



## Reviewer Assessment

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

<https://doi.org/10.1515/iss-2018-0008>

Received February 11, 2018; accepted April 11, 2018

**\*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 manoeuvre. They conclude that intermittent Pringle manoeuvre may be beneficial especially in Ablation Areas with Major vessels Close to the area of interest.

Comments:

Title. complex title, why not: Intermittent Pringle manoeuvre may be beneficial for radiofrequency ablations in situations with tumor-vessel proximity

page 3: you write about an intravessel flow 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 abbreviations 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.<sup>1</sup>

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 the application of the Pringle maneuver concurrently with extended liver RFA aggravates gut barrier dysfunction with more aggressive translocation of endotoxins and intestinal bacteria.<sup>2</sup> Extended liver RFA causes SIR and multi-organ injury, which are exacerbated when a concurrent Pringle maneuver is applied.<sup>3</sup>

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 mm<sup>2</sup>”. However, in Tab. 1 it’s 936 mm<sup>2</sup>? 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 manoeuvre. They conclude that intermittent Pringle manoeuvre may be beneficial especially in Ablation Areas with Major vessels Close to the area of interest.

Comments:

Title. complex title, why not: Intermittent Pringle manoeuvre may be beneficial 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 abbreviations 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.<sup>1</sup>

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 the application of the Pringle maneuver concurrently with extended liver RFA aggravates gut barrier dysfunction with more aggressive translocation of endotoxins and intestinal bacteria.<sup>2</sup> Extended liver RFA causes SIR and multi-organ injury, which are exacerbated when a concurrent Pringle maneuver is applied.<sup>3</sup>

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 mm<sup>2</sup>”. However, in Tab. 1 it’s 936 mm<sup>2</sup>? Please clarify!

- Thank you for recognizing this important issue. 936 mm<sup>2</sup> 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

---

<b>Reviewer Recommendation Term:</b>	Accept
<b>Overall Reviewer Manuscript Rating:</b>	75
<b>Custom Review Questions</b>	<b>Response</b>
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
<b>Comments to Authors:</b>	
none	

---

### Reviewer 3: anonymous

Mar 26, 2018

---

<b>Reviewer Recommendation Term:</b>	Accept
<b>Overall Reviewer Manuscript Rating:</b>	85
<b>Custom Review Questions</b>	<b>Response</b>
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.

---