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Multispectral fluorescence imaging as a tool to separate healthy and disease related lymphatic anatomies during robot-assisted laparoscopic procedures.

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ABSTRACT

To reduce the invasive nature of extended pelvic lymph node dissections (ePLND) in prostate cancer, we have developed a multicolor fluorescence guidance approach that enables the discrimination between prostate draining lymph nodes (LNs) and lower limb draining LNs.

Methods

In five porcine models multispectral-fluorescence guidance was performed using da Vinci Si- and Xi-robot consoles. They received fluorescein into the lower limb(s) and indocyanine green (ICG)-nanocolloid into the prostate.

Results

Fluorescein was detected in 29 LNs (average of 3.6 LNs/template); ICG-nanocolloid visualized 12 LNs (average of 1.2 LNs/template). Signal intensities appeared equal for both dyes and no visual overlap in the lymphatic drainage patterns was observed. Furthermore, fluorescein supported both the identification of leakage from damaged lymphatic structures and the identification of ureters.

Conclusion

We demonstrated that the different lymphatic flow patterns from the prostate and lower limbs could be intraoperatively distinguished using multispectral-fluorescence imaging approaches.

Keywords:

Image guided surgery, prostate cancer, fluorescence, multiplexing, robotic surgery

INTRODUCTION

The metastatic pattern of prostate cancer to the lymphatic system has resulted in the European Association of Urology guidelines recommendating extended pelvic lymph node dissection (ePLND) for intermediate- and high risk prostate cancer patients with an estimated risk of nodal metastases exceeding 5% based on Briganti or Kattan nomogram (*1*). Unfortunately, ePLND induces surgical damage to the natural lymphatic flow in the pelvis resulting in an increased complication rate (*2-4*), with lymphoceles (10.3%) and lymphedema (4.1%) being the most common (*5,6*).

For indications such as breast and penile cancer, minimally invasive sampling of the so-called first tumor draining lymph nodes, i.e. sentinel nodes (SNs), has helped reduce the number of patients requiring a LND by >75% (*7-9*). However, pelvic SN dissections in intermediate- and high-risk prostate cancer patients is a one-stop-shop procedure that includes ePLND, since surgical re-exploration of the pelvic nodal basins after SN sampling is cumbersome and relying solely on SN sampling would leave tumor bearing non-SN in situ in 27.1% of LN-positive patients (*7*). This setting creates the demand for technologies that preserve the oncological outcome of an ePLND, but that reduces its impact on healthy lymphatic structures not related to the target organ. When available, such an approach could create a paradigm shift for the surgical treatment of lymphatic disease, changing the focus from “removing nodes that count” to “sparing nodes that are not involved”.

We hypothesized that real-time fluorescence multiplexing could allow us to differentiate the pelvic lymphatic drainage profiles of healthy tissues (lower limb) from those of the primary tumor (prostate), respectively (Fig. 1). For this we used the spectrally differentiated lymphangiographic tracer fluorescein and the SN specific tracer indocyanine green (ICG)-nanocolloid (*10*). We evaluated this concept during robotic pelvic LND procedures in porcine models to demonstrate that: 1) the ICG-tailored da Vinci fluorescence laparoscopes are also capable of imaging the clinically approved visible dye fluorescein and support multispectral-imaging applications, 2) multicolor- or multispectral-fluorescence imaging supports real-time intraoperative separation of the lower limb- and prostate-related lymphatic anatomies, and 3) imaging of fluorescein helps visualize damage to the lymphatic network and highlights ureters.

MATERIALS AND METHODS

Camera Setup

The study was performed using two generations of clinical grade surgical-robotic systems (da Vinci Si and Xi; Intuitive Surgical Inc.) and their integrated Firefly fluorescence laparoscopes. In the surgical console, with both systems processed fluorescent signals were displayed as artificially colored bright green over a grayscale background image. Only with the older Si system it was also possible to display the raw, unprocessed video signal.

Preclinical Evaluation Setup

The multispectral-fluorescence imaging setup was evaluated in five male porcine models during surgical training (Orsi-Academy). The studies were allowed by the Ethical Committee for animal experiments of Gent University (EC2015-152).

To identify the lymphatic drainage of the lower limbs, 5mL fluorescein was administered (100 mg/mL solution) in 2.5mL subcutaneously and 2.5mL intramuscular into the right hind leg (pigs one and two) or deposits of 1.25mL in both hind legs (pigs three, four and five)(Fig. 1). Following fluorescein administration (after 70 – 150 minutes), though preceding ICG-nanocolloid injection, the pelvic area was evaluated for fluorescein positive LNs using both white light and fluorescence imaging.

To allow for lymphatic mapping of the prostate ICG-nanocolloid was used. This tracer was prepared similarly as was previously described for the radiolabeled analogue (*11*), resulting in a 2 mL solution with 0.125 mg/mL ICG and was injected intra-operatively using a 2mL syringe attached to an injection needle using flexible tubing. After insertion, the surgeon then placed two to four tracer deposits into the prostate using the robotic arms (Fig. 1). Following ICG-nanocolloid injection, the pelvic area was examined for both tracers during a 15-30 minute period.

RESULTS

Overall Performance Fluorescein Imaging versus ICG Imaging

In the processed images of the Si and Xi set-ups, both the fluorescent signals of fluorescein and ICG-nanocolloid were displayed with a green coloration (Fig. 2). Differentiation between the two fluorescent signals required use of the white light images to define the location of fluorescein and/or sequential administration and imaging of fluorescein and ICG-nanocolloid. The ability to use the unprocessed images of the Si system, which displays the two fluorescent signals as yellow/green or red/pink coloration, respectively, made differentiation between the signals much more straight forward (Fig. 3).

In Vivo Experiments

During surgery (~2h post injection) fluorescein was detectable in leg-draining LNs (3.6 LNs/template) in the white light images but was more easily defined in the fluorescence setting (Figs. 2 and 3). Fluorescein leakage provided a valuable indicator for surgical damage of fluorescein containing lymphatics (Fig. 4). Further, the biological (renal) clearance of fluorescein supported fluorescence-based visualization of the ureters (Fig. 4)(*12*). When using the fluorescence setting, prostate draining SNs (1.2 SNs/template) and lymphatic ducts became visible within minutes after administration of ICG-nanocolloid. Similar to the clinically used ICG-^{99m}Tc-nanocolloid (*10*), ICG-nanocolloid did not contaminate the surgical field when lymphatic resections were performed.

A clear dividing line was observed between the lymphatic drainage pattern of the prostate and lower limbs; no signal overlap was observed between the lymphatic drainage profiles of the two dyes imaged (Table 1). Differentiation could even occur in the same template (Fig. 3).

DISCUSSION

To our knowledge, this study is the first to demonstrate the fluorescein and multispectral-imaging capabilities of the da Vinci Si and Xi robots. With this technology, a successful distinction was made between ICG-nanocolloid and fluorescein positive lymphatics. Despite the theoretical advantages of near-

infrared dyes, both dyes could be clearly visualized in vivo (Figs. 2 and 3). With this multicolor guidance concept, healthy lymphatic structures could potentially be spared, converting to a reduction of lymphedema of the lower limb. The fact that commercially available and clinically approved robotic systems allow such a multispectral-imaging approach, facilitates the translational aspect of these studies. Further clinical follow-up studies are needed to validate the impact of the proposed imaging concept on the outcome in clinical trials.

Next to visualizing the lymphatic drainage patterns of the lower limbs, fluorescein imaging also supported the identification of damage to these same lymphatics; a similar contamination was reported in studies where "free" ICG was used (*13*). In line with literature, the ureters could also be visualized during surgery using fluorescein (Fig. 4)(*14*).

Combined with previous reports (*10,15,16*), the favorable *in vivo* visualization of fluorescein using the da Vinci robotic platform again debates the monopoly of near-infrared approaches in the field of fluorescence-guided surgery.

CONCLUSION

The multispectral-imaging properties of the fluorescence laparoscopes of the da Vinci robotic platform helped realize an intraoperative differentiation between prostate-related (ICG-nanocolloid) and lower limbs-related lymphatic structures, lymphatic damage, and ureters (fluorescein). With that a technology has become available that supports a further improvement of the balance between cure and surgically induced side-effects.

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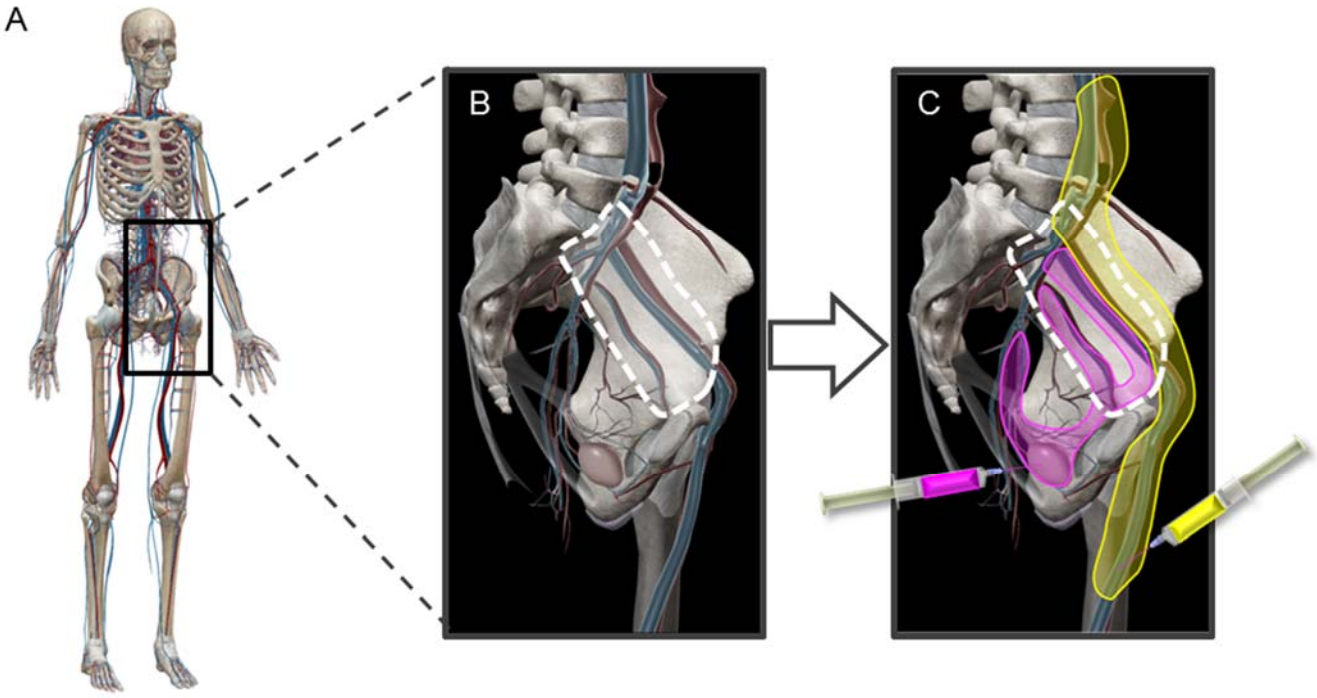

FIGURE 1. Schematic illustration of relevant anatomical structures within the patient (A). (B) All lymphatic structures within the template (white dotted line) get resected during a lymph node resection. (C) Schematic illustration of lower limb (yellow; fluorescein) and prostate (pink; sentinel nodes; ICG-nanocolloid) drainage patterns that pass through the template. The schematic anatomical images were generated with Visible Body software (Argosy Publishing Inc.).

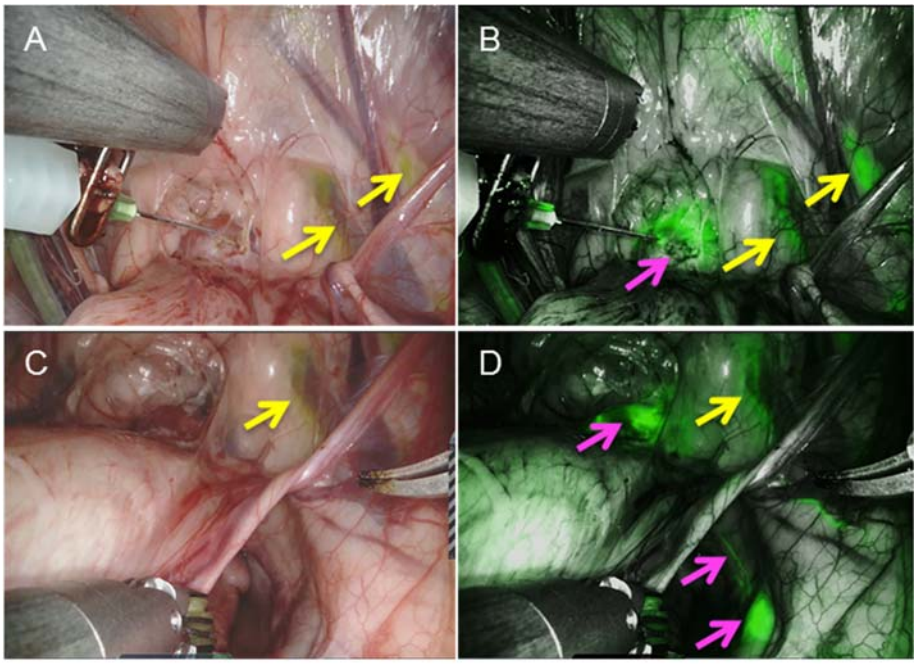

FIGURE 2. Multispectral-fluorescence imaging with the da Vinci Xi system. **(**A) Imaging during the intraprostatic administration of indocyanine green (ICG-)nanocolloid, depicting fluorescein in the white light image, (B) while the fluorescence image presents both fluorescein and ICG. (C) Imaging in white light following the lymphatic drainage of ICG-nanocolloid shows fluorescein positive areas (yellow arrow), (D) while fluorescence imaging clearly depicts an ICG-draining lymphatic duct and a sentinel node (pink arrows).

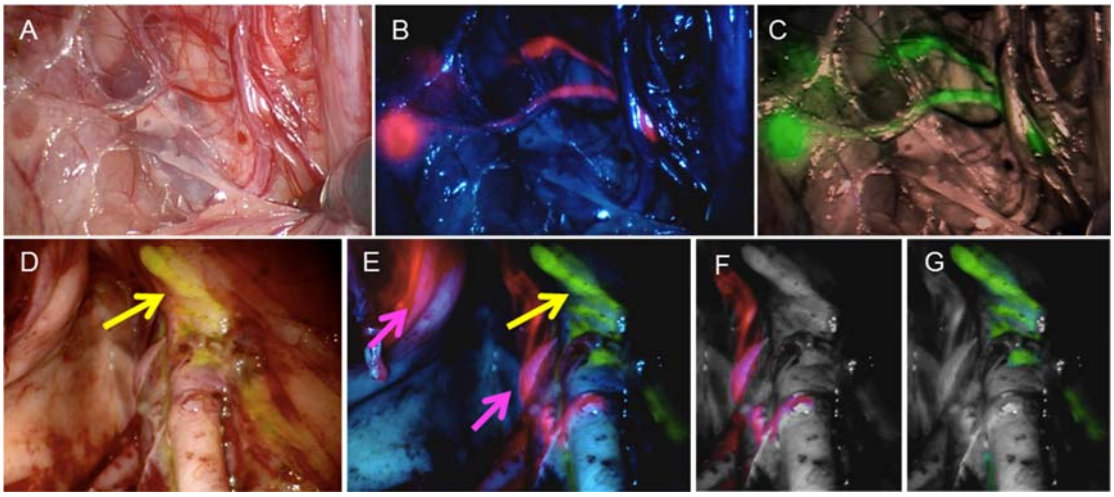

FIGURE 3. Multispectral-fluorescence imaging with the da Vinci Si system. Image series of lymphatic ducts and two sentinel nodes containing indocyanine green (ICG-)nanocolloid; (A) white light, (B) unprocessed image (ICG pink), (C) processed image (ICG green). Lymphatics in the external iliac region; (D) white light imaging displaying a fluorescein containing lymph duct (yellow arrow), (E) unprocessed multispectral-imaging simultaneously displays a SN (pink arrow) and the fluorescein stained lymph duct (yellow arrow), with the two signals digitally separated in (F) ICG and (G) fluorescein.

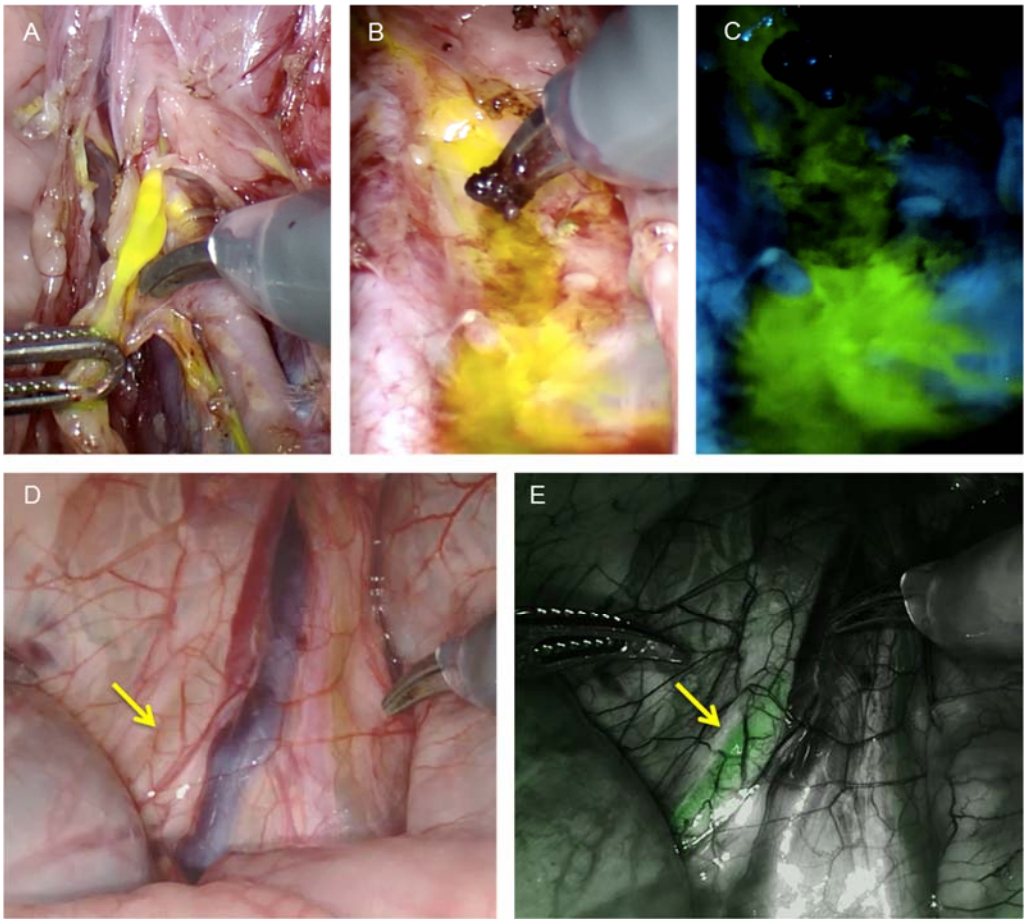

FIGURE 4. Additional values offered by fluorescein usage. (A) Surgical damage of a fluorescein containing lymph duct (Si-system). (B) As a consequence of the damage, the lymphatics leak fluorescein, staining the surgical field in white light and (C) fluorescence mode. (D) Depiction of a fluorescein containing ureter (yellow arrow; Xi-system) in white light and (E) fluorescence mode.

TABLE 1: Overview findings

Surgical system (# case)	**Time between fluorescein injection and intraoperative imaging**	**Massage fluorescein injection site required for detection**	**Recording unprocessed images**	**Injection leg(s) fluorescein**	**Fluorescein visualization ureter**	**Fluorescein visualization lymphatic ducts**	**Number of LNs visualized using fluorescein**	**ICG-nanocolloid visualization lymphatic ducts**	**ICG-nanocolloid visualization SNs**
Da Vinci Si									
2	150 min	yes	yes	R	Yes	yes	- LNs (L) 3 LNs (R)	yes	2 SNs (L) 1 SN (R)
3	80 min	N/A	yes	L+R	Not evaluated	yes	2 LNs (L) 3 LNs (R)	yes	1 SN (L) 1 SN (R)
4	92 min	N/A	yes	L+R	Not evaluated	yes	5 LNs (L) 4 LNs (R)	yes	1 SNs (L) 1 SNs (R)
Da Vinci Xi									
1	145 min	N/A	no	R	Yes	yes	- LNs (L) 5 LNs (R)	yes	2 SNs (L) 1 SN (R)
5	70 min	N/A	no	L+R	Yes	yes	4 LNs (L) 3 LNs (R)	yes	1 SN (L) 1 SNs (R)
Average	107 min						3.6 LNs/leg (L) 3.6 LNs/leg (R)		1.4 SNs (L) 1.0 SNs (R)

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