Renal tumor ablation: beyond limitations of biopsy and follow-up

Barwari, K.

Citation for published version (APA):
Barwari, K. (2012). Renal tumor ablation: beyond limitations of biopsy and follow-up

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

Download date: 13 Dec 2018
Differentiation between normal renal tissue and renal tumors using functional optical coherence tomography: a phase-I in-vivo human study

Kurdo Barwari
Daniel M. de Bruin
Dirk J. Faber
Ton G. van Leeuwen
Jean J. de la Rosette
M. Pilar Laguna

Published in British Journal of Urology 2012 May 10 (e-pub ahead of print)
ABSTRACT

Objective: To determine the ability of optical coherence tomography (OCT) in differentiating human renal tumors in an in-vivo setting by assessing differences in attenuation coefficient ($\mu_{\text{OCT}}$; mm$^{-1}$) as a quantitative measurement.

Methods: Consecutive patients undergoing nephrectomy (partial/radical) or cryoablation for an enhancing solid renal tumor were included in our center between September 2010 and May 2011. In-vivo OCT-images and attenuation-coefficients were obtained from renal tumor and normal parenchyma. Ex-vivo OCT-images of internal tissue were obtained after longitudinal dissection of the extirpated specimen. Attenuation-coefficients of the OCT-images were compared between normal renal parenchyma vs. renal tumors (grouped per tissue-type and per individual patient); and between OCT-images recorded from tissue surface vs. internal (sub-capsular) tissue.

Results: In-vivo OCT was performed in 16 cases (11 RCC, 3 benign tumors, 1 non-diagnostic biopsy and 1 not-accessible tumor). Median attenuation-coefficient of normal renal parenchyma was 5.0 mm$^{-1}$ vs. 8.2 mm$^{-1}$ for tumor tissue (p<0.001) with normal parenchyma differing significantly from malignant tumor (9.2 mm$^{-1}$, p<0.001) and non-significantly from benign tumor (7.0 mm$^{-1}$, p=0.050). Benign tumors differed from that of malignant tumors (p=0.139).

Using patients as their own control, attenuation-coefficients of normal renal parenchyma differed significantly from malignant tumor (p<0.001) and non-significantly from benign tumor (p=0.109). Assessed in 10 patients, no significant difference between attenuation-coefficient of tumor surface vs. attenuation-coefficient of internal tumor was seen (8.5 vs. 9.7 mm$^{-1}$ respectively, p=0.260).

Conclusion: In this first in-vivo study on OCT for differentiation of renal tumors in humans the attenuation-coefficients (as a quantitative assessment) differed significantly between normal renal parenchyma and malignant tumor. Tumor surface and internal tumor did not differ significantly suggesting that superficial OCT attenuation-coefficient reliably assess tissue composition inside the tumor. These results justify further research on OCT for various clinical applications in the diagnosis of renal tumors.
INTRODUCTION

The incidence of kidney and renal pelvis cancer is still rising, but a fast and reliable minimally invasive diagnostic method to establish a pre-operative diagnosis of renal tumors is not readily available. In contrast to malignancies in most other organs, the high number of non-diagnostic results following renal tumor biopsy currently prevents general use of pre-operative biopsies in the diagnostic workup of (small) renal tumors. In a recent ex-vivo pilot study we showed that Optical Coherence Tomography (OCT) successfully distinguished normal renal parenchyma from malignant renal tumors, based on the optical properties extracted from the OCT images[1]. OCT is the optical equivalent of B-mode ultrasonography. Whereas ultrasonography is based on the intensity of time delayed reflected sound pulses, OCT is based on the intensity of back reflected light. The intensity in both modalities is mapped to a spatial coordinate in the imaged specimen. OCT provides micrometer-scale resolution, cross-sectional images up to a depth of about 2 mm in renal tissue. Owing to this high resolution, OCT is usually visually correlated to structural information in histological images and therefore bear the potential to be the optical equivalent of normal biopsy (see figure 1) [2]. The imaging depth is limited by scattering of light by organelles and other cellular structures as the light penetrates the tissue, which hinders reflections to return to the receiver. This attenuation of the OCT signal can be observed in images by decreased signal from larger depth and can be quantified by measuring the decay of signal intensity per unit depth using Lambert-Beer’s law after careful calibration of the OCT system, resulting in an attenuation coefficient ($\mu_{\text{OCT}}$, mm$^{-1}$ )[3]. Because malignant tissue displays an increased number, larger and more irregularly shaped nuclei with a higher

Figure 1 Example of an OCT-image (A) of renal tissue with the corresponding histology as seen by microscopy (B). The comparable size of the blood vessels (black holes on OCT, white holes on microscopy) demonstrates the similar order of resolution magnitude of both techniques.
refractive index and more active mitochondria, the attenuation of light is expected to be higher compared to normal and benign tissue. We hypothesize that by OCT signal analysis, measurable differences in $\mu_{\text{OCT}}$ between tissue types can be assessed\[4;5\].

This pilot study is to our knowledge the first to assess the ability of OCT to differentiate renal tumor tissue from normal renal parenchyma in an \textit{in-vivo} setting in humans. Furthermore, we aim to assess whether benign tumors can be differentiated from malignant tumors and whether superficial imaging by OCT is representative for the tissue located below the renal capsule, given the limited penetration depth of the technique.

\textbf{METHODS}

From October 2010 to May 2011, consecutive patients scheduled for nephrectomy (radical or partial) or laparoscopic cryoablation (for a solid enhancing renal mass suspect for renal cell carcinoma), were eligible for the study. Inclusion was based on informed consent approval by the patient and availability of the OCT-device at time of surgery. Institutional Review Board (IRB) approval for this study was acquired.

The commercially available Santec Innervision 2000 OCT system used for this study acquired X images per second of 2 mm by 4 mm with 9 $\mu$m (depth) by 20 (lateral) $\mu$m resolution. The system was interfaced with a rotating sample arm probe that was developed in our institute for this study. The outer diameter of the probe was 2.3 mm and is therefore applicable with most common surgical trocars and endoscopes. The imaging direction of the probe is perpendicular to its axis of rotation (i.e. “sideways looking”). Prior to OCT imaging, the system response vs. depth was carefully calibrated as described by Faber et al.[3].

\textit{In-vivo measurements}

Surgery was performed without deviation of the clinical protocol of the department. In case of a radical nephrectomy, a small window was made in Gerota’s fascia at the confluence of the tumor and normal renal tissue in order to provide access to the tumor surface. The \textit{in-vivo} OCT-probe was inserted in an optically transparent endo-ultrasound cover under sterile conditions (see figure 2). After access to the tumor and normal renal parenchyma was provided, the surgeon (MPL) placed the tip of the OCT-probe in contact with the normal renal parenchyma and the tumor. Five OCT-images
In-vivo OCT for renal tumor differentiation

Figure 2 The in-vivo OCT-probe covered in a sterile endo-ultrasound cover used to obtain images from a renal tumor during laparoscopic surgery.

were recorded and stored, labeled for patient ID and type of tissue. In case of a laparoscopic approach, the covered OCT-probe was introduced in the abdominal cavity through one of the trocars. By using the laparoscopic instruments, the tip of the probe was placed in contact with the kidney and tumor in a similar fashion as during open surgery and OCT-imaging was performed as described above (figure 2). The overall image acquisition process took approx. 5 minutes after which the normal surgical procedure continued.

Ex-vivo measurements

After extirpation, the specimen was transferred to the department of Pathology where the surface of the specimen was inked and cut longitudinal through the tumor to provide access to the tumor internal tissue (see figure 3). Then, internal (sub-capsular) areas of both tumor and normal parenchyma were imaged 5 times each by OCT, analogous to the in-vivo procedure.

Figure 3 Renal tumor longitudinally dissected in order to perform ex-vivo OCT-imaging of the internal tumor tissue.
Quantitative analysis

The attenuation-coefficient ($\mu_{\text{OCT}}$) was determined by one investigator (DMdB) who was blinded for pathology, by selecting a region of interest (ROI) in the OCT image. In short, an average OCT signal vs. depth was calculated (figure 4). By using the depth-dependent response of the OCT system from the calibration measurement, the $\mu_{\text{OCT}}$ of the ROI was determined by curve fitting of Beers law.

Statistical analysis.

From the five OCT-images per tissue type per patient, the mean attenuation-coefficient was stored in a PASW 18.0.2 database and combined with clinical parameters and demographic data of the corresponding patient. The standard pathological report as issued for clinical purposes was considered as the gold standard for tissue classification.

Statistical analysis was based on:

1) The difference of $\mu_{\text{OCT}}$ of normal tissue vs. $\mu_{\text{OCT}}$ of tumors, including sub-comparison of: normal tissue vs. malignant tumor (1a); normal tissue vs. benign tumor (1b); and malignant tumor vs. benign tumor (1c), all using a Mann Whitney U test (MWU) due to non-normal distribution.

2) The difference of $\mu_{\text{OCT}}$ of normal tissue vs. the $\mu_{\text{OCT}}$ of tumor per patient (e.g. patients as their own control), subdivided in: normal tissue vs. malignant tumor (2a) and

![Figure 4](image-url)

**Figure 4** OCT-images of normal and malignant tumor tissue and the quantitative analysis procedure. First, a region of interest (ROI) is selected in the OCT-image indicated with the vertical blue lines. Second, the OCT signal vs. depth within this ROI is plotted in a graph. Finally, a mathematical model describing is fitted to this graph (transparent blue line) yielding the attenuation-coefficient ($\mu_{\text{OCT}}$) for both normal and tumor tissue. A steep slope will result in a high attenuation-coefficient.
normal tissue vs. benign tumor (2b), using a Wilcoxon Signed Ranks test (WSR) because of not normally distributed paired measurements.

3) The difference of $\mu_{OCT}$ obtained from tumor surface vs. internal tumor obtained after extirpation, using a WSR-test.

For all tests differences were considered statistically significant if the two-sided p-value was <0.05.

RESULTS
From all cases that underwent radical nephrectomy (RN) or nephron sparing surgery (NSS, partial nephrectomy or laparoscopic cryoablation) in our department in the study period, 16 cases were included and underwent per-operative in-vivo OCT imaging. Overall, 3 tumors were benign (1 oncocytoma, 1 leiomyoma, 1 benign cyst) and in one case no definitive pathological diagnosis of the tumor could be made (non-diagnostic biopsy result during laparoscopic cryoablation). In one case, the tumor could not be imaged during laparoscopy due to dense perirenal fat. Further details of the tumors and patients are shown in table 1. For each OCT-image the $\mu_{OCT}$ is determined as shown in figure 4.

<table>
<thead>
<tr>
<th>Table 1 Demographic data of patients and pathology of the 16 tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean age (years)</strong></td>
</tr>
<tr>
<td><strong>Mean tumor size (cm)</strong></td>
</tr>
<tr>
<td><strong>Male:female</strong></td>
</tr>
<tr>
<td><strong>Pathology</strong></td>
</tr>
<tr>
<td>Clear cell RCC</td>
</tr>
<tr>
<td>Papillary RCC</td>
</tr>
<tr>
<td>Chromophobe</td>
</tr>
<tr>
<td>Benign</td>
</tr>
<tr>
<td>Non-diagnostic (biopsy during LCA)</td>
</tr>
</tbody>
</table>

RCC: Renal Cell Carcinoma
LCA: Laparoscopic Cryoablation
Figure 5: Boxplot showing the median attenuation-coefficient ($\mu_{\text{oct}}$) and IQR's of in-vivo acquired OCT-images of the different tissue types. Normal $\mu_{\text{oct}}=5.0$ mm$^{-1}$ (4.3-5.4), Benign $\mu_{\text{oct}}=7.0$ mm$^{-1}$ (6.7-N.A.), Malignant $\mu_{\text{oct}}=9.2$ mm$^{-1}$ (7.4-9.9). The circle displays an outlier (pt. 16, chromophobe RCC).

Analysis 1: In-vivo comparison of normal renal parenchyma vs. tumor (per group).

The tumor with a non-diagnostic biopsy result was excluded from this analysis, resulting in inclusion of $\mu_{\text{oct}}$ of 16 normal, 3 benign and 11 malignant OCT-images. Median $\mu_{\text{oct}}$ of normal renal parenchyma was lower (5.0 mm$^{-1}$) compared to tumor tissue (8.2 mm$^{-1}$), $p<0.001$. Among the 3 groups, (1a) median $\mu_{\text{oct}}$ of normal tissue (5.0 mm$^{-1}$) was lower than that of malignant tumor (9.2 mm$^{-1}$), $p<0.001$. (1b) Median $\mu_{\text{oct}}$ of normal tissue (5.0 mm$^{-1}$) was lower than that of benign tumor (7.0 mm$^{-1}$), $p=0.050$. 

Figure 6: Attenuation-coefficients of in-vivo acquired OCT-images of normal- and tumor tissue displayed per individual patient. Pts 4, 10 and 11 had a benign tumor (oncocytoma, benign cystic lesion and leiomyoma respectively). Pt. 8 had a non-diagnostic biopsy result. In pt. 12 the tumor was not accessible and pt. 16 had a chromophobe RCC.
(1c) Median $\mu_{\text{oct}}$ of benign tumor (7.0 mm$^{-1}$) was lower than that of malignant tumor (9.2 mm$^{-1}$), $p=0.139$. The last two comparisons failed to reach statistical significance. The results are depicted in figure 5.

**Analysis 2: In-vivo comparison of normal renal parenchyma vs. tumor (per patient).**

Two cases were excluded from this analysis (non-diagnostic biopsy and the case without a tumor measurement), resulting in 14 patients with a $\mu_{\text{oct}}$ of both normal tissue and the tumor. The results are depicted in figure 6.

(2a) The median $\mu_{\text{oct}}$ of normal renal parenchyma (5.0 mm$^{-1}$) was lower than that of malignant tumor (9.2 mm$^{-1}$), $p<0.001$.

(2b) Median $\mu_{\text{oct}}$ of normal renal parenchyma (5.4 mm$^{-1}$) was lower than that of benign tumor (7.0 mm$^{-1}$), $p=0.109$.

**Analysis 3: Comparison of superficial and internal recorded OCT-images**

Three cryoablation cases were excluded (no OCT imaging possible because no tumor was extirpated) from this analysis. Three other cases were excluded because pathology could not be processed immediately after extirpation. In the remaining 10 patients, no significant differences were observed between superficial and internal $\mu_{\text{oct}}$ for both normal (5.0 and 5.8, respectively, $p=0.169$) and tumor tissue (8.5 and 9.0 respectively, $p=0.260$). The results are depicted in figure 7.

![Figure 7](image-url) Boxplot showing the median attenuation-coefficient ($\mu_{\text{oct}}$) and IQR’s of superficially and internal (sub-capsular) acquired OCT-images for normal renal parenchyma and tumors. Normal Superficial $\mu_{\text{oct}}$=5.0 mm$^{-1}$ (4.2-5.4), Normal Internal $\mu_{\text{oct}}$=5.8 mm$^{-1}$ (3.8-7.8), Tumor Superficial $\mu_{\text{oct}}$=8.5 mm$^{-1}$ (7.4-9.5), Tumor Internal $\mu_{\text{oct}}$=9.0 mm$^{-1}$ (7.6-14.4). The circle displays an outlier.
DISCUSSION

In this study we report the first results of in-vivo performed OCT in renal tumors. Using quantitative assessment of the OCT-images a statistical significant difference between normal renal parenchyma and malignant renal tissue was established, proving the ability of OCT to differentiate in-vivo normal and malignant renal tissue in real-time without violation of the tissue.

In order to overcome obstacles in the diagnostic process of renal tumors mentioned in the introduction, optical techniques are likely to be of additional value as they are light-based and therefore harmless to human tissue, providing real-time information and are suitable for miniaturization enabling integration with existing clinical procedures and instruments. Optical Coherence Tomography [2;6] is of specific interest because it provides a ‘functional optical biopsy’: cross sectional images that can be correlated to histopathology in combination with quantification of optical properties that can be related to tissue physiology and cellular organization. Publications on OCT in kidneys are scarce. In addition to ex-vivo studies demonstrating that renal microstructures can be visualized by OCT in order to assess ischemic damage as an indicator of transplant kidney viability[7;8], we demonstrated the ability of OCT to differentiate normal and malignant renal tissue by quantitative OCT-assessment in an ex-vivo pilot study[1].

Shortly thereafter, Linehan et al.[9] described microstructural differences of several renal tumors in ex-vivo acquired OCT images which allowed differentiation of several tumor types (e.g. AML and TCC). Unfortunately, clear cell-RCC and other renal cell carcinoma subtypes showed a heterogeneous appearance in the images, which precludes distinction of RCC from normal renal parenchyma based on the images alone. In a very recent ex-vivo multi-observer qualitative study Lee et al. demonstrated that OCT combined with confocal microscopy could differentiate normal from neoplastic renal tissue with a high sensitivity and specificity. Furthermore they observed a marked decrease of imaging depth in tumor tissue caused by a higher degree of scattering, which is fully compatible with our hypothesis[10].

Translation of these promising ex-vivo results to clinical measurements has never been proven and is not straightforward due to a variety of factors involved[11]. In more detail, the availability of suitable OCT probes is limited. We therefore developed our
own prototype imaging probe for this study. The present probe configuration allowed
2-dimensional imaging with a small field of view which compromises image-based
interpretation and comparison between tissue sites but did not hamper the ability to
extract $\mu_{\text{OCT}}$.

Moreover, most ex-vivo results were obtained in the center of the tumor (specimen)
whereas in an in-vivo setting only superficial measurements can be obtained. We
therefore assessed whether superficial OCT-imaging of tissue is representative for
tissue which lays deeper. Comparison of $\mu_{\text{OCT}}$ obtained from tissue surface with internal
(sub-capsular) areas after longitudinal dissection of the extirpated specimen showed
no significant differences. This representativeness of superficial assessment using
optical diagnostic tools with a limited penetration depth is also suggested by Bensalah
et al. using Optical Reflectance Spectroscopy[12]. Interestingly, the spread in $\mu_{\text{oct}}$ values
is higher for internal tissues, which we attribute to inhomogeneity of the central tumor.
The current in-vivo study demonstrates a statistically significant difference between
$\mu_{\text{OCT}}$ for normal and malignant renal tissue, albeit still in a modest sample population.
The small number of benign tumors precluded conclusions on OCT’s ability to
differentiate benign from malignant tumors and normal tissue. Since the percentage
(19%, 3 out of 16 extirpated tumors) is compatible with reports in the literature[13;14],
this limitation is hard to avoid in this primary phase of clinical research. Moreover,
the biology of the benign tumors varied widely which makes interpretation of the
determined $\mu_{\text{OCT}}$ challenging for these cases.

In spite of this limitation the current preliminary data on the ability of OCT to
differentiate between malignant and normal renal tissue as well as the recently showed
synergistic effect between OCT and Raman spectroscopy reinforce the pursuit of the
investigation in quantitative OCT measurements in renal tumors. The ultimate goal
should be to identify discriminatory cut-offs in the attenuation-coefficients between
malignant and benign tumors.

If the diagnostic value of OCT is confirmed, the thin currently available OCT-probes can
be integrated with biopsy needles enabling the incorporation of OCT into the diagnostic
algorithm of renal tumors complementing or substituting percutaneous or operative
biopsies. Furthermore OCT could be used in the assessment of renal parenchyma
margins after partial nephrectomy preventing frozen-section analysis.
CONCLUSION

OCT provides high-resolution non-invasive cross sectional images suitable for quantitative analysis. This phase-I study showed that OCT can be safely employed in humans. A significant difference between attenuation-coefficients of normal renal parenchyma and malignant renal tissue was found, proving the ability of OCT to distinguish malignant and normal renal tissue. Expansion of the population and validation of the results is needed in order to assess OCT as a clinically valuable new diagnostic tool.

ACKNOWLEDGEMENTS

This project was supported by the Cure for Cancer Foundation.
REFERENCES


