Pathological features of radiofrequency (RF) renal scar CT-imaging of in a porcine model

Poster No.: C-1363
Congress: ECR 2010
Type: Scientific Exhibit
Topic: Genitourinary
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Keywords: radiofrequency, kidney, experimental
DOI: 10.1594/ecr2010/C-1363

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Purpose

Radiofrequency (RF) is a tumor ablation technology based on molecular agitation using radiofrequency current, causing thermally induced coagulation and necrosis. The results [1] and the complication rate for this technique [2] are equivalent to those of partial surgery and have thus given it a definite indication in the treatment of renal tumors [3]. The efficacy of the treatment is assessed on the imaging as being no significant enhancement after the injection of contrast medium [4] without comparing the imaging and the histological specimens to validate this criterion. Our study analyzed the radiofrequency ablation areas in the kidneys of a swine model.

Taking into account the results of this comparison, how long after the procedure should the first CT-scan be performed to check the results (usually four weeks [5]), and what can we expect from this examination?

Methods and Materials

The faculty institutional review board approved this prospective study which was carried out between September and December 2008 in the L2PTV research lab (Timone Hospital medical school).

a) Radiofrequency and CT_scan protocols :

The same surgeon operated all the animals, using the same radiofrequency protocol used to treat human renal tumors on each end of both kidneys, treating in all 6 male pigs (6 months, 40±3kg) : the pigs were given general anesthesia, intubated and ventilated then placed in a ventral decubitus position in the scanner with the four return electrodes connected to the generator (RF3000 ® Boston Scientific), and a peripheral venous line was placed to infuse fluids, drugs and the contrast medium. The position of the needles (Needle LeVeen Superslim ® 2cm Boston Scientific) and their deployment in the renal cortex were checked on ultrasound and CT scan.
The thermal protocol was based on the algorithms proposed by the manufacturer: 10 watt increments every minute starting from 20 watts until the first roll-off was obtained, followed by the second roll-off occurred (starting at half the power of the first roll-off).

The pigs were given three CT-scans (under general anesthesia and apnea): the first before RF with spontaneous contrast, then two surveillance CT-scans were performed, the first immediately after the procedure (D0) and the second at 4 weeks (D28) without injection and with injection at the nephrographic stage (1.5 cc/kg per injection [6] to study the shape, the size and the enhancement of the ZA and check that there were no local or regional complications.

The pigs were euthanized 4 weeks after the procedure with a bolus IV injection of 15 mg of midazolam and 25 mg of chlorpromazine with 20 ml of KCl at 15%. The kidneys were harvested and set in formol for 48 hours.

A 4 week deadline was selected because the histological lesions are considered as definitive this time-point [7, 8] and it is also the recommended time-frame for performing the first CT-scan to check the effectiveness of the treatment [5]. Goldberg [9] defines the area of parenchyma treated as the ablation zone (AZ), without any reference to its histological nature.

b) Pathology:

The pathology slides were examined by a specialized renal pathologist using hematoxylin-eosin-saffron staining (HES) and two immuno-histochemical markers, anti-CD10 (marker of the microvilli of the proximal convoluted tubuli) and Purified Rabbit Anti-Active Caspase-3 (an anticaspase apoptosis marker).

c) Statistical analysis:

The results were reported in accordance with the Standards for Reporting of Diagnostic Accuracy, or STARD, criteria [10]. Quantitative variables were expressed as means (± standard deviations), and categorical variables were expressed as percentages. After the descriptive phase, a comparative analysis was carried out using Chi² tests or Fisher exact tests for the qualitative variables and non parametric tests (Mann-Whitney, Spearman’s Rho, paired Wilcoxon tests) for the quantitative variables. For all the bilateral tests a p value a degree below 0.05 was considered as statistically significant. The statistical data were processed with SPSS V15 software.
Fig. 1: CT-scan 4 weeks after radiofrequency treatment (injected scan): Fluid effusion of the lower end of the right kidney of pig 831 with enhancement of its walls showing an abscess in the ablation zone (white arrow) communicating with an abscess of the psoas (black arrow).
Results

All the RF procedures were correctly performed and 2 roll-offs were obtained, except for one hit which was not completed because of a problem with the electrical supply to the generator. The pigs’ general status remained stable. One pig died of complications of the anesthesia at the end of the RF: its kidneys were explanted after the procedure and only the pathology slides were examined. The other kidneys were explanted 4 weeks after RF. The series of bipolar bilateral RF procedures on 6 pigs thus enabled us to study 23 AZ: 4 « acute » AZ at D0 and 19 « chronic » AZ at D28.

Unfortunately we had two complications: an abscess in an AZ (Figure 1) and an inhalation related lung pathology (Figure 2).

a) On the post-RF CT-scans, 84% (16/19) of the AZ displayed triangular areas of enhancement defect based in the peripheral cortex (Figure 3), the other three AZ were circular. The mean enhancement of the 19 zones studied was 14HU [30.3]. The mean volume of renal parenchyma that was less enhanced than the normal cortex was 3.73 cm³ [1, 46]. RF-induced reorganization due to cavitation was present in 69.4% of the AZ (Figure 4).

b) On the CT-scans four weeks after RF using the commonly accepted CT-scan success criteria (enhancement below 10 HU after injection) [11, 12], we successfully completed 10 treatments, a 52% success rate, 8 treatments were incomplete (48%) (in case of enhancement a repeat RF procedure is not considered as a failure) (13) and an abscess on an AZ which communicated with an abscess of the psoas (Figure 1). The mean enhancement of the AZ was 19HU [22.5], their mean volume at D28 was 3.5 cm³ [1.35].

c) The pathology findings in the ZA immediately after RF: the macroscopic examination revealed whitish circular or triangular areas showing the AZ (Figure 5). The microscopic examination revealed histological changes in all four AZ: elevated cytoplasmic eosinophil levels, the cell walls had lost their clarity and the nuclei had become hazy with interstitial hemorrhage and hemorrhages along the needle tracks, inflammatory infiltration and necrosis (in some cases a sub-capsular or perirenal hematoma). There was also intravascular coagulation in the injured tissue. The anticaspase apoptosis marker was positive mainly in the necrotic areas but also around the edges of the necrosis.
These lesions were heterogeneously distributed on the different AZ. On some slides the necrotic areas were mixed with areas of inflammatory and healthy tissue, whereas on others the areas were clearly circumscribed as if they had been cut out with a knife (Figure 6). In one AZ, areas of mixed tissue and clearly circumscribed areas were combined, with no systematic distribution pattern.

d) The AZ pathology findings four weeks post-RF described three areas (Figure 7):

-1) Areas of central or peripheral necrosis associating a loss of architecture, disappearance of the pyknotic nuclei, misshapen cells and ruptured membranes in the tissue. These are typical features of thermally-induced coagulation necrosis.

-2) Ischemic or damaged tissue featuring intravascular coagulation and irregular nuclei, although the architecture was intact in 100% of the AZ.

-3) An inconsistent mesenchymatous area (50% of the AZ studied) made up of fibrous connective tissue located between the necrotic areas and the ischemic tissue.

The immuno-histochemical assays revealed over-expression of the anticaspase in different areas, indicating RF-induced apoptosis. This reaction occurred constantly in 100% of the AZ and was visible predominantly in the centre and also in the peripheral areas.

Discussion

1) The CT-scan performed immediately after RF was of no interest in predicting the efficacy of the procedure (as suggested by Lui) because 26.3% of the AZ were enhanced early, whereas necrosis was constantly found in all the AZ in the histology findings. The enhancement on D0 did not significantly correlate with the enhancement of the AZ on D28 (rho=0.016; p=0.951). The only value of a CT-scan with spontaneous contrast at the end of the procedure is probably to research early complications [15].

2) Vascular infarction and ischemia: the pathology examination (Fig. 3-5-7) confirmed that the triangular hypodense areas with clearly circumscribed edges around the peripheral cortex on the CT-scan, were in fact infarction areas.
Most authors perform their first CT-scan four weeks after the procedure [5] because they consider that at this time-point the lesions are definitive. (Goldberg [16] describes the architectural distortion of the necrotic areas after only 30 days).

The devascularisation of the treated zones on the arteriogram described by Inderbir [4] explains the presence of ischemic tissue in the peripheral area immediately after RF. The persistence of ischemic tissue in 100% of the AZ on D28 is surprising because one might have expected it to develop into necrosis or reperfusion. The persistence of ischemic tissue one month post-RF shows that the lesions were not yet definitively healed.

Taking into account the pathology findings, the first CT-scan to check the results of the radiofrequency treatment performed at one month after the procedure seems to us to be programmed too early to assess an AZ that is still in the process of healing.

3) In theory the spherical volume of tissue treated by a LeVeen needle is 4.2 cm³. At the end of the treatment the volume treated, calculated by measuring the hypodense areas, was 3.73 cm³, and 3.5 cm³ à D28.

Is this due to the fact that the needle cannot treat the theoretical volume because of a "cooling" phenomenon or does the treated parenchyma retract due to infarction?

There are also morphological variations in the AZ with a systematic decrease in their volume, the initially conical areas become spherical on D28.

4) Heterogeneity:

The borders of the areas of reorganized tissue in the AZ were either hazy at D0 (with transitional areas of mixed histology) or they were clear as if they had been cut with a knife. Logically, this suggests that the changes were caused by a combination of two mechanisms: ischemia, inducing the clear borderlines (by vascular thrombosis) and RF waves creating the hazy borders through their thermal effect. However, the statistical study in our results does not reveal any significant relationship between the type of tissue reorganization (necrotic, ischaemic or mesenchymatous), the borders, the size and their aspect on the CT-scan (at D0 and D28) in these different types of tissue. We have simply learned that 100% of the AZ that were circular and 50% of the AZ that were triangular on D0 developed a mesenchymatous area, with no significant connection (p=0.228).

5) Value of the enhancement for constant necrosis:

The first CT-scan to check the result of the RF treatment is usually performed one month after the procedure [5] to avoid the immediate coagulation and hemorrhage phenomena and analyze the final architectural changes. The persistence of ischemic tissue in 100% of our AZ means that the post-RF changes were not finalized, the one-month deadline thus seems too early to judge the effectiveness of the treatment.
The acknowledged success criteria are based on enhancement of the AZ zone below 10 HU after injection of a contrast medium [11, 12]. This success criterion was selected on the basis of the follow-up studies for liver tumors treated by RF, although no histological study has ever been carried out to prove it [17], Clark [12] even states that it is arbitrary.

Our study did not find any statistical correlation between no enhancement and necrosis since 26% of the AZ showed enhancement on the first CT-scan, whereas central necrosis was observed in 100% of the AZ.

Because there was necrosis in all the AZ we were unable to calculate the sensitivity of the « no enhancement » test to predict the absence of necrosis, however, the specificity was low at 44.4% (VPP=0%; VPN=100%). Enhancement does not therefore mean that there is no tissue necrosis. The persistence of an inflammatory reaction four weeks after treatment (or of granulation tissue as described by Rowland [18] after hepatic RF) is a hypothesis which may explain the enhancement despite tissue necrosis.

According to our comparisons between the pathology findings and the CT-scan enhancement does not indicate that there is no tissue necrosis. The persistence of an inflammatory reaction four weeks after treatment is a hypothesis that might explain the enhancement despite tissue necrosis.

Although no enhancement can be considered as a success criterion for the treatment (because it always correlates with necrosis), enhancement of an AZ one month later cannot be considered as a failure and the AZ enhancement study is not a criterion of effectiveness or failure of RF treatment; this parameter should probably be considered in association with the volumetric progression [19] and heterogeneity of enhancement.

We were surprised to discover the heterogeneity of the apoptotic areas which had no consistent distribution pattern: the markers were positive in the centre and on the edges of the treated areas, in the necrotic and ischemic tissue and in the healthy tissues: this discovery shows that ischemia and induced heat may not be the only mechanisms of cell destruction caused by radiofrequency and this aspect should be the subject of a complementary study.

This study had limitations imposed by its goals and our materials:

- The RF hits were performed on healthy renal parenchyma. Because of this, the decrease in volume of the tumor, which is often used as a second success criterion [19], was not studied.

- The CT-scan in our laboratory did not offer acquisition times short enough to analyze the enhancement kinetics of the AZ. However, Wile [20] obtained equivalent results in detecting tumors and recurrences in the nephrographic and excretory stages.
Despite a strict rationale: same procedure, same materials, same radiologist, with precise positioning, avoiding the blood vessels and the urinary cavities to ensure the homogeneity and reproducibility of the AZ, there was significant disparity in the size and distribution of the enhanced areas after injection of the AZ (D0 14HU [30.3], 3.73 cm³ [1.46] and D28 19HU [22.5], 3.5 cm³ [1.35]).

Images for this section:

Fig. 1: CT-scan 4 weeks after radiofrequency treatment (injected scan): Fluid effusion of the lower end of the right kidney of pig 831 with enhancement of its walls showing an
abscess in the ablation zone (white arrow) communicating with an abscess of the psoas (black arrow).

**Fig. 2:** lung pathology due to inhalation seen on the thoracic CT-scan after radiofrequency treatment
Fig. 3: post-procedure CT-scan. As the macroscopic examination does, the CT-scan (Figure 5) shows triangular enhancement defects with their base in the peripheral cortex, corresponding to areas of renal infarction.
**Fig. 4:** end of procedure CT-scan with cavitation changes in the 2 ablation zones.
**Fig. 5:** macroscopic aspect of the AZ immediately after RF Axial and sagittal slices of a kidney: area of renal infarction with its base in the peripheral cortex (white arrow) and pericapsular hematoma (black arrow) secondary to puncture with the radiofrequency needle. This kidney belongs to pig 828 and was explanted immediately after the radiofrequency procedure.

**Fig. 6:** histo-immunochemical assays of the ablation zone with the apoptosis marker. The brown colored areas are marked with anticaspase 3 and reveal apoptosis. On the two slides the following phenomena can be seen (zoom ×100 and ×200): 1- healthy renal tissue combined with damaged tubules (showing ischemia) 2- a mesenchymatous area 3- tissue necrosis 4- intra vascular coagulation 5- apoptosis markers in the nuclei

**Fig. 7:** slides of the ablation zones four weeks post-procedure : ( HES staining, zoom ×50 and ×100) 1- tissue necrosis 2- mesenchymatous zone 3- healthy renal tissue combined with damaged tubules (showing ischemia)
Conclusion

The goal of our study was to compare the CT-scan data and the histological findings pertaining to tissue viability and the homogeneity of the ablation area after renal radiofrequency treatment. These parameters had never been studied although radiofrequency is a proven ablation technique.

Six pigs were treated with renal radiofrequency. The ablation areas were monitored by CT-scan at D0 and at four weeks. The kidneys were explanted four weeks later and the ablation zones were analyzed in the path lab. All the radiofrequency protocols were carried out normally.

For the first time, the path lab findings compared with the CT-scan data in our study reveal the irreversible, heterogeneous lesions on the renal parenchyma caused by RF treatment. This study thereby challenges the dogmatic beliefs concerning radiofrequency such as the usefulness of post-procedure CT-scans, the time that should elapse before the first check-up and enhancement of the ablation area as a criterion of insufficient treatment.

It proposes that the first CT-scan should be delayed, taking place later than one month after the procedure and systematically be associated with a study of the enhancement of the ablation area and the distribution of the enhancement and volumetric progression of the contrast medium. The fact that we have shown that there is an RF-induced apoptotic reaction means that necrosis caused by elevated local temperatures is not the only tissue destruction mechanism and opens up new avenues of research.

References


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