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Indocyanine Green Cannot Predict Malignancy in Partial Nephrectomy: Histopathologic Correlation with Fluorescence Pattern in 100 Patients

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Abstract

Introduction: Indocyanine green (ICG) is emerging as a potential adjunct to robot-assisted partial nephrectomy by its ability to aid in the real-time identification of renal vasculature, renal masses, and the renal mass-parenchymal margin. The fluorescence patterns of renal masses have not been adequately described according to histology, and it remains unknown if fluorescence pattern can reliably predict histology or malignancy. We therefore describe the ICG fluorescence pattern of our first 100 robot-assisted partial nephrectomies (RAPN) and correlate with histology.

Materials and Methods: We reviewed our prospective RAPN database and categorized fluorescence pattern as isofluorescent (same as surrounding parenchyma), hypofluorescent (less than surrounding parenchyma, but with uptake), or afluorescent (no visible uptake of dye). Descriptive statistics were applied.

Results: All 14 cystic lesions were afluorescent and comprised 9 malignant and 5 benign masses. Eighty-six lesions were solid, of which 3 were isofluorescent including two clear-cell and one translocation tumor. The remaining 83 solid lesions were hypofluorescent and included 65 malignant and 18 benign lesions. Clear-cell was the most common histology of which 96% were hypofluorescent and 4% isofluorescent. In determining malignant *vs* benign lesions, hypofluorescence had a positive predictive value of 87%, negative predictive value of 52%, sensitivity of 84%, and specificity of 57%.

Conclusions: A three-grade classification of renal mass ICG fluorescence pattern is correlated with some histologic findings but unable to reliably predict malignant *vs* benign lesions.

Introduction

Indocyanine green (ICG) is a nontoxic compound that exhibits maximum near infrared fluorescence (NIRF) when stimulated by light of 780 nm wavelength.[1] ICG is approved by the United States Food and Drug Administration for human clinical applications and has been shown to have reduced expression in the majority of renal neoplasms relative to normal parenchyma.[2] We have previously explored the use of ICG during robot-assisted partial nephrectomy (RAPN) and found it to be objectively associated with decreased warm ischemia time and subjectively enhanced visualization of renal vasculature and the mass-parenchymal interface.[3]

It has been hypothesized based on a small pilot series that ICG hypofluorescence may predict malignant histology compared with other fluorescence patterns that may predict benign lesions.[4] If correct, this hypothesis could potentially alter the treatment algorithm of renal masses to include ICG fluorescence as a potential diagnostic modality to help distinguish which masses should be treated *vs* observed. To test this hypothesis further, we performed analysis of ICG fluorescence pattern and histopathologic correlation.

Materials and Methods

One hundred consecutive patients undergoing RAPN from April 2011 to May 2012 were included for analysis. All patients were evaluated preoperatively with abdominal axial imaging, chest radiography, and laboratory evaluations and were found to have clinically localized disease. All masses had preoperative imaging features worrisome for renal malignancy or were consistent with angiomyolipoma (AML) >4 cm. Non-AML solid lesions lacked macroscopic fat and had either contrast enhancement or displayed restricted diffusion. Cystic lesions were Bosniak 3 or more. Masses were assessed using the R.E.N.A.L. nephrometry score and

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Table 1. Clinical Characteristics of 100 Consecutive Patients Undergoing Robot-Assisted Partial Nephrectomy with Use of Indocyanine Green

Male:female	46:54
Median age (range)	60 (24–83)
Median BMI (range)	29 (19–46)
Median ASA (range)	3 (1–4)
No. previous abdominal surgery	49
Median tumor diameter, cm (range)	3 (1–9)
Median nephrometry (range)	6 (4–11)
No. low nephrometry	50
No. medium nephrometry	40
No. high nephrometry	10
No. nonclamped	22
No. clamped	78
Median warm ischemia time, min (range)	15 (7–30)
Median blood loss, mL (range)	75 (10–1100)

BMI = body mass index; ASA = American Society of Anesthesiologists.

categorized based on complexity as low (score 4–6), moderate (score 7–9), or high (>10) (Table 1). Curative-intent RAPN was then performed using ICG dye (IC-Green, Akorn Pharmaceuticals, Lake Forest, IL) as described previously.[3] Key points include a single intravenous dose of 5 to 7.5 mg ICG before vascular clamping, with clamping performed after the hilar vessels were confirmed and renal parenchyma visualized as fluorescent. Twenty-two cases were performed without vascular clamping but similar ICG dose. NIRF was performed with the FireFly (Novadaq Technologies Inc, Bonita Springs, FL) enabled Da-Vinci Si robot (Intuitive Surgical, Sunnyvale, CA); this uses a laser light source at 803 nm and NIRF camera to visualize fluorescence at 805 nm, which is overlaid onto the screens of the surgeon console (Fig. 1). NIRF mode and white light mode were toggled back and forth at surgeon discretion after ICG dosing.

Fluorescence pattern was determined with a subjective three-grade scale of afluorescent (no visible uptake of dye), hypofluorescent (uptake of dye, but less than parenchyma), or isofluorescent (uptake of dye at intensity indistinguishable from surrounding parenchyma) (Fig. 1). Two surgeons independently graded fluorescence pattern intraoperatively. Descriptive statistics were applied. To test the potential performance of ICG hypofluoresence as a test of malignancy, we determined sensitivity, specificity, positive and negative predictive values considering hypofluorescence as a positive test and isofluorescence or afluorescence as negative.

Data were prospectively recorded and stored in a secured Institutional Review Board approved database of RAPN.

Results

All 100 patients underwent RAPN without conversion to an open procedure or radical nephrectomy. There were no adverse reactions attributable to dye administration. The dye was easily reconstituted and injected by rotating anesthesia providers without problems. There were no difficulties with specimen tissue processing or pathologic interpretation of frozen or permanent specimens. There were no discrepancies in fluorescence grading between surgeons.

Clinical characteristics of patients and masses are listed in Table 1. Fluorescence patterns and tissue histology of all masses are listed in Table 2.

Mass hypofluorescence with ICG NIRF was able to predict malignancy with a sensitivity of 84%, specificity of 57%, and positive predictive value of 87% and negative predictive value of 52%.

Discussion

Tobis and associates[4] were the first to perform histopathologic correlation of renal cortical masses and ICG fluorescence pattern.

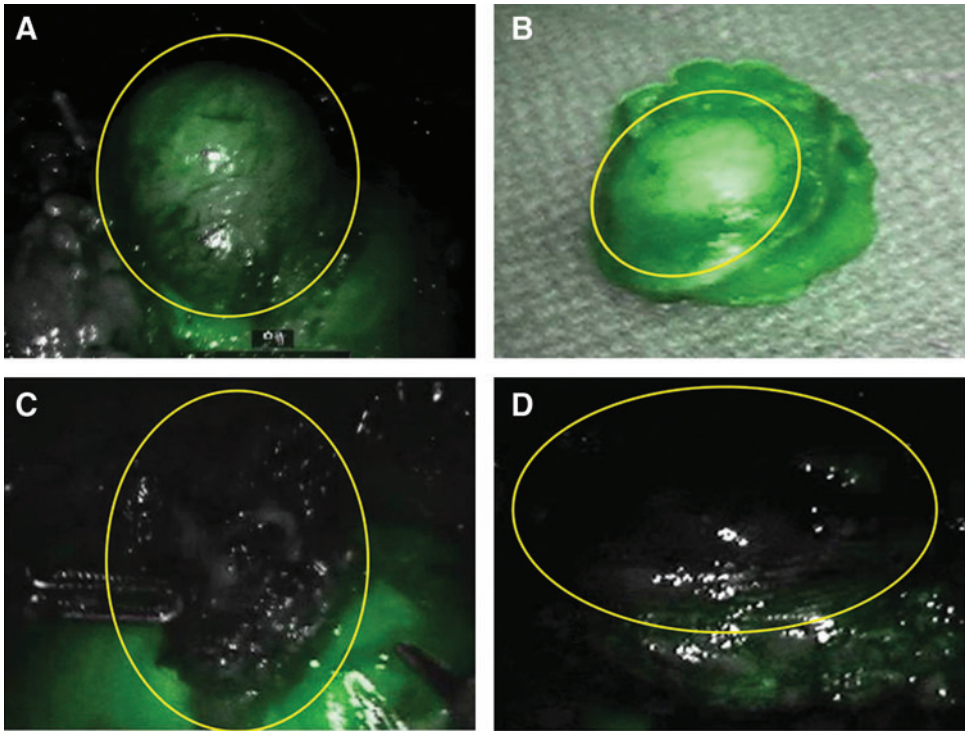

FIG. 1. Indocyanine green fluorescence patterns of representative renal masses. **(A)** Hypofluorescent clear cell; **(B)** isofluorescent clear-cell; **(C)** afluorescent cystic clear-cell; **(D)** afluorescent angiomyolipoma.

Table 2. Histopathologic Correlation of Fluorescence Pattern Observed in 100 Consecutive Patients Undergoing Robot-Assisted Partial Nephrectomy with Use of Indocyanine Green

	Histology	*Isofluorescent*	*Hypofluorescent*	*Afluorescent*
Solid	Angiomyolipoma			8
	Chromophobe		6	
	Other benign		2	
	Clear-cell	2	46	
	Oncocytoma		8	
	Papillary		13	
	Translocation tumor	1		
Cystic	Simple cyst			3
	Cystic chromophobe			1
	Cystic papillary			1
	Cystic clear-cell			7
	Multilocular cyst			2
All benign			10	13
All malignant		3	65	9

In their series, the authors used a similar injection technique, ICG source, and dosing, but a different NIRF visualization system, different grading scale of fluorescence, and reached very different conclusions. In their study, the authors divided masses into three fluorescence categories by subjectively comparing the intensity of the mass to surrounding parenchyma and found all 13 malignant tumors were hypofluorescent *vs* 6 benign lesions that were either isofluorescent or hyperfluorescent.

In the present study, again using a different visualization system than Tobis and colleagues,[4] we found no hyperfluorescent masses and could not reliably correlate hypofluorescence with malignancy. The only two reliable patterns of fluorescence involved AMLs and cystic lesions that were all afluorescecnt (Table 2). Seven of the AMLs were of the common triphasic lipid-rich variety, while one AML was an epitheloid variant that we have described previously.[3] Subjectively, it seemed that the lipid component of the AMLs was responsible for the lack of dye uptake, as it appeared as a nearly identical hue to surrounding perirenal fat during NIRF. Therefore, it is possible that lipid-poor varieties of AML may demonstrate a pattern other than afluorescence.

Cystic lesions, both benign and malignant, were all afluorescent (Table 2). This comprised a variety of cystic malignancies including cystic papillary, cystic clear-cell, and cystic chromophobe variants as well as benign entities including simple and multilocular cysts.

Compared with modern preoperative imaging techniques such as CT and MRI, intraoperative ICG fluorescence pattern may yield some additional information in terms of mass characterization, but it is unclear if the additional information is clinically useful. ICG afluorescence was consistently associated with AML or cystic lesions in the present series. These histologic characteristics are also reliably detectable with imaging, because CT and MRI are up to 100% specific for the detection of AML when macroscopic fat is present[5]. CT and MRI are also highly accurate in the determination of cystic *vs* solid lesions, with ultrasonography being nearly 100% accurate in determination of cystic components.

It is difficult to compare ICG hypofluorescece as a test for malignancy with the ability of preoperative imaging, because all patients in this series were already evaluated with imaging and had worrisome features. This study population is therefore a screened population. As such, ICG hypofluorescence with a sensitivity of 84% and specificity of 57% to predict malignancy does not apply to all-comers with renal masses. Although interesting, there is little rationale for ICG to replace preoperative imaging modalities because of its invasive nature and the relatively high accuracy of preoperative imaging in the detection of renal malignancy at up to 100% sensitive and 96% specific using techniques such as combined contrast-enhanced and diffusion-weighed MRI.[6]

Additional noncapital costs associated with ICG administration are approximately $28 to $80 per case. Our present cost for a 10 mL vial of ICG obtained through the pharmacy wholesale market is approximately $80. Once reconstituted, the dye may be stored for up to 6 hours, allowing a single vial to be used for multiple cases. Using doses of 2 mL (5 mg), we routinely use a single vial for two cases and sometimes three cases before encroaching on the 6-hour limit for storage. Other costs, such as disposable syringes to inject the dye, are negligible. Capital costs, such as FireFly capable scopes, light source, and da Vinci Si robot, vary widely according to institutional and amortization practices, are much more difficult to quantify, and beyond the scope of the present study.

Chief among the potential pitfalls of the current study include the subjective nature of the fluorescence grading scale. Neither of the commercially available laparoscopic NIRF platforms, FireFly or SPY (Novadaq Technologies Inc, Bonita Springs, Fl), allow for quantification of fluorescence strength in absolute or relative terms. Future advancements to the imaging technology could incorporate such features, which may allow for more precise histopathologic correlation. In addition, while this is the largest published series to date of ICG RAPN using the Firefly system, it is still relatively small and therefore subject to underreporting bias.

Despite the inability of ICG fluorescence pattern to reliably predict malignancy, we do think the technology has merit during RAPN. We have previously shown a small, but significantly decreased warm ischemia times in ICG *vs* non-ICG techniques in a case-control series.[3] We also think ICG has an excellent application in identification of hilar vessels, identification of tumor-specific vessels for selective clamping, and providing more visual contrast between mass and tumor parenchyma in most cortical lesions. In the present study,

however, we cannot support the use of ICG fluorescence to make a diagnosis of malignancy *vs* benignity.

Conclusions

A three-grade classification of renal mass ICG fluorescence pattern is correlated with some histologic findings, but unable to reliably predict malignant *vs* benign lesions. Further study is necessary to confirm these findings. Future refinements to NIRF technologies that incorporate objective measures of fluorescence may increase its utility and allow for more standardized reporting of outcomes.

Disclosure Statement

No competing financial interests exist.

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Abbreviations Used

AML = angiomyolipoma
ICG = indocyanine green
NIRF = near infrared fluorescence
RAPN = robot-assisted partial nephrectomy