November 11, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 5395-review.doc).

Title: Intraductal endoscopic radio-frequency ablation (RFA) for the treatment of non-resectable malignant bile duct obstruction

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The manuscript has been improved according to the suggestions of reviewers:

1) Format and language have been updated according to the journals policy

2) Revision has been made according to the suggestions of the reviewer:

Reviewer 1

1. Delete form the title "a feasibility study" and from Methods "This is an open label pilot study". Both statements refer to prospective study design while authors present their experience retrospectively.
   The title "a feasibility study" and the Methods' section denoting "This is an open label pilot study" have been removed according to the reviewer's comment.

2. In the methods section, authors should define "technical success" and how was it measured
   The technical success of RFA was defined as positioning the RFA catheter at the region of interest and applying coagulation current as intended with consecutive successful stent insertion.

3. In the results, it is not reported the settings of the ablations (what power? 7 or 8 or 9 or 10 W) for each patient. Please report also how many treatment cycles were applied per patient.
   Thank you for this helpful comment. Table 1 depicts clearly the number of RFA treatment cycles that have been applied to the respective patient; these were 17 in 11 patients. The number of RFA applications within one intervention ranges from 1 to 4 according to stricture length and bilateral or unilateral applications (as mentioned on page 4 under the topic 'Methods'). We added this information into the methods and results part of our manuscript.
   We did not apply 7 Watts in the patients, this range was optional for the endoscopists; this has been corrected now. 8 Watts was used for the left or right intrahepatic biliary ducts and 10 W was used for the subhilar section of the common hepatic or common bile duct, respectively. This information has been inserted into the 'Methods' part of our manuscript.

4. The first paragraph of the discussion can be omitted. All the relevant info is included in the introduction.
   This is true, we therefore removed this paragraph.

5. Please respect the authors' guidelines when resubmitting the manuscript.
   This has thoroughly been considered.

6. Experiment animals are not killed but euthanized.
   This has been corrected.

Reviewer 2

1. It wasnt apparent in the methods section that this was a pre-clinical and clinical study involving both swine and human.
   Thank you for this comment. As it is our intention to completely clarify that we performed both, a pre-clinical and a clinical study, we changed arrangement of the methods part and now start with explaining the pre-clinical part followed by the human RFA application. We moreover added an explanation on our study concept.

2. Also the outcome parameters and duration of the study needs to be described.
   Outcome of this study was
   - pre-clinical study part: technical feasibility of RFA in a pig model, realization of inducing necrosis in respect to differing application mode and power.
   - clinical study part: technical feasibility of RFA in patients, evaluation of periinterventional complications and follow-up.
   The period of treatment was added in the Methods part"between February 2012 and April 2013".

3. The conclusion that hemobilia resulted from RFA is a postulation. There are so many confounding factors that could result in these complications eg stage of tumour, setting of RFA and thus, this should be amended.
   This is completely true; we are aware of the fact, that we could not prove the coincidence of RFA application and bleeding. Nevertheless, our group has an extensive experience in performing ERCP in Klatskin tumors being a well renowned superregional referral center for surgery and interventional therapy of these tumors. E. g. we have been doing photodynamic therapy (PDT) for more than ten years, now. We never experienced live threatening hemobilia in any of our Klatskin or PDT patients, though, and were heavily surprised from the reported incidences.
   Nevertheless, as we don't know the exact reason we rewrite the text to"…to be potentially associated to RFA application in our series". Of course we discuss these events in the 'discussion' section of our paper.

4. Introduction: I assume that the ex-vivo study was performed first to assess the appropriate setting first before the human study? If yes, the authors need to describe this more clearly in both the introduction and methods section Methods.
   This has been corrected.

5. The ex-vivo study should be described more clearly. So was it an ex-vivo non survival study? How many swine liver was used? How was this determined? If only one was used. How sure are the authors that a similar effect could be repeated in each swine? How was the site of rfa selected? How was the catheter introduced into the bile duct? Was histological assessment used to delineate the extent of necrosis? If not, how accurate was the macroscopic assessment? Was it just by the change in colour of the tissue? If yes how does this correlated with histological damage?
   As now mentioned in the methods part we used five fresh swine livers from euthanized animals. This part of the study was to assess the correlation between electrosurgical parameter and ablation areal. The catheter was introduced directly into liver tissue and not into the bile duct as this would have been filled with air. Necrotic tissue was assessed macroscopically and measured by an investigator with a caliper

6. Results: Figure 2 was not referenced in the results section. Also the figure needs more explanation. So from left to right, a higher energy was used. How high was the energy? How was the depth of necrosis measured? If only one swine was used, so was a different IHD cannulated for each application?
   Any overlapping between each application?
   This was corrected. Figure 2 is now referenced in the methods part. Figure 2 is just an example for the results of figure 3 were electrosurgical parameter and ablation area are shown in detail. For the rest of the questions see answer of comment 5 and the method part.

7. In the porcine study, the authors mentioned was a power of 8-10 was most appropriate. How was this determined? Is this measured in relationship to the usual thickness of cholangioca in human?
   The preclinical study was a feasibility study, demonstrating effect of RFA to the investigators who planned to perform RFA in patients. We were orientating on the manufacturers recommendations, of course. We considered deep induction of necrosis with applying >10 W as potentially penetrating any biliary duct.
   A widely accepted definition of a usual thickness of CCA is not known to us.

8. For the human study, the authors need more explanation on the selection of cases. I noticed that some of them are not suffering from cholangioca, is it possible that the biliary obstruction in these patients are from extrinsic compression? If so, can this be one of the reasons of bleeding as you are potentially applying RFA to the native bile duct?
   Every patient eligible for RFA of the biliary tract was included to this analysis. As it is a new method we wanted to evaluate safety and follow up without focusing on one tumor entity. Biliary obstruction was examined with ultrasound, CT-scan and ERCP. Extrinsic compression could therefore be excluded
   .

9. Also, the postprocedural course of the patients need more explanation? Did any patients suffered from 30 day morbidities? I seldom attribute complications occurring after 30 days after treatment as treatment-related as many factors can be responsible eg tumour progression, chemo or radiotherapy.
   It is true that none of the patients died within less than four weeks after RFA application. Nevertheless, a potential relationship might not be excluded in our opinion.
   Cp. answer to comment No. 3!
   In our opinion, RFA should not be applied outside from controlled studies until any potential relation between postinterventional bleeding and RFA has been excluded. It is vital to consider that on the 'pro' side of offering RFA to the patient, this method has no controlled studies to show proving efficacy / improving outcome of the patients. We (who are or have been performing RFA) are all hoping that effects

of RFA might be similar effective as PDT has been shown to be…

10. Figure 3 needs an english translation on the side bar Figures 4&5 are not referenced in the manuscript.
    Translation was done; Figure 4 is referenced now on page 5 in the methods part. Figure 5 is mentioned in the results part on page 6.

11. Discussion Pls add the reference for the austrian study referred to in paragraph 2.
    This has been corrected. The Austrian study was meanwhile published and cited:
    Dolak W, Schreiber F, Schwaighofer H, et al. Endoscopic radiofrequency ablation for malignant biliary obstruction: a nationwide retrospective study of 84 consecutive applications. Surg. Endosc. 2013.

Reviewer 3

1. Since the authors documented in 3/11 patients severe side effects from RFA treatment (two of those patients died consequently) the report mainly raise severe concerns about the clinical significance of RFA ablation in this indication (despite the proven efficacy). This point should be more stressed out in the Abstract and the Discussion.
   We profoundly discuss this potential relation of RFA and life-threatening hemobilia in the 'discussion' part now.

2. Further it may be interesting to know if those patients with post-RFA hemobilia received concomittant chemotherapy or not.
   Added on page 6: "None of these patients underwent chemotherapeutical treatment".

3. Additionally some words about the technical success of the RFA-procedure are necessary: Did you observe an effect on the stricture diameter or on the ease to insert the plastic endoprosthesis ?
   The technical success of RFA was defined as positioning the RFA catheter at the region of interest and applying coagulation current as intended with consecutive successful stent insertion (cp. Reviewer 1, comment No. 2).
   We indeed observed increasing ease to insert plastic endoprosthesis and increase of diameter of the tumor stenosis as an effect of RFA, but we did not consequently measure diameters in the patients for this study.

4. What do you think is the additional effect of RFA beside the stent placement (in the light that you observed 4/11 cases with recurrent cholangitis after RFA)?
   RFA is a local treatment for palliative Situations. It should prevent cholangitic episodes, provide longer stent patencies, show less hospital residencies and should be seen as a cost effective treatment option. At this time we cannot say if there is a potential benefit in any of these points in comparison to the gold standard. As we are not sure why the haemobilia occurred, different hypothesis are possible.
   Added in the discussion part: "Another possibility could be that the strong necrotic effect of the RFA triggers a strong angiogenic response inside the tumor causing the recruitment of new vessel branches within the treated tissue. This hypothesis should be confirmed and analysed by immunohistochemical stainings and biochemical processing of the damaged tissue.
   In this hypothetical case, a consecutive chemotherapy to prevent those angiogenic stimuli could show better results

3) Language, references and typesetting were corrected

Thank you again for giving us the opportunity to publish our manuscript in the World Journal of Gastrointestinal Endoscopy.

Sincerely yours,

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