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ORIGINAL ARTICLE

Diagnostic value of imprint cytology testing in kidney tumors: review of 200 cases

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Introduction Previous investigations have studied the importance of imprint cytology (IC) testing of core needle biopsy (CNB) from various organs. We have presented the largest series, to the best of our knowledge, of IC testing of CNB for patients with kidney tumors.

Materials and methods The present retrospective study (January 1, 2015, through January 30, 2016) identified laboratory information through a computer search of the cytology archived reports for 200 consecutive IC testing with CNB for renal tumors cases. A board-certified cytopathologist and cytology-trained fellow reviewed the IC testing and CNB slides and rendered them as nondiagnostic, positive for malignancy, negative for malignancy, positive for neoplasm, or atypical. The tumors were graded using the International Society of Urological Pathology grading system. The sensitivity, specificity, positive predictive value, and negative predictive value were calculated.

Results The IC testing cases classified as atypical (n = 53) or positive for neoplasm (n = 28) were evaluated separately because of the ambiguous morphologic characteristics. Of the other 119 cases, IC testing classified 95 (80%) as positive for malignancy, 5 (4%) as negative for malignancy, and 19 (16%) as nondiagnostic. The corresponding CNB histologic diagnoses showed that 85 of 95 cases (89%) were true positive for malignancy. Of these 85 cases, 45 (53%) were low grade, 21 (25%) were high grade, and 19 (22%) were ungraded. The corresponding sensitivity, specificity, and accuracy were 85%, 11%, and 58%, respectively. The 53 IC-identified atypical cases were more likely to be malignant (n = 40; 75%). Of the remaining IC testing atypical cases, 12 (23%) were negative for malignancy and 1 (2%) was nondiagnostic. Of the 28 cases positive for neoplasm using IC, 13 (46%) were positive and 15 (54%) were negative for malignancy.

Conclusions The relatively low diagnostic value of IC testing for renal tumors showed it to be less powerful for screening than its use in other organs.

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Introduction

Imprint cytology (IC) testing is a tool that can be used in the rapid preliminary diagnosis of malignant and benign lesions, with the histopathologic evaluation of the core needle biopsy (CNB) continuing to be the reference standard.[1] IC testing is a simple technique for diagnosis. The tissue is touched on a slide and leaves its imprint in the form of cells. Although many studies have discussed this technique and compared its accuracy in different organs, few of these studies have included the renal tumor.[1-5]

The increasing use of cross-sectional imaging techniques has detected more cases of renal cell carcinoma (RCC) at an early stage.[6] The increased incidence of small renal masses has resulted in sparing surgical techniques aimed at preserving kidney function. Partial nephrectomy, when possible, either open or laparoscopic, has become the reference standard treatment for this subgroup of patients.[7] Recently, the ablation procedure has become more popular owing to the lower incidence of complications and a success rate of $\leq$97%.[8] The most common 2 methods to ablate the tumor are radiofrequency with electrodes to produce heat and destroy the tumor cells and cryoablation with a low temperature (−40°C) to cause cell death. The ideal renal tumor size for ablation is <4 cm.[9] Tumor seeding has been minimal during removal of the probe after ablation.[10] However, the most common complication for both methods has been hemorrhage, which can require blood transfusion or an open surgical technique.[11]

We assessed the diagnostic utility and value of IC testing of CNB specimens performed on renal masses at our institution. In addition, we evaluated whether the diagnosis rendered with IC testing could be a potential triage tool to facilitate the ordering of the necessary ancillary immunohistochemical (IHC) stains on the CNB specimen in advance, because this could potentially decrease the turnaround times in these cases.

Materials and methods

Study population

The Mayo Clinic Institutional Review Board approved the present study. All the patients had provided written informed consent for the procedures and interventions performed. The patients with a diagnosis of renal tumors were identified through a retrospective review of the cytology archived reports at the Mayo Clinic (Rochester, MN) from January 1, 2015, through January 30, 2016, with the keywords "kidney" and "renal." We identified 200 consecutive cases in which CNBs with their IC testing of renal masses had been obtained, including primary and metastatic lesions.

Pathologic studies

The IC testing and CNB slides with the ancillary IHC studies on all renal biopsies were reviewed by a board-certified cytopathologist (Y.H.) and a cytopathology-trained fellow (S.T.A.), who rendered the IC testing diagnosis of nondiagnostic, positive for malignancy, negative for malignancy, positive for neoplasm, or atypical. No significant discrepancy was identified in the IC testing and CNB diagnoses during the review process. The finding of negative for malignancy included benign non-neoplastic lesions and normal renal parenchyma. The finding of positive for neoplasm included all benign neoplastic and malignant lesions (Table 1 and Supplementary Table S1). The cytology criteria for the diagnosis and classification of the different types of renal tumors have been summarized in Supplementary Table S2.

Sample collection

The CNBs were performed primarily by radiologists, with their technique guided by ultrasonography or computed tomography. They used an 18- or 20-gauge needle attached to a 20-mL syringe. To obtain diagnostic material for predominantly solid lesions, 1 to 4 passes were made, depending on the quantity of the core biopsy, which is totally dependent on the radiologist evaluating the number of passes.

In our institution, the interventional radiologists have been trained to prepare IC testing slides on-site by gently touching the CNB tissue to glass slides. The CNB tissue should not be pushed hard on the glass to avoid producing a crush artifact as much as possible. However, a touch that is too light should also be avoided because it will result in hypocellular smears or, even, nondiagnostic material.

Before smear preparation, the blood was removed by gently touching the surface of the biopsy tissue on dry gauze. The smears were immediately fixed in 95% alcohol for a minimum of 15 minutes before staining with Papanicolaou stain and sending the smear to the cytology department, where it was read by a certified cytopathologist who recorded the IC testing diagnosis and informed the performing radiologist. After preparation of the imprint smears, the specimens were sliced and fixed in 10% formalin. After proper

Table 1 IC testing correlating with CNB findings.

IC testing result	Nondiagnostic (n)	CNB result (n)[a]		
		Positive	Negative	Total
Nondiagnostic	1	10	8	19
Negative	0	2	3	5
Atypical	1	40	12	53
Positive for neoplasm	0	13	15	28
Suspicious	0	28	6	34
Positive for malignancy	0	57	4	61
Total	2	150	48	200

Abbreviations: CNB, core needle biopsy; IC, imprint cytology.
[a]Indicative of reference standard/true results.

fixation, the samples were processed by routine histopathologic processing and the sections stained with hematoxylin-eosin. All CNB specimens with IC slides were retrieved from the tissue registry and were reviewed independently by 2 pathologists (1 board-certified cytopathologist [Y.H.] and 1 cytopathology fellow trainee [S.T.A.]), to whom the core tissue pathology and cytology diagnoses were masked. The 2 reviewers rendered an IC testing diagnosis.

We combined the positive and suspicious for malignancy cases in the "positive for malignancy" category. Atypical and positive for neoplasm categories were evaluated separately because of the ambiguous and overlapping morphologic features with benign and malignant conditions. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated. The equations for these calculations were as follows:

$$\text{Sensitivity(TPR)} = \text{TP}/(\text{TP} + \text{FN})$$

$$\text{Specificity(TNR)} = \text{TN}/(\text{TN} + \text{FP})$$

$$\text{PPV(precision)} = \text{TP}/(\text{TP} + \text{FP})$$

$$\text{NPV} = \text{TN}/(\text{TN} + \text{FN})$$

$$\text{Accuracy} = (\text{TP} + \text{TN})/(\text{TP} + \text{FP} + \text{TN} + \text{FN})$$

where FN, false negative; FP, false positive; TN, true negative; TNR, true negative rate; TP, true positive; and TPR, true positive rate.

The demographic data were collected for each patient, including age and sex. Other collected information included the number of cores, size of the renal mass, laterality, IC testing and CNB diagnoses, and findings from ancillary IHC studies.

Statistical analysis

The Fisher exact test was used to compare the significant differences. All analyses were performed using the SAS, version 9.4, system and JMP, version 10 (SAS Institute Inc, Cary, NC, for both). $P < 0.05$ was considered to indicate statistical significance.

Results

In total, 200 cases of CNB specimens with the corresponding IC testing preparation were identified. No significant discrepancy was identified between the reported cytology diagnosis and the review results. Of the 200 patients, 79 (40%) were men and 121 (60%) were women. The mean age was 67.1 years (range, 20.0-107.0 years). Of the 195 cases with a known tumor size, the mean size was 4.1 cm (range, 0.9-19.0 cm). No statistically significant difference was

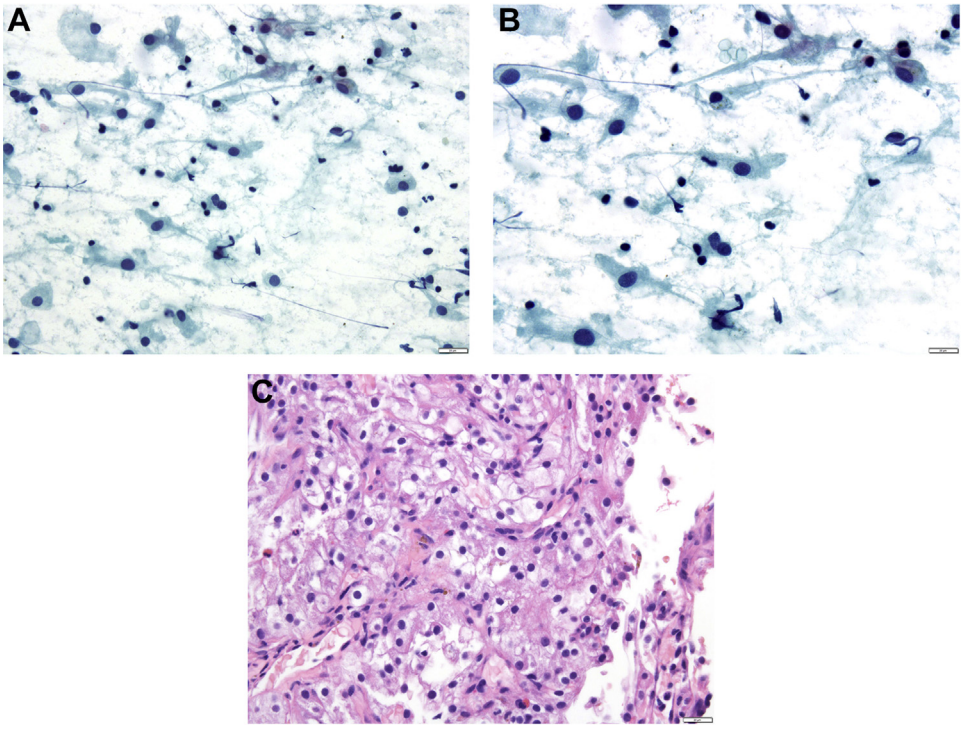

Figure 1 A 64-year-old man with 8.8-cm right renal mass. (A and B) Imprint cytology testing positive for malignancy, with cellular smear, pleomorphic nucleus, irregular nuclear membrane, inconspicuous nucleoli, moderate-to-low nuclear/cytoplasm ratio, and moderate homogeneous clear to slightly granular cytoplasm (Papanicolaou stain, original magnification ×400 and ×600, respectively). (C) Corresponding core needle biopsy confirmed the diagnosis of renal cell carcinoma, clear cell type, International Society of Urological Pathology nuclear grade 2 (hematoxylin-eosin stain, original magnification ×400).

observed ($P = 0.63$) comparing the negative and positive malignancy CNB cases dichotomized at 4 cm in tumor size. Of the 200 cases, 53 had been classified as atypical and 28 as positive for neoplasm on the basis of the IC testing slide review. These 2 groups were evaluated separately.

Of the other 119 cases, IC testing analysis classified 95 (80%) as positive for malignancy, 5 (4%) as negative for malignancy, and only 19 (16%) as nondiagnostic. The corresponding CNB histologic diagnosis showed that 85 of 95 cases (89%) positive for malignancy and 10 of 95 (11%) were negative for malignancy (Table 1). The PPV was 89.5%, NPV was 60% (Supplementary Table S3). The sensitivity, specificity, and accuracy were 85%, 11%, and 58%, respectively (Supplementary Table S4).

Of the 150 cases positive for malignancy from the final CNB examination, 96 (64%) had been classified using the International Society of Urologic Pathology (ISUP) as low grade (grades 1 and 2; Fig. 1), 28 (19%) as high grade (grades 3 and 4; Fig. 2), and 26 (17%) as ungraded/unclassified (Table 2).

Of the 24 cases of high-grade renal tumor, we found that 21 (88%) were positive for malignancy on IC. In contrast, 45 of 66 low-grade renal cases (68%) were positive for malignancy on IC. The difference between the high- and low-grade lesions among the malignant renal tumors with IC testing was statistically significant ($P = 0.04$). No statistically significant difference was observed ($P = 0.74$) using IHC compared with CNB (Supplementary Tables S5 and S6).

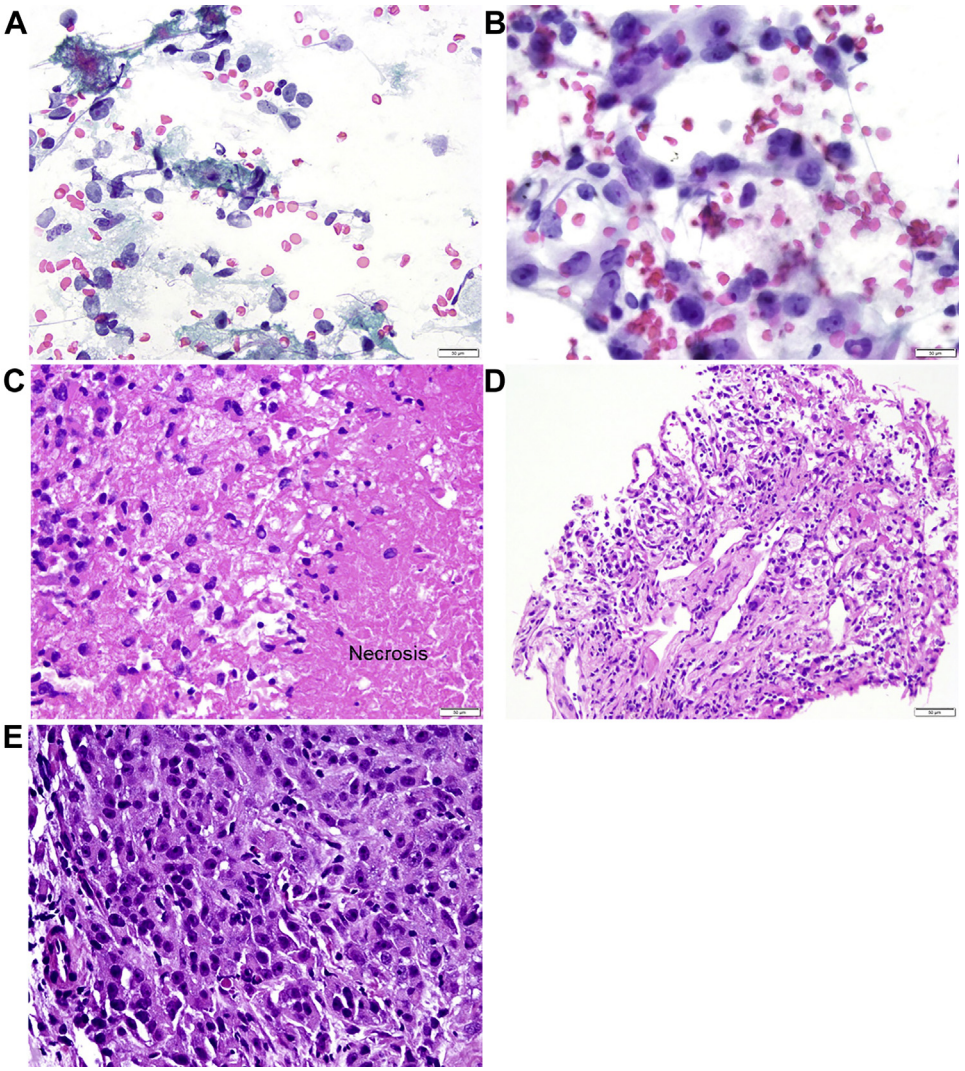

Figure 2 A 78-year-old man with 5.3-cm left renal mass. (A and B) Imprint cytology testing positive for malignancy, with cellular smear showing overt malignant characteristics, including pleomorphic nuclei, prominent nucleoli, and a high nuclear/cytoplasm ratio with low cytoplasm (Papanicolaou stain, original magnification ×60 and ×40, respectively). (C-E) Corresponding core needle biopsy showing proliferation of highly pleomorphic cells with irregular nuclear membrane and prominent nucleoli and necrosis, in keeping with high-grade (International Society of Urological Pathology grades 3-4) renal cell carcinoma, favoring clear cell type (hematoxylin-eosin stain, original magnification [C] ×40, [D] ×10, and [E] ×60, respectively).

Table 2 IC testing in correlation with tumor grade of positive CNB.

IC testing	Tumor CNB (n)				Cases (n, %)	
	Low grade	High grade	Ungraded	Total	IHC study to reach diagnosis	No IHC study
Nondiagnostic	8	2	0	10	3/10 (30)	7/10 (70)
Negative for malignancy	2	0	0	2	1/2 (50)	1/2 (50)
Atypical	30	4	6	40	12/40 (30)	28/40 (70)
Positive for neoplasm	11	1	1	13	7/13 (54)	6/13 (46)
Suspicious	16	7	5	28	9/28 (32)	19/28 (68)
Positive for malignancy	29	14	14	57	24/57 (42)	33/57 (58)
Total	96	28	26	150	NA	NA
IHC used to reach diagnosis (n, %)	14/96 (15)	16/28 (57)	26/26 (100)	NA	NA	NA

Abbreviations: CNB, core needle biopsy; IC, imprint cytology; IHC, immunohistochemical; NA, not applicable.

Of the 200 patients, 93 (47%) had undergone imaging-guided radiofrequency ablation of the renal masses. Of these 93 cases, 49 (53%) were clear cell RCC on CNB. The size of the renal masses in the patients who had undergone an ablation procedure averaged 2.3 cm (range, 0.9-3.5 cm).

Atypical cases

The 53 cases diagnosed as atypical on IC testing had been evaluated separately. Cytomorphologically, most smears showed scant to low cellularity with mild atypical features, including a mild irregular nuclear membrane, a moderate amount of cytoplasm with a moderate nuclear/cytoplasmic (N/C) ratio, infrequent prominent nucleoli, and an absence of necrotic and hemorrhagic background (Figs. 2 and 3). In our experience, these few atypical cells do not meet the criteria to fit them into a more definitive category (benign vs malignant). Of the 53 cases diagnosed as atypical on IC testing in the present study, 40 (75%) showed true positivity for malignancy on CNB, and 12 (23%) were negative for malignancy. Only 1 case (2%) was nondiagnostic using both IC testing and CNB. Of the 40 atypical cases, IHC ancillary studies had been performed for 12 (30%) to reach a definitive diagnosis (Table 2).

Neoplastic cases

Of the 200 cases, 28 (14%) had been classified as positive for neoplasm on IC testing. The smears were moderately cellular with bland-appearing cytology features, including a round to mild irregularity of the nuclear membrane, moderate homogeneous to granular cytoplasm, and a moderate-to-low N/C ratio (Fig. 4). Of these 28 cases, 13 (46%) had a diagnosis of malignancy by the final CNB examination, and 15 (54%) had a benign diagnosis found on the CNB examination. Of the 13 malignant cases, 9 (69%) were low-grade RCC (ISUP grade 1 or 2; Table 3). Of the 15 benign cases, oncocytoma was the most common final diagnosis (n = 12; 80%) on CNB, with 2 cases (13%) of normal renal parenchyma and 1 (7%) metanephric adenoma (Supplementary Table S1). Of the 13 malignant cases, 7 (54%) had required IHC studies to reach a definitive diagnosis (Table 2).

Exact diagnosis using IC testing

Of the 200 IC testing cases, 46 (23%) had a definitive diagnosis confirmed by CNB examination without further ancillary studies (ie, the IC testing diagnosis matched the

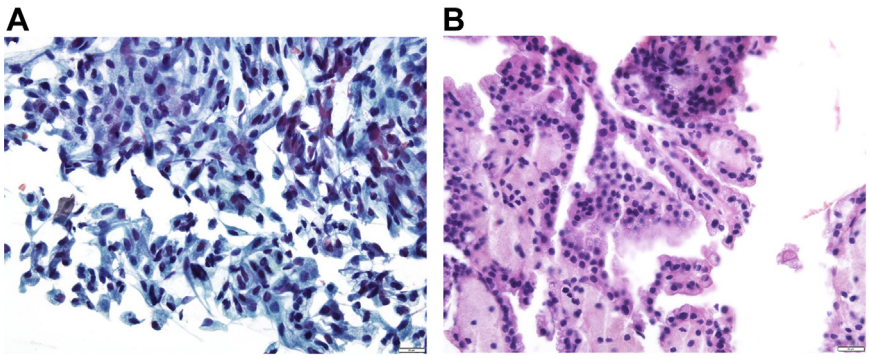

Figure 3 A 67-year-old man with 1.7-cm left renal mass. (A) Atypical imprint cytology diagnostic for a cellular smear with bland-appearing spindled-shaped nucleus with a moderate amount of homogeneous cytoplasm (Papanicolaou stain, original magnification ×400). (B) The corresponding core needle biopsy finding confirmed the diagnosis of renal cell carcinoma, papillary type 1, International Society of Urological Pathology nuclear grade 2 (hematoxylin-eosin stain; original magnification ×400).

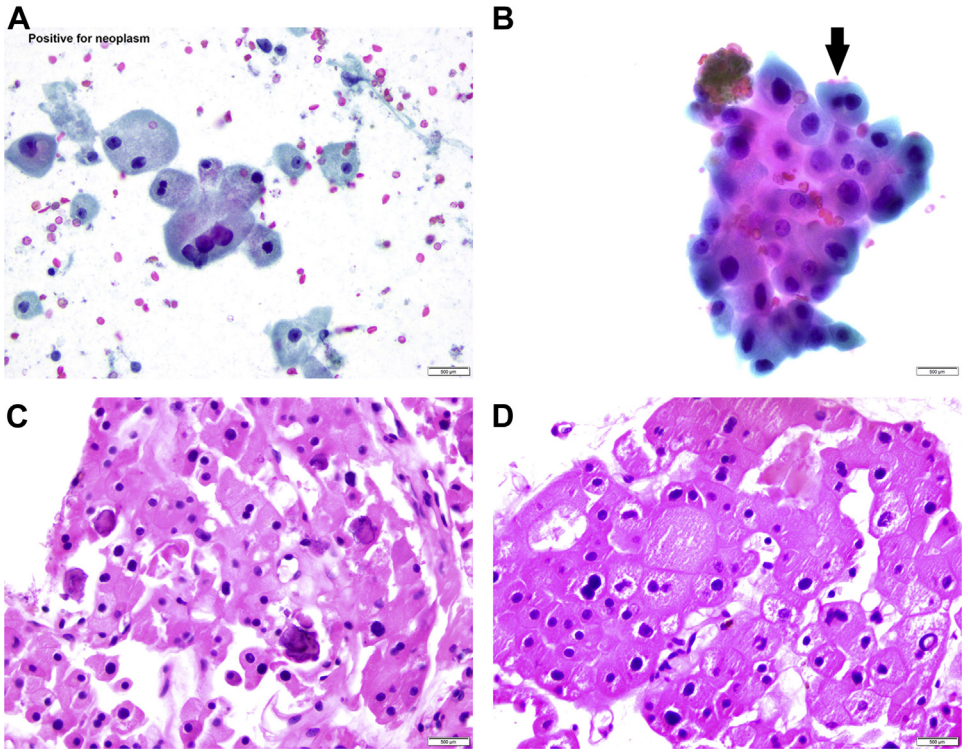

Figure 4 A 63-year-old man with 3.0-cm right renal mass. (A) Imprint cytology positive for neoplasm showing moderately hypercellular smears of cells with sharp cell borders, pale cytoplasm, and round nuclei with dense cytoplasm (Papanicolaou stain, original magnification ×600). (B) A few cells have binucleation (arrow; Papanicolaou stain, original magnification ×400). (C) Corresponding core needle biopsy showing solid growth pattern with distinct cell borders (hematoxylin-eosin stain, original magnification ×400). (D) Irregular, wrinkled, and angulated nuclei with perinuclear halos (hematoxylin-eosin stain, original magnification ×400).

CNB diagnosis; Table 4). Generally, 28% of the IC testing cases (56 of 200) had required IHC studies to reach a definitive diagnosis. The number of ordered IHC stains ranged from 1 to 15 stains. In addition, 26 cases (100%) with ungraded renal tumors were evaluated using IHC to reach a definitive diagnosis (Table 2).

Discussion

IC testing is simple and quick and does not require a sophisticated technique. The importance of the intraoperative IC testing analysis has been reported and validated for different organs.[12-15] To the best of our knowledge, no previous large study has evaluated the importance of IC testing for renal tumors. For most discrete renal lesions, the decision to perform nephrectomy is dependent on the radiologic features of the lesion. Small renal masses will often be biopsied before they are ablated.[16]

Nevertheless, the use IC testing analysis and CNB remains valuable. One of the most acceptable indications for biopsy occurs when a patient is not a good candidate for a major operation and a malignant lesion is presumed. Recently, the number of partial nephrectomy procedures has increased compared with radical nephrectomy for various reasons.[17] The main benefit of partial nephrectomy is preservation of

Table 3 Renal tumors classification for patients with imprint cytology testing positive for neoplasm.

RCC classification by CNB	Patients (n = 13)	ISUP nuclear grade
Clear cell carcinoma	6 (46)	1 or 2
Chromophobe type	3 (23)	Not graded
Papillary type 1	3 (23)	1 or 2
Clear cell carcinoma	1 (8)	3

Abbreviations: CNB, core needle biopsy; ISUP, International Society of Urological Pathology; RCC, renal cell carcinoma.
Data presented as n (%).

Table 4 Correlation of exact imprint cytology diagnosis of 200 patients with different renal tumor subtype.

Renal tumor diagnosis	Patients (n = 46)
RCC, clear cell carcinoma subtype	18 (9.0)
RCC, papillary type	14 (7.0)
RCC, chromophobe	5 (2.5)
Oncocytoma	8 (4.0)
Urothelial carcinoma	1 (0.5)

Abbreviation: RCC, renal cell carcinoma.
Data presented as n (% [of total 200 patients]).

the remaining renal function by as much as possible, especially for a young patient with a relatively small lesion. The procedure should also be considered for patients who might not be able to tolerate radical nephrectomy.[6]

We have presented a retrospective study of 200 consecutive cases of renal masses from our institution. We found that, regardless of renal lesion type, the accuracy and sensitivity of IC testing were 58% and 85%, respectively, after exclusion of the atypical cases. Although no large series has reported the accuracy and sensitivity of IC testing for renal tumors, these values are relatively less than those from studies of other organs.[3,18] According to our data, the screening role of IC testing triaging for renal masses will be less effective than for other organs, such as lung malignancy.[19] The reason for the low diagnostic triage power of these lesions, especially with low-grade malignancy, might be the large number of cases considered atypical by the IC testing evaluation. The low cellularity and strong cohesiveness of tumor cells will prevent them from exfoliating well on the smear.

However, most of the atypical IC testing cases (75%) were undercalled on IC. We believe that the cellularity of the smear, in addition to the mild nuclear atypia that morphologically overlaps with benign renal tubules, will preclude a definitive classification. However, for the same reasons, some cases were overcalled on IC. Proximal renal tubules on the smear are one of the common pitfalls in overcalling the diagnosis. Oncocytoma (positive for neoplasm) can be classified because both have oncolytic cytoplasm that can be easily mistaken as tumor cells. In our experience, the cellularity of IC testing is an important factor contributing to the wrong estimation. However, no practical method is available to avoid an underdiagnosis on IC testing with a low-cellularity smear. However, a helpful approach is to be vigilant and conservative in determining positivity in these situations, unless considerable cytology atypia is present.

Cytomorphologically, low-grade tumors will have a low nuclear grade, a moderate N/C ratio, and a moderate amount of cytoplasm with fewer pleomorphic features. These features make the IC testing diagnosis a challenging mission for cytopathologists to render a definitive diagnosis in such cases. Thus, most cytopathologists will tend to give a categorical diagnosis of atypia for this type of lesion on IC testing. The other atypical cases usually required additional advanced ancillary studies such as IHC (30%) to render a definitive diagnosis. However, the sensitivity of IC testing will be significantly increased with upgrading of the tumor; thus, high-grade tumors had a sensitivity of 88% compared with that (68%) of low-grade tumors, depending on the ISUP grading system used. That difference might result from the cellularity. With the loosely noncohesive nature of high-grade tumors, it will be easy to exfoliate the tissue during the swirling of it on the slide.

Regarding the positive for neoplasm category, most benign cases were found to be oncocytoma on the final CNB examination. This was another interesting category in our series. IC testing smears cytomorphologically had moderate cellularity with monotonous cell proliferation, giving the impression of neoplastic biology for this proliferation. Some of these lesions were low-grade malignant renal cell tumor on the final CNB examination, with different subtypes, for the same reasons cited (Table 4).

Because of the morphologic overlap between oncocytoma in IC testing and low-grade oncocytic renal malignancy, such as chromophobe, it is not uncommon to require the use of IHC to render a definitive diagnosis for IC testing cases positive for neoplasm (54%; 7 of 13; Table 2). The crucial role of reaching a definitive diagnosis in these cases has future prognostic and management sequences.

However, we had 26 cases of ungraded renal tumor found on the CNB specimens (Table 2), including RCC unclassified, RCC chromophobe, urothelial carcinoma, sarcoma/sarcomatoid tumor, and metastatic tumors. For all the ungraded/unclassified cases (100%), IHC was used to reach the definitive diagnosis because of the ambiguous morphologic characteristics of these lesions.

In our institution, we do not perform instant on-site IC testing evaluation of renal masses. All specimens will be sent immediately after fixation in alcohol to the cytology laboratory to be read by a cytopathologist within 1 to 2 hours after the procedure, with the IC testing diagnosis reported to the radiologist the same day. IC testing serves as a tool to correlate with the radiologists' and clinicians' diagnostic impression from the imaging and clinical features. However, for patients with lymphoma, we found an IC testing diagnosis of positive or suspicious in 66% of the cases (4 of 6), which allowed us to order a battery of basic lymphoma IHC testing in advance (CD3, CD20, and IgG) to decrease the turnaround time. It is advisable to order flow cytometry for these cases at any institution that does performed rapid in situ evaluation for renal IC.

Our study has shown the diagnostic value of IC testing, with a PPV of 89.5%, making it a useful and valuable tool to correspond with the CNB findings in rendering an accurate diagnosis in the malignant cases. The sensitivity was significantly increased in detecting high-grade malignancy compared with low-grade lesions. Our cytopathologists were usually confident in giving an exact diagnosis, which matched the final CNB findings without the use of ancillary studies for 23% of the tumor cases, a relatively low percentage compared with those from other studies for different organs.[3,4]

Conclusions

To the best of our knowledge, our study is one of the largest to report on the implementation of IC testing for renal tumors. In contrast to tumors of other organs, our data showed relatively low accuracy and sensitivity for making an IC testing diagnosis owing to cytomorphologic and technical reasons. However, the accuracy and sensitivity for rendering an exact diagnosis was relatively valuable for the malignant category.

Supplementary data

Supplementary data accompanying this article can be found in the online version at https://doi.org/10.1016/j.jasc.2018.12.005.

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Conflict of interest disclosures

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