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

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Chapter 3

Introduction

Unlike for other solid tumors, biopsy has not played an important role in the diagnosis of renal tumors and it was not included in diagnostic algorithms [1, 2]. Classically, a biopsy was indicated in those cases where there was a reasonable clinical doubt as to the origin of the tumor (primary renal or metastases of other cancers) or when the differential diagnosis was between a renal cell carcinoma and other causes of a renal mass (e.g. lymphoma or infection). However, in the most recent version of the American Urological Association (AUA) guidelines, biopsies are also recommended in patients with a wide range of treatment options varying from observation to surgery [1]. Another indication for a renal mass biopsy is the presence of metastatic disease, where there is a role for targeted therapy such as immunotherapy or angiogenesis inhibitors. Studies have shown that the determination of renal cell carcinoma (RCC) subtype is of vital importance since there are different treatment strategies for clear-cell RCC and nonclear cell RCC [3].

Surveys among urologists show that the majority do not perform a renal mass biopsy even in cases of indeterminate lesions [4, 5]. The main reasons given for this were the presence of false negative results and that biopsy in most cases will not change management. A major concern was the low predictive negative value of the biopsy. The post-test probability of a diagnosis of RCC in cases with a negative renal mass biopsy has been estimated at 18% [6]. A recent comprehensive review shows that in approximately three-quarters of cases with nondiagnostic biopsies, malignancy is diagnosed at re-biopsy or surgery [7].

Currently, due to the profuse use of abdominal imaging, up to 70% of kidney tumors are accidentally discovered before symptoms arise [8, 9]. The mean size of tumors that are operated on has steadily decreased in the last two decades [10–12] and the absolute number of small renal masses (≤ 4cm) diagnosed has increased, although their relative impact on the total number of tumors is still unknown. Additionally, pathologic assessment of small renal masses has evolved and the classical cut-off of 3 cm to distinguish a renal carcinoma from a benign adenoma is no longer valid. While 20 years ago every renal mass smaller than 3 cm was considered as benign, with increasing knowledge, identification of the different types of RCC and broad application of immunohistochemistry (IHC) techniques, it is known that a high percentage of
these small renal masses are carcinomas [13]. The real problem does not arise in the differential diagnosis of large renal masses, where cross-sectional imaging has been proven to have a high accuracy, but in the proper assessment and differentiation of the small renal masses [13, 14]. Doubts remain about the aggressiveness of small renal masses. In an autopsy study between 1984 and 1995 it was demonstrated that 47% of all renal tumors was not clinically known. The stages of the nonclinically diagnosed tumors were mainly of T1 and T2 [9]. Another autopsy study showed a decrease in the size of nonclinically discovered renal tumors found at autopsy from 4.63 to 1.65 cm between 1955–1960 and 1991–2001, respectively [15]. Surgical contemporary series show a benign tumor incidence of 20–30% among small renal masses and this incidence is inversely proportional to size [13, 14, 16]. A definitive cut-off for aggressiveness can be established at the 3 cm diameter. While bias cannot be ruled out in the surgical series, mainly from the inclusion of cysts with different Bosniak grades, the percentage of benign masses in this size range is too high to justify an intervention without histologic confirmation of malignancy or a reasonable doubt that the mass is benign. However, interventional policies based on surgical excellence might not be

![Figure 1](A) Diagnostic and (B) nondiagnostic (fibrin) core biopsy of a renal tumor
ideal for the evaluation of the potential use of a test in the wider medical community. Furthermore, partial nephrectomy, which should be considered the gold standard for small renal masses, is unfortunately underused [17], leading to overtreatment of small benign masses [18]. At the other extreme of the management spectrum, ablation or surveillance, small renal masses deserve to be biopsied for diagnosis and follow-up purposes.

**Biopsy nomenclature**

Before reviewing the results of renal mass biopsy, it is necessary to critically assess the nomenclature. The accuracy of a diagnostic test is measured by its sensitivity and specificity. Strictly speaking, only those studies with histologic confirmation on a surgical specimen can give accurate figures for these. Basically, when a renal tumor is evaluated, a biopsy can deliver one of two results: diagnostic (benign or malignant) or nondiagnostic (Figure 1).

When the biopsy is diagnostic, other characteristics such as histopathologic type and grade can also be assessed. There are different reasons for a nondiagnostic biopsy (Table 1). Conceptually a failed biopsy means that there is no tumor tissue available for assessment in the biopsy specimen, although other types of tissue might be present in the sample. The reason for a failed biopsy could be a technical failure of the puncture method (e.g. misfire or malfunctioning of the biopsy gun) or an incorrect sampling. Incorrect sampling is sometimes unavoidable due to the nature of the renal tumors: these may contain necrotic and fibrotic tissue, or be mixed in nature with solid and cystic components. Also, the presence of normal renal tissue implicates that the sampling is incorrect as very few renal masses are composed of normal renal tissue. The presence of fibrotic, inflammatory, fatty or necrotic tissue in the specimen will mean that a diagnosis cannot be made from it.

In indeterminate (or inconclusive) biopsies, tumor tissue is present in the biopsy specimen but it is impossible to determine the biology of the cells. In this case, IHC techniques may be of help if enough tissue is available. Definitions in the literature are confusing and a certain overlap between failed, incorrect sampling and indeterminate biopsies is unavoidable. Diagnostic yield is the rate at which biopsies establish a diagnosis. Diagnostic biopsies can also give false results, both positive and negative,
Renal Mass Biopsy

and the sum of these is the rate of inaccurate biopsies. Accuracy is then calculated from the total number of diagnostic biopsies.

Besides the technical failures and the indeterminate biopsies, benign nontumoral conditions (e.g. normal renal parenchyma) found in the biopsy specimen will be classed as diagnostic by some and nondiagnostic by others. Variations in results are conditioned by the lack of standardization of the taxonomy but also by the well-proven interobserver variability among pathologist. Interobserver variability may be responsible for an up to 11% difference in the rate of nondiagnostic biopsy, both for core biopsy (CB) and fine needle aspiration (FNA) [6, 19–21].

Renal biopsy technique

There are two aspects when considering the technique of percutaneous renal biopsy: the type of image guidance and the type of biopsy performed [CB or cytologic aspiration (FNA)].

Image guidance

Most renal biopsies are performed percutaneously and are supported by image guidance using computed tomography (CT) or ultrasound. The biopsies are normally performed under local anesthesia in an outpatient setting. When performing biopsies during surgery or ex vivo, direct vision can be used. Compared with ex vivo renal mass biopsies, recent studies with imaging-guided percutaneous renal mass biopsies have shown better outcomes in terms of failed or indeterminate biopsies [22]. The outcomes of biopsies performed using ultrasound, CT or magnetic resonance imaging (MRI) are similar and therefore the choice between the radiologic modalities should be made on

| Table 1 Nomenclature of the nondiagnostic biopsies based on the sample findings, and reasons leading to a nondiagnostic result. |
|---|---|---|
| Nomenclature | Tissue in the sample | Reasons |
| Failed biopsy | Absence of tumor cells (other types of tissue may be present) | Technical failure (e.g. misfiring) Erroneous sampling (e.g. normal kidney, fibrosis, fat, inflammation, necrosis, blood) |
| Indeterminate (inconclusive) biopsy | Impossibility to differentiate benign from malignant cells | Insufficient cells Morphologic overlap Cellular heterogeneity |
other grounds [23–26]. MRI-guided biopsy is not widespread as it entails the use of the more costly nonferromagnetic needles [23].

**Ultrasound**

Ultrasound guidance appears to have several advantages: it is generally available and less costly, results in less radiation, most urologists can perform it themselves, provides real-time and multiplanar imaging, and the device is portable. However, not all lesions can be visualized on ultrasound and anatomic structures, such as the ribs, and gas can obscure visibility. Finally, performing ultrasound-guided renal biopsies requires a significant learning curve. Visualization of the needle tip on ultrasound can be improved by several methods [27]. Biopsies can be performed using a guide or “freehand.”

**Computed tomography** (Figure 2)

Advantages of CT guidance are that most renal tumors can be identified after contrast injection and that the puncture of intratumoral changes can be avoided to optimize the results of the biopsy (e.g. hemorrhagic zones that can result in necrosis). Adjacent organs can be optimally identified and the technique is easier to master. These advantages are even more significant in obese patients. However, real-time CT imaging is potentially hazardous for the investigator and patient due to radiation exposure. It is possible due to manipulation of the needle that the biopsy is performed outside the selected target, resulting in false-negative of nondiagnostic biopsies.

**Type of biopsy**

**Fine needle aspiration**

FNA or cytologic aspiration is performed under image guidance. When the patient is properly positioned, the most suitable needle path is chosen. A guiding cannula is advanced onto the tumor surface and the needle is passed through the cannula into the tumor, trying to avoid possible necrotic areas. The cannula is used to avoid direct contact between the tumor and surrounding retroperitoneal tissue, decreasing the risk of tract seeding [27, 28]. The needle is then moved back and forth within the tumor to collect the cells. Traditionally, negative pressure with a syringe is applied on the needle in order to collect more cells. Another method that does not use negative pressure is Zajelda’s fine needle capillary technique. This tends to reduce the amount of blood in
the sample, but may result in fewer cellular smears [23]. If a CB and FNA are to be performed in the same session, the FNA should be performed first, to reduce the amount of blood in the cytologic specimen [23]. The aspirate is placed on slides and smeared directly. An on-site cytotechnologist can immediately determine the quality of the specimen, which should increase the rate of diagnostic FNAs and subsequent CBs since correct needle placement is ensured [23]. Cytologic examination can provide cytologic detail that is sometimes superior to that seen in CBs (Figure 3 and 4). FNA samples can be used to prepare cell blocks which are helpful to identify specific histological features and to perform IHC studies. A recent study has shown excellent results with an improved agar microscopy which comprises processing the aspirate of FNA by centrifuging it in agar. This technique results in concentrated cell blocks composed of fragments and loose cells that are suitable for slicing and subsequent histologic interpretation with IHC [29]. Additional testing, such as flow cytometry, fluorescent in situ hybridization (FISH), apoptosis assays, and picrosirius red F3BA staining, are also possible with FNA [23].
Core biopsy
A CB is performed with a hollow needle, allowing it to remove small but solid samples of tissue suitable for fixation and histologic examination (Figure 3). The needle has a cutting edge that allows tissue samples larger than 1 cm in length to be removed. Thicker needles than for FNAs are used (17G or 18G). The risk of complications does not increase with the use of these needles [25, 30–39]. Usually, 18G or 17G needles are used with an automatic biopsy gun to obtain tissue samples of 15–22 mm in length. The quality of the core should be checked at the time of the biopsy, in order to repeat it if there are doubts about its quality.

Technical considerations
There are technical considerations with both FNA and CB to improve the quality of the sampling and to maximize the results. The choice of radiologic imaging depends on tumor location, size, access feasibility, visibility and individual preference. Imaging is used to avoid areas suggestive of pathologic necrosis or cystic areas. The same considerations apply to the choice of biopsy type, FNA or CB. In general higher accuracy has been shown for CB, although experienced groups achieve the same results with FNA [40]. The ideal is to perform both types of biopsy during the same procedure. Larger needles (see figure 5) have shown better results in terms of sample quality, and there is no increased risk for complications when comparing 18G to 22G needles [23, 25, 29, 36–39]. Most recently, Breda et al. compared the ex vivo performance of three distinct needles (14G, 18G, and 20G) in a blinded study [41]. One core biopsy was obtained with each needle from 31 tumors. Biopsies were analyzed by H&E and a standard IHC panel. All the biopsies performed with the 14G and 18G needles provided sufficient tissue for diagnosis, whereas the technical failure rate for the 20G needle was 14%. Sensitivity to distinguish between benign and malignant tumor was high for the three needle calibers, but specificity was lower for the 20G needle. Histologic accuracy was 92%, 97% and 81% for 14G, 18G, and 20G needles, respectively (see table 2). Grade accuracy was consistently low for the three needles and in the range 40–56%.
Figure 3 Histopathologic core biopsy samples (H&E staining) of different subtypes of renal cell carcinoma and oncocytoma: (A) clear cell; (B) papillary; (C) chromophobe; (D) oncocytoma.

Figure 4 Fine needle aspiration biopsy samples (Giemsa staining) of different subtypes of RCC and oncocytoma: (A) clear cell; (B) papillary; (C) chromophobe; (D) oncocytoma.
These authors concluded that at least one core biopsy with an 18G needle offers the highest accuracy in histologic diagnosis [41]. The change to thicker needles could be at least partly responsible for the increasing accuracy and decreasing failure rate of the biopsies [22].

Different type of needles are available to perform a CB. Automatic Tru-cut needles are easier to manage than the original manual Tru-cut needles.

There are no studies in the literature comparing the results for different number of samples. However, the number of CBs advised in the literature is at least two [22, 23, 33]. For FNA, as many passes as necessary should be performed to ensure a diagnostic smear. As mentioned above, ideally the cytologist should prepare the smear directly after puncturing (“on site”) and examine it immediately to ensure the presence of cells and to give the opportunity to repeat the cytologic aspiration if necessary. In both CB and FNA it is recommended to use a cannula that is wider than the biopsy needle to be
Table 2 Results of *ex vivo* biopsies. Pathologic surgical specimen available in all cases.

<table>
<thead>
<tr>
<th>Study</th>
<th>Mean or median tumor size [cm range]</th>
<th>Number of tumors</th>
<th>Accuracy (biology)</th>
<th>Nondiagnostic biopsies*</th>
<th>Accuracy RCC type</th>
<th>Accuracy RCC grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nurmi et al. (1984) [55]</td>
<td>–</td>
<td>150</td>
<td>100%</td>
<td>2%</td>
<td>–</td>
<td>76%</td>
</tr>
<tr>
<td>Dechet et al. (1999) [21]b</td>
<td>4.6 (1-18)</td>
<td>106</td>
<td>Overall 76–80%</td>
<td>Spec 60–73%</td>
<td>11–17%</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sens 77–84%</td>
<td>Spec 60–73%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PPV 94–96%</td>
<td>NPV 69–73%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dechet et al. (2003) [6]b</td>
<td>NR</td>
<td>100</td>
<td>Overall 72–77%</td>
<td>Sens 81–83%</td>
<td>20–21%</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Spec 33–60%</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Wunderlich et al. (2005) [42]c</td>
<td>4.97 (2-20)</td>
<td>50</td>
<td>98% (diagnostic yield)</td>
<td>2%</td>
<td>70%</td>
<td>83%</td>
</tr>
<tr>
<td>Barocas et al. (2006) [30]</td>
<td>5.3 (1.1–15.5)</td>
<td>77</td>
<td>Overall 90%</td>
<td>Sens 87–100%</td>
<td>4%</td>
<td>90%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Spec 96.5–96.5%</td>
<td>PPV 96.4–96.9%</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>NPV 87.5–100%</td>
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<tr>
<td>Barocas et al. (2007) [31]</td>
<td>4.5 (1.3–11.3)</td>
<td>36</td>
<td>69–79%</td>
<td>14% (H&amp;E)</td>
<td>87%</td>
<td>NA</td>
</tr>
<tr>
<td>Kummerlin et al. (2008) [19]f</td>
<td>5.5 (2–12)</td>
<td>62</td>
<td>Overall 77–90%</td>
<td>Sens 79–100%</td>
<td>8–19%</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Spec 100%</td>
<td>PPV 100%</td>
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<td></td>
<td></td>
<td></td>
<td>NPV 29–100%</td>
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<tr>
<td>Kummerlin et al. (2009) [20]g</td>
<td>5.5 (2–12)</td>
<td>66</td>
<td>Overall 73–91%</td>
<td>Sens 72–97%</td>
<td>3–14%</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Spec 63–100%</td>
<td>PPV 93–100%</td>
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<td></td>
<td></td>
<td></td>
<td>NPV 24–75%</td>
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<tr>
<td>Breda et al. 2010 [41]i</td>
<td>6.3 (0.8–17)</td>
<td>31</td>
<td>Sens 96–100%</td>
<td>Spec 67–75%</td>
<td>0% (14G needle)</td>
<td>92% (14G)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PPV 96%</td>
<td>0% (18G needle)</td>
<td>97% (18G)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NPV 75–100%</td>
<td>16% (20G needle)</td>
<td>81% (20G)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>48% (14G)</td>
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<td></td>
<td></td>
<td></td>
<td>40% (18G)</td>
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<td>56% (20G)</td>
<td></td>
</tr>
</tbody>
</table>

* Nondiagnostic biopsies includes failed biopsies and indeterminate biopsies.

a Grade as low, intermediate and high.

b Two pathologists independently evaluated all samples.

c Five core biopsies per tumor.

d Addition of molecular diagnosis increased sensitivity and NPV.

e By adding FISH to conventional H&E staining.

f Core biopsy evaluated independently by five pathologists.

g FNA evaluated independently by five pathologists.
h For malignant tumors.
i Three different biopsy needle calibers compared (14G, 18G, and 20G).

NA, not assessed; NR, not reported; PPV, positive predictive value; NPV, negative predictive value.
used. This cannula is advanced under imaging onto the surface of the tumor and remains stable during the whole procedure. The biopsy needle is advanced through the cannula, which allows a number of passes or biopsies to be performed without the risk of contamination or seeding, and avoids manipulation of the chosen tract. Tumor size does affect the results of the biopsy. Since larger tumors more frequently have central necrosis, it is recommended to perform a peripheral and a central biopsy in tumors smaller than 4 cm and two peripheral biopsies for tumors larger than 4 cm [42]. Although bleeding complications are rare (see below), coagulation abnormalities should be corrected prior to biopsy, anticoagulants should be stopped when possible, and some hours of rest are recommended after biopsy. CBs are best preserved in formalin until fixation and analysis.

**COMPLICATIONS**

Potential complications of percutaneous renal biopsies are bleeding, tumor seeding along the needle tract, arteriovenous fistula, infection, and pneumothorax. There is enough evidence to affirm that minor complications are infrequent, occurring in less than 5% of all biopsies performed for renal masses. Catastrophic complications are extremely rare and mortality has not been described in the recent literature [22]. The most frequent complication is hemorrhage [22, 43, 44]. There are controversial reports on the correlation between needle size and rate of complications [39, 45, 46]. The correlation is highest when 15G needles are compared with 21G needles; however, as mentioned above there is no significant difference in complication rate when 18G and 21G needles are compared [46].

The most feared complication of renal biopsies is tumor seeding. However, in all recent series (from 1994 onwards) of renal tumor biopsy, no cases of tract seeding have been reported. This might be explained by the widespread use of a guiding cannula for renal biopsy [23, 47]. Urothelial carcinomas have a higher tendency to seed along the tract than RCCs. Therefore, when urothelial carcinoma is suspected, a percutaneous biopsy for diagnostic purposes is not recommended [47, 48]. Other complications occur very rarely. An arteriovenous fistula should be considered in cases of persistent bleeding [49]. When the renal biopsy is performed by a posterior approach, pneumothorax has been reported in 14–29% of cases, although clinically significant pneumothorax
is uncommon (<1%) [44, 48]. To further minimize the risk of a pneumothorax, the puncture should be performed in expiration and the needle should be positioned subcostally. Some upper pole tumors can be punctured using the paravertebral approach, which involves the injection of saline in the paravertebral space to displace the pleura laterally [50]. There is no evidence that a renal mass biopsy can complicate a subsequent partial or radical nephrectomy [39, 51, 52].

RESULTS
From the 1970s onwards, many studies have been performed on the diagnostic accuracy of renal mass biopsy. Early studies focused on cytologic aspiration with Chiba needles, but the incorporation of automatic fine CB needles minimized trauma and allowed core samples to be obtained that were easier to analyze. In 2008 Lane et al. published an extensive review on the subject [22]. They divided the studies chronologically between those conducted before and from 2001 onwards. Before 2001, 27 studies were considered, in two of which “ex vivo” tumor biopsy was performed. After 2001, seven clinical studies and three ex vivo studies were considered. An overall diagnostic accuracy of 88.9% was reported for the earlier period (including ex vivo biopsies) and a 96% clinical accuracy (excluding ex vivo biopsies) for the later period. Although no statistical comparison was available, these figures suggest an improving trend in the diagnostic performance of the biopsy. Whether a 7% difference in accuracy is of clinical significance remains unknown, but it is worthwhile. Furthermore, false-negative and -positive rates both decreased between periods. Accuracy of the biopsy was calculated based on the number of successful biopsies. Biopsy failures defined as “the inability to obtain an amount of tissue sufficient for diagnosis” were excluded from the accuracy assessment. Consequently, in clinical practice, the physician has to be aware that diagnostic yield is different from accuracy, as the biopsy failures accounted still for 9% and 5.2% for the periods before 2001 and from 2001 onwards, respectively. Indeterminate biopsies were included in the accuracy assessment in the review. However, an indeterminate biopsy is in fact a nondiagnostic biopsy. The sum of technical failures plus indeterminate biopsies (“no definitive diagnosis possible using the available techniques”) was 19% and 10% in the respective periods. In summary, biopsy has a high accuracy in the modern era, but nondiagnostic biopsies still account
for an overall 10% in general series irrespective of the tumor size.

When considering these results, some limitations of the review, most of which were pointed out by the authors, must be mentioned. First, the studies included in this review compared the biopsy results to numerous different gold standards. These index tests varied from pathologic examination of the specimen after surgical excision to radiologic follow-up of nonextirpated tumors [7]. For example, in the period before 2001, surgery and consequently surgical specimen available for comparison was noted in only 49.5% of the cases previously biopsied, excluding the *ex vivo* biopsy studies for which a surgical specimen was obviously available in all cases. The number of cases with surgical specimen available for comparison increased to 54% in the second study period. Together with the recent knowledge that the lack of radiologic growth does not necessarily mean absence of malignancy [53], this means that the results of the biopsies were compared to a strict index test in only half of the cases. Second, the percentage of pathologically confirmed renal carcinomas was 70.5% before 2001 and 82% from 2001 onwards, which could suggest that selection criteria based on imaging could have improved over time. Third, heterogeneity among series in both periods in terms of selection criteria, technical issues (e.g. needle used, histopathologic type of biopsy), and the different number of cases included precludes sound statistical comparison.

All the above mentioned reasons may be sources of bias in the interpretation of the analysis, and overestimation of the biopsy accuracy cannot not be ruled out, but neither can the improving trend be denied. In an attempt to clarify the results, we further examined those series where pathologic confirmation was obtained in the form of a surgical specimen in 100% of cases, whether *ex vivo* or in the clinical setting, including with a preoperative percutaneous biopsy. Results of biopsy of small renal masses are also considered separately, as results from general series may not be extrapolatable to these masses.

*Ex vivo biopsies*

Several studies have evaluated the accuracy of the *ex vivo* (in bench) biopsy for the diagnosis of renal tumors [6, 20, 21, 30, 31, 41, 42, 54, 55]. The accuracy of surgical excision (nephrectomy or partial nephrectomy) biopsies taken under direct vision were
compared to the definitive pathology of the surgical specimen in 100% of the cases. In most of the studies pathologists evaluating the biopsy were blinded to the definitive histopathologic results. Ex vivo CBs were sufficiently accurate to be able to differentiate between a malignant and a benign renal mass (Table 2). Overall accuracy varied from 72% to 90%, and was not lower for FNAs [20]. The rate of nondiagnostic biopsies varied from 2% to 20% with a trend to be higher than in the modern percutaneous biopsy studies [22]. Using the same nomenclature as in the article by Lane et al. [22] is used, Kummerlin et al. described a rate of failed biopsies (called nondiagnostic) between 8% and 16% for the five pathologists involved in the study, and a rate of indeterminate biopsies (called nonconclusive) between 0% and 8% [19]. This fact may be explained by the lack of visualization of the entire tumor, as can occur during imaging or in the absence of needle stabilization during percutaneous puncture. Subtype differentiation between oncocytoma and chromophobe RCC remains problematic (see Figures 3 and 4). Kummerlin et al. found a good overall accuracy for ex vivo FNA in differentiating between malignant and benign masses [18, 20]. However, again there was a substantial interobserver variation regarding the subtype differentiation other than for clear-cell RCC.

When interpreting the results of in-bench biopsy studies, it has to be taken into account that this ex vivo setting is only partially comparable to clinical practice, where percutaneous biopsies will be the standard.

**Percutaneous biopsy compared with 100% surgical specimens**

The number of studies on the accuracy of percutaneous renal mass biopsy based on 100% comparison of the preoperative samples with the surgical specimen is very low. Before 2001 only three such studies were identified in the review of Lane et al. [56–58]. In these studies accuracy for the diagnosis of RCC varied from 40% to 94%; the rate of biopsy failures varied from 0% to 22% and the rate of indeterminate biopsies from 4.3% to 36%. False positives were almost nonexistent (0–2.2%), but false negatives accounted for 0–24%. These results are clearly insufficient to justify the systematic use of percutaneous biopsy in the diagnostic setting. However, comparison with modern data is precluded as different needles, mostly 21G and 22G, were used and in two of those studies biopsy was guided by old ultrasound devices. After 2001, only three
more reports are available in the literature in which the percutaneous renal mass biopsy has been compared in all cases with the surgical specimen [54, 59, 60] (Table 3). The sensitivity of percutaneous core biopsy to detect malignancy proven at surgical specimen varies from 91.4% to 93.5% and the negative predictive value from 50% to 81.3%. The rate of nondiagnostic biopsies was very low in these three modern studies, in the range of 2.4–7.1%, although in some cases a repeat biopsy was necessary to reach a diagnosis. Correct RCC subtype determination varied from 77.5% to 91% and correct Fuhrman nuclear grade from 51.5% to 76%, with the grade mainly being underestimated on CB.

**Biopsy in small renal mass**

The performance of renal mass biopsies in small renal masses (SRMs; ≤4cm) is of utmost importance for a number of reasons. There is a high percentage of benign masses among the small renal tumors [13, 61], radiologic distinction between malignant and benign may be extremely difficult in this size range [14], and in spite of the low biologic potential of small RCC, some will be of high grade [16, 18]. The clinical scenario becomes even more complicated as a considerable number of those SRMs are accidentally found in older patients with comorbidity. Especially in SRMs, a preoperative histologic diagnosis may lead to a change in management.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Modern series on percutaneous renal mass biopsy with pathological confirmation (surgical specimen) in all cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumors</td>
<td>Mean tumor size in cm (range)</td>
</tr>
<tr>
<td>Schmidbauer (2008) [60]</td>
<td>78</td>
</tr>
<tr>
<td>Blumenfeld (2010) [54]</td>
<td>81</td>
</tr>
<tr>
<td>Sofkerim (2010) [59]</td>
<td>42</td>
</tr>
</tbody>
</table>

*Nondiagnostic biopsy includes failed and indeterminate biopsies.
*Nondiagnostic rate lowered to 4.7% after repeated biopsy.
CT, computed tomography; US, ultrasound; CB, core biopsy; NR, not reported.
In general, the sensitivity of renal biopsy in SRM is lower when compared with general series on renal biopsy (including all tumor sizes). The biopsy failure rate increases in SRMs. A rate of biopsy failure as high as 37% has been reported in renal masses smaller than 3 cm versus 9% in tumors larger than 3 cm [52].

Table 4 gives an overview of published studies on biopsies in SRM. As expected, biopsy results for SRMs are less accurate than in larger masses, with histologic confirmation by means of surgical specimen in 30–78% of cases [22]. The rate of nondiagnostic biopsies is also higher in SRM, either because of technical failure or because of indeterminate results. It was demonstrated that when the result of a biopsy is malignant, subtype

<table>
<thead>
<tr>
<th>Tumor size (cm)</th>
<th>Imaging guide</th>
<th>No. of tumors</th>
<th>ND biopsy*</th>
<th>Pathologic confirmation**</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuzillet (2004) [51]</td>
<td>2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CT</td>
<td>88</td>
<td>9%</td>
<td>70.4%</td>
</tr>
<tr>
<td>Rybikowski (2008) [63]</td>
<td>≤ 4</td>
<td>CT</td>
<td>66</td>
<td>18%</td>
<td>78%</td>
</tr>
<tr>
<td>Thuillier (2008) [64]</td>
<td>2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NS</td>
<td>53</td>
<td>23%</td>
<td>60%</td>
</tr>
<tr>
<td>Wang (2008) [65]</td>
<td>2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CT/US</td>
<td>110</td>
<td>9%</td>
<td>34%</td>
</tr>
<tr>
<td>Volpe (2008) [62]</td>
<td>2.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>CT/US</td>
<td>100</td>
<td>16%</td>
<td>20%</td>
</tr>
<tr>
<td>Shannon (2008) [66]</td>
<td>2.9&lt;sup&gt;b&lt;/sup&gt; (&lt;5)</td>
<td>CT</td>
<td>222</td>
<td>22%</td>
<td>59%</td>
</tr>
<tr>
<td>Kummerlin (2009) [20]</td>
<td>3.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>In bench&lt;sup&gt;c&lt;/sup&gt;</td>
<td>30</td>
<td>7–17%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>100%</td>
</tr>
</tbody>
</table>

*Includes failed and indeterminate biopsies.
**Cases with surgical specimen available.
<sup>a</sup>Mean size.
<sup>b</sup>Median size.
<sup>c</sup>Five pathologists’ independent results.
CT, Computed tomography; US, ultrasound; NS, not specified.
determination is possible in 93% of the biopsies; however, IHC is necessary in a substantial number of cases. Concordance with the surgical specimen is high (91–100%) [62, 63]. Fuhrman nuclear grade was correct in 68%, with a lower concordance than for subtype (60–100%), as was the case in larger renal masses [62–64].

**Accuracy for histology and grade**

Kidney tumors are known to show intratumoral heterogeneity and therefore subtype identification can be misleading when biopsies are assessed. However, in the modern series an overall accuracy of 94% in identifying the correct histologic subtype was reported [22]. Similar results for grade accuracy have been reported for SRMs, although IHC was necessary in most cases [62]. One of the major problems in differentiating subtypes of RCC is the distinction between oncocytoma and chromophobe RCC, since hybrid tumors containing areas of benign oncocytoma and malignant chromophobe RCC exist [60]. This issue remains a challenge for the pathologist, especially when limited tissue is available, as is the case with biopsies. New auxiliary techniques, such as genetic profiling, polymerase chain reaction (PCR), and FISH are under development to distinguish chromophobe RCC, oncocytomas, and other subtypes of RCC with more accuracy [32, 33, 67, 68]. Heterogeneity of renal tumors is also hindering correct determination of Fuhrman’s nuclear grade by biopsy. In two large recent series, Fuhrman’s nuclear grade was correctly determined in 70% [51] and 83% [42] respectively. In all the discordant cases, the actual grade found in postoperative pathology was within one grade of the grade found at biopsy. A higher accuracy (76–100%) is obtained when the nuclear grade of tumors is grouped into “low grade” (Fuhrman I–II) and “high grade” (Fuhrman III–IV) [51, 52].

**Biopsy during thermal ablation**

When performing thermal ablation of small renal tumors, such as cryoablation (CA) or radiofrequency ablation (RFA), information on the pathology of the ablated mass can only be gained through biopsy of the tumor, since the specimen is not extirpated. This information has not only diagnostic purposes but may impact follow-up policy. An additional use of biopsies in the frame of ablation therapy is in determining the presence of residual tumor. Two recent meta-analysis have compared CA with RFA [69] and percutaneous ablation with surgical ablation, including laparoscopy [70].
Biopsy of the mass was performed in 82.3% of the CA, in 62.2% of the RFA, in 84% of the percutaneous procedures and in 88% of the surgically assisted ablations [69, 70]. Overall, between 54% and 64% of the biopsies confirmed RCC, 12.7% showed benign pathology and 33.5% had unknown or undetermined pathology [69]. With the limitations derived from the lack of well-designed comparative studies it becomes evident that biopsy of the tumors is widely adopted during ablation treatments but in the case of RFA where still 40% of the patients are treated without information on tumor biology. The rate of nondiagnostic biopsy varies from 0% to 23% in those series of percutaneous ablation [71–74] and from 0% to 30% when tumor biopsy is performed during laparoscopic ablation [75–80]. These figures are similar to the nondiagnostic rates described for SRMs (see Table 4) as the tumors treated by ablation are in this size range. At least during laparoscopy-assisted ablation, modification of the biopsy technique, by activating the firing mechanism of the biopsy gun externally to the target tissue, led to a higher diagnostic yield in a small clinical series [77]. The criteria classifying a biopsy as nondiagnostic or benign varies between groups, which may explain the broad range of nondiagnostic or benign results. As an example, normal renal tissue, fibrotic tissue or necrotic tissue are distinctly classed as benign by some and nondiagnostic by others [78, 80]. In fact, the stricter the nondiagnostic criteria are, the higher the nondiagnostic rate of the biopsy. Tumor biopsies can also be taken immediately after ablation to minimize risk of bleeding or tract seeding. In this situation, the pathologist can identify RCC architecture both after RFA and CA and the diagnostic yield of the pre-ablation and immediate post-ablation biopsies is not statistically different [81–83].

Success after ablation therapy is mainly determined using cross-sectional imaging with contrast. Lack of contrast enhancement indicates absence of tumor recurrence [84]. However, the presence of viable tumors in the ablated area is not completely ruled out by the lack of enhancement on CT or MRI [85]. Therefore, some centers have performed an additional post-ablation biopsy to assess the success of the ablation. A study on the correlation of radiographic imaging and histopathology showed that in 24% of patients treated by RFA, the 6-month post-ablation biopsy showed viable renal cancer cells even though there were no signs of radiologic enhancement at that
time [86]. The 6-months post-cryoablation biopsy was consistently negative in all non-enhancing masses. Therefore, the sensitivity of nodular enhancement at 6 months after RFA to detect a positive biopsy was only 38.4%, with a specificity of 91.3%. The results for cryoablation were superior, with a sensitivity and specificity of 77.8% and 95.1%, respectively. In contrast, a study on RFA lesion biopsy more than 1 year after ablation showed no vital lesion [87]. It is therefore advisable to use NADH or other oxidative stress stains when assessing biopsy after ablation [88].

Additional pathologic methods

RCC subtyping was originally performed using a light microscope and standard H&E staining. The diagnostic accuracy in differentiating benign from malignant renal mass is substantial, but for determining RCC subtypes the accuracy is lower, since complex and overlapping morphologic features exist. As described earlier, this results in the interobserver and intraobserver variability of subtyping [19, 20]. For a long time research to improve subtyping of renal tumors had no priority since there were no clinical consequences. However, there has been interest in subtype determination of RCC following the incorporation in clinical practice of targeted therapies. Correct determination of RCC subtype can be of capital importance since paradigms for specific RCC subtypes may not apply to other subtypes. Therefore, a more accurate differentiation of RCC subtypes is desirable. Differences among RCC subtypes at the DNA or RNA and the protein level have been explored in order to improve the accuracy of subtype differentiation. Although different subtypes of RCC show overlapping histologic features they are biologically distinct. This is obvious when the cytogenetic abnormalities of different subtypes are observed: different subtypes have characteristic abnormalities, such as chromosome 3p aberrations in clear-cell RCC and trisomy 17 in chromophobe RCC [89]. These characteristic differences imply that the different subtypes are distinguishable by mapping this genetic expression. One method to perform a molecular gene profiling analysis is DNA microarray. This has shown differences in gene expression profiles between subtypes of RCC and therefore it differentiates between the histologic subtypes of RCC [90–92]. By analyzing the mRNA expression ratios of different genes in different subtypes of RCC, the individual subtypes can be differentiated: high CA9 expression in clear-cell RCC,
AMACR in papillary RCC, and CLCNKB in chromophobe RCC and oncocytoma [93] which facilitates a molecular diagnostic algorithm. It has been demonstrated that renal masses can be accurately classified on CBs using a combination of histopathology and molecular gene profiling analysis [30]. In order to obtain sufficient material for investigation, the genetic material harvested with percutaneous biopsy should be amplified by PCR [23, 30]. Another auxiliary technique for subtype differentiation is IHC, which localizes specific antigens or proteins in a tissue sample by binding the antigens with labeled antibodies. The formed antigen–antibody complex can be visualized by (fluorescent) staining and therefore the presence of the antigen can be demonstrated. Several antigens have been found to be useful as a marker for subtype determination of RCC, and they are currently used in combination with histologic investigation to improve the diagnostic accuracy. It is likely that in the near future these techniques will contribute more to the diagnostic process [94–96].

**Conclusions and recommendations**

There is an increasing interest and trend to incorporate the percutaneous biopsy of a renal mass into the diagnostic algorithm of small renal tumors, when treatment depends on histologic subtype determination, and for ablative procedures. CB is more commonly performed than FNA. Complications are rare and mostly of low grade. Modern series on percutaneous biopsy of renal masses show a high accuracy and a lower rate of failed or undetermined biopsies than older series. Although still scarce, those recent series with pathologic confirmation by means of surgical specimen support these encouraging results. The accuracy of histologic subtype determination in the biopsy specimen may be up to 94% and of Furman’s grade up to 70%. Oncocytic features may overlap with those of chromophobe RCC. However, when considering a percutaneous biopsy in an SRM, there needs to be an awareness that accuracy will be inferior to that reported in general series. The overall rate of nondiagnostic biopsy, either failed or inconclusive, is still high in the SRM. Biopsies are not consistently performed during ablation therapy. When performed, the literature shows a similar rate of nondiagnostic biopsies as in the case of SRM. Biopsy immediately performed after ablation leads to the same diagnostic yield as before ablation as architectural structure is still recognizable. Current percutaneous renal
biopsy is recommended when there is a suspicion of nonprimary RCC, when an infectious cause is suspected, during ablation therapy, to decide on treatment of SRMs, for therapeutic purposes in metastatic RCC, and for documentation and follow-up purposes when a RCC is submitted to active surveillance.
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