Review

"Real-time" Assessment of Surgical Margins During Radical Prostatectomy: State-of-the-Art

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Abstract

Histopathologic examination of the pathologic specimens using hematoxylin & eosin stains represents the backbone of the modern pathology. It is time-consuming; thus, "real-time" assessment of prostatic and periprostatic tissue has gained special interest in the diagnosis and management of prostate cancer. The current study focuses on the review of the different available techniques for "real-time" evaluation of surgical margins during radical prostatectomy (RP). We performed a comprehensive search of the Medline database to identify all the articles discussing "real-time" or intraoperative assessment of surgical margins during RP. Several filters were applied to the search to include only English articles performed on human subjects and published between January 2000 and March 2019. The search revealed several options for pathologic assessment of surgical margins including intraoperative frozen sections, confocal laser endomicroscopy, optical spectroscopy, photodynamic diagnosis, optical coherence tomography, multiphoton microscopy, structured illumination microscopy, 3D augmented reality, and ex vivo fluorescence confocal microscope. Frozen section represents the gold standard technique for real-time pathologic examinations of surgical margins during RP; however, several other options showed promising results in the initial clinical trials, and considering the rapid development in the field of molecular and cellular imaging, some of these options may serve as an alternative to frozen section.

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Introduction

Prostate cancer (PCa) is among the most commonly diagnosed cancers in the male population worldwide (13.5% of all male cancers), especially in northern and western Europe.[1] Several adverse events in the pathologic report may affect the outcomes and prognosis after radical prostatectomy (RP), including extracapsular extension (ECE) and positive surgical margins (PSMs).[2,3] PSM is defined as the presence of cancer cells in contact with the inked surface of the RP specimen. It may occur as a result of the migration of the tumor cells beyond the confines of the prostate (a phenomena known as ECE), or the intraprostatic surgical dissection (usually known as capsular incision).[4] PSM is associated with an increased risk of biochemical recurrence (BCR)[5]; however, its effect on the metastatic-free survival and cancer-specific mortality is still debatable.[6] This controversy about the actual clinical impact of PSMs may be attributable to multiple factors. The high inter-observer variability in the assessment of surgical margins (SMs) between pathologists during RP ($\kappa = 0.45$) is among the main factors causing this controversy.[7,8] Furthermore, the PSM rate is greatly affected by the experience of the surgeons and pathologists, which in turn, can result in highly variable rates of PSMs between different studies.[9] Thus, the International Society of Urological Pathology (ISUP) published their recommendation about SM assessment to standardize the pathologic reporting of SM status.[10] Moreover, the surgical approach may affect the rates of PSMs and BCR. In their systematic review and meta-analysis, Srougi et al[11] reported that robotic RP was associated with a statistically significant lower risk of PSMs and BCR when compared with open RP. In addition, different surgical techniques were introduced to reduce the risk of PSMs, like the collar technique, which significantly decreased the risk of apical PSMs (odds ratio, 0.05; $P = .009$).[12] Several other

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pathologic patterns and characteristics may affect the clinical impact of PSMs, like the extent and location of positive margins (extensive apical or bilateral PSMs may be associated with increased risk of BCR),[13,14] length of PSMs, and Gleason score at the margin.[15,16] On the other hand, PSMs may be associated with the subsequent need for further treatment and thus increase the cost of management. The main treatment options in this case are either adjuvant radiotherapy to the prostatic bed immediately after surgery or salvage radiotherapy offered only to men with BCR. The choice between these treatment options still relies on the surgeon's preference as there is no strong evidence supporting one strategy over the other.[4] Despite the ongoing debate about the oncologic impact of PSMs, surgeons should try to achieve maximum surgical radicality, without compromising the functional outcomes, as PSMs stimulate anxiety and require additional therapy that may have a subsequent effect on the patients' quality of life.[4,17] In these settings, several options have been proposed for prediction of ECE to provide the balance between oncologic radicality and functional outcomes without increasing the risk of PSMs, like the statistical tools based mainly on the clinical stage, prostate-specific antigen, and biopsy Gleason score; however, the predictive performance of such tools is still questionable.[18,19] Recently, a systematic review and external validation of the statistical tools used for ECE prediction showed that their predictive performance during external validation was not reliable.[18] Thus, "real-time" assessment of surgical margins has evolved as an urgent need to reduce the subsequent risk of BCR and avoid overtreatment.[20-24]

Histopathologic examination of the pathologic specimens using hematoxylin & eosin (H&E) stains represents the backbone of modern pathology; however, it was introduced in the late 1800s.[25] Moreover, this technique is time consuming, with a minimum turnaround time for surgical pathology reports of 2 days for the highest quality pathology laboratories.[26] In the current era of precise medicine and patient-tailored surgeries, this time-consuming approach does not fulfill the need for optimized surgical planning and improved surgical precision.[27]

Thus, "real-time" assessment of prostatic and periprostatic tissue has gained special interest in the diagnosis and management of PCa.[22,28-30] The current study focuses on the review of the different available techniques for "real-time" evaluation of surgical margins during RP.

Methodology

A comprehensive search of the PubMed database was done for all the articles discussing "real-time" or intraoperative assessment of surgical margins during RP. The search was done using a combination of the following keywords: prostatic neoplasms; prostate cancer; prostate carcinoma; prostatic carcinoma; prostate adenocarcinoma; prostatic adenocarcinoma; real time; real-time; intraoperative; intra-operative; pathological examination; frozen sections; NeuroSAFE; cryoultramicrotomy; frozen section; light reflectance spectroscopy; spectroscopy; Cellvizio; confocal laser endomicroscopy; gigapixel; structured illumination microscopy; confocal microscope; optical coherence tomography; augmented reality; 3D reconstruction; MRI; and magnetic resonance imaging. Several filters were applied to the search to include only English articles performed on human subjects and published between January 2000 and March 2019. Furthermore, a manual search in the references of the included articles was performed to identify all the possible technologies reported in the literature. The search was performed by 3 of our authors (A.E., A.Z., and S.P.).

Our search identified different options for assessment of surgical margins during RP that will be discussed in the current review including frozen section (FS), multiparametric magnetic resonance imaging (mpMRI), confocal laser endomicroscopy (CLE), optical spectroscopy, photodynamic diagnosis (PDD), optical coherence tomography (OCT), multiphoton microscope, video rate-structured illumination microscopy (VR-SIM), ex-vivo fluorescence confocal microscope (FCM), 3D augmented reality (AR), photoacoustic imaging (PAI), and histoscanning (HS).

Clinically Available Methods

FS

FS is a relatively old technique that dates back to the late 1800s. William Welch was the first to introduce the concept of FS in the surgical field, when he examined a specimen taken from breast tissues during surgery; however, Thomas Cullen was the first to publish the description of intraoperative FS technique in 1895.[31]

FS is based on the concept of rapid freezing of tissue samples using a cryostat machine (−16°C to −20°C for prostatic tissue specimens); thus, the water component of the specimen is converted to ice, allowing the tissue to be cut into multiple sections. The tissue can then be stained using H&E for microscopic examination of the specimen.[32]

FS is the most commonly used technique for assessment of surgical margins during RP.[20,21,28,33-59] Table 1 shows a summary of the studies published between January 2000 and March 2019 discussing the use of FS for assessment of surgical margins during RP.

The prostate is surrounded by a fibromuscular fascial layer known as the prostatic capsule; however, this capsule is deficient at some parts of the prostate, including the apex of the prostate,[60] thus rendering the dissection of the prostate at this part challenging owing to the proximity of the urethral sphincter, which must be preserved.[51] The urethral stump (the apex of the prostate) is one of the most common sites of PSMs.[6,14,16] Several authors studied the value of FS analysis of apical margins during RP, showing sensitivity and specificity ranging from 57% to 67% and 99% to 100%, respectively.[51-53] Interestingly, Ye et al[52] evaluated the oncologic value of FS analysis and apical margin re-excision by comparing the BCR-free survival at 60 months in patients with negative apical margins after tissue re-excision versus patients who had positive apical margins at the final pathology (93.75% vs. 80%, respectively), reporting a limited value for routine apical FS analysis.

The second most common site for PSMs is the posterolateral margins of the prostate (neurovascular bundle [NVB]).[6] Walsh introduced the NVB-sparing RP in 1983, and it has become the standard care for appropriately selected patients with PCa to improve the postoperative functional outcomes. A recent systematic review and meta-analysis showed that nerve sparing (either unilateral or bilateral) was not associated with a significant increase in the risk of PSMs in patients with pT2 or pT3 disease[61]; however, this is still debatable.[62] In these settings, several authors assessed the value of FS analysis of the posterolateral margins of the

Table 1 Summary of the Studies Published Between January 2000 and March 2019 Discussing the Use of FS for Assessment of Surgical Margins During RP

Site	Author	No.	Procedure	FS Time, min	PSM FS, %	False Negative, %	False Positive, %	PSM Conversion, %	Sensitivity, %	Specificity, %	Accuracy, %	PSM at Pathology, %	PSM Reduction, %	NS Increase
Apical														
	Shah et al, 2001[51]	95	RRP	NA	4.2	3.2	0	50	57	100	NA	- Apical (16.8) - Overall (26)	NA	NA
	Ye et al, 2011[52]	1669	NA	NA	6.7	2.8	0.2	45.5	59.1	99.8	NA	- Apical (5.5) - Overall (15)	NA	NA
	Wambi et al, 2013[53]	329	RARP	30	2.7	0.9	0.9	NA	67	99	98	- Overall (11)	NA	NA
Neurovascular bundle (main site)														
	Goharderakhshan et al, 2002[40]	101	NA	20	14.9	5	4	80	69	95	91	14.9	11.8	NA
	Fromont et al, 2003[37]	100	LRP	10-15	24	1	NA	NA	96	100	NA	12	21	NA
	Eichelberg et al, 2006[36]	83	RRP	NA	42.2	6	NA	85.7	NA	NA	NA	15.7	4.9	52
	Heinrich et al, 2010[42]	130	RRP	NA	6.9	0.8	NA	100	NA	NA	90	3.1	NA	NA
	Lavery et al, 2010[43]	177	RARP	NA	6	0.6	0.6	54.5	NA	NA	98	7	11[b]	NA
	Schlomm et al, 2012[50]	5392 (2567)[a]	RRP, LRP, RARP	35	27.2	2.5	NA	63.5	93.5	98.8	97.3	15	7[b]	16[b]
	Beyer et al, 2014[20]	1040	RARP	35	29.6	0.2	NA	NA	NA	NA	NA	- NeuroSAFE region (0.2) - Unifocal (10.97) - Multifocal (4.93)	7.8	16.2
	Petralia et al, 2014[49]	134	RARP	30	13.4	3.7	NA	72.2	NA	NA	NA	7.5	11.2[b]	NA
	Vasdev et al, 2015[35]	40	RARP	31	25	NA	10	60	75	75	NA	7.8	16.9	42.2
	Hatzichristodoulou et al, 2015[41]	471	RRP	30	29.1	3.3	NA	92.7	NA	NA	NA	4.9	19.6	37.2
	Bianchi et al, 2016[45]	254	RARP	35	29.1	9.8	NA	79.9	NA	NA	NA	15.75	NA	NA
	Mirmilstein et al, 2017[46]	120	RARP	NA	NA	1.5	NA	NA	82.4	91	NA	9.2	8.6[b]	6.1[b]
	Preisser et al, 2019[59]	156	RARP RRP	NA	NA	NA	NA	NA	NA	NA	NA	15.4	14.1[b]	40.2[b]

Table 1 Continued

Site	Author	No.	Procedure	FS Time, min	PSM FS, %	False Negative, %	False Positive, %	PSM Conversion, %	Sensitivity, %	Specificity, %	Accuracy, %	PSM at Pathology, %	PSM Reduction, %	NS Increase
Bladder neck														
	Nakamura et al, 2007[47]	51	NA	NA	6	NA	NA	100	NA	NA	NA	- Overall (20) - Bladder neck (0)	NA	NA
Apical and/or bladder neck and/or neurovascular bundle														
	Lepor et al, 2003[44]	500	RRP	NA	6.9	NA	NA	NA	57.7	98.2	96	NA	3.6 (apical)	NA
	Dillenburg et al, 2005[39]	198	LRP	7.5	13	NA	NA	NA	Apical (70)	Apical (97)	Apical (96)	- Apical (6) - NVB (1) - Bladder neck (0.5)	6	NA
	Tsuboi et al, 2005[54]	259	NA	NA	8.9	13.6	NA	73.9	42	100	NA	17.4	NA	NA
	Gillitzer et al, 2010[38]	178	RRP	NA	10.7	NA	NA	NA	- NVB (11.5) - Apex (29.2) - BN (18.2)	- NVB (98) - Apex (97.4) - BN (100)	NA	- Overall (27.5)	NA	NA
	Fasolis et al, 2006[57]	259	RRP	NA	24.3	11.9	NA	NA	NA	NA	NA	12.4	NA	NA
	Emiliozzi et al, 2010[58]	270	LRP	NA	24.8	NA	NA	NA	NA	NA	50.7	12.6	12.2	NA
	Kakiuchi et al, 2012[21]	1128	RARP	NA	5.3	1.7	5	63.3	NA	97.3	89.7	9.7	NA	NA
	Akin et al, 2013[33]	66	RARP	NA	34.8	34.8	NA	4.3	NA	NA	NA	37.9	NA	NA
	Almeida et al, 2013[34]	128	RARP	NA	18.7	8	NA	58.3	NA	NA	NA	16.4	10.9	NA
	Von Bodman et al, 2013[56]	236	RRP, RARP	35	22	1.6	NA	92.3	NA	NA	NA	3	19	NA
	Nunez et al, 2016[55]	71	LRP, RARP	NA	15.5	NA	NA	NA	85	100	99	NA	NA	NA
	Obek et al, 2018[28]	170	RARP	57	33	1.8	NA	85	NA	NA	NA	7.6	14.9	4.9
	Pak et al, 2018[48]	2013	NA	15	10.6	2.7	NA	65	78.1	97.8	NA	24.9	NA	NA

Abbreviations: BN = bladder neck; FS = frozen section; LRP = laparoscopic radical prostatectomy; min = minutes; NA = not available; NS = nerve sparing; No. = number; PSM = positive surgical margins; RP = radical prostatectomy; RRP = open retropubic radical prostatectomy; RARP = robotic-assisted laparoscopic radical prostatectomy.

[a]Propensity score matched sample.

[b]Difference between frozen section group and non-frozen section group.

prostate.[20,35-37,40-43,45,46,49,50] In 2012, Schlomm et al[50] developed the neurovascular structure-adjacent frozen section examination (NeuroSAFE) to improve the sensitivity and specificity of frozen section in the NVB region. The NeuroSAFE is based on the concept of dissection of the entire neurovascular structure-adjacent prostatic tissue and assessing it using FS. This approach showed a sensitivity of 93.5%, specificity of 98.8%, and accuracy of 97.3%; however, the BCR-free survival was not significantly different compared with the non-NeuroSAFE group.[50] Beyer et al[20] and Mirmilstein et al[46] further assessed the NeuroSAFE technique, reporting on the feasibility and applicability of this approach with a reduced PSM (from 24% to 16% and 17.8% to 9.2%, respectively) and increased NVB sparing (from 81% to 97% and 69% to 75.1%, respectively). Interestingly, Bianchi et al[45] and Petralia et al[49] reported that mpMRI-directed FS was able to reduce the risk of PSM and predict the risk of upgrading and upstaging in patients undergoing RP.

Regarding the oncologic value of FS conversion to negative in the posterolateral margins of the prostate, the BCR-free survival was not significantly affected by the FS conversion to negative.[40,41,43]

On the other hand, PSMs at the base of the prostate (bladder neck) are infrequent.[6] Nakamura et al[47] demonstrated that FS analysis of the bladder neck was able to reduce the PSMs at this location to 0%.

Considering the limitations of the NeuroSAFE technique represented in the FS evaluation of only NVB-adjacent tissues that may result in missing PSMs in other locations, Öbek et al[28] introduced the intrasurgical total and reconstructible pathologic prostate examination for safer margins and nerve preservation (Istanbul preserve) approach. This approach includes the examination of the entire prostate for margins (including the apex and bladder neck) with easier reconstruction, reducing the tissue loss. Istanbul preserve demonstrated a 14.9% reduction in the PSM with a 4.9% increase in the NVB-sparing approach; however, it was associated with a longer time of FS (57 minutes) owing to the assessment of the urethral margins that delay the initiation of the anastomosis.[28]

Furthermore, Almeida et al[34] tried to overcome the time consumed in the undocking of the robot during robotic-assisted laparoscopic prostatectomy by using the ALEXIS trocar, reporting easy and fast retrieval of the prostate with good cosmetic outcomes and low complications.

Overall, FS analysis is considered the gold standard diagnostic approach for intraoperative pathologic evaluation of surgical margins during RP, which is associated with a 3.6% to 21% reduction of the PSMs and a 4.9% to 52% increase in the nerve-sparing rates; however, its value is still debatable. Some authors argue against its value because it has no statistically significant BCR-free survival benefits.[38,39] Furthermore, it is considered time- (ranging from 7.5-57 minutes) and resource-consuming (because it requires a dedicated team).[28,39] On the other hand, supporters of the approach state that it significantly reduces the PSM rates and increases the number of patients eligible for the NVB-sparing approach.[20,50] Moreover, Pak et al[48] reported that FS may significantly improve the BCR-free survival in low- and intermediate-risk patients but not in high-risk patients.

MRI

MRI has shown promising results in the preoperative local staging of prostate cancer; however, regarding ECE, a recent meta-analysis reported a high specificity (91%) and only moderate sensitivity (57%), which was increased to 68% when considering only 3 Tesla studies.[63] A more recent systematic review and meta-analysis showed that the apparent diffusion coefficient MRI had a pooled sensitivity and specificity of 80.5% and 69.1%, respectively, for ECE prediction.[64] The main difference between MRI and the other options included in the current review is that MRI is used for preoperative detection of ECE, not for the "real-time" assessment of the surgical margins during RP; yet we decided to add it to the available options as there is a growing interest in the use of MRI for local staging of prostate cancer, and it may be used in combination with other technologies to facilitate the "real-time" assessment of surgical margins. Several authors studied the clinical value of preoperative MRI on surgical planning and PSM rates showing that it may influence the surgical decision in 26% to 59% of patients.[65-70] Recently, Kozikowski et al[71] reported, in their systematic review and meta-analysis, that MRI modified the NVB dissection on 1 or both sides in 35% of patients, of which 63% performed a more aggressive resection of the bundle and 37% had more preservation of the bundle. Furthermore, they showed that the appropriateness of the surgical decision modification based on the MRI findings was 77%.[71] Similarly, Druskin et al[70] showed that the use of preoperative MRI in patients undergoing RP resulted in a 5.6% decrease in the PSM rate.

Overall, MRI plays an important role in the diagnosis and management of PCa. Moreover, it may be used to enhance the predictive performance of statistical tools and nomograms used for ECE prediction.[72]

Experimental Technologies

CLE

The CLE is a fiber-optic system that uses a blue laser (488-nm) together with fluorescein to provide a high-resolution cellular image of the examined tissue similar to histopathology.[73] The CLE has been used in urology for the in vivo grading of upper tract urothelial carcinomas and bladder tumors.[74]

The commercially available CLE used in urology is the probe-based system known as Cellvizio (ManuaKea Technologies, Paris, France). This device has different imaging probes ranging from 0.85 to 2.6 nm. The 0.85-nm probe is a small probe that is compatible with nearly all the available endoscopes used in urology, whereas the 2.6-nm probe requires larger working ports.[74] The spatial resolution, penetration depth, and field of view of the CLE are 1 μm and 3.5 μm, 60 μm and 50 μm, and 240 μm and 320 μm for the 2.6- and 0.85-nm probes, respectively.[22] Lopez et al[22] demonstrated the feasibility of intraoperative application of CLE during robot-assisted RP (RARP) and its ability to identify important anatomical and structural landmarks, which may render it a potential alternative for intraoperative pathologic examination of prostatic and periprostatic tissues.

Furthermore, Panarello et al[73] created an atlas of CLE in prostate, thus forming the base for identification and interpretation of different prostatic pathologic characters during RARP, which may enhance the use of this technology in achieving cancer-free surgical margins during RP.

Optical Spectroscopy

Optical spectroscopy is the technique of molecular analysis of tissues based on the interpretation of specific interactions between

Table 2 Summary of the Performance of Optical Spectroscopy in Differentiation Between Benign and Malignant Prostate Tissues and PSMs

Study	Cross Validation	Spectroscopy	Sensitivity, %	Specificity, %	Accuracy, %	AUC
Crow et al, 2005[75]	LOOCV	Raman spectroscopy	NA	NA	85	NA
Salomon et al, 2008[77]	LOOCV	Triple spectroscopy	75	87.3	NA	NA
Baykara et al, 2014[76]	LOOCV	Elastic scattering spectroscopy	86	97	NA	0.87
Morgan et al, 2015[78]	RCV	Elastic scattering spectroscopy	86	85	86	0.95
Lay et al, 2016[79]	RCV	Elastic scattering spectroscopy	65.5	88.1	83.3	0.86
Aubertin et al, 2018[24]	LOOCV	Raman spectroscopy (FP + HWN)	85	89	88	0.91
	LOPOCV		81	90	88	0.91
Aubertin et al, 2018[80]	LOOCV	Raman spectroscopy	87	86	86	0.93
Pinto et al, 2019[81,a]	NA	Raman spectroscopy	90.5	96	91	0.96

Abbreviations: AUC = area under the curve; FP = fingerprint region; FTIR = fourier transform infrared spectroscopic imaging; HWN = high wave number; LOOCV = leave one out cross validation; LOPOCV = leave one patient out cross validation; PSM = positive surgical margin; RCV = random cross validation.
[a]Differentiation between prostatic and extraprostatic tissues.

light and tissues.[75] These techniques can be used to produce distinct scattering spectral signatures that reflect structural changes in tissue architecture, cellular morphology, and biochemical distribution, allowing them to differentiate between benign and neoplastic tissues based on the different biological and molecular characteristics between them.[76,77] Different types of spectroscopies have been used in the field of PCa (Table 2), including fluorescence spectroscopy,[77] elastic scattering spectroscopies,[76,78,79] and inelastic scattering spectroscopy.[24,75,80,81] Most of the tissues' light scattering is elastic, where there is no exchange of energy between the photons and the molecules (Rayleigh scattering); however, only very few photons show inelastic light scattering with energy exchange.[82]

Raman spectroscopy (RS) is a molecular tissue characterization technique that depends on inelastic scattering of light after excitation of tissues with monochromatic light.[81] Crow et al[75] showed that fiber-optic near-infrared RS was able to differentiate between benign and malignant bladder samples and prostate samples with an overall accuracy of 84% and 86%, respectively; however, this study was performed in vitro on snap frozen samples of bladder and prostate, and they were unable to determine accurately the biochemical constitutes that allowed this differentiation. Most of the bimolecular data obtained from the RS is contained in the fingerprint (FP) spectral region; nonetheless, the high wave number region (HWN) includes data that can be used for differentiation between benign and malignant tissues.[24] Aubertini et al[24] demonstrated that combining the information from both regions (FP and HWN) improves PCa detection with an area under the curve (AUC) of 0.91 compared with 0.89 and 0.86 for the FP alone and HWN alone, respectively. In a trial to study the potential therapeutic and diagnostic application of the in vivo fiber-optic RS, Aubertini et al[80] acquired 947 Raman spectra that were correlated with the corresponding histopathologic examinations of the interrogated tissues. They reported 82% sensitivity, 83% specificity, 83% accuracy, and an AUC of 0.9 for distinguishing prostatic from extraprostatic tissues. Furthermore, they examined the ability of RS to differentiate benign from neoplastic tissues with 87% sensitivity, 86% specificity, 86% accuracy, and 0.93 AUC. Moreover, Pinto et al[81] presented a dual excitation RS (680- and 785-nm excitation) that can be used in vivo during RARP. They performed an ex vivo analysis of 20 whole prostate specimens, which were analyzed showing 90.5% sensitivity, 96% specificity, 91% accuracy, and 0.96 AUC. Later, they performed in vivo analysis in 4 patients reporting similar FP spectra between in vivo and ex vivo analysis; however, the HWN showed lower intensities compared with the ex vivo analysis.[81] Furthermore, Fourier transform infrared spectroscopic imaging can be used for identifying PCa cells in tissues with a resolution of 6.25 μm × 6.25 μm on FSs.[83]

Elastic light single scattering spectroscopy is a single fiber optical probe that is capable of delivering light to the tissue and measures the intensity of the light backscattered from the tissue.[76] It consists of an optical probe of 1 mm in diameter and a penetration depth of 2 mm. The probe is connected to a tungsten-halogen light source and a spectrometer.[76,78,79] It has been used for detection of PSMs during RP, showing an AUC ranging from 0.87 to 0.96 for prediction of PSM on ex vivo RP specimens.[76,78,79]

Salomon et al[77] demonstrated that the combination between auto-fluorescence spectroscopy with electrical impedance measurement increased the sensitivity and specificity for discrimination between benign and malignant prostatic tissues to 93.8% and 92.4%, respectively. Finally, the desorption electrospray ionization mass spectorometric imaging that can rapidly assess the molecular characteristic of tissue in-situ may be used together with the FS or alone for assessment of surgical margins during RP.[84]

PDD

5-Aminolevulinic acid (ALA) is the precursor of porphyrin in the biosynthesis of heme. Administration of 5-ALA results in the accumulation of protoporphyrin (Pp) XI, which is a potent photosensitizer in the mitochondria. PpIX accumulation is increased in neoplastic tissues; thus it can be used for identification

Table 3 Summary of the Performance of 5-ALA PDD in Detection of PSMs During RP

Study	No. Patients	Surgery	Sensitivity, %	Specificity, %	False Negative, %
Adam et al, 2009[88]	15	Open RP	75	100	50
	24	Endoscopic RP	38	88.2	25
Ganzer et al, 2009[89]	24	Endoscopic RP	75	88.2	8.3
Fukuhara et al, 2011[87]	16	Open RP	81.8	68.8	31.2
Fukuhara et al, 2015[23]	52	Open RP Endoscopic RP	75	87.3	3.8

Abbreviations: 5-ALA PDD = 5-aminoeluvelnic acid photodynamic diagnosis; PSMs = positive surgical margins; RP = radical prostatectomy.

of malignant tissues during surgery.[23] PDD using 5-ALA is a known concept in oncologic surgery that is used for "real-time" identification of malignant tissues; it has been used in urology in the diagnosis of bladder urothelial carcinoma using violet light.[85] Zaak et al[86] were the first to demonstrate, in their ex vivo clinical study, the feasibility of 5-ALA PDD in the detection of PSMs using the RP specimens of 16 patients with PCa. Several other authors demonstrated the feasibility and safety of 5-ALA PDD in the assessment of surgical margins during open RP[23,87] and endoscopic RP.[88,89] However, Fukuhara et al[23] reported in their study that heat degeneration by electrical device and the length of positive margins are among the limitations of 5-ALA PDD in assessing surgical margins during RP. Heat degeneration may result in the damage of the accumulated PpIX; thus the fluorescence light is unable to detect it. Furthermore, PSMs < 3 mm in length could not be detected by 5-ALA PDD.[23] Table 3 summarize the results of the studies that reported the use of 5-ALA PDD in detecting PSMs during RP.

OCT

OCT is a tissue-imaging technique that is capable of providing real-time high resolution images of tissue microstructures.[90] It is similar to B-mode ultrasonography, but it depends on the differences in infrared waves (1300 nm) scattering from different tissue structures instead of acoustic waves.[91,92]

D'Amico et al[90] demonstrated the ability of OCT to differentiate between benign and malignant prostatic tissues using tissue samples obtained from RP specimens of 7 men with clinically localized PCa. In 2006, Aron et al[92] examined the feasibility of in vivo OCT for identification of NVBs during laparoscopic and robotic RP in 24 patients with PCa. OCT was capable of differentiation between different prostatic and periprostatic structures, including nerves, prostatic capsule, adipose tissue, lymphatics, and NVBs.[92] These studies suggested that OCT could be used for ensuring cancer-free surgical margins during RP.[90,92]

In these settings, Dangle et al[91] used RP specimens from 100 patients with PCa undergoing robotic RP to assess the value of OCT in the evaluation of surgical margins, ECE, and seminal vesical invasion. With regard to PSMs, OCT showed a sensitivity of 70%, a specificity of 84%, a positive predictive value (PPV) of 33%, and a negative predictive value (NPV) of 96%. Similarly, it showed a sensitivity of 46% and 33%, a specificity of 84% and 97%, a PPV of 50% and 33%, and an NPV of 92% and 97%, for prediction of ECE and seminal vesicles invasion, respectively.[91] Furthermore, several authors confirmed the feasibility of needle-based OCT for differentiation between benign and malignant prostatic tissues.[93-96]

Multiphoton Microscopy

Multiphoton microscopy mainly depends on the non-linear excitation resulting from the simultaneous absorption of 2 (or more) near-infrared photons (700-800 nm range), thus producing intrinsic optical sectioning similar to the conventional confocal microscopy.[97,98] It is capable of providing cellular information about the tissues without any tissue preparation or processing, and without the need for any exogenous contrast agent. Furthermore, it is characterized by a deep penetration depth up to 0.5 mm.[97] Despite showing promising results on ex vivo tissue samples obtained from RP specimens, this technology is still under development, and it has never been applied in vivo in real surgical settings for the assessment of surgical margins during RP.[97,98]

VR-SIM

VR-SIM combines a fast, ferroelectric spatial light modulator for pattern production with a 4.2-megapixel, high-speed scientific complementary metal-oxide semiconductor camera to produce high-resolution cellular images of fluorescently stained tissues.[29,99] Wang et al[29] applied this technology for the examination of 34 unprepared PCa core biopsies, showing an AUC of 0.82 to 0.88, a sensitivity of 62.5% to 87.5%, a specificity of 77.8% to 83.3%, and accuracy of 76.5% to 82.4% for the diagnosis of PCa. In another study, VR-SIM was used for the ex vivo assessment of the entire margins of 19 RP specimens instantly after resection of the prostate; this study reported that VR-SIM was able to detect 3/4 patients with PSMs, and the only missed patient had a circumferential PSM of < 500 μm. However, the main disadvantage of this technique is the time as the process took nearly 1 hour.[99]

Ex Vivo FCM

Ex vivo FCM (VivaScope 2500M-G4; Mavig GmbH, Munich, Germany; Caliber I.D, Rochester, NY) combines 2 types of lasers to allow pathologic examination of freshly excised unfixed specimens with reflectance (785 nm) and fluorescence (488 nm) modes. This technology is characterized by a vertical resolution of 4 μm, penetration depth of 200 μm, and magnification of 550×. Furthermore, it is capable of providing fast H&E-like digital images that can be sent electronically to distant pathologists for interpretation. It has been applied in the examination of different visceral organs, showing promising results.[100] FCM has been used for "real-time" pathologic examination of prostatic tissues, with a 91% diagnostic accuracy compared with histopathologic examination and 83.33% sensitivity and 93.53% specificity.[101] Furthermore, FCM has been applied for the first time in the assessment of the surgical margins during robotic RP in the European Section of Uro-Technology (ESUT) - Italian

Endourological Association (IEA) meeting held in Modena, Italy, in May 2018.[102] However, further studies are required to prove the feasibility of this technology in patients with PCa.

3D AR

3D reconstruction and AR are new technologies that have been applied in different medical fields.[103-105] In the field of PCa, Ukimura et al[105] used 5 anatomic aspects of the prostate (prostate surface, trans-rectal ultrasound or MRI-detectable biopsy-proven index lesion, NVBs, urethra, and color-coded biopsy paths) to create a 3D model that can be displayed during robotic RP using the TilePro function of the DaVinci surgical system. This 3D model was used to facilitate intraoperative navigation and dissection in 10 patients; however, it was not superimposed over the prostate. This initial experience showed negative surgical margins in 9 patients, whereas PSMs were detected in only 1 patient with extensive ECE.[105] Recently, with the increased interest in AR technology in the medical field, several studies used a combination of HyperAccuracy 3D reconstruction of mpMRI data and AR to allow more precise surgical dissection.[104,106-108] Porpiglia et al[104] used the AR-superimposed 3D-reconstructed model in 30 patients undergoing robotic RP to assess the accuracy of the AR-reconstructed model compared with the final histopathology, showing that the index lesion was successfully localized using this technology in 100% of patients. Moreover, there was 79% concordance between the AR model-suspected ECE and the final histopathology with a mismatch between the AR model and whole mount specimen of less than 3 mm in 85% of the gland. Interestingly, Puliatti et al[108] reported the use of the same technology, but instead of only using the mpMRI data in the 3D image reconstruction, they used a combination of mpMRI results and a statistical tool that is used for prediction of ECE[109] in a live surgery during the ESUT congress on May 2018. A further step forward in the use of this technology was reported by Porpiglia et al,[110] where they successfully demonstrated the use of an elastic 3D virtual reality model superimposed over the prostate during robotic RP. The elasticity of the model allowed its bending and stretching based on the traction applied to the prostate during the procedure. This new elastic AR 3D showed a sensitivity and PPV of 100% for identification of capsular invasion.[110]

Others

Several other options has been used in the literature for the identification of the NVBs and assessment of the surgical margins during RP. PAI is among these technologies. PAI is a novel technology that utilizes optical and ultrasonic waves for real-time identification of vascular structures, while using hemoglobin as an endogenous contrast agent. It has shown promising results in the in vivo identification of the NVBs during RP; however, it was not used for the detection of PSMs.[111]

Moreover, HS is another non-invasive technology that has been applied in the assessment of surgical margins during RP. This technology depends on processing the backscattered ultrasonic waves to detect neoplastic foci. HS has shown promising results in the detection of PCa foci ($\geq$ 0.5 mL) with a sensitivity and specificity of 100% and 82%, respectively.[112,113] Salomon et al[114] evaluated the accuracy of HS in the preoperative preparation of NVB-sparing RP showing that the AUC for PSM prediction is 0.76.

Conclusion

PSMs represent one of the surgically controlled limitations of RP. FS still represents the gold-standard technique for the assessment of surgical margins during PCa surgery; however, it was introduced in the late 1800s, and its results are debatable. Several other options showed promising results in the initial clinical trials, and considering the rapid development in the field of molecular and cellular imaging, further options with better outcomes may help in overcoming this problem; however, most of these technologies are still experimental and are not yet routinely used in clinical practice.

Disclosure

A. Eissa has a temporary contract of consultation with MAVIG GmbH. The remaining authors have stated that they have no conflicts of interest.

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