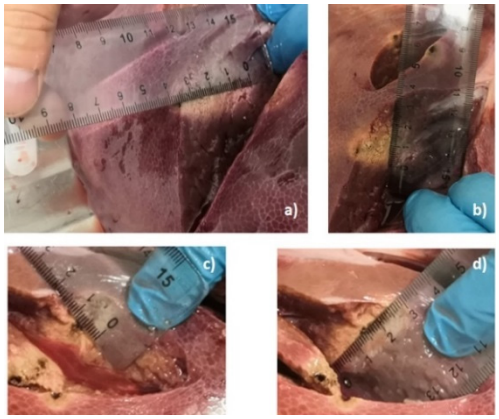

Figura 4: Comparison between 17 Gauge ablation in non-perfused (Fig. a and b) and in perfused (Fig. c and d) liver.

The ablation volumes for the 14G needle were 24 cm3 and 9.4 cm^3, in non-perfused and perfused mode, with a volumetric ratio of 0.66 and 0.59 respectively.

The ablation volumes for the 17G needle were 10 cm3 and 3.4 cm^3 in non-perfused and perfused mode, with a volumetric ratio of 0.55 and 0.45 respectively.

The data acquired with thermal cameras allowed to measure with great accuracy the temperature distribution on the entire liver and therefore to verify in real time the correct perfusion state of the parenchyma during the two ablation procedures with active perfusion. Within the limits of the small number of ablations performed, the preliminary results indicate that an isolated spherical tumor with a diameter up to 1.5 cm can be thermally ablated with an adequate margin using a single NIC 14 G. applicator.

If the tumor diameter exceeds 1.5 cm for its complete thermal ablation it will be necessary to use multiple NIC applicators or a single adequately cooled high power IC device.

Moreover, as shown in figure 4, the thermal behavior highlights the differences produced by the perfusion in terms of maximum temperature reached by the metallic shaft and consequently by the tissue in direct contact with the MW tools.

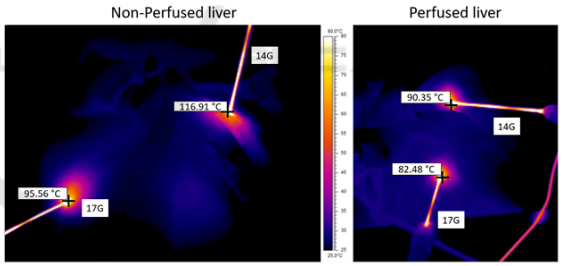

Figure 5: Maximum Temperature comparison between ablation in non perfused and in perfused liver at the end of the termoablation (10 min).

6 DISCUSSION

With the exception of special situations such as a neoplasia localized in unresectable positions, a high number of lesions, inadequate hepatic reserve or multiple comorbidities that contraindicate anesthesia, percutaneous thermal ablation techniques (TPA) are not yet considered the gold standard in the treatment of liver neoplasms. This is mainly due to the fact that liver transplantation and hepatic resection have shown their superiority in the

treatment of primary and secondary liver neoplasms demonstrating higher long-term disease-free survival rates than TPA (F. Romano 2012). In the oncological TPA one of the main problems facing doctors is the uncertainty about the actual size of the ablations, mainly as a direct consequence of the well-known "heat sink effect".

The prediction of this effect and of the 3D dimensions of the ablation zone in relation to the amount of applied microwave energy is a crucial aspect since an incorrect prediction can lead to an insufficient volume of ablation and a relapse of the neoplasm. Since it is very hard to predict in practice the exact size of the ablation for each combination of time, power and size of the needle before the execution of the procedure, these data are often supplied to the doctor by the manufacturers of thermal ablation systems in the form of ablation charts or algorithms, but in our opinion these information, together with the operator's personal experience does not always provide the expected results because they are often based on experiments on ex-vivo organs at ambient temperature that present serious deficiencies, preventing reliable translation into the clinical practice.

In order to overcome these limitations, the Department of Industrial Engineering (DIEF) of the University of Florence has developed an experimental platform (patent pending) to keep the explanted liver in a state of normal physiological thermal perfusion, capable to simulate the actual heat sink effect during a TPA procedure. We need to underline that the platform can simulate physiological liver perfusion but is not able to keep the liver cells alive and this aspect has been evaluated by the authors before performing experimental tests. Recent studies have underlined that a warm ischemia time up to 60min does not generate any irreversible cellular change and is acceptable even for hepatic transplant (Kalisvaart, 2018).As a consequence of that, if the ablation experiment is performed in 60min beginning from the liver explant, the effect of liver warm ischemia on ablation shape and dimensions is negligible or even absent. The aim of the study was therefore to extract preliminary data to be compared with the literature data in vivo. In our opinion, the ex-vivo pig perfused liver test should represent the gold standard for the definition of truly reliable algorithms and ablation charts for the following reasons:

1. The test is easily reproducible and allows a definitive evaluation of the ablation volume in the presence of the heat sink effect;
2. The proposed approach could allow the standardization of the experimental procedure to extract reliable algorithms and ablation charts;
3. The economic burden is lower than the costly in vivo animal procedures.
4. Important ethical implications can be overcome.

In order to confirm experimentally this belief, the ablation experiments where made in two different perfusion inflow states: active and blocked using the UniFi Hepatic Flow platform.

Ablation results of the two inflow state are depicted in Tab1.

Preliminary results obtained by comparing ablation performed in blocked and active perfusion states show a reduction of about 30% in the ablation radius from one to the other state, regardless of the size of the applicator.

The ablation zones in this study where commensurate with those of previous studies (M. Dimitri 2018) obtaining results that confirm the substantial equivalence between in-vivo and ex-vivo normothermic perfused liver for the same energy delivery.

It is important to note that the electromagnetic and thermal properties of the ex vivo liver at ambient temperature normally used by manufacturers to extract the ablation chart, are very different from those of the ex vivo normothermic perfused liver.

7 CONCLUSION

The preliminary results obtained with this study are too limited to have a statistical relevance but if this result will be confirmed even in future tests this model could constitute the best procedure to evaluate the effectiveness of TPA without the use of in vivo animal models. The availability of a standardized ablation model based on ex-vivo perfused liver opens the way to a more in-depth investigation of the heat sink effect at peripheral and central vessel locations. The UNIFI Perfusion Platform is very versatile and it allows to easily change the composition and flow rate of the perfusion solution of the liver parenchyma.

Moreover this model could be used to emulate an open surgical ablation, allowing the surgeon to rotate the liver within its anatomical surrounding, manually protect heat-sensitive organs (bowel) and easily insert clustered applicators to treat large non spherical tumors.

The preliminary results obtained with this study are too limited to have a statistical relevance, therefore the final validation of the proposed approach will require a further and more in-depth experimental activity.

8 FUTURE WORK

Future work will have the main focus trying to statistically validate the actual results. If the new tests will confirm them, a compact and autonomous platform for the normothermic perfusion of porcine livers will be engineered.

With this new device in the future we would like to carry out:

- the comparisons between TPA in a swine in-vivo model and TPA in a swine ex-vivo perfused model.
- the comparison between ablations performed by high power (120W) cooled applicator and low power single or multiple uncooled applicator in term of energetic efficiency and procedural security;
- the optimization of multi-applicator ablation procedures and the editing of more realistic and affordable ablation charts;
- the study and optimization of a pulsed microwave ablation technology (PMWA) (M.Bedoya 2012) in order to obtain an increase of the ablation volume with the same input average power.

REFERENCES

Ruiter S.J.S., Heerink W.J., De Jong K.P., (2019). Liver microwave ablation: a systematic review of various FDA-approved systems. *Eur Radiology 29(8):4026-4035*

Meloni M.F. et alii, (2017). Microwave ablation in primary and secondary liver tumours: technical and clinical approaches. *International Journal of Hyperthermia 33(1): 15–24.*

Yung J., Liang P., (2017). Status and advancement of microwave ablation in China. *International Journal of Hyperthermia Vol. 33, 2017 - Issue 3*

Kodama H., (2018). High power microwave ablation of normal swine lung: impact of duration of energy delivery on adverse event and heat sink effects. *International Journal of Hyperthermia 34(8):1186-1193.*

Biffi Gentili G, Ignesti C, Tesi V. (2014). Development of a novel switched-mode 2.45 GHz microwave multi-applicator ablation system. *International Journal of Microwave Science and Technology*, *Article ID 973736*

De Cobelli F et alii, (2017). Microwave ablation of liver malignancies: comparison of effects and early outcomes of percutaneous and intraoperative approaches with different liver conditions. *Medical Oncology 34(4)*

R. Ravikumar, et alii, (2016). Liver transplantation after ex-vivo normothermic machine preservation: a phase 1 (first-in-man) clinical trial. *American Journal of Transplantation,* pp. 1779-1787.

F. Romano et alii, (2012). Bleeding in hepatic surgery: sorting through methods to prevent it. *HPB Surgery, vol. 2012, 12pg, Article ID 169351.*

Kalisvaart M, et alii, (2018).The impact of combined warm ischemia time on development of acute kidney injury in donation after circulatory death liver transplantation: stay within the golden hour. *P. Transplantation. 102(5):783-793.*

M. Dimitri et alii, (2018). A new microwave applicator for laparoscopic and robotic liver resection. *Internal Journal of Hyperthermia, vol 36: 75-86.*

M.Bedoya et alii (2012). Microwave ablation energy delivery: Influence of power pulsing on ablation results in an ex vivo and in vivo liver model. *Medical Physics 41 (12).*