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Autofluorescence imaging can identify preinvasive or clinically occult lesions in fallopian tube epithelium: A promising step towards screening and early detection

J.N. McAlpine [a,*], S. El Hallani [b], S.F. Lam [b], S.E. Kalloger [c], M. Luk [c], D.G. Huntsman [d], C. MacAulay [b], C.B. Gilks [c], D.M. Miller [a], P.M. Lane [b]

[a] University of British Columbia, Department of Gynecology and Obstetrics, Division of Gynecologic Oncology, 2775 Laurel St., 6th Floor, Vancouver, Canada BC V5Z-1M9
[b] Department of Integrative Oncology, BC Cancer Agency, Vancouver, BC, Canada
[c] Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada
[d] Centre for the Translational & Applied Genomics, BC Cancer Agency, Vancouver, BC, Canada

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ABSTRACT

Background. Optical imaging systems are robust, portable, relatively inexpensive, and have proven utility in detecting precancerous lesions in the lung, esophagus, colon, oral cavity and cervix. We describe the use of light-induced endogenous fluorescence (autofluorescence) in identifying preinvasive and occult carcinomas in *ex vivo* samples of human fallopian tube (FT) epithelium.

Methods. Women undergoing surgery for an i) ovarian mass, ii) a history suggestive of hereditary breast-ovarian cancer, or iii) known serous ovarian cancer following neoadjuvant chemotherapy (NAC) were approached for informed consent. Immediately following surgery, FT's were photographed in reflectance and fluorescence at high resolution. Images included: (1) white-light reflectance of luminal/epithelial surface; (2) narrow-band green reflectance (570 nm) (3) green autofluorescence (405/436 nm excitation); and (4) blue autofluorescence (405 nm excitation). Areas revealing a loss of natural tissue fluorescence or marked increase in tissue microvasculature were recorded and compared to final histopathologic diagnosis (SEE-FIM protocol).

Results. Fifty-six cases involving one or both fallopian tubes underwent reflectance and fluorescence visualization. Nine cases were excluded, either secondary to non-ovarian primary pathology (7) or excessive trauma (2) rendering tissue interpretation impossible. Of the 47 cases remaining, there were 11 high grade serous (HGS) and 9 non-serous ovarian carcinomas undergoing primary debulking surgery, 5 serous carcinomas having received NAC, 8 benign ovarian tumors, and 14 women undergoing risk-reducing bilateral salpingo-oophorectomy (RRBSO). Methodology was feasible, efficient, and reproducible. TIC or carcinoma was identified in 7/11 HGS, 3/5 NAC, and 1/14 RRBSO. Optical images were reviewed to determine test positive or negative based on standardized criteria. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for the entire cohort (73%; 83%; 57%; 91%) and in a subgroup that excluded non-serous histology (87.5%; 92%; 78%; 96%).

Conclusions. Abnormal FT lesions can be identified using *ex vivo* optical imaging technologies. With this platform, we will move towards genomic interrogation of identified lesions, and developing *in vivo* screening modalities via falloposcopy.

Introduction

High grade serous (HGS) carcinoma comprise 70% of all epithelial ovarian carcinomas [1–5] and are believed to originate in the epithelium or inner lining of the fallopian tube [6–11]. Precursor lesions have been identified, termed Tubal Intraepithelial Carcinoma or TIC. Identification has thus far been by pathology *ex vivo*, after surgical removal of the fallopian tubes (and ovaries) for risk reduction [6,12–17], for known cancer [7,18,19], and even isolated in cases where there is no known personal or familial predisposition for serous carcinoma [20]. Modeling for the growth and progression of these precancerous or occult fallopian tube lesions suggests we may have a window of detection encompassing several years [21]. Identifying lesion *in vivo* during this period would enable surgical intervention and/or the administration of chemotherapy and result in cure rates in the range of >95%. This is in stark contrast to our current clinical scenario where the majority (70%) of patients with ovarian cancer are diagnosed with advanced stage (stage III/IV) disease and five year survival is in the range of 15–20% [22–31].

Understanding the role of the fallopian tube in HGS carcinomas and appreciation of the limitations of currently available screening modalities [32–36] has prompted our center to direct research in

* Corresponding author. Fax: +1 604 875 4869.
E-mail address: jessica.mcalpine@vch.ca (J.N. McAlpine).

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prevention and early detection of ovarian cancer to this anatomical structure. Even with this focus, however, novel tactics are needed to detect these tiny lesions, projected to be 200-fold smaller [21] than a clinically apparent ovarian cancer.

Autofluorescence imaging is a relatively new modality, which enables real time, high resolution imaging of epithelial tissue. Cancerous or even precancerous changes in the epithelium lead to loss of normal tissue autofluorescence (AF) [37]. Coupled to handheld devices or endoscopic tools direct fluorescence visualization (FV) has been described in many anatomic locations [37–52]. Change to the pattern of tissue AF may be secondary to breakdown of the collagen matrix, tissue remodeling, or increased metabolism, all of which can occur during the process of malignant transformation. These changes can be compared to normal adjacent epithelium.

A familiar and successful application of this technology can be seen in your dentist's office. Direct fluorescence visualization of the oral cavity is now commercially available and used to identify severe dysplasia and invasive carcinoma in an outpatient setting [43,45]. A handheld unit projects excitation light onto the oral mucosa and provides a viewing port coaxial with illumination for fluorescence visualization. This technology has changed clinical practice for resection of lesions in BC. Recurrence rates (severe dysplasia or neoplasia) at the treatment site have dropped from approximately 25% to 0% since implementation [53].

Although AF has been described in other gynecologic malignancies [37,47,51,52,54,55] to the best of our knowledge this is the first investigation using this technology to assess the lumen of the fallopian tube(s). We report on our initial experience with AF inspection of mucosal FT epithelium and demonstrate that both precancerous and cancerous lesions can be detected, with promising ability to discern benign vs. malignant change. We believe this technology has huge potential impact in enabling further characterization of preinvasive FT lesions, and in the development of novel screening tools.

Methods

Patient selection and specimen handling

Following approval by the Institutional Review Board at the BC Cancer Agency, patients are identified by the gynecologic oncology team preoperatively in order to obtain informed consent prior to surgery. Enrollment targeted i) women with an ovarian mass (query malignancy), ii) women with known BRCA mutations carriers or with a strong personal or family history suggestive of hereditary breast-ovarian cancer (HBOC) per BC Cancer Agency referral criteria, and iii) women who had been diagnosed with advanced high grade serous ovarian cancer, status post three to four cycles of neoadjuvant chemotherapy (NAC) and undergoing delayed or interval debulking surgery. Surgery could be by minimally invasive (laparoscopy) or open (laparotomy) technique. Immediately following surgical extirpation of the fallopian tubes the tubes are accessioned, oriented (cornual vs. fimbriated end), and opened along the tubal axis exposing the full length of mucosal surface. They are lavaged with saline to remove any blood or debris and kept moist while photographing (detailed below). After completion of imaging, the fallopian tube(s) are formalin fixed, paraffin-wax embedded and sectioned according to SEE-FIM protocol [9]. Sections are stained with H&E and examined by a gynecological pathologist from Vancouver General Hospital. Of note, SEE-FIM protocol was done on all fallopian tube specimens, even in the absence of a known BRCA mutation or perceived HBOC.

Reflectance and autofluorescence imaging

We collected a series of four images of the fallopian tube mucosal surface, including (1) white-light reflectance; (2) narrow-band green reflectance (570 nm) (3) green autofluorescence (405/436 nm); and (4) blue autofluorescence (405 nm). Narrowband reflectance imaging increases the contrast of vasculature while autofluorescence imaging identifies structural changes in the extracellular matrix. Both are associated with early lesions. Narrow-band reflectance imaging at wavelengths corresponding to hemoglobin absorption maxima increases the contrast of microvasculature relative to the surrounding tissue. We performed reflectance imaging at 570 nm (green) illumination to increase the contrast of deep vessels. Narrowband illumination at 410 nm (blue) enhances the contrast of superficial vessels but was not employed here. Blue-violet excitation induces autofluorescence in the structural proteins (primarily collagen and elastin) of the extracellular matrix. Foci where this endogenous fluorescence is lost are used in other epithelial tissues to identify precancers understood to be due to the restructuring of the extracellular matrix as a prelude to invasion. Fluorescence imaging was performed at two excitation wavelengths (405 nm and 436 nm) that have been shown clinically effective for the detection of early lesions in the lung, uterine cervix and oral cavity.

The illumination system consisted of a bench-top light source coupled through a liquid light guide to a filter wheel containing illumination/excitation filters. The light source (X-Cite 120, EXFO) used a 120 W metal-halide arc lamp with an integral elliptical reflector. The light intensity coupled into the light guide was adjustable in 4 steps by rotating a variable iris. The fluorescence excitation light was provided by filtering the light from the light guide with either a 10 nm bandpass filter centered at 405 nm (Z405/10×, Chroma Technology) or a 60 nm bandpass filter centered at 425 nm (D425/60×, Chroma). Narrow-band reflectance images were acquired using a green illumination produced through a 30 nm bandpass filter centered at 570 nm (D570/30 m, Chroma). White light reflectance images were produced using a 400 nm longpass filter (E400LPv2, Chroma) to block UV illumination. Digital images of tissue reflectance and fluorescence were used to classify the lesions. Images were acquired using the illumination system described above and a digital SLR camera (105 mm AF-S Micro Nikko lens; Nikon D700, Japan) equipped with specific emission filters that minimize reflection at the tissue surfaces and block the excitation wavelengths: 405 blocking filter for 405 nm excitation, Y(K2) filter (Hoya, Japan) for 436 nm excitation, and a polarizer PL-CIR filter (Hoya, Japan) for both green and white-light illumination.

Categorization of positive vs. negative screening of the fallopian tube

Autofluorescence images from the mucosal surfaces of one, or where available both, fresh fallopian tubes were reviewed by two members of the imaging team (SE and PL) who were blinded to the history, surgical approach, and known or suspected pathology. Consistent with the categorization of precancerous or cancerous lesions in other epithelial tumors [42,43] a “test positive” was assigned if in one or both fallopian tubes loss of fluorescence and/or significant neovascularity was observed. “Test negative” was assigned to fallopian tubes where bright fluorescence or brown fluorescence (no loss) was noted over the entire visualized surface. The presence of blood pooling, evidence of trauma, the appearance of “leaky vessels” or suspected extravasation of blood and fluid from tissue were recorded as possible confounders. Test positive areas were mapped to corresponding paraffin embedded tissue sections to allow correlation to final pathology. Pathology review was performed on all cases in this cohort. For the purposes of calculating sensitivity, specificity, positive predictive value and negative predictive value “disease positive” was considered to be the presence of either TIC or carcinoma in either the right, left or (where available) both tubal specimen and “disease negative” the absence of preinvasive or invasive tubal pathology [56,57].

Table 1
Clinicopathology characteristics of 47 cases evaluated with optical imaging of the fallopian tubes. Cases are grouped in broad categories based on histopathology. Indication for surgery, age, stage, grade, histology, a personal or family history suggestive of hereditary breast ovarian cancer, side(s) of fallopian tube examined, and whether or not the tubal specimen was removed by laparoscopy (i.e., laparotomy unless stated) are detailed. Where BRCA mutation status is known it is recorded. Pending refers to cases where referral to the hereditary cancer program has been initiated but we are awaiting germline testing results. HGS = high grade serous, NAC = neoadjuvant chemotherapy undergoing interval debulking surgery, NS = non-serous histology, RR = risk reducing bilateral salpingo-oophorectomy (yellow), B9 = benign (blue). IDS = interval debulking surgery. R = right, L = left, B = bilateral, U = unknown. LSC = laparoscopy. Green denotes serous histology on final pathology.

Group	Reason for OR	Age	Stage	Grade	Histology	HBOC?	Side	LSC?
HGS1	Mass	51	IIIC	3	Serous	None	R	
HGS2	Mass	64	IIIC	3	Serous	None	R	
HGS3	Mass	72	IC	3	Serous	Family hx	B	
HGS4	Mass	61	IIIC	3	Serous	None	R	
HGS5	Mass	41	IIIC	3	Serous	Personal breast ca, pending	B	
HGS6	Mass	56	IIC	3	Serous	None	L	
HGS7	Mass	58	IIIC	3	Serous	None	B	
HGS*	Mass	83	IIIC	3	Serous	None	L	
HGS9	Mass	79	IIIC	3	Serous	None	B	
HGS10	Mass	74	IV	3	Serous	Family hx	B	
HGS11	Mass	72	IIIC	3	Serous	Family hx	B	
NAC1	IDS	71	X	3	Serous	Personal breast ca, pending	B	
NAC2	IDS	52	X	3	Serous	None	B	
NAC3	IDS	64	X	3	Serous	None	B	
NAC4	IDS	63	X	3	Serous	Family hx	B	
NAC5	IDS	49	X	3	Serous	None	L	
NS1	Mass	54	IIC	3	Clear cell	None	R	
NS2	Mass	58	IC	2	Endometrioid	None	L	LSC
NS3	Mass	75	IIIA	3	Undifferentiated	None	L	
NS4	Mass	54	IC	3	Clear cell	None	R	
NS5	Mass	68	IA	3	Clear cell	None	L	
NS6	Mass	48	IC	3	Clear cell	None	R	
NS7	Mass	52	IV	3	Endometrioid	None	L	
NS8	Mass	67	II	3	Endometrioid	None	R	
NS9	Mass	60	IIC	3	Transitional cell	None	U	
RR1	RR	44	NA	NA	NA	BRCA1, personal breast cancer	R	LSC
RR2	RR	41	NA	NA	NA	BRCA1	B	LSC
RR3	RR	39	NA	NA	NA	Personal breast ca, pending	B	LSC
RR4	RR	54	NA	NA	NA	Personal breast ca, pending	B	LSC
RR5	RR	47	NA	NA	NA	BRCA1	B	LSC
RR6	RR	54	NA	NA	NA	BRCA1	B	LSC
RR7	RR	53	NA	NA	NA	BRCA2	B	LSC
RR8	RR	65	NA	NA	NA	BRCA2	0	LSC
RR9	RR	58	IC	3	Serous	BRCA1 and2	B	LSC
RR10	RR	41	NA	NA	NA	BRCA1	B	LSC
RR11	RR	53	NA	NA	NA	Personal breast ca, pending	B	LSC
RR12	RR	42	NA	NA	NA	BRCA1	B	LSC
RR13	RR	50	NA	NA	NA	BRCA2, personal breast ca	B	LSC
RR14	RR	60	NA	NA	NA	BRCA1	B	LSC
B9-1	Mass	60	NA	NA	NA	None	R	LSC
B9-2	Mass	59	NA	NA	NA	None	R	LSC
B9-3	Mass	38	NA	NA	NA	None	R	LSC
B9-4	Mass	70	NA	NA	NA	Family hx	L	
B9-5	Mass	41	NA	NA	NA	None	B	LSC
B9-6	Mass	43	NA	NA	NA	None	L	LSC
B9-7	Mass	56	NA	NA	NA	None	R	
B9-8	Mass	52	NA	NA	NA	None	U	

Table 2
Disease status and optical imaging test designation of fallopian tube specimens for the entire cohort of 47 cases. Final pathology of the fallopian tubes (one or both) is categorized as having identified a tubal intraepithelial carcinoma (TIC), carcinoma (CA), both, or the absence of any pathology (ABSENT). Disease and test positivity are given. TP = true positive, TN = true negative, FP = false positive, FN = false negative. Correct assessments (TP or TN) are shown in pink.

Group	TIC or CA	Disease	Test	Classification
HGS1	TIC	Positive	Positive	TP
HGS2	CA	Positive	Positive	TP
HGS3	Absent	Negative	Positive	FP
HGS4	TIC, CA	Positive	Positive	TP
HGS5	TIC, CA	Positive	Positive	TP
HGS6	TIC	Positive	Positive	TP
HGS7	CA	Positive	Positive	TP
HGS8	Absent	Negative	Negative	TN
HGS9	CA	Positive	Negative	FN
HGS10	Absent	Negative	Negative	TN
HGS11	Absent	Negative	Negative	TN
NAC1	TIC, CA	Positive	Negative	FN
NAC2	Absent	Negative	Positive	FP
NAC3	Absent	Negative	Negative	TN
NAC4	CA	Positive	Positive	TP
NAC5	CA	Positive	Negative	FN
NS1	Absent	Negative	Negative	TN
NS2	Absent	Negative	Positive	FP
NS3	Absent	Negative	Negative	TN
NS4	Absent	Negative	Positive	FP
NS5	Absent	Negative	Negative	TN
NS6	Absent	Negative	Negative	TN
NS7	Absent	Negative	Negative	TN
NS8	Absent	Negative	Negative	TN
NS9	Absent	Negative	Positive	FP
RR1	Absent	Negative	Negative	TN
RR2	Absent	Negative	Negative	TN
RR3	Absent	Negative	Negative	TN
RR4	Absent	Negative	Negative	TN
RR5	Absent	Negative	Negative	TN
RR6	Absent	Negative	Negative	TN
RR7	Absent	Negative	Negative	TN
RR8	Absent	Negative	Negative	TN
RR9	TIC, CA	Positive	Positive	TP
RR10	Absent	Negative	Negative	TN
RR11	Absent	Negative	Positive	FP
RR12	Absent	Negative	Negative	TN
RR13	Absent	Negative	Negative	TN
RR14	Absent	Negative	Negative	TN
B9-1	Absent	Negative	Negative	TN
B9-2	Absent	Negative	Negative	TN
B9-3	Absent	Negative	Negative	TN
B9-4	Absent	Negative	Negative	TN
B9-5	Absent	Negative	Negative	TN
B9-6	Absent	Negative	Negative	TN
B9-7	Absent	Negative	Negative	TN
B9-8	Absent	Negative	Negative	TN

Results

Fifty-six patients consented to optical imaging of one or both fallopian tubes for ovarian masses ($n = 37$), RRBSO ($n = 14$) or with known serous ovarian cancer having received 3 or 4 cycles of NAC and undergoing interval debulking surgery (IDS) ($n = 5$). Final

Table 3
Sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) with optical imaging of the fallopian tube. Calculations were done i) on the entire cohort ($n = 47$), ii) in the risk reducing surgical group who underwent BSO for known or suspected HBOC ($n = 14$), and iii) excluding the non-serous cases and cases where pathology was known prescreening (i.e., excluding NS and NAC group) ($n = 33$).

	Total cohort	Risk reducing surgery	Non-serous excluded
Sensitivity	73%	100%	88%
Specificity	83%	92%	92%
PPV	57%	50%	78%
NPV	91%	100%	96%

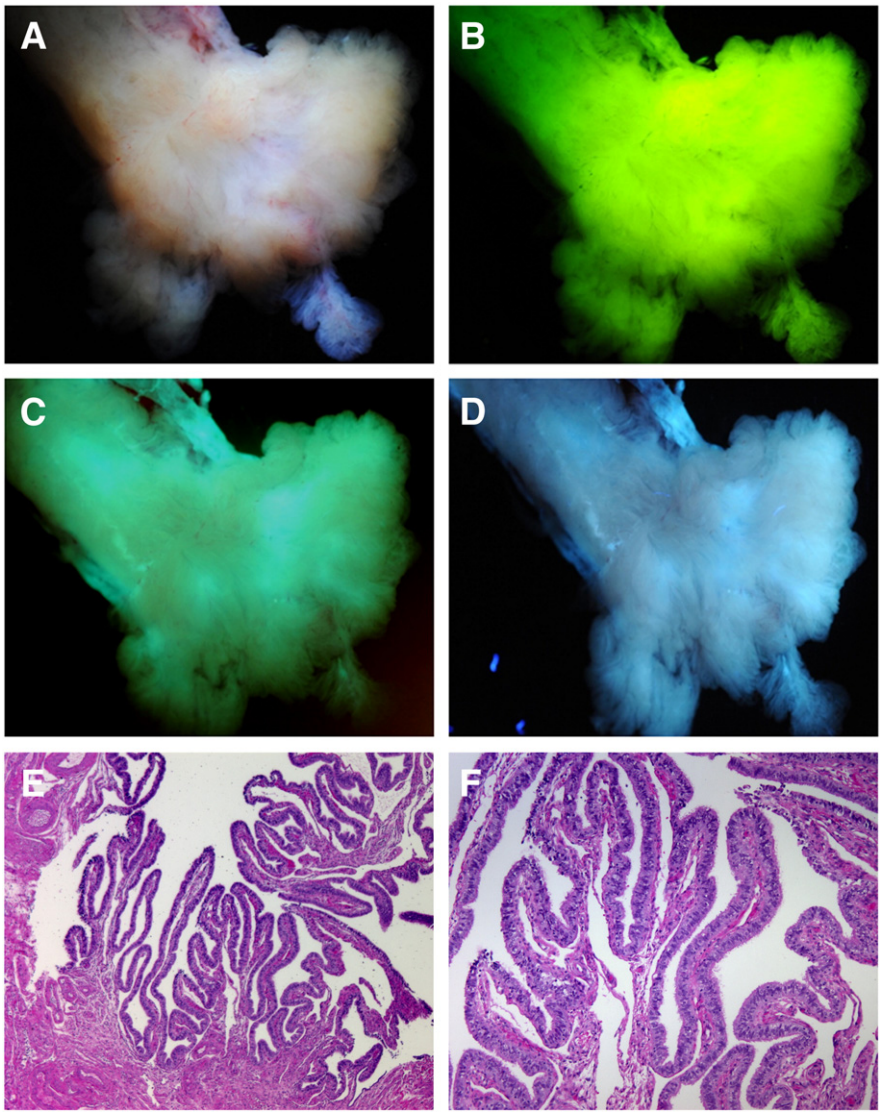

Fig. 1. Normal pattern of reflectance and fluorescence corresponding to normal fallopian tube epithelium from case RR10 fimbriated end. (A) white light reflectance of luminal/epithelial surface; (B) narrow band green reflectance (570 nm) (C) green autofluorescence (405/436 nm); and (D) blue autofluorescence (405 nm); Hematoxylin and eosin (H&E) stained sections are shown at (E) 4× and (F) 10× magnification (TIFF format; 300dpi).

pathology revealed eleven HGS, nine non serous ovarian malignancies, ten benign ovarian masses, and six cases that had abnormal appearing ovaries prompting surgery but final pathology revealed non-ovarian primary. In this last category were three endometrial cancers, three gastrointestinal primaries and one high grade and widely metastatic cervical adenocarcinoma, all of which were removed from final analysis. In addition, two cases of benign ovarian pathology were excluded as surgical removal via laparoscopy had grossly distorted the entire specimen rendering it non-interpretable. Further details of the 47 evaluable cases are described in Table 1.

Table 2 categorizes the final pathology found on serial sectioning of the fallopian tube(s) for each case. Carcinoma (CA) or tubal intraepithelial carcinoma (TIC) was recorded, referring to the FT, independent of disease at other sites. Presence of either CA or TIC was considered positive disease. If neither CA nor TIC was observed (ASBSENT) the case was designated negative. TICs or serous carcinoma was found in 7/11 of the HGS carcinomas, 3/5 of the NAC group, and 0/9 of the non serous ovarian carcinomas. Of the risk reducing surgeries one woman with both a BRCA1 and BRCA2 mutation was found to have a concurrent TIC and occult cancer (1/14 RRBSO cases positive pathology).

There was a high level of agreement between the two reviewers (43/47 cases or 91.5%). Where there was a discrepancy, it was decided that the final designation of category (positive or negative) would be that assigned by the more experienced reviewer. The most common incorrect categorization was false positive, where loss of fluorescence that was secondary to tissue trauma or blood pooling (vs. true abnormal pathology) had been incorrectly assigned a "test positive." This was true for both reviewers but particularly true for the less experienced reviewer and in his review of earlier cases. The risk reducing surgery and the benign ovarian mass groups seemed particularly vulnerable to potentially elevated false positive rates, with comments recorded by the reviewers of hemorrhage, blood, trauma etc. Within these two subgroups, the majority (19/22 or 86%) of surgeries were performed laparoscopically. Fallopian tubes and ovaries were extracted via a 12 mm (sometimes extended) suprapubic trochar incision within an Endo Catch ™ bag. Of note, the two benign cases that were excluded from evaluation secondary to uninterpretable

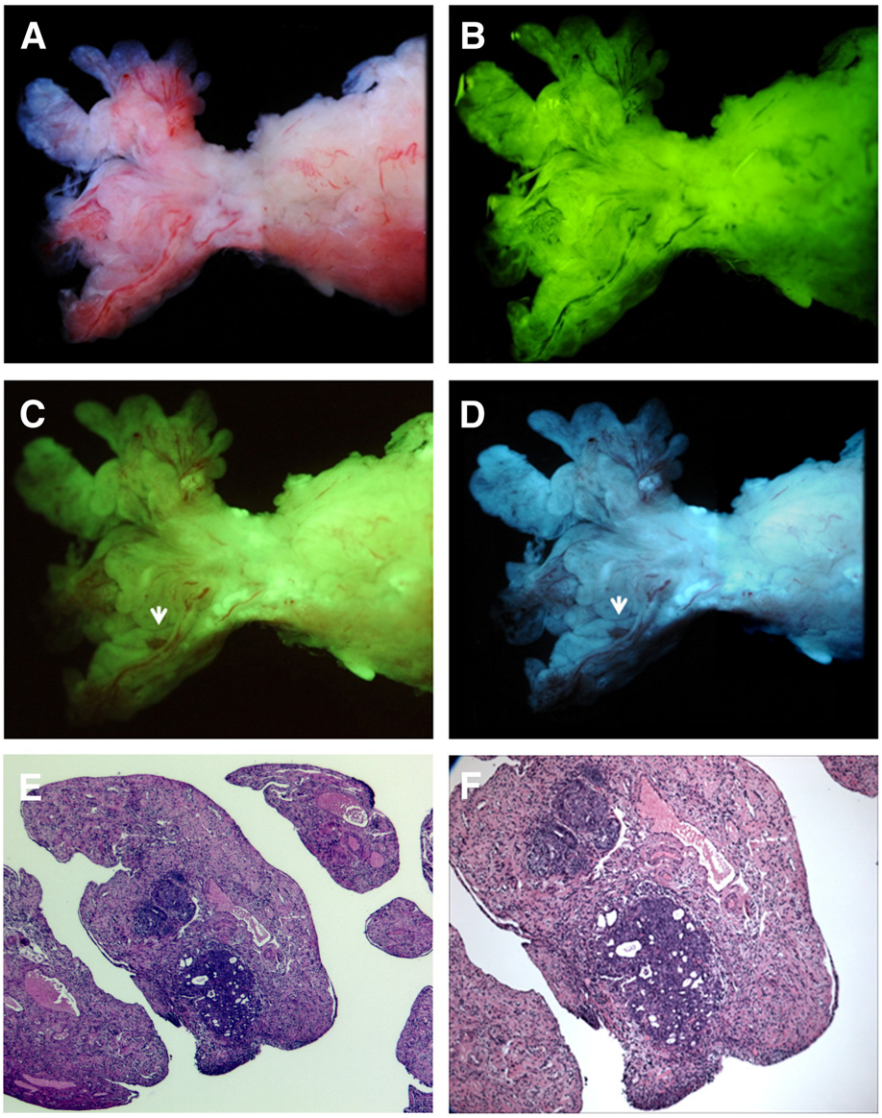

Fig. 2. Loss of fluorescence (white arrow) and isolated TIC found in case HGS4. (A–E) as above.

epithelial surface (trauma) had been laparoscopic procedures with the ovaries morecellated within the catch bag. Encouragingly, both reviewers noted that consistent with other epithelial cancers there is often a characteristic appearance of cancer adjacent to prominent neovascularity (and quite different than hemorrhage from tissue trauma).

Categorization of test negative and test positive is given in Table 2. Standard definitions of true positive (TP), false positive (FP), false negatives (FN), and true negatives (TN) are used [56,57]. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated for the entire cohort. In addition, we calculated the above for subgroups considered to be of high interest/consideration for this technology and its screening potential; i) the RRBSO group who were known or suspected to be at increased risk of developing FT, ovarian, or peritoneal serous cancer, and ii) women undergoing surgery for a mass or RRBSO with non serous histology excluded. Table 3 outlines these results for each group described above.

An example of a normal fallopian tube (disease negative, RR10) with the previously described fluorescence and reflectance images and its corresponding Hematoxylin and Eosin (H&E) histopathology is shown in Fig. 1.

Fig. 2 demonstrates loss of fluorescence in a focal area of the fimbria identified by the optical imaging reviewers and designated as test positive (white arrow). This corresponded to an isolated tubal intraepithelial carcinoma confirmed on final pathology (disease positive, HGS4) and shown under 4× and 10× magnification (images A-F specifics as above).

In Fig. 3, diffuse loss of fluorescence is noted on the fimbriated end and flagged as positive by the imaging team. This corresponded both with areas of blood artifact (potential false positive) but also with neovascularity and serous carcinoma (disease positive, HGS5) in multiple sections shown on histopathology.

Discussion

Risk reducing surgery for BRCA 1/2 mutation carriers can reduce woman's lifetime risk for “ovarian cancer” from as high as 56% (range 14–27% lifetime risk for women with germline BRCA2 mutations, 40–56% for germline BRCA1 mutations) to less than 4 % [58–60]. However, many women may be unaware of their risk of HBOC, never undergo testing, and never initiate protective measures. In addition, precancerous lesions have been identified

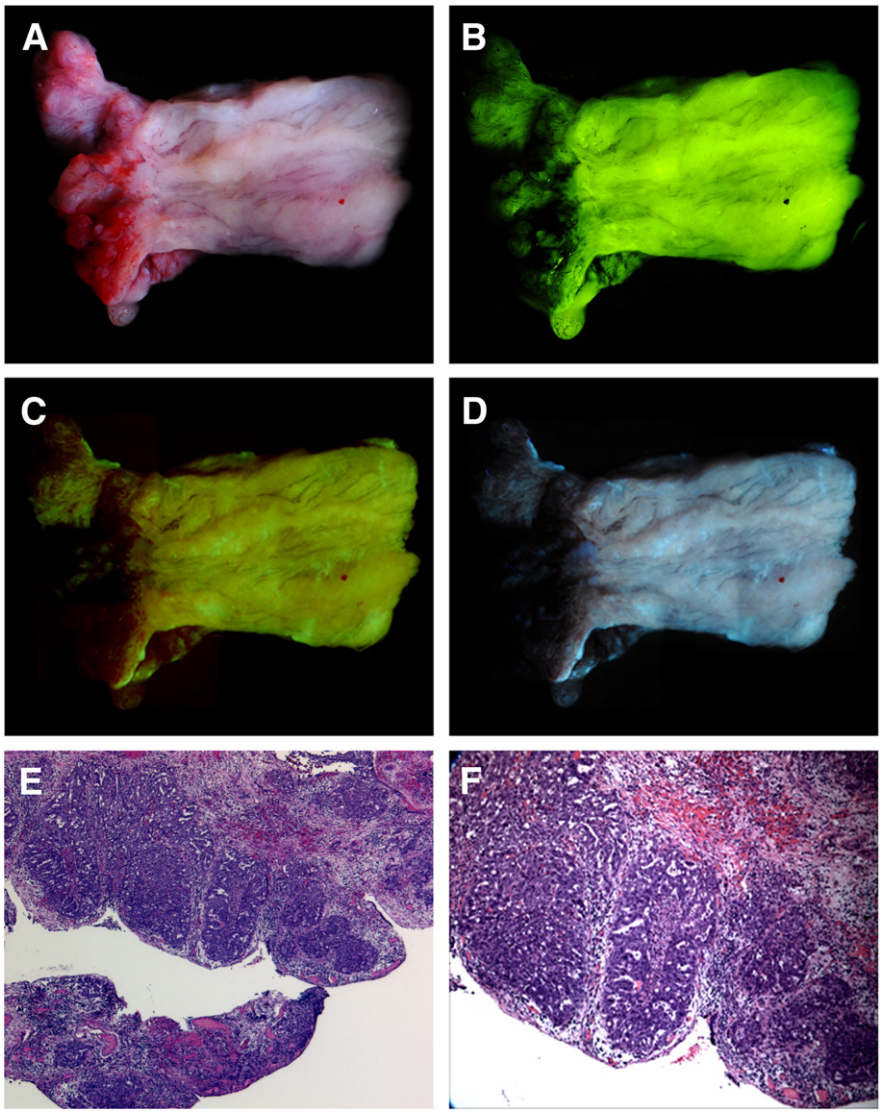

Fig. 3. Loss of fluorescence with blood artifact and serous carcinoma at the tubal fimbriae in case HGS5. (A–E) as above.

in patients with no known personal or family history to suggest HBOC. Although the frequency of this finding has yet to be validated in a larger series, it raises the issue that not only do we need to consider serial sectioning in all cases to identify and possibly treat these lesions in women who have had their fallopian tubes removed, but perhaps we need to be looking at screening or early detection modalities that examine the fallopian tubes *in vivo* in all women, regardless of suspected familial risk. At our center, we have shifted our focus in developing screening tools to detect HGS cancers to the fallopian tube. We have demonstrated that optical technologies can identify precancerous and cancerous lesions with promising levels of sensitivity (73–100%), specificity (83–92%), PPV (50–78%) and NPV (91–100%). We believe our predictive ability will improve, both as we gain experience and familiarity in interpreting the convoluted and extensive surface area of the tubal mucosa and by working to minimize surgical manipulation and contortion of the fallopian tubes in order to reduce potentially confounding hemorrhage/trauma. Mapping of lesions identified with AF in order to allow correlation with pathology has been challenging. Systematic cataloging of FT images in a pattern that mirrors SEE-FIM [9] serial pathology sectioning has been successful but the complexity of the fallopian tube fimbriae requires continuous readjustment.

Success of this pilot phase of our project, with the consistent identification of the lesions *ex vivo* enables us to initiate several new directions of investigation. We have already begun testing other optical technologies on the distal fallopian tube. Optical coherence tomography (OCT) provides *in vivo* images of tissue morphology with resolution approaching that of histopathology (10–20 μm) and penetration of 2–3 mm [61–66]. The thickness of bronchial epithelium as measured by OCT *in vivo* has been shown to correlate with dysplastic progression from normal to cancer [61].

Identification of these lesions in fresh tissue specimens provides an opportunity for detailed genomic analysis. *Ex vivo* tubal epithelium will be surveyed with AF and/or additional optical technologies. Abnormal areas will be marked for immediate microdissection. Both RNA and DNA sequencing will be performed in a series of TIC's and compared with normal adjacent epithelium as well as advanced serous cancers. Through this we hope to characterize differences in these precursor lesions and identify proteins that might be labeled (nanoprobes) to aid in AF identification and/or that might be picked up in serum samples or lower genital tract secretions. Our group has previously demonstrated that lower genital tract secretions yielded proteins, DNA and RNA, sometimes at concentrations several fold higher than in serum [67,68]. It is not inconceivable that in the near

future we will have a pap smear-like office test to probe for a panel of markers, and if a woman has a positive screen she will go on to have further testing i.e., falloposcopy as outlined below.

The lumen of the fallopian tube is accessible via endoscopy with historic and current devices utilized and well described in the fertility literature [69–75]. The diameter of the fallopian tube, even towards the convoluted distal fimbrial end is not considered an insurmountable barrier, with many devices capable of cannulating structures of lesser or equal diameter (i.e., carotid artery, 2.7 F (900 um)). Many of these anatomic locations have also had optical imaging systems incorporated in their endoscopic assessment in the research and clinical trial setting [61]. We are in the development phase of such a tool for the fallopian tube that with will have the capability to take AF images *in vivo*. These could be interpreted in real time. *In vivo* imaging will essentially eliminate the tissue trauma and hemorrhage challenges we have encountered in post-surgical specimens. Pathognomonic FT changes would prompt discussion with the patient and consideration of surgical intervention (+/- chemotherapy depending on final pathology).

In summary, autoflourescence imaging has proven valuable tool in many epithelial tumors and this technology has now successfully been transferred to the fallopian tube epithelium. Precancerous and cancerous FT lesions can be consistently identified in *ex vivo* samples providing many opportunities for further characterization of these lesions with the ultimate goal of developing a screening or early detection tool in EOC.

Conflict of interest statement
The authors have no conflict of interest to declare.

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