CHAPTER 6
INTERPRETATIONS OF ALTERNATIVE IMAGING METHODS FOR THE POST-CRYOSURGICAL RENAL MASS:

CONTRAST-PULSE SEQUENCE ULTRASONOGRAPHY
Parts of this chapter are published in:


Abstract

Cryotherapy is a curative treatment option for patients with small (<4cm) renal cell cancers. For the follow-up of ablated lesions, imaging is the current standard, but the best imaging tool has yet not been determined. The method selected should be able to determine the presence of viable tissue in the area and measure the size of the lesion. The aim of this chapter is to evaluate the characteristics and perfusion patterns of contrast-enhanced ultrasonography using contrast-pulse sequence imaging (CPS) in renal masses treated with cryoablation (CA). A Siemens Acuson Sequoia device with contrast pulse sequence imaging and Sonovue (Bracco) as a contrast agent was used. The perfusion characteristics in the lesions were described and scored. The first experiences with CPS-imaging for the follow-up of renal mass CA show that this technique can be used to characterize local perfusion defects at various times after CA.
Introduction

Nephron-sparing procedures for the management of small renal tumors have become increasingly accepted during the last decade, including partial nephrectomy, and thermal ablation procedures \(^1,2\).

Currently, the most widely used energy modalities for thermal ablative techniques are radiofrequency (RF) ablation and cryoablation (CA). They can be performed percutaneously assisted by image-guidance or under direct visualization at laparoscopic (LCA) or open surgery. Image-guided percutaneous thermal ablation techniques have potential advantages over surgical ablation, including a decreased convalescence with reduced morbidity and appropriate oncological efficacy. However, the availability of large-cohort long-term results is limited. For the most part, the patients who can benefit from thermal ablation procedures are those who are poor surgical candidates because of compromised renal function and/or comorbid disease.

Because the tumor remains in situ after ablative treatment of a proven malignant renal mass, the efficacy can be evaluated only by means of imaging or biopsies. Histological evaluation of the ablated tissue at follow-up would be an option for assessing the efficacy of the treatment \(^3,4\). However, this method is not routinely used because of its invasive character, sampling error, failure to detect small recurrences, risk for complications when carried out regularly, and the non-confirmed histological accuracy.

Vascular damage and consequently ischemic injury is significant in CA. The current mainstay of follow up is the assessment of vascular flow in and around the ablated area. Therefore, the recommendation for follow up of CA for small renal tumors is based on the imaging of blood flow \(^5\). The imaging method selected should be able to evaluate the presence or absence of perfusion in the ablated area and measure the size of the lesion. Routinely, contrast-enhanced Computed Tomography (CT) or Magnetic Resonance (MR) imaging is used \(^6\). However, allergic reactions and renal toxicity are the disadvantages of the contrast-agent used. Therefore, complementary adjustments need to be arranged in order to avoid these risks. In practice, according current guidelines, there is no consensus on which set of diagnostic tools, time and frequency of follow up of the cryoablated renal mass is recommended \(^1,2\). The Society of Interventional Radiology Technology Assessment Committee and the International Working Group on Image-Guided Tumor Ablation published a framework for standardizing the method and reporting of follow up findings and complications \(^5\).

Alternative imaging methods can focus either on the vascular or the molecular changes in the cryoablated zone. Vascular patterns can be
recognized by contrast-enhanced ultrasonography (CEUS) in kidneys. CEUS depicts perfusion in a real-time setting in which all phases of enhancement can be evaluated. Various CEUS techniques have been developed during the last decades using the linear and the non-linear reflections of the microbubble contrast agent in an ultrasound wave. The technique used offers a highly sensitive and selective depiction of the contrast agent. Because the microbubbles remain inside the vasculature, selective perfusion imaging is possible. This method has the advantage of easy access and low costs. Furthermore, the contrast-agent used has a low risk for adverse events. However, the limitation of this modality at follow up is that it is only used for the assessment of the local ablation status.

In this chapter CEUS-imaging characteristics and perfusion patterns of the contrast-agent in renal mass before and after LCA are evaluated and discussed. The feasibility study of Wink et al is used to describe these phenomena in more detail.

Materials and methods

Patient preparation for CEUS

Patients are prepared with intravenous access before the investigation. Because of small number of cardiac events after the use of Sonovue (Bracco, Milan, Italy) in patients with serious cardiac morbidity, patients with unstable cardiac conditions cannot be investigated with this microbubble agent. Otherwise, adverse events are rare.

CEUS technique

For these investigations, contrast pulse sequence (CPS) imaging was used. All harmonic imaging techniques exploit the fact that microbubbles react in a nonlinear way to an ultrasound wave, whereas tissue mainly reflects linear signals. This CEUS technique separates the signal from the tissue from that reflected by the microbubbles by applying multipulse sequences, each pulse having a different amplitude and phase. Phillips and Gardner describe the technique in detail. For ultrasonography, the Acuson Sequoia (Siemens Medical, Mountain View, CA, USA) and a 4C1 transducer, equipped with CPS, was used. The two images that are created, a tissue-only and a contrast-only image, can be viewed simultaneously, which offers selective perfusion imaging making it possible to localize the abnormality on the B-mode image. Target areas are localized using the B-mode ultrasonography. A bolus of 2.4 mL of Sonovue is administered intravenously. While the target area is kept within the ultrasound range of imaging, the microbubbles arrive in the renal blood flow about 15 seconds after administration.
Cryoablation

Cryoablation was carried out under transperitoneal or retroperitoneal laparoscopic assistance using 1.47 mm diameter (Seednet Gold®, Oncura, Plymouth Meeting, PA, USA) sharp and closed-tip cryoprobes and thermosensors. Depending on the tumor size, several cryoprobes were placed percutaneously and 2 freezing cycles were used. The freezing process was monitored by laparoscopic ultrasound (EUB-6500, Hitachi, Tokyo, Japan), temperature registration, and the endoscopic camera overview.

Study group

In the study of Wink et al, 7 patients treated with LCA for small solid renal tumors were included⁹. A researcher with experience in CPS did all the investigations. Renal mass CPS-CEUS imaging was performed in the follow up of LCA. Data were stored for off-line analysis. Two independent readers measured perfusion defects three times each. Perfusion characteristics were described using a scale from 0 to 4:

- 0 - no perfusion;
- 1 - increased enhancement in the rim of the lesion;
- 2 - diffuse enhancement throughout the lesion;
- 3 - localized enhancement;
- 4 - no perfusion defect visible.

Inter- and intraobserver variability of the measurements was determined. The results of the CEUS imaging were compared to the most recent standard follow up imaging consisting of contrast-CT or MRI every three months during the first year after cryoablation and every six months thereafter.

Results

CEUS of renal mass

Regular grey-scale ultrasonography performs the identification and localization of the renal mass. After switching to the contrast-only image, the same lesion is without any signal. About 15 seconds after intravenously administrating 2.4 mL Sonovue, the micro bubbles arrive in the renal parenchyma. Enhancing of the normal renal tissue will appear as a regular pattern. However, using CEUS, a renal mass can be identified by its inhomogeneous enhancement pattern. Panels a, b, and c of figure 1 show examples of three types of imaging of a small renal mass in patient number
3. Grey-scale US (panel A) demonstrates a solid exophytic renal mass. The same lesion at contrast-CT imaging (panel B) shows only a slight enhancement. However, performing CEUS (panel C), this lesion is clearly demarcated by the enhancement of an inhomogeneous vascular pattern imbedded in normal renal parenchyma appearing as a regular vascular pattern. At grey-scale US, the perirenal fat will appear as hyper-echoic as compared to the renal parenchyma. However, using CEUS the same fat will demonstrate a lower vascular density, or at least a different pattern than the renal parenchyma and the renal mass.

**CEUS in the follow up of LCA**

The patients, studied by Wink et al., were investigated at different times after ablation (29-527 days). In all but one patient the cryoablation lesion could be detected and described. Figure 1 shows all the relevant follow up imaging data from patient number 3 (panels D, E, and F). At one month after cryoablation, a new CPS investigation was performed and the cryoablation lesion could be visualized as a complete perfusion defect (perfusion score 0). Figure 1, panel F shows the complete absence of perfusion in the contrast-only image. The first CT-scan three months after cryoablation shows the same lesion with no enhancement after contrast administration.

Patient number 6 was investigated with CPS imaging twice, once at 29 days and again 8 months after cryoablation; Figure 2 shows the result of both investigations. Whereas the first CPS shows no enhancement in this area (perfusion score 0), during the second investigation some reflections inside this area were visible, suggesting reperfusion in the cryoablation lesion (perfusion score 2-3). The size of the defect had not changed over time. The latest contrast-CT, 9 months after cryoablation, showed no enhancement. During the investigations, the grey-scale US images (at the right of panel A and B) demonstrated that the ablated zone could not be clearly defined. Another example of this phenomenon is seen in figure 1, panel D.

Patient number 5 was examined 18 months after cryoablation. MR imaging was performed because of renal insufficiency. The latest MR imaging before CPS imaging visualized the cryoablation lesion as a retracted parenchyma and interpreted as an extrarenal fibrotic area of 31mm. During CPS imaging the lesion could not be identified and thus no obvious perfusion defect was objectified in the kidney (perfusion score 4). The other 4 patients showed comparable perfusion defects with those found in patient number 3. A summary of the findings is presented in table 1.

In the patients who were investigated within 5 weeks after the ablation (patients numbers 2, 3 and 6), the perfusion defect was larger than the initial tumor as measured in the pre-treatment CT in two of the three patients (see
In the third patient, the lesion was 2mm smaller than the initial tumor.

In patients numbers 1, 6a and 7, the measured dimension of the lesion without perfusion at CPS imaging was still larger than the dimension of the tumor before cryoablation, despite the fact that imaging was performed between 196 and 266 days after treatment. In patient number 4, the lesion was smaller than the tumor at 71 days after ablation. The lesion in patient number 5 was not visualized. The mean standard deviation (SD) of the sizes in the CPS imaging was 1.1mm. The mean (SD) difference between the two investigators was 0.7mm (1.4mm).

**Figure 1.**

Patient 3; Panels A+B: US and CT images of a small renal tumor (21 mm, panel B) before cryoablation. Panel C: CEUS image of this tumor before cryoablation. Neo-vascularization in renal tumors causes alterations in perfusion patterns that were demonstrated in this tumor using CEUS: the perfusion patterns in the area of the tumor are clearly different and less homogeneous than the perfusion pattern in the normal renal parenchyma. Panel D: Unenhanced ultrasound image one month after cryoablation. The lesion has a solid aspect and measures 17 mm. Panel E: Contrast enhanced CT scan after three months. A clear perfusion defect is visible. Panel F: The same perfusion defect as seen during CEUS investigation 36 days after surgery.
Figure 2.

Patient 6; Panel A: CEUS investigation of a renal tumor 29 days post cryoablation. No perfusion can be seen in the ablated area (Score 0). Panel B: The same patient, the same tumor location, 8 months after surgery. Some signs of perfusion (Score 2-3) can be observed in the tumor area.
Table 1.

The patient’s tumor and lesion characteristics using CT/MR and CPS-CEUS imaging after cryoablation. *) The CT/MR results closest to the CPS investigation; **) CEUS perfusion scores: 0, no perfusion; 1, increased enhancement in the rim of the lesion; 2, diffuse enhancement throughout the lesion; 3, localized enhancement; 4, no perfusion defect visible.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>6a</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56</td>
<td>60</td>
<td>54</td>
<td>78</td>
<td>78</td>
<td>52</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>Tumor diameter (mm)</td>
<td>11</td>
<td>32</td>
<td>21</td>
<td>30</td>
<td>24</td>
<td>18</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Post-ablation Contrast-CT/MR *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesion diameter (mm)</td>
<td>21 28 23 29 31 20 19 27</td>
</tr>
<tr>
<td>Time since ablation (days)</td>
<td>168 111 97 99 485 105 205 270</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>CEUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesion size (mm) (SD)</td>
<td>22.4 (0.6) 30.0 (2.1) 25.5 (1.2) 22.4 (1.0) 0 21.6 (0.8) 20.9 (1.4) 27.9 (0.7)</td>
</tr>
<tr>
<td>Time since ablation (days)</td>
<td>196 31 36 71 527 29 233 266</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Investigator perfusion score **</th>
</tr>
</thead>
<tbody>
<tr>
<td>Investigator 1</td>
<td>0 0 0 0 4 0 3 0</td>
</tr>
<tr>
<td>Investigator 2</td>
<td>0 0 0 0 4 0 2 0</td>
</tr>
</tbody>
</table>
The advantage of CPS over other CEUS techniques is its greater specificity for microbubble detection. Signals in the contrast-only image correspond to perfusion. Without the gray-scale signal of the tissue interfering, enhancement patterns can be evaluated selectively. Correlation of these patterns with the histological findings is necessary to evaluate the diagnostic accuracy.

The major benefits of CEUS are its easy applicability, low cost, and safe contrast medium. Furthermore, the high sensitivity to micro-vascular perfusion might enable CEUS to image the short-term dynamic process of vascular injury after cryoablation. Clearly, CEUS can detect blood flow in tissue. After cryoablation, blood flow is expected to have ceased in the cryoablative area, and therefore CEUS can be performed to demonstrate absence of blood flow in certain areas. The study of Wink et al shows that this technique is capable of doing so. However, the resolution and the interpretation of the images is the key issue to the success of this method. So far, this study proved that perfusion defects could be detected and discriminated and this is compatible with the findings at standard contrast-CT imaging. Simply put, both methods are capable of detecting and showing the existence of blood flow.

Two investigators measured the size of the lesions three times. The mean SD of all six measurements was 1.1mm, and the mean (SD) difference between the investigators was 0.7mm (1.4mm). To the best of our knowledge, the variation in lesion measurements after cryoablation using CT or MRI has not been investigated. However, information is available on the size of the kidney tumors measured in CT/MRI and pathological evaluations and this fails to correspond in approximately 10 % of cases. Furthermore, Hopper et al describe an inter- and intra-observer variation for CT tumor measurements of 15% and 6%, respectively. Therefore, we conclude that the inter- and intra-observer variability of CPS imaging measurements is acceptable. Due to the design of this feasibility study, CPS and CT/MRI were not performed on the same day. Therefore, an accurate comparison between the measurements was not possible. Comparing the measurements of the CT/MRI closest to the CEUS date to CPS measurements shows differences of approximately 10%. Barwari et al have studied the concordance between the detection of enhancement assessed by contrast CT/MR and CEUS. At three months follow-up assessed in 32 patients, they found a specificity and negative predictive value of 92% and 77% respectively. At one-year follow-up assessed in 21 patients, the specificity found was 90% with a negative predictive value of 100%. However, the absence of focal recurrences in this series limits the ability to
draw conclusions on the sensitivity and positive predictive value of CEUS used as method for follow up.

Perfusion defects after renal mass cryoablation were identified and scored in 6 out of 7 patients investigated (perfusion score 0-4). In most patients (n=5), no CPS signals were detected inside the lesion (perfusion score 0), and thus according to CPS imaging, no perfusion was present. In all these five patients these findings corresponded with the contrast CT/MRI results. In patient number 6, one month after cryoablation, no perfusion was detected using either CPS or CT imaging. However, 8 months after the ablation using CPS imaging, perfusion was observed inside the lesion (perfusion score 3 and 2), whereas no enhancement was found performing contrast CT. The implication of this finding could not be that CPS imaging is a more sensitive technique for detecting reperfusion than CT. However, the other option is a false-positive CPS result.

Haendl et al studied the perfusion patterns of suspected malignant renal masses at CT-scan using CPS-CEUS imaging. They compared the findings of vascularisation patterns in the early phase (<30 seconds) and the late phase (60-120 seconds) to the histopathological results. All 25 proven renal cell carcinomas showed a chaotic vascularisation pattern at both phases. In the early phase, compared to the perfusion of surrounding normal renal parenchyma, 12 tumors showed hyperperfusion, 3 showed isoperfusion, and 9 showed hypoperfusion. During the late phase, 5 tumors showed hyperperfusion, 9 showed isoperfusion, and 10 showed hypoperfusion. One cystic lesion did not indicate contrast enhancement at any time. Since hypoperfusion of malignant tumors was found at the two phases, it can be questioned how the perfusion patterns of focal recurrences of ablated tumors would appear.

Currently, in order to define cryoablative treatment success, the follow up contains contrast-enhanced CT or MR imaging. However, ionizing radiation, allergic reactions and the renal toxicity are the downside of the contrast-agent used. Therefore, complementary adjustments need to be arranged in order to avoid these risks. Descriptive evaluations of the use of contrast enhanced CT and MR imaging for follow up of renal mass cryoablation have been published. A successful ablation has been described as one in which the lesion showed less than 10 Hounsefield Units of contrast medium enhancement on CT or no qualitative evidence of enhancement after gadolinium contrast enhanced MR imaging. Cryoablated renal tumors usually decrease in size over time. In a study of 56 patients treated with laparoscopic cryoablation for solid renal masses, Gill et al observed a gradual involution in the size of the ablation zone by an average of 75% 3 years after cryoablation, and 38% of ablation zones were undetectable on MR imaging. CT and MRI can reveal a thin rim of peripheral enhancement around the ablation zone in 17-30% of cases at 1 month after treatment.
This peri-ablational enhancement is considered benign. It is suggested that this is a benign physiologic response to thermal injury and it appears as a relatively concentric, symmetric, and uniform process with smooth inner margins. It seems that during the first months after ablation this finding is of no consequence, however, if it occurred later in the follow-up, that can be interpreted as a sign of recurrence and will typically appear as irregular peripheral enhancement. This peri-ablational enhancement has not been described as such when performing CEUS. However, at three-month follow-up, Barwari et al reported in 2 cases signs of enhancement performing CEUS, whereas no enhancement was found at the corresponding CT-scan. In one of the two cases it was described as rim enhancement.

The search for the best method to assess the presence of recurrent and persistent vital tumor tissue after therapy is ongoing. However, this first investigational study using CPS imaging for follow up of cryoablation has, as have most feasibility studies, many limitations. Only a few patients, at different times after cryoablation, were studied to examine the possibility of using CPS imaging in order to characterize lesions. No longitudinal data were collected and no direct comparison with CT/MRI or histology was intended. However, the results of the present study showed that CPS imaging could be used to describe the perfusion characteristics of the cryoablation lesions. This justifies, therefore, the implementation of larger prospective studies with long term follow-up in order to determine the exact value of this technique for detecting remnant or recurrent tumors or reperfusion of the scar tissue.

In one patient, 1.5 years after cryoablation, the lesion was not recognized. This might be due to the fact that the initial tumor was mainly exophytic and the cryoablation lesion was thus mostly located outside the contour of the kidney. Over 1.5 years, the lesion shrank and the contour of the kidney was reinstated. The MRI of this patient showed a slightly retracted parenchyma and extra-renal scar tissue with no enhancement. As minimal enhancement is seen in the perirenal fatty tissue during CPS imaging, this is probably the reason that the lesion had not been recognized as such. Therefore, long term follow up with CEUS needs to be studied as well. However, the risk for local recurrence at the ablation site is relatively low (8.5%) and it has still not been determined what the best imaging method is for detecting a local recurrence. So far, for long term follow up, still contrast CT and MR imaging are used to assess treatment success of the renal mass. However, since most recurrence appear at the first years of follow up, it can be debated whether CT or MR is the preferred imaging mode for the long term follow up.

A limitation of this CEUS technique for the follow up of renal mass cryoablation is that it only assesses the local situation. The risk for the development of distant or seeding metastases after clinical T1a renal cancer.
cryoablation is not high (1%)\textsuperscript{24}. However, if CEUS investigation is only performed in the local region bearing the ablated tumor, it will miss possible metastases. CEUS scanning range and scanning time is limited and is dependent on microbubble dosage and stability. To assess a larger scanning area than only the ablation area, more than one dose of microbubbles is needed. The scanning area covered by CT or MR imaging is dependent on the range it is set for. Even though the scanning range is covering a larger area the dose of intravenous contrast medium does not have to be increased or repeated. Only the ionizing radiation dose will increase when the scanning range is enlarged.

CEUS is not likely to replace standard CT or MR imaging but can be a valuable alternative in selected cases. For patients with a small renal mass treated with CA and with relative or imperative contra-indications for the use of contrast-agents at CT or MRI imaging, CEUS may be a reasonable alternative technique for imaging at follow-up.

**Conclusion**

This study of the CEUS technique for the follow up of renal mass cryoablation shows that CPS imaging can be used to characterize perfusion defects at different times after cryoablation. A longitudinal prospective study comparing CEUS results to those of CT/MRI and histology are needed in order to establish the exact diagnostic value at long-term follow-up of renal mass cryoablation.
References


