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Are there parameters that predict a non-diagnostic biopsy outcome taken during laparoscopic assisted cryoablation of small renal tumours?

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ABSTRACT

Introduction: The histopathological diagnosis of small renal mass (SRM) treated with cryoablation relies on pre- or intraoperative biopsies. Since a considerable number of these SRM are benign, accurate diagnosis has prognostic and follow-up implications. Main problem in SRM is the high rate of non-diagnostic biopsies.

Purpose: To assess whether certain tumour and biopsy characteristics are correlated with a diagnostic biopsy outcome.

Methods: One hundred tumours smaller than 4.5 cm in 94 patients were treated with laparoscopic cryoablation. After dissection of the perirenal fat and identification of the tumour by intra-abdominal ultrasound, one or more biopsies were obtained before freezing. Using the Student-t/Mann Whitney U-test the following parameters were evaluated for predicting biopsy outcome: tumour size, location and exophytic part of the tumour, size of the biopsy needle, the number of biopsies taken and presence of non-enhancing areas compatible with necrosis inside the tumours. Correlations among parameters were assessed using a Spearman correlation or Kruskal-Wallis test.

Results: 22 biopsies (22%) were non-diagnostic and consisted of normal kidney tissue, connective tissue, fat, fibrosis, necrosis and/or blood. There were no significant differences in parameters between the diagnostic and non-diagnostic group. There was a positive correlation between tumour size and number of biopsies (p=0.029) and between the presence of non-enhancing areas and both size (p<0.001) and the number of biopsies taken (p<0.001).

Conclusion: No statistical significant correlation was found between biopsy outcome and tumour- or biopsy characteristics. More biopsies were taken in larger tumours, and larger tumours contained more non-enhancing areas suspect for necrosis.
INTRODUCTION
Renal cryosurgery is a treatment option for small renal masses (SRM) in which the tumour is ablated in situ by lethal freezing injury. Consequently, there is no surgical specimen for pathological examination and therefore the histopathological diagnosis relies exclusively on biopsies. A large study has shown that 23% of operated renal tumours smaller than 4 cm turn out to be benign and even when cancer is present in these tumours a considerable percentage is of low grade[1]. Preoperative diagnosis can be obtained by performing a percutaneous core biopsy or fine needle aspiration, however concerns about e.g. the accuracy have prevented their routine use[2-4]. Of percutaneous core biopsies up to 21% are non-diagnostic[4], and reports on intra-operative biopsies taken before cryoablation show a similar range of non-diagnostic biopsy outcome[5-8]. Non-diagnostic biopsies are usually due to either an insufficient amount of tissue or erroneous sampling of necrotic/fibrotic areas or normal kidney tissue[4].

Since determination of treatment success after cryoablation is mainly based on cross-sectional imaging, obtaining a histopathological diagnosis should be warranted in order to withhold patients with a benign tumour from an intense follow up. Moreover it has been demonstrated that a vast amount of non-diagnostic biopsies appears to be false negative in small renal mass[9]. This study evaluated whether tumour and biopsy characteristics can predict biopsy outcome in a series of patients treated with laparoscopic renal cryoablation in our centre.

METHODS
We identified consecutive patients treated with laparoscopic cryoablation between July 2004 and May 2010 for one or more solid enhancing SRM that underwent an intraoperative biopsy prior to freezing. The cryoablation was done using a 3rd generation argon-based system with 1.47 mm (17G) cryoprobes (type SeedNet® or IceRods®, Seednet Gold System™, Galil, Tel Aviv, Israel).

After mobilization of the kidney and dissection of the peritumoural fat, real-time laparoscopic ultrasound (US) was used to identify and measure the size of the tumour. Percutaneous biopsies were taken under laparoscopic vision using a 16 or 18 gauge core biopsy system (18G Topnotch™ and TruPath™, Boston Scientific, USA and 16G...
Quick-Core® Biopsy Needle, Cook Medical, Denmark) without the use of a guiding sheath. To ensure an accurate position of the needle the tumour was first penetrated for 1 or 2 mm before the biopsy gun was fired. The number of biopsies taken was determined by the number of shots needed to obtain a proper core of tissue as identified visually by the surgeon. All biopsies were fixed in formalin, embedded in paraffin and stained using haematoxylin and eosin (H&E). All biopsies were viewed and multidisciplinary discussed by specialized GU-pathologists. Pathology reports were retrieved from the hospital computer system and results were marked in a database as either diagnostic or non-diagnostic. Biopsies were considered diagnostic when a definitive diagnosis of benign or malignant tumour was possible. A biopsy was considered non-diagnostic if no tumour cells were present in the biopsy tissue, or insufficient tumour cells were present to differentiate between a benign and malignant tumour. The histopathological diagnosis was determined according to the WHO 2004 classification[10]. If necessary, additional immunohistochemical staining was done at the discretion of the pathologist.

The following tumour and biopsy characteristics were evaluated for their influence on biopsy outcome:

1. Tumour size; the maximum diameter as measured on preoperative computerized tomography (CT) or magnetic resonance imaging (MRI).
2. The exophytic part of the tumour; the percentage tumour protruding outside the surface of the kidney as measured on CT or MRI.
3. Tumour location; the location of the tumour epicentre was recorded as upper, middle or lower pole of the kidney, assessed on the preoperative CT or MRI.
4. The presence of non-enhancing areas compatible with necrosis inside the tumours on preoperative CT or MRI (categorised as present of absent).
5. The number of biopsies taken (i.e. number of shots, see above).
6. The size of the biopsy needle (16 or 18 gauge).
7. The quality of the biopsies (after preparation for pathological assessment).

In order to assess the quality of the biopsies the slides used for microscopical investigation by the pathologist were retrieved from our clinics pathological archive.
Figure 1 A solid tumor (A) with a good quality biopsy specimen consisting of a compact core (C). Below, a tumor with nonenhancing areas suggestive for necrosis (B) with a poor quality biopsy specimen consisting of multiple short fragments (D).

and scored by two investigators (KB and MH) according to the following standards (also see figure 1):

- **Good**: One or more compact cores > 10 mm in length
- **Intermediate**: One or multiple fragments between 5 and 10 mm
- **Poor**: multiple or solitary, separate fragments smaller than 5 mm.

To evaluate differences in tumour- and biopsy characteristics between diagnostic and non-diagnostic biopsy outcome we used the Student t-test or in case of a skewed distribution the Mann Whitney U-test for numerical parameters and the chi-square test for the categorical parameters.

To analyse correlations between individual parameters we performed a Spearman’s correlation for numerical and ordinal parameters and the Kruskal-Wallis test for numerical vs. multicategorical parameters. For all analyses the level of significance was set at 5%.
Table 1 Characteristics of patients and tumors

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>94</td>
</tr>
<tr>
<td>Tumours</td>
<td>100</td>
</tr>
<tr>
<td>Patients with multiple tumours</td>
<td>5</td>
</tr>
<tr>
<td>Side of tumour L / R</td>
<td>49 / 51</td>
</tr>
<tr>
<td>Diagnostic biopsy outcome</td>
<td>78 (78%)</td>
</tr>
<tr>
<td>RCC</td>
<td>56 (72%)</td>
</tr>
<tr>
<td>Oncocytoma</td>
<td>20 (26%)</td>
</tr>
<tr>
<td>Angiomyolipoma</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Non-diagnostic biopsy outcome</td>
<td>22 (22%)</td>
</tr>
<tr>
<td>Normal renal tissue</td>
<td>7 (32%)</td>
</tr>
<tr>
<td>Fibrous tissue or sclerosis</td>
<td>8 (36%)</td>
</tr>
<tr>
<td>(chronic) Infection</td>
<td>3 (14%)</td>
</tr>
<tr>
<td>Fat tissue</td>
<td>2 (9%)</td>
</tr>
<tr>
<td>Necrosis or blood</td>
<td>2 (9%)</td>
</tr>
<tr>
<td>Mean tumour size (SD) (cm)</td>
<td>2.58 (0.77)</td>
</tr>
<tr>
<td>No. of tumours 1.0 - 1.9 cm</td>
<td>23 (23%)</td>
</tr>
<tr>
<td>No. of tumours 2.0 – 2.9 cm</td>
<td>38 (38%)</td>
</tr>
<tr>
<td>No. of tumours 3.0 - 3.9 cm</td>
<td>36 (36%)</td>
</tr>
<tr>
<td>No. of tumours 4.0 – 4.5 cm</td>
<td>3 (3%)</td>
</tr>
<tr>
<td>Exophytic part of tumours</td>
<td></td>
</tr>
<tr>
<td>No of tumours 0-24 % exo</td>
<td>18 (18%)</td>
</tr>
<tr>
<td>No of tumours 25-49 % exo</td>
<td>14 (14%)</td>
</tr>
<tr>
<td>No of tumours 50-74 % exo</td>
<td>54 (54%)</td>
</tr>
<tr>
<td>No of tumours 75-99 % exo</td>
<td>14 (14%)</td>
</tr>
<tr>
<td>Tumour location</td>
<td></td>
</tr>
<tr>
<td>Upper pole</td>
<td>33 (33%)</td>
</tr>
<tr>
<td>Middle pole</td>
<td>41 (41%)</td>
</tr>
<tr>
<td>Lower pole</td>
<td>26 (26%)</td>
</tr>
<tr>
<td>Mean no. of biopsies taken (SD)</td>
<td>2.68 (1.00)</td>
</tr>
<tr>
<td>1</td>
<td>4 (4%)</td>
</tr>
<tr>
<td>2</td>
<td>47 (47%)</td>
</tr>
<tr>
<td>3</td>
<td>30 (30%)</td>
</tr>
<tr>
<td>4</td>
<td>9 (9%)</td>
</tr>
<tr>
<td>5</td>
<td>4 (4%)</td>
</tr>
<tr>
<td>(missing data)</td>
<td>6 (6%)</td>
</tr>
<tr>
<td>Biopsy needle size 16G / 18G</td>
<td>17 / 83</td>
</tr>
</tbody>
</table>

SD = standard deviation; G= gauge
RESULTS

A total number of 100 tumours in 94 patients were identified. The mean age of the 57 (61.3%) men and 36 women was 67 years (range 38-91). In five patients multiple tumours were treated. Characteristics of the tumours and the biopsy results are shown in table 1. Our series showed 22 non-diagnostic biopsies (22%). In 47% of tumours immunohistochemistry was used to differentiate benign from malignant tumours and/or to further distinguish subtypes of RCC.

Table 2 shows comparison of different variables (radiological and procedural) between the groups of diagnostic and non-diagnostic biopsy results. There was no statistically significant difference between the groups of diagnostic and non-diagnostic biopsies. There was a positive correlation between tumour size and the number of biopsies (Spearman r = .191, p-value = .029), between tumour size and exophytic part of the tumour (Spearman r = .209, p-value = .018) and between the size of the biopsy needle...
and the quality of the biopsy (Spearman r = .342, p-value = <0.001). Furthermore central non-enhancing regions were significantly more present in larger tumours with a mean tumour size of 2.11 cm for tumours without non-enhancing intratumoural areas and 2.88 cm for tumours with this feature (Student t-test, p-value < 0.001) and more biopsies were taken when non-enhancing areas were present in the tumour (Spearman r = .227, p-value < 0.001).

DISCUSSION
In our series on laparoscopic cryoablation of small renal masses, 22% of intra-operatively taken biopsies were non-diagnostic. This is within the range reported in other studies on renal cryoablation although figures vary widely with 6% to 32% for laparoscopic cryoablation and 11% to 23% for percutaneous cryoablation[6;7;11-18]. The wide range can be caused by different reasons as tumour characteristics, procedural details, variation in the definition of a diagnostic biopsy, intraobserver variability[19;20] and inherent pathological diagnostic difficulties in RCC. In the present study, a diagnostic biopsy was strictly defined by tissue that would explain a renal mass on an imaging study, such as carcinoma or benign renal tumours. In some studies, biopsies consisting of normal renal tissue are also categorized as a diagnostic biopsy. However, we strongly believe that the presence of normal renal tissue usually implies a sampling error since theoretically renal masses are not composed of normal renal tissue. In other studies the presence of fibrosis and necrotic tissue is regarded as a sign of tumour and categorized as a diagnostic biopsy. We also regarded this as non-diagnostic because of the absence of tumour cells and therefore the inability to determine a definitive diagnosis.

When comparing the ranges of non-diagnostic biopsies between laparoscopic and percutaneous cryoablation series it is remarkable that these numbers differ so little; one would expect that the laparoscopic vision would be an advantage in obtaining a biopsy and therefore the non-diagnostic biopsy rate would be lower. However we experienced that with the biopsy needle suspending freely in the abdominal or retroperitoneal cavity during laparoscopy it is difficult to aim the exact location of the biopsy, something that is not encountered when the biopsy is taken percutaneously. Perhaps the use of a guiding sheath during laparoscopy could be helpful and improve biopsy results.
In the present study none of the studied parameters on radiological tumour characteristics, on biopsy needle size, number of biopsies, or quality of biopsies was found to influence the percentage of non-diagnostic biopsies significantly. The size of the tumour (all tumours in our study were ≤ 4.5 cm) did not influence biopsy outcome. However Wunderlich and colleagues did find tumour size to affect biopsy outcome when including larger sized tumours[21]. In their series 250 ex-vivo biopsies were taken from 50 tumours (range 2 – 20 cm), 1 central and 4 peripheral biopsies from each tumour. For the 30 tumours smaller than 4 cm, 16.7% of the central biopsies and 25% of the peripheral biopsies consisted of necrosis and/or fibrosis as a result of which the tumour biology (benign or malignant) was indefinable. For the 20 tumours larger than 4 cm this was the case for 30% of the central biopsies and 32.8% of peripheral biopsies. In our study the percentage of biopsies (all our biopsies were taken centrally) containing mostly necrosis and/or fibrosis was 10% (see table 1). We found that significantly more biopsies were taken in larger tumours. In our setting the number of biopsies depended on the macroscopic appearance of the obtained biopsy assessed by the surgeon (i.e. the biopsy gun was fired until a proper core of tissue was yielded). Furthermore there was a positive correlation (p = < 0.001) between tumour size and the presence of intratumoural non-enhancing areas on imaging suggestive for necrosis. This is compatible with the conclusion of Wunderlich et al. that larger tumours result in a higher amount of non-diagnostic biopsies especially when taken centrally from the tumour, as was done in our series.

After dissection of the perirenal fat, an exophytic tumour protruding from the kidney surface is more easily recognizable than a predominantly intrarenal tumour. We expected that under laparoscopic vision it might be easier to take a diagnostic biopsy from a more exophytic tumour than from a less exophytic tumour. However, this is not demonstrated by our results. A possible explanation is the use of intra-abdominal ultrasound; in this way even scarcely exophytic tumours can also easily be identified intra-operatively provided they are not isoechoic[22]. Furthermore we found tumour size to be correlated with a larger exophytic part of the tumour which is expected since large tumours are more likely to deform the kidney surface.

Laparoscopically, the upper pole is usually more difficult to reach with a biopsy needle than the middle and lower pole; the reason why we included this parameter into our
study. Nonetheless, our analysis shows that this did not significantly influence the biopsy outcome.

Breda et al. compared the accuracy of three different sized biopsy needles in a prospective, ex-vivo study[23]. Their study shows that 100% of the biopsies obtained with the 14- and 18G biopsy needles were diagnostic, while this number decreased to 84% with the 20G needle. The histological accuracy of the 14-, 18, and 20G needles was 92%, 97% and 81% respectively. They therefore advise to use an 18G biopsy needle as a minimum size. This was the case in our centre where intraoperative biopsies were performed with an 18G needle. Proven safety of larger size needles[24] together with an interim analysis of our results prompted us to use a 16G needle in an attempt to improve our diagnostic rate. Therefore we were able to assess potential advantages of a larger calibre needle in an in-vivo setting. Needle size did not turn out in a statistical significant increase in the rate of diagnostic biopsies, but larger needle size positively influenced the quality of the biopsies. In concordance with this, Hruby et al. found that larger calibre needles (12-14 gauge vs. 16-20 gauge) resulted in improved tissue specimens in terms of width and number of glomeruli and vessels captured in a porcine model[25]. To our knowledge there is no standardized way to score the quality of a biopsy. We based our scoring system on the length and number of (fragmented) cores after preparation for pathological assessment. According to our definition, a total of 42 biopsies were of poor quality (32 of the diagnostic biopsies (76%) and 10 of the non-diagnostic biopsies (24%)). Apparently, the macroscopic appearance of the biopsies says little about the microscopic appearance and the ability of the pathologist to make the diagnosis.

Main limitation of this ‘ad hoc’ study is the retrospective collection of some of the variables and the lack of randomisation. Consequently the number of biopsies and the needle size reflect clinical practice and as mentioned the attempt of improving diagnostic results. The sample size might seem insufficient to show any statistical significant difference in an observational study, however indications to perform laparoscopic renal cryoablation are limited. A randomized controlled trial shall definitively answer the question although a big sample would be necessary to adjust for the number of core biopsies related to tumour size and biopsy needle calibre.
A certain percentage of non-diagnostic biopsies is ultimately unavoidable and inherent to the sampling of small renal masses. Efforts should be made to improve the diagnostic rate in ablation of SRM since decreasing the non-diagnostic rate has significant impact on follow up, health care costs, and patient apprehension.

CONCLUSIONS

In our setting 22% of intraoperative biopsies are non-diagnostic during laparoscopic cryoablation. Radiological tumour characteristics (tumour size, exophytic part, tumour location or non-enhancing parts in the tumour) or the number of biopsies taken, the size of the biopsy needle or the quality of the biopsies did not predict the biopsy outcome.
REFERENCES


