Original Article

Franz G.M. Poch*, Christina A. Neizert, Ole Gemeinhardt, Beatrice Geyer, Katharina Eminger, Christian Rieder, Stefan M. Niehues, Janis Vahldiek, Stefan F. Thieme and Kai S. Lehmann

Intermittent Pringle maneuver may be beneficial for radiofrequency ablations in situations with tumor-vessel proximity

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Abstract

Background: Radiofrequency ablation (RFA) represents a treatment option for non-resectable liver malignancies. Larger ablations can be achieved with a temporary hepatic inflow occlusion (Pringle maneuver – PM). However, a PM can induce dehydration and carbonization of the target tissue. The objective of this study was to evaluate the impact of an intermittent PM on the ablation size.
Methods: Twenty-five multipolar RFAs were performed in porcine livers *ex vivo*. A perfused glass tube was used to simulate a natural vessel. The following five test series (each n = 5) were conducted: (1) continuous PM, (2–4) intermittent PM, and (5) no PM. Ablations were cut into half. Ablation area, minimal radius, and maximal radius were compared.
Results: No change in complete ablation size could be measured between the test series ($p > 0.05$). A small rim of native liver tissue was observed around the glass tube in the test series without PM. A significant increase of ablation area could be measured on the margin of the ablations with an intermittent PM, starting without hepatic inflow occlusion ($p < 0.05$).
Conclusion: An intermittent PM did not lead to smaller ablations compared to a continuous or no PM *ex vivo*. Furthermore, an intermittent PM can increase the ablation area when initial hepatic inflow is succeeded by a PM.

Keywords: cooling effect; hepatic; liver; multipolar; radiofrequency ablation.

Introduction

Radiofrequency ablation (RFA) is an important therapy option for the treatment of non-resectable malignant liver tumors [1, 2]. Electrical current administered through RFA applicators induces thermal heating of the target tissue around the applicator and therefore causes tumor necrosis [3]. The vascular cooling effect ("heat sink") of adjacent liver vessels restricts the ablation size. A temporary hepatic inflow occlusion of both the hepatic artery and portal vein ("Pringle maneuver") can reduce vascular cooling effects in RFA [4]. Higher local temperatures are observed when a Pringle maneuver is performed. Therefore, larger ablations can be achieved [5]. However, temperatures >100 °C result in dehydration and carbonization of the target tissue. Carbonization leads to an increase in electrical resistance, which results in an electrical isolation of the tissue and a reduction of energy transmission. RFA is then limiting itself in regard to the ablation size [6].

Internal cooling of radiofrequency applicators is used in some RFA devices to reduce ablation temperatures around the applicators and consequently prevent carbonization of the target tissue adjacent to the applicators [6, 7]. A similar effect may exist around hepatic vessels. Although a positive effect on ablation size could be demonstrated with a complete Pringle maneuver, no study has investigated the effect of an intermittent Pringle maneuver so far [5]. An intermittent Pringle maneuver may lead to a

***Corresponding author: Franz G.M. Poch,** Department of General, Visceral and Vascular Surgery, Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Campus Benjamin Franklin, Hindenburgdamm 30, 12200 Berlin, Germany,
Phone: +49 30 450-522712, E-mail: franz.poch@charite.de
Christina A. Neizert, Beatrice Geyer, Katharina Eminger and Kai S. Lehmann: Department of General, Visceral and Vascular Surgery, Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Campus Benjamin Franklin, Berlin, Germany
Ole Gemeinhardt, Stefan M. Niehues, Janis Vahldiek and Stefan F. Thieme: Department of Radiology, Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Campus Benjamin Franklin, Berlin, Germany
Christian Rieder: Fraunhofer MEVIS, Institute for Medical Image Computing, Bremen, Germany

more even distribution of thermal energy within the target tissue as carbonization of the tissue is reduced. Larger ablation volumes may result.

The objective of this study was to compare the impact of an intermittent hepatic inflow occlusion to that of a complete inflow occlusion and a no inflow occlusion in RFA *ex vivo*. Total ablation area and vascular cooling effects were evaluated.

Materials and methods

Experimental setup

Multipolar RFA was performed in porcine liver *ex vivo*. A multipolar radiofrequency generator (CelonLab POWER System; Olympus Surgical Technologies Europe, Hamburg, Germany) was used with three internally cooled bipolar applicators (CelonProSurge T-20, Olympus Surgical Technologies Europe). A triple peristaltic pump (CelonAquaflowIII, Olympus Surgical Technologies Europe) ensured the internal cooling of each applicator. Applicators were set parallel to each other at a distance of 20 mm (Figure 1A). A preinstalled resistance controlled automatic power mode regulated the energy delivery into the liver tissue. The starting power was set to 60 W (according to manufacturer specifications) [8]. Ablations were stopped at an energy input of 40 kJ [9]. A glass tube (hereafter referred to as "vessel") was used to simulate a natural liver vessel (inner/outer diameter: 3.4/5.0 mm). The vessel was set parallel to the applicators in the ablation center point. The vessel was perfused with water at room temperature with a peristaltic pump and a continuous flow rate of 100 mL/min (physiological parameters of a human with 70 kg: blood flow in the hepatic artery is 300 mL/min, blood flow in the portal vein is 1150 mL/min [10]). In previous experiments, we could demonstrate that a cooling effect already occurs at a flow rate of 100 mL/min [9]. Stopping the peristaltic pump simulated a Pringle maneuver. The test

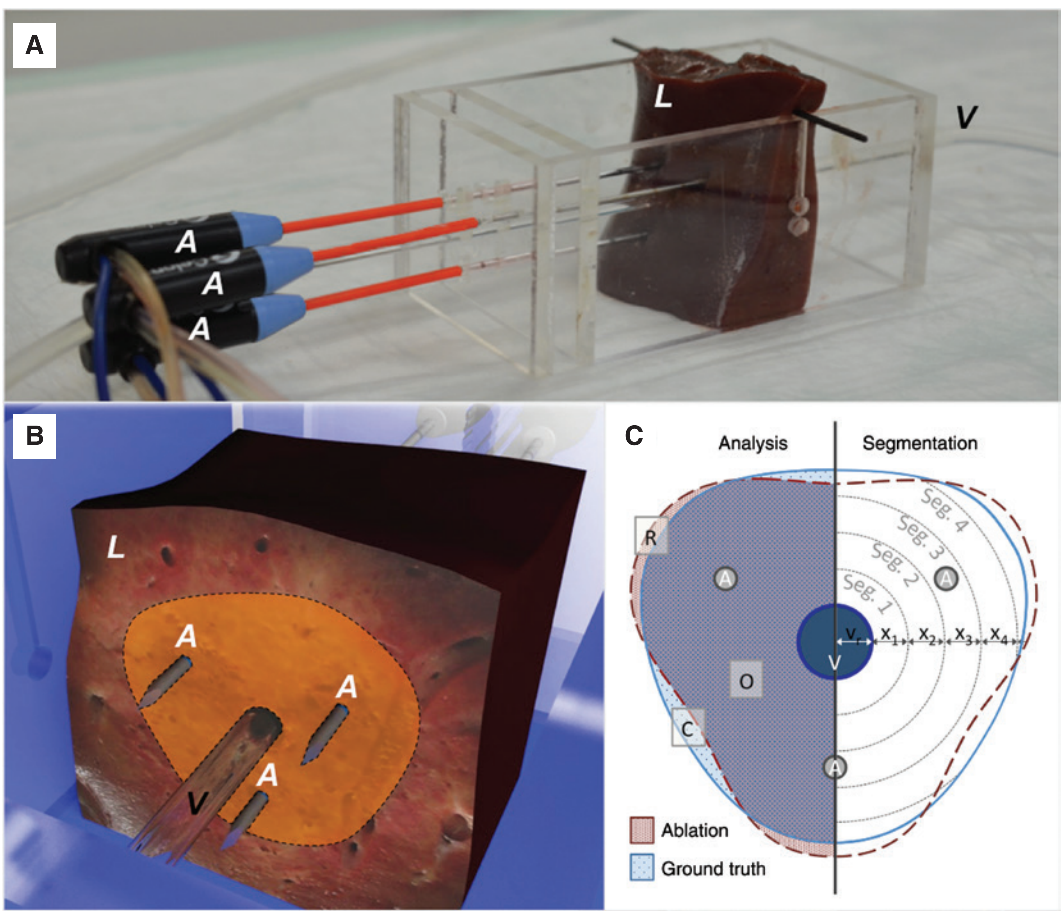

Figure 1: Experimental implementation of the *ex vivo* test series.
(A) Three internally cooled bipolar radiofrequency applicators *(A)* were used in porcine livers *(L) ex vivo*. A perfused glass tube *(V)* was used to simulate a natural liver vessel. The glass tube was situated in the center of the ablations, running parallel to the applicators. (B) Ablations were cut into half, orthogonally to the applicators on the height of the isolators (located at the tip of the applicator). Ablation areas were measured (dotted line). (C) An annular segmental model (Seg 1–4, …) with an adjustable segment width of "x" mm was used to compare ablations areas to an averaged mask (consisting of ablations with continuous Pringle maneuver reffered to as "ground truth"). Three areas have to be distinguished in analysis: (1) ablations are congruent ("*O*"); (2) an increase of ablation area compared to ground truth exists ("*R*"); and (3) the ablation is smaller than ground truth (cooling effects: "*C*").

settings, which are specified above, corresponded to parameters that were established within our research group [9, 11, 12].

The following abbreviations are used to visualize the different test settings (Figure 2):
- "≈": vascular flow,
- "≠": no vascular flow (Pringle maneuver),
- "/": alteration in perfusion ("flow→no flow"; respectively "no flow→flow").

Five experimental settings were planned with five ablations each (Figure 2). For each ablation, energy input was set to 40 kJ (respectively 4 equal units of 10 kJ or 2 equal units of 20 kJ):
- V_p: ≠≠≠≠ (continuous Pringle maneuver),
- V_1: ≠≠/≈≈ (intermittent Pringle maneuver: 20 kJ no flow/20 kJ flow),
- V_2: ≈≈/≠≠ (intermittent Pringle maneuver: 20 kJ flow/20 kJ no flow),
- V_3: ≈/≠/≈/≠ (intermittent Pringle maneuver: 10 kJ flow/10 kJ no flow/10 kJ flow/10 kJ no flow),
- V_4: ≈≈≈≈ (no Pringle maneuver).

Liver tissue was obtained from a slaughterhouse. Ablations were performed within 6 h after euthanasia of the animals at room temperature to minimize the effects of autolysis [11]. The livers were randomly assigned to the experimental settings.

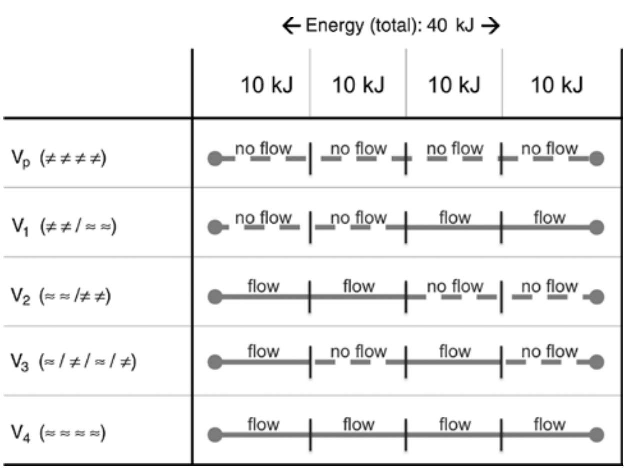

Figure 2: Test series with corresponding energy input.
Five test series (n = 5) were planned: (1) a continuous Pringle maneuver (V_p); (2–4) intermittent Pringle maneuvers (V_1–V_3); and (5) no Pringle maneuver (V_4). Total energy input was set to 40 kJ ("≈": perfusion; "≠": Pringle maneuver).

Analysis

Ablations were cut in half after ablation along a plane defined by the three isolators situated between the two electrodes, located at the tip of the applicators (Figure 1B). Deformations caused by the cutting process of the soft liver tissue were subsequently adjusted using a thin spline landmark registration [13, 14]. The ablation area as well as minimal and maximal ablation radii were measured within this plane [15, 16]. Ablations were compared to a geometrically averaged mask ("ground truth"), comprising the ablations without perfusion, where no cooling effects are expected. The impact of a (temporary) blood flow occlusion was analyzed with circular segments defined by the vessel as center point (Figure 1C). Segment width was set to 2.5 mm (equivalent to the vessel radius). Ablation areas were compared within each circular segment to the averaged ground truth mask [9, 14]. Three different cases can occur in this analysis:
- Areas between ablation and ground truth are identical (Figure 1C: "*O*").
- The ablation outreaches ground truth: the ablation area is larger than expected (Figure 1C: "*R*").
- Ground truth outreaches the ablation: the ablation area is smaller than expected ("cooling effect"; Figure 1C: "*C*").

Statistical analysis

Analyses were conducted with a statistical software (SPSS version 20; SPSS Inc., Chicago, IL, USA). Data are expressed as median (minimum – maximum). The Kruskal-Wallis test was used for comparisons between more than two independent groups; the Mann-Whitney U-test was used for two independent groups. The level of significance was 0.05 (two sided) for each statistical testing.

Results

A total of 25 ablations were performed in 10 porcine livers. Figure 3 exemplarily shows cross sections of each

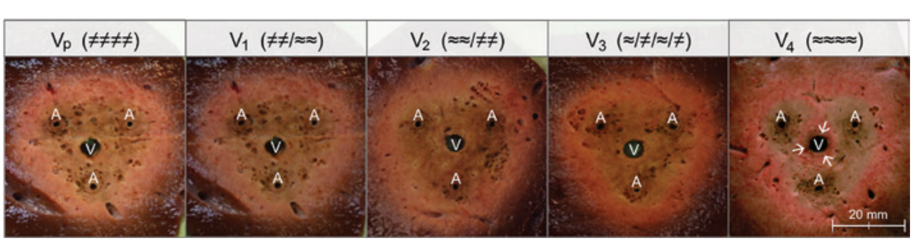

Figure 3: Exemplary cross-sectional areas of all five test series.
Homogeneous ablation areas could be observed in ablations in which a (temporary) Pringle maneuver was performed (V_p, V_1–V_3). A vascular cooling effect is seen around the vessel in the test series with continuous perfusion (V_4) (V, vessel; A, applicator).

Table 1: Mean values of ablation area and ablation radii (min–max) for each test setting.

	V_p (≠≠≠≠)	V_1 (≠≠/≈≈)	V_2 (≈≈/≠≠)	V_3 (≈/≠/≈/≠)	V_4 (≈≈≈≈)	p-Value
Area, mm^2	936 (812–1186)	1136 (878–1351)	953 (885–1141)	1058 (863–1194)	897 (795–935)	>0.05
R_{min}, mm	14 (14–17)	15 (15–16)	17 (14–19)	16 (15–17)	13 (13–17)	>0.05
R_{max}, mm	20 (19–23)	20 (19–22)	22 (19–22)	22 (20–22)	20 (17–21)	>0.05

No differences in ablation area and ablation radius were observed between the five test series.

experimental setting. All ablations led to rounded and homogeneous lesions. No difference in ablation area could macroscopically be observed between ablations with continuous and intermittent Pringle maneuver. However, a macroscopic small rim of native liver tissue could be detected immediately around the artificial vessel in all ablations of the test series without Pringle maneuver (Figure 3; V_4 "→"). This "native" rim did not occur in ablations with continuous or intermittent Pringle maneuver (V_p, V_1–V_3).

The characteristics of the test series are presented in Table 1. Five ablations without perfusion (V_p ≠≠≠≠) were carried out (cf. Table 1 and Figure 3). The geometrical averaged area of this experimental setting without any perfusion was 936 mm^2. No difference in ablation area and ablation radius (minimum and maximum) could be observed between the five experimental settings ($p > 0.05$).

Additionally, ablation areas were measured within segments of 2.5 mm width to analyze the impact of vascular cooling effects with higher precision (Figure 4). A vascular cooling effect of almost 80% could be measured within 2.5 mm around the vessel in the test setting with continuous perfusion (V_4 ≈≈≈≈). This cooling effect was not significant ($p > 0.05$). No significant cooling effects could be observed in the test settings with intermittent Pringle maneuver. Additionally, no change of complete ablation area could be discovered between a continuous Pringle maneuver and the test settings without perfusion in the beginning (V_1 ≠≠/≈≈). However, an increase of ablation area was measured in the periphery of the ablations in the test settings with intermittent Pringle maneuver, starting without blood flow occlusion (V_2 ≈≈/≠≠, V_3 ≈/≠/≈/≠).

Discussion

In the actual study, we could demonstrate that an intermittent Pringle maneuver did not lead to smaller ablations compared to a continuous or even no Pringle maneuver *ex vivo*. Furthermore, an intermittent Pringle maneuver can increase the ablation area when initial hepatic inflow is succeeded by a Pringle maneuver.

No change of ablation area, minimal radius, and maximal radius could be observed between test series

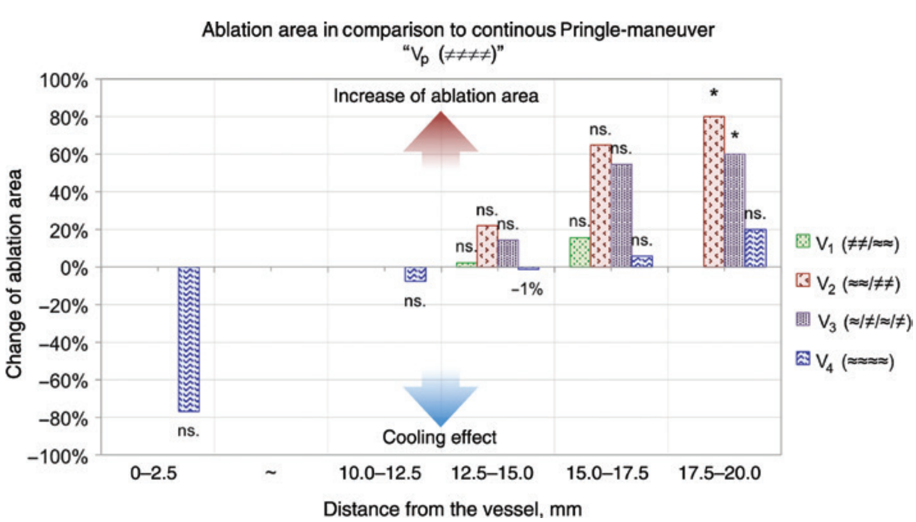

Figure 4: A vascular cooling effect could be observed around the vessel in all ablations of the test series without Pringle maneuver (V_4). However, this cooling effect was not significant ($p > 0.05$). An increase of ablation area could be observed on the margin of the ablations in the test series with intermittent Pringle maneuver, starting without hepatic inflow occlusion (V_1–V_3). In between 2.5 and 10.0 mm ("~"), no change in ablation area could be observed between the five test series (*, significant; ns., not significant; $p > 0.05$).

with continuous (V_p), intermittent (V_1–V_3), or without (V_4) Pringle maneuver in our *ex vivo* study. A small rim of native liver tissue was observed macroscopically around the vessel in the test series without Pringle maneuver (V_4). This "cooling effect" was not significant, because its size was too small/inhomogeneous in comparison to the total ablation area. However, an increased risk for local tumor recurrence after RFA appears to be likely around the vessel in these cases. Therefore, a Pringle maneuver seems to be a reasonable method for ablations close to major hepatic vessels. No cooling effect was observed in the test series with intermittent Pringle maneuver. Although the study was performed *ex vivo*, it demonstrated that intermittent vascular inflow occlusion is not inferior to continuous inflow occlusion. Moreover, we could observe that an intermittent Pringle maneuver increases the ablation area in the periphery of the ablations. This occurs especially when an initial hepatic inflow is succeeded by a Pringle maneuver during the course of the ablation (V_2, V_3). High temperatures >100 °C within the ablation zone limit the efficiency of RFA due to dehydration and carbonization of the target tissue. An early carbonization of the tissue in the ablation center may be prevented by a persisting hepatic inflow in the beginning of an ablation. Residual tumor/liver tissue around major hepatic vessels may be finally destructed by a Pringle maneuver performed in the further course of an ablation. In summary, this so-called intermittent Pringle maneuver seems to ensure a more uniform distribution of thermal energy within the target tissue, resulting in larger ablation sizes. To our knowledge, no study examining the effect of an intermittent Pringle maneuver on hepatic RFA has been performed so far.

The effect of a Pringle maneuver in surgical hepatic resection has been controversially discussed in the literature. A Pringle maneuver is often used in patients with poor parenchymal conditions, larger tumors, or longer surgeries. A Pringle maneuver may have protective effects in surgical hepatic resection for high-risk cases by minimizing perioperative blood loss [17]. In several clinical studies, no difference in perioperative complications, postoperative liver function, tumor recurrence, or overall survival could be shown between hepatic resection and a Pringle maneuver/an intermittent Pringle maneuver [17–21]. However, an aggravation of gut barrier dysfunction with more aggressive translocation of endotoxins and intestinal bacteria has been described while performing a Pringle maneuver during RFA [22]. In an *in vivo* study by Kim et al., a Pringle maneuver resulted in severe pathologic changes in the portal vein, bile ducts, and liver parenchyma surrounding the ablation zone. Therefore, a Pringle maneuver should be performed with caution to avoid unintended thermal injuries [23]. An increased risk of ischemia-reperfusion injury was reported after RFA when performing a continuous Pringle maneuver *in vivo* [24]. However, a prolonged Pringle maneuver may lead to undesired thermal injuries. Therefore, an intermittent Pringle maneuver can represent an opportunity to combine the advantages of a Pringle maneuver with the disadvantages of a prolonged hepatic inflow occlusion.

The limitations of our study are the lack of a tumor model and the simulation of a natural liver vessel with a glass tube. The evaluation of vascular cooling effects *in vivo* is challenging due to the complexity of the existing natural vascular hepatic blood supply [25]. The cooling effect of a single hepatic vessel can hardly be estimated *in vivo*. Therefore, the study was performed with a glass tube that simulated a liver vessel to achieve a standardized setup. The isolating properties of the glass tube can be neglected, as it was situated outside of the electrical field of the applicators [9, 11, 12, 26]. Energy transmission took place at the position of the glass tube by direct temperature transfer. Glass has thermal properties that are similar to those of liver tissue and does not interfere with heat conduction [11, 26]. The thermal properties of the vascular wall itself were disregarded within this study. A standardized and reproducible test setting was used in our study. The perfusion of the glass tube was the only variable that was altered. Therefore, the impact of an intermittent Pringle maneuver could be exactly evaluated in this *ex vivo* model. The experiments were conducted at room temperature. Previous tests have demonstrated that a hepatic heat sink effect with similar extent to the heat sink effect at body temperature can occur at room temperature [12]. Ablations were performed in native porcine liver due to a lack of an adequate porcine tumor model. Porcine liver tissue has physiological properties similar to those of human liver tissue [27].

Our study suggests that an intermittent Pringle maneuver has a positive effect on RFA. In comparison to a continuous Pringle maneuver and to uninterrupted blood flow, an intermittent approach has favorable results regarding the ablation size. Functional disorders of the remnant liver may be reduced when performing an intermittent Pringle maneuver. *In vivo* experiments will be necessary to confirm the results of this *ex vivo* study.

Author Statement

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of interest. Informed consent: Informed consent is not applicable. Ethical approval: The conducted research is not related to either human or animal use.

Author Contributions

Franz G.M. Poch: conceptualization; formal analysis; investigation; methodology; project administration; software; supervision; writing – original draft. Christina A. Neizert: data curation; formal analysis; investigation; methodology; project administration; writing – original draft. Ole Gemeinhardt: conceptualization; formal analysis; writing – review and editing. Beatrice Geyer: formal analysis; investigation; methodology; project administration; supervision; writing – review and editing. Katharina Eminger: supervision; writing – review and editing. Christian Rieder: software; validation; visualization; writing – review and editing. Stefan M. Niehues: conceptualization; writing – review and editing. Janis Vahldiek: conceptualization; writing – review and editing. Stefan F. Thieme: writing – review and editing. Kai S. Lehmann: conceptualization; funding acquisition; methodology; project administration; supervision; writing – review and editing.

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Supplementary Material: The article (https://doi.org/10.1515/iss-2018-0008) offers reviewer assessments as supplementary material.

Reviewer Assessment

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***Corresponding author: Franz G.M. Poch,** Department of General, Visceral and Vascular Surgery, Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Campus Benjamin Franklin, Hindenburgdamm 30, 12200 Berlin, Germany, Phone: +49 30 450-522712, E-mail: franz.poch@charite.de

Reviewers' Comments to Original Submission

Reviewer 1: Andreas Schnitzbauer

Feb 25, 2018

Reviewer Recommendation Term:	Revise with Major Modification
Overall Reviewer Manuscript Rating:	65
Custom Review Questions	**Response**
Is the subject area appropriate for you?	3
Does the title clearly reflect the paper's content?	3
Does the abstract clearly reflect the paper's content?	3
Do the keywords clearly reflect the paper's content?	3
Does the introduction present the problem clearly?	3
Are the results/conclusions justified?	3
How comprehensive and up-to-date is the subject matter presented?	3
How adequate is the data presentation?	3
Are units and terminology used correctly?	3
Is the number of cases adequate?	3
Are the experimental methods/clinical studies adequate?	3
Is the length appropriate in relation to the content?	3
Does the reader get new insights from the article?	3
Please rate the practical significance.	3
Please rate the accuracy of methods.	3
Please rate the statistical evaluation and quality control.	3
Please rate the appropriateness of the figures and tables.	3
Please rate the appropriateness of the references.	3
Please evaluate the writing style and use of language.	3
Please judge the overall scientific quality of the manuscript.	3
Are you willing to review the revision of this manuscript?	Yes

Comments to Authors:

The authors present an experimental ex vivo study of 25 RFAs in porcine livers simulating Perfusion and intermittent Pringle maneuvre. They conclude that intermittent Pringle maneuvre may be beneficial especially in Ablation Areas with Major vessels Close to the area of interest.
Comments:
Title. complex title, why not: Intermittent Pringle maneuvre may be benficial for radiofrequency ablations in situations with tumor-vessel proximity
page 3: you write about an intravesselflow of 100ml/min. What is the common intraportal, intraarterial and intravenous flow in physiologically perfused livers? There may be a difference in dependence of the tumors location within the liver that you want to treat.
page 5 Vp#### confused me. You should explain under methods what all These abreviations mean. I first thought it is a correction remnant.
page 6: first paragraph of discussion. do not repeat your introduction. This is redundant. Start with your strongest finding!
page 7: in the Cochrane Analysis investigating the effect of pringle on survival, there was no effect detectable fro randomized controlled studies. Therefore your arguments are somewhat irritating to me. Did you look for publications that fit your findings or did you use the best evidence that you discussed in correlation to your findings (the better way!). It is questionable, whether the Pringle-maneuvre per se leads to worse outcome. In my opinion, Pringle reflects complex patients, severe belleing, impaired immunology and an increased risk of dying and Tumor recurrence due to hemorrhage and the complicated course. Therefore I would highly recommend to re write this section.

Reviewer 2: anonymous

Feb 26, 2018

Reviewer Recommendation Term:	Reject
Overall Reviewer Manuscript Rating:	20

Custom Review Questions	**Response**
Is the subject area appropriate for you?	1 - Low/No
Does the title clearly reflect the paper's content?	2
Does the abstract clearly reflect the paper's content?	2
Do the keywords clearly reflect the paper's content?	2
Does the introduction present the problem clearly?	1 - Low/No
Are the results/conclusions justified?	1 - Low/No
How comprehensive and up-to-date is the subject matter presented?	1 - Low/No
How adequate is the data presentation?	1 - Low/No
Are units and terminology used correctly?	2
Is the number of cases adequate?	1 - Low/No
Are the experimental methods/clinical studies adequate?	1 - Low/No
Is the length appropriate in relation to the content?	3
Does the reader get new insights from the article?	1 - Low/No
Please rate the practical significance.	1 - Low/No
Please rate the accuracy of methods.	1 - Low/No
Please rate the statistical evaluation and quality control.	N/A
Please rate the appropriateness of the figures and tables.	N/A
Please rate the appropriateness of the references.	1 - Low/No
Please evaluate the writing style and use of language.	2
Please judge the overall scientific quality of the manuscript.	1 - Low/No
Are you willing to review the revision of this manuscript?	No: To poor article

Comments to Authors:
The issue developed is very important. Radiofrequency is one of the essential tools in the treatment algorithm of malignant hepatic tumors.
The research objective and hypothesis were not clearly defined.
The objective of this study should be to evaluate the impact of a Pringle maneuver on the ablation size.

It should be interesting to measure liver tissue necrosis by a blinded gastrointestinal histopathologist. Instead of analyzing the ablation area using a thin spline landmark registration that conclude with no differences in ablation area and ablation radius were observed between the five test series.
In the introduction, asseverate that radiofrequency ablation (RFA) is an important therapy option for the treatment of non-resectable malignant liver tumors. Also, the vascular cooling effect ("heat sink") of adjacent liver vessels restricts the ablation size. A temporary hepatic inflow occlusion of both the hepatic artery and portal vein ("Pringle maneuver") can reduce vascular cooling effects in RFA.

Nevertheless, the ablation technique is not usually associated with a temporary hepatic flow occlusion (Pringle maneuver). Radiofrequency ablation with the Pringle maneuver created more severe pathologic changes in the portal vein, bile ducts and liver parenchyma surrounding the ablation zone compared with RF ablation without the Pringle maneuver.1
The isolating properties of the glass tube and thermal properties that are similar to liver tissue but not similar to a real vessel. And we know that he application of the Pringle maneuver concurrently with extended liver RFA aggravates gut barrier dysfunction with more aggressive translocation of endotoxins and intestinal bacteria.2 Extended liver RFA causes SIR and multi-organ injury, which are exacerbated when a concurrent Pringle maneuver is applied.3
Therefore, many variables were not taken into account when analyzing the vascular cooling effects.
1- Kim SK, Lim HK, Ryu J, et al. Radiofrequency Ablation of Rabbit Liver In Vivo: Effect of the Pringle Maneuver on Pathologic Changes in Liver Surrounding the Ablation Zone. Korean Journal of Radiology. 2004;5(4):240-249.
2- Ypsilantis P., Lambropoulou M., Grapsa A., et al. Pringle maneuver deteriorates gut barrier dysfunction induced by extended-liver radiofrequency ablation. Digestive Diseases and Sciences. 2011;56(5):1548-1556.
3- Ypsilantis P., Lambropoulou M., Anagnostopoulos C., et al. Pringle maneuver exacerbates systemic inflammatory response and multiple-organ injury induced by extended liver radiofrequency ablation. Human & Experimental Toxicology. 2011;30:1855-1864.

Reviewer 3: anonymous

Mar 16, 2018

Reviewer Recommendation Term:	Revise with Major Modification
Overall Reviewer Manuscript Rating:	70

Custom Review Questions	**Response**
Is the subject area appropriate for you?	4
Does the title clearly reflect the paper's content?	1 - Low/No
Does the abstract clearly reflect the paper's content?	4
Do the keywords clearly reflect the paper's content?	4
Does the introduction present the problem clearly?	5 - High/Yes
Are the results/conclusions justified?	3
How comprehensive and up-to-date is the subject matter presented?	4
How adequate is the data presentation?	3
Are units and terminology used correctly?	5 - High/Yes
Is the number of cases adequate?	3
Are the experimental methods/clinical studies adequate?	3
Is the length appropriate in relation to the content?	4
Does the reader get new insights from the article?	4
Please rate the practical significance.	3
Please rate the accuracy of methods.	3
Please rate the statistical evaluation and quality control.	3
Please rate the appropriateness of the figures and tables.	2
Please rate the appropriateness of the references.	4
Please evaluate the writing style and use of language.	4
Please judge the overall scientific quality of the manuscript.	3
Are you willing to review the revision of this manuscript?	Yes

Comments to Authors:
The authors used an ex vivo porcine liver model to analyze the effects of different Pringle maneuver (PM) settings on the ablation size of radiofrequency ablation (RFA). Of interest, they found that an intermittent PM increases the ablation area when initial hepatic inflow is succeeded by PM. Although this should be further confirmed in additional studies, this observation may be of clinical relevance and contribute to improve the efficiency of future RFA protocols.
I have several major and minor comments, which should be addressed to further improve the quality of this interesting manuscript:
Major comments:
1. The title does not reflect the content and conclusions of the manuscript. In fact, the authors could not only show that intermittent PM "is not inferior to continuous vascular inflow occlusion", but may even improve the outcome of RFA due to an increase in ablation area (as nicely discussed at the end of the manuscript). Therefore, I suggest that the authors change the title and emphasize this interesting positive observation.

2. To improve the clarity of the MM section, the authors should include more information about the five experimental settings in the section “Experimental setup” (although they are shortly explained in the legend to Fig. 2). This information should include: 1) Nomenclature and total number of ablations per group (see also Abstract; please also transfer first sentence of the Results section to this paragraph). 2) Length of time intervals shown in Fig. 2 (please also include a time scale in this figure). 3) How were the 10 porcine livers assigned to the different groups?
3. Why did the authors perfuse the livers with water at room temperature (i.e. 20-22°C) and not at 37°C (this would better mimic the in vivo conditions). This point needs clarification and should ideally be discussed on page 8. In fact, this difference in temperature may markedly affect the outcome of the entire study!
4. The segmental analysis of ablation areas is confusing and needs more explanation. In Fig. 1c the authors show 4 different segments with a diameter of 2.5 mm. However, in Fig. 4 they present data from more segments “0-2.5; ~; 10-12.5; 12.5-15.0; 15.0-17.5; 17.5-20.0). Moreover, it is not clear why a cooling effect of almost 80% is not significant, whereas the increase of ablation area of 80% is significant for V2 and V3 at 15.5-20.0 mm. Is this due to a large heterogeneity of the data? Why are the data not presented as mean +/- SD?
Minor comments:
1. Page 3, line 3: The abbreviation “RFA” has already been introduced in the first sentence of the introduction.
2. Page 3, line 10: “…power) power….” Please correct!
3. Page 5, line 13: “The geometrical averaged area of this experimental setting….was 958 mm²”. However, in Tab. 1 it’s 936 mm²? Please clarify!
4. The first paragraph of the discussion section is a simple repetition of the introduction. Please remove this redundant paragraph and start the discussion with more specific information.
5. Legend to Fig. 4: Statistical analysis was defined as $p < 0.05$ (see MM section). The differentiation between “significant” and “highly significant” is not explained and also makes no sense from a statistical point of view.
6. Fig. 3: Please include scale bars.

Authors’ Response to Reviewer Comments

Mar 23, 2018

Reviewer #1:

The authors present an experimental ex vivo study of 25 RFAs in porcine livers simulating Perfusion and intermittent Pringle maneuvre. They conclude that intermittent Pringle maneuvre may be beneficial especially in Ablation Areas with Major vessels Close to the area of interest.
Comments:
Title. complex title, why not: Intermittent Pringle maneuvre may be benficial for radiofrequency ablations in situations with tumor-vessel proximity
- Thank you for this valuable suggestion, we have adjusted the title.
page 3: you write about an intravesselflow of 100ml/min. What is the common intraportal, intraarterial and intravenous flow in physiologically perfused livers? There may be a difference in dependence of the tumors location within the liver that you want to treat.
- Blood flow in a 70 kg human:
o Liver: 1450 ml/min
o Hepatic artery: 300 ml/min
o Portal vein: 1150 ml/min
- In previous studies we could demonstrate, that the vascular cooling effect is independent of blood flow volume or vessel diameter. We could demonstrate, that a cooling effect already occurs in multipolar RFA at a flow rate of 100 ml/min (cf. p. 4). Exceptional cases include small vessels (< 2 mm), which may occlude during RFA and will not take part in vascular cooling effects in vivo.
page 5 Vp#### confused me. You should explain under methods what all These abreviations mean. I first thought it is a correction remnant.
- Thank you for this hint. We have initially introduced the abbreviations in Figure 2. However, we added an explanation in the text in order to avoid confusion.
page 6: first paragraph of discussion. do not repeat your introduction. This is redundant. Start with your strongest finding!
- Thank you for this comment, we have revised this section.
page 7: in the Cochrane Analysis investigating the effect of pringle on survival, there was no effect detectable fro randomized controlled studies. Therefore your arguments are somewhat irritating to me. Did you look for publications that fit your findings or did you use the best evidence that you discussed in correlation to your findings (the better way!). It is questionable, whether the Pringle-maneuvre per se leads to worse outcome. In my opinion, Pringle reflects complex patients, severe belleing, impaired immunology and an increased risk of dying

and Tumor recurrence due to hemorrhage and the complicated course. Therefore I would highly recommend to re write this section.
- Thank you for addressing this important issue. We have rewritten this section!

Reviewer #2:
The issue developed is very important. Radiofrequency is one of the essential tools in the treatment algorithm of malignant hepatic tumors. The research objective and hypothesis were not clearly defined.
The objective of this study should be to evaluate the impact of a Pringle maneuver on the ablation size.
- Temperatures above 100 °C lead to carbonization of the tissue. Since carbonized tissue acts as an electric isolator, temperatures above 100 °C should be avoided in RFA for a homogenous temperature distribution within the target tissue. An intermittent Pringle maneuver may combine the advantages of a Pringle maneuver (no cooling effect), while avoiding temperatures above 100 °C. Therefore, the objective of this study was to compare the impact of an intermittent hepatic inflow occlusion to a complete inflow occlusion and to no inflow occlusion in RFA ex vivo.
It should be interesting to measure liver tissue necrosis by a blinded gastrointestinal histopathologist. Instead of analyzing the ablation area using a thin spline landmark registration that conclude with no differences in ablation area and ablation radius were observed between the five test series.
- This recommendation would be of great interest. However, the study was performed ex vivo in porcine livers from a slaughterhouse. Therefore a histopathological examination was not possible. The lesions were evaluated along the accepted macroscopic borders of the ablation´s white zone.
In the introduction, asseverate that radiofrequency ablation (RFA) is an important therapy option for the treatment of non-resectable malignant liver tumors. Also, the vascular cooling effect (“heat sink”) of adjacent liver vessels restricts the ablation size. A temporary hepatic inflow occlusion of both the hepatic artery and portal vein (“Pringle maneuver”) can reduce vascular cooling effects in RFA.
- Thank you for this recommendation, we have added an additional comment in the introduction.
Nevertheless, the ablation technique is not usually associated with a temporary hepatic flow occlusion (Pringle maneuver). Radiofrequency ablation with the Pringle maneuver created more severe pathologic changes in the portal vein, bile ducts and liver parenchyma surrounding the ablation zone compared with RF ablation without the Pringle maneuver.1
The isolating properties of the glass tube and thermal properties that are similar to liver tissue but not similar to a real vessel. And we know that he application of the Pringle maneuver concurrently with extended liver RFA aggravates gut barrier dysfunction with more aggressive translocation of endotoxins and intestinal bacteria.2 Extended liver RFA causes SIR and multi-organ injury, which are exacerbated when a concurrent Pringle maneuver is applied.3
Therefore, many variables were not taken into account when analyzing the vascular cooling effects.
- Thank you for this comment, we have rewritten this section in the manuscript.
1- Kim SK, Lim HK, Ryu J, et al. Radiofrequency Ablation of Rabbit Liver In Vivo: Effect of the Pringle Maneuver on Pathologic Changes in Liver Surrounding the Ablation Zone. Korean Journal of Radiology. 2004;5(4):240-249.
2- Ypsilantis P., Lambropoulou M., Grapsa A., et al. Pringle maneuver deteriorates gut barrier dysfunction induced by extended-liver radiofrequency ablation. Digestive Diseases and Sciences. 2011;56(5):1548-1556.
3- Ypsilantis P., Lambropoulou M., Anagnostopoulos C., et al. Pringle maneuver exacerbates systemic inflammatory response and multiple-organ injury induced by extended liver radiofrequency ablation. Human & Experimental Toxicology. 2011;30:1855-1864.

Reviewer #3:
The authors used an ex vivo porcine liver model to analyze the effects of different Pringle maneuver (PM) settings on the ablation size of radiofrequency ablation (RFA). Of interest, they found that an intermittent PM increases the ablation area when initial hepatic inflow is succeeded by PM. Although this should be further confirmed in additional studies, this observation may be of clinical relevance and contribute to improve the efficiency of future RFA protocols.
I have several major and minor comments, which should be addressed to further improve the quality of this interesting manuscript:
Major comments:
1. The title does not reflect the content and conclusions of the manuscript. In fact, the authors could not only show that intermittent PM “is not inferior to continuous vascular inflow occlusion”, but may even improve the outcome of RFA due to an increase in ablation area (as nicely discussed at the end of the manuscript). Therefore, I suggest that the authors change the title and emphasize this interesting positive observation.
- Thank you for this comment, we have adjusted the title.
2. To improve the clarity of the MM section, the authors should include more information about the five experimental settings in the section

“Experimental setup” (although they are shortly explained in the legend to Fig. 2). This information should include: 1) Nomenclature and total number of ablations per group (see also Abstract; please also transfer first sentence of the Results section to this paragraph). 2) Length of time intervals shown in Fig. 2 (please also include a time scale in this figure). 3) How were the 10 porcine livers assigned to the different groups?

1. Nomenclature and number of ablations were included

2. The length of one interval was set to 10 kJ, since the energy input is most important for a complete ablation. Ablation time is less relevant. This has been clarified in the MM section.

3. The porcine livers were randomly assigned to the ablations. A statement was added into this section.

3. Why did the authors perfuse the livers with water at room temperature (i.e. 20-22°C) and not at 37°C (this would better mimic the in vivo conditions). This point needs clarification and should ideally be discussed on page 8. In fact, this difference in temperature may markedly affect the outcome of the entire study!

- In previous studies we could demonstrate, that the cooling effect is independent of the tissue temperature. Therefore, we have chosen 22 °C for the perfusion of the vessel and the temperature of the tissue. A corresponding remark has been added in the manuscript.

4. The segmental analysis of ablation areas is confusing and needs more explanation. In Fig. 1c the authors show 4 different segments with a diameter of 2.5 mm. However, in Fig. 4 they present data from more segments “0-2.5; ~; 10-12.5; 12.5-15.0; 15.0-17.5; 17.5-20.0). Moreover, it is not clear why a cooling effect of almost 80% is not significant, whereas the increase of ablation area of 80% is significant for V2 and V3 at 15.5-20.0 mm. Is this due to a large heterogeneity of the data? Why are the data not presented as mean +/- SD?

- Figure 1c represents a schematic representation of the segmental analysis. In the previous schematic representation (Figure 1c) the diameter of the segments was “x”. In the final evaluation “x” was set to “2.5 mm”. No change in ablation area could be observed between 2.5 and 10.0 mm (“~”).

The section was revised.

The data is represented as median, due to the small sample. The heterogeneity of the cooling effect in V4 is large. Therefore, the cooling effect is not significant in V4. However, a macroscopic rim of native tissue is observed, a increased risk of tumor recurrence exists around the vessel.

Minor comments:

1. Page 3, line 3: The abbreviation “RFA” has already been introduced in the first sentence of the introduction.

- Thank you for this hint.

2. Page 3, line 10: “…power) power….” Please correct!

- Thank you for this suggestion. We have rectified it.

3. Page 5, line 13: “The geometrical averaged area of this experimental setting….was 958 mm²”. However, in Tab. 1 it’s 936 mm²? Please clarify!

- Thank you for recognizing this important issue. 936 mm2 is correct.

4. The first paragraph of the discussion section is a simple repetition of the introduction. Please remove this redundant paragraph and start the discussion with more specific information.

- Thank you for this comment, we have adjusted the section.

5. Legend to Fig. 4: Statistical analysis was defined as $p < 0.05$ (see MM section). The differentiation between “significant” and “highly significant” is not explained and also makes no sense from a statistical point of view.

- Thank you for this comment. “Highly significant” was removed.

6. Fig. 3: Please include scale bars.

- We have included scale bars.

Reviewers' Comments to Revision

Reviewer 1: Andreas Schnitzbauer

Apr 04, 2018

Reviewer Recommendation Term:	Accept
Overall Reviewer Manuscript Rating:	75
Custom Review Questions	**Response**
Is the subject area appropriate for you?	3
Does the title clearly reflect the paper's content?	3
Does the abstract clearly reflect the paper's content?	3
Do the keywords clearly reflect the paper's content?	4
Does the introduction present the problem clearly?	3
Are the results/conclusions justified?	3
How comprehensive and up-to-date is the subject matter presented?	3
How adequate is the data presentation?	3
Are units and terminology used correctly?	3
Is the number of cases adequate?	3
Are the experimental methods/clinical studies adequate?	3
Is the length appropriate in relation to the content?	4
Does the reader get new insights from the article?	4
Please rate the practical significance.	4
Please rate the accuracy of methods.	3
Please rate the statistical evaluation and quality control.	4
Please rate the appropriateness of the figures and tables.	4
Please rate the appropriateness of the references.	4
Please evaluate the writing style and use of language.	4
Please judge the overall scientific quality of the manuscript.	4
Are you willing to review the revision of this manuscript?	Yes

Comments to Authors:
none

Reviewer 3: anonymous

Mar 26, 2018

Reviewer Recommendation Term:	Accept
Overall Reviewer Manuscript Rating:	85
Custom Review Questions	**Response**
Is the subject area appropriate for you?	4
Does the title clearly reflect the paper's content?	4
Does the abstract clearly reflect the paper's content?	4
Do the keywords clearly reflect the paper's content?	4
Does the introduction present the problem clearly?	5 - High/Yes
Are the results/conclusions justified?	3
How comprehensive and up-to-date is the subject matter presented?	4
How adequate is the data presentation?	4
Are units and terminology used correctly?	5 - High/Yes
Is the number of cases adequate?	3
Are the experimental methods/clinical studies adequate?	3

Is the length appropriate in relation to the content?	4
Does the reader get new insights from the article?	4
Please rate the practical significance.	3
Please rate the accuracy of methods.	3
Please rate the statistical evaluation and quality control.	3
Please rate the appropriateness of the figures and tables.	2
Please rate the appropriateness of the references.	4
Please evaluate the writing style and use of language.	4
Please judge the overall scientific quality of the manuscript.	4
Are you willing to review the revision of this manuscript?	No: The authors have adequately responded to all my comments.

Comments to Authors:
The authors have revised their manuscript according to my comments. I have no further comments.