Review Article

Development of intraoperative assessment of margins in breast conserving surgery: a narrative review

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Objective: We intend to provide an informative and up-to-date summary on the topic of intraoperative assessment of margins in breast conserving surgery (BCS). Conventional methods as well as cutting-edge technologies are analyzed for their advantages and limitations in the hope that clinicians can turn to this for reference. This review can also offer guidance for technicians in the future design of intraoperative margin assessment tools.

Background: Achieving negative margins during BCS is one of the vital factors for preventing local recurrence. Conducting intraoperative margin assessment can ensure negative margins to a large extent and possibly relieve patients of the anguish of re-interventions. In recent years, innovative methods for margin assessment during BCS are advancing rapidly. And there is a lack of summary regarding the development of intraoperative margin assessment in BCS.

Methods: A PubMed search with keywords "intraoperative margin assessment" and "breast conserving surgery" was conducted. Relevant publications were screened manually for its title, abstract and even full text to determine its true relevance. Publications on neo-adjuvant therapy and intraoperative radiotherapy were excluded. References from the searched articles and other supplementary articles were also looked into.

Conclusions: Conventional methods for margin assessment yields stable outcome but its use is limited because of the demand on pathology staff and the trade-off between time and precision. Conventional imaging techniques pass the workload to radiologists at the cost of a significantly low duration of time. Involving artificial intelligence for image-based assessment is a further improvement. However, conventional imaging is inherently flawed in that occult lesions can't show on the image and the showing ones are ambiguous and open to interpretation. Unconventional techniques which base their judgment on cellular composition are more reassuring. Nonetheless, unconventional techniques should be subjected to clinical trials before putting into practice. And studies regarding comparison between conventional methods and unconventional methods are also needed to evaluate their relative efficacy.

Keywords: Breast cancer; breast conserving surgery (BCS); margin assessment

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Introduction

In terms of treatment for breast cancer, multiple randomized trials have proven breast conserving surgery (BCS) followed by irradiation to be equally safe as mastectomy, assuming that the margin is free of tumor and the cosmetic outcome is satisfactory (1-5). An increasing amount of early-stage, impalpable breast cancer is diagnosed due to the popularized implementation of breast screening and people's ever-growing health awareness, enabling more patients to become eligible as candidates for BCS. In addition, the application of neo-adjuvant therapy also played a role in increasing rates of BCS (6).

A BCS can be considered successful only when two criteria are met at the same time, that is to retain as much healthy tissue as possible while resecting all cancerous tissue. A higher volume of breast tissue left accounts for better cosmetic outcome and patient satisfaction (7). Positive margin is one of the strongest indicators of local recurrence (8). Meanwhile, a clean margin status can significantly lower the risks of local recurrence (9). Balancing the aforementioned goals at once can be a demanding task, however the solution lies in making accurate judgment on margin status.

Generally speaking, the golden standard for margin assessment is the pathology performed post-operatively, yet relying solely on this method may subject patient to the extra cost and discomfort of a second operation once the pathology come back with a positive result (10). A number of studies have reported the reoperation rate after the publication of the 2014 SSO-ASTRO Consensus Guideline ranging as high as 16.5–23.1% (11-13). It is quite obvious that the existing intraoperative margin assessment methods cannot live up to expectations. Not only does margin assessment techniques lack standardized procedure, the most frequently practiced ones such frozen section analysis (FSA) and imprint cytology (IC) are both labor and time-extensive and demand for an experienced pathologist, limiting its spread. The ideal intraoperative margin assessment methods should meet all of the following clinical requirements: capable of diagnosing with great precision, quick to perform, easy to operate, applicable to the majority of patient group, cost-effective and above all, effectively lowering reoperation rates. To our delight, this field has witnessed a boom in innovative and experimental margin assessment methods in recent years. Therefore, this narrative review will focus on the limitations and performance and hence the prospect of the existing margin assessment methods by dividing it in to four parts: pathological methods, conventional imaging methods, unconventional imaging techniques and methods involving novel imaging agents. We present the following article in accordance with the Narrative Review reporting checklist (available at https://gs.amegroups.com/article/view/10.21037/gs-21-652/rc).

Methods

A PubMed search with keywords "intraoperative margin assessment" and "breast conserving surgery" was conducted. Relevant publications were screened manually for its title, abstract and even full text to determine its true relevance. Publications on neo-adjuvant therapy and intraoperative radiotherapy were excluded. References from the searched articles and other supplementary articles were also looked into.

Pathological methods

The most well-established margin assessment methods are gross inspection, FSA and IC. Gross inspection usually entails visual examination and palpation of freshly excised and unprocessed specimen to determine if there is tumor left on the margins. Since it examines the specimen on a macroscopic level, it is only natural to assume that it cannot match the precision of a microscopic assessment. Consequently, gross inspection is rarely used single-handedly and instead mostly serves a complementary purpose (14). Still, one study pointed out that after assigning gross inspection and FSA respectively to two groups of patients undergoing BCS, the difference between their reoperation rates was statistically insignificant. However, it is note-worthy that the sensitivity of FSA performed in this single-centered trial was only 5.3% which was considerably lower than that reported by other authors (15). FSA can be subdivided into perpendicular shaved-margin technique, tangential shaved-margin technique and cavity shave method depending on how and where the tissues were resected. In the perpendicular shaved-margin technique, each surface of the specimen is inked with a special color for orientation. Then the specimen is sliced perpendicular to its major axis and submitted for microscopic inspection. In the tangential shaved-margin technique, the specimen is oriented and dyed with a single color. The shavings were taken tangentially at a depth of 2–3 mm from all six surfaces and then sent for pathological examination of its inner surface. Interestingly, Wright *et al.* found that even

though tangential shaved-margin technique can conduct a more thorough inspection of the margin surface, it actually yielded a higher reoperation rates than the perpendicular shaved-margin technique (16). One major difference between cavity shave method and shaved-margin techniques is self-explanatory in that the cavity shave method takes additional tissues from residual cavity after lumpectomy instead of the specimen itself. Superior, inferior, medial, and lateral shave margins compassing the entire cavity or in some case only selective biopsies from cavity margins were removed, along with anterior and posterior margins if the resection had not extended to the dermis and pectoralis fascia, respectively (17). A research on the impact of different FSA method on the breast volume excised and reoperation rates has found that the cavity shave method resulted in the lowest rates of positive margin while its effect on tissue volume varied among surgeons. More specifically, for small tumors without extensive intra-ductal component (EIC), cavity shave method could lead to unnecessary resections (18). Meanwhile, there were also studies stating that cavity shave method could more than halved the positive margin rates without taking any toll on cosmetic outcomes (17,19). Notably, patients with ductal carcinoma in situ (DCIS) which is characterized by its discontinuous distribution and multifocality, more frequently fall victim to positive margin and reoperation when using other margin assessment methods (20). Fortunately, that is not the case with cavity shave method which has been proven to reduce positive margin rates by 65% among DCIS patients undergoing BCS (21). One systematic review encompassing 37 articles showed that compared with abandoning intraoperative margin assessment, FSA and IC could reduce reoperation rates from 35% to 10% and 11% respectively, which clearly testified for its clinical significance. Despite that, the pooled sensitivity of FSA and IC were 83% and 72% which would translated into quite a few cases of miss diagnosis, particularly in cases of DCIS (22). In order to overcome this flaw, Osako *et al.* came up with a solution based on FSA called intraoperative entire-circumferential frozen section analysis (IEFSA). The margins of the tumor-containing specimen were shaved into 6–12 pieces with sharp scissors around the entire circumference of the tumor, marking each as a clock position (the direction of the nipple was the 12 o'clock position) and the inner surface of each pieces were submitted for pathological inspection as was done in conventional FSA (23). Having taken into account the extremely low reoperation rates of 0.1% after BCS, IEFSA is still impractical and impossible to put into routine use due to its time assumption of approximately 110 min—about thrice the time of a conventional FSA, not to mention the increased workload for the pathology staff. In short, improving prognosis at the cost sacrificing efficiency makes IEFSA unsuitable for most medical facilities. A more reasonable attempt was to combine the two methods. IC was performed on all margins and FSA only on those deemed suspicious or positive by initial IC. The margin assessment method in their study had 87.6% sensitivity, 97.5% specificity and 95.8% accuracy. It turned out that while IC were easier and faster to perform than FSA, adding FSA could reduce its false positive rate from 13.4% to 2.5%, thus avoiding unnecessary re-excision (24). Unlike gross inspection which can be performed by surgeons, both FSA and IC strictly demand the presence of pathologists and preferably well-trained ones. Yet some medical facilities aren't equipped with pathology staff. And even if they do, the quality of examination differs because of sampling error—pathologists have to make subjective calls based on their experience and intuition as to which spots are mostly likely to contain residual cancer cells.

Conventional imaging methods

Ultrasound is utilized for intraoperative margin assessment in three forms: (I) localization of lesions by providing real-time visualization; (II) *ex vivo* ultrasound to verify the tumor edge; (III) application of both. In contrast to pathological methods, intraoperative ultrasound (IOUS) is more rapid and economical and it doesn't dictate the coordination between pathology unit and surgeons. Moreover, IOUS can be mastered by surgeons with shorter amount of time and it's also safe without radiation, easily accommodated in operating room which gives it edge over other imaging techniques. As a result, IOUS has received much recognition ever since it was introduced into the field of breast cancer treatment. According to a study spanning ten years, involving 945 BCS patients, only 5% patients received margin revision owing to inadequate resection. And the routine margin assessment method was IOUS guided lumpectomy followed by *ex vivo* ultrasound (25). Numerous studies have unanimously demonstrated IOUS's excellence at achieving negative margins, lowering resection tissue volume and improving overall aesthetic result and patient-satisfaction (26-28). Other than that IOUS has displayed even better performance when combined with other intraoperative margin assessment method such as FSA or gross inspection (29,30). IOUS for margin assessment

can be considered well-established when it comes to both palpable and impalpable lesions after years of try-out in the field (31). But one problem remains which still looms over is how to achieve margin negative resection in sonographically invisible breast cancer. It was proposed that taking advantage of the iatrogenic hematoma resulting from vacuum-assisted breast biopsy to localize the lesion was feasible (32,33). The downside to ultrasonography lies in its poor sensitivity to micro-calcification for micro-calcification is of vital importance to early detection of DCIS (34). So it can be inferred that ultrasound won't offer any distinct advantages in attaining adequate margin status in DCIS cases. Research designated for IOUS's diagnostic accuracy on DCIS margin status is inadequate. One study elucidated that the choice between IOUS-guided and wire-guided localization of DCIS lesions made no impact on final positive margin rates and reoperation rates of BCS whatsoever (35). A few ultrasound-based novel techniques permitted better recognition of micro-calcification with small samples, including MicroPure (MicroPure, Canon Medical Systems, Tustin, CA, USA), which is based on speckle reduction technique with a constant false alarm rate (CFAR) filter. By combination of CFAR and strain-compounding technique, MicroPure was able to effectively reduce speckles and elevate signal-noise ratio (SNR) to a higher level. Similarly, high-frequency ultrasound whose spatial resolution is less than 1 mm can easily make out micro-calcifications against background signals in *ex vivo* specimen (36-38). However, these experimental techniques await for further evidence.

Intraoperative specimen mammography has become the standard procedure for margin assessment during BCS in a proportion of western medical institutions. Perhaps, the most adopted ones are digital mammography and Two-dimensional (2D) Faxitron high-resolution specimen mammography (Faxitron X-Ray LLC, Lincolnshire, IL, USA). After excision, all specimens are oriented grossly by sutures and then handed over to the radiologist present who seals the specimen in a plastic bag and places it under the X-ray immediately. Cranio-caudol and latero-lateral (LL) views or shots from other directions are obtained. Radiologist and surgeon work side by side to analyze the breast imaging and a mutual decision is made by estimating the distance between lesion and closest margin. In various literature, the reported sensitivity of specimen mammography for intraoperative margin assessment ranged from 20.6% to 45.45% and pooled specificity ranged from 85.25% to 94.6% (39-41). Potential causes for poor sensitivity are: (I) the resolution is insufficient to discern minor lesions; (II) the specimen is compressed or distorted leading to a mismatch between specimen and lumpectomy. So margin revision might be done at the wrong spot on cavity wall, leaving residual cancer cells at large; (III) 2D imaging is inherently flawed in depicting 3D specimens comprehensively; (IV) dense breasts is another obstacle to identifying lesions by intraoperative mammography (42). Faxitron improved the detection rate of micro-calcifications by increasing the resolution of 2D image which translated into slimmer chance of reoperation (43,44). Vacuum apparatus for specimen could help prevent overlaps and underestimation of the surgical margin, even in the case of micro-calcifications. However, this vacuum technique is quite experimental with insufficient clinical data (45). To solve the dilemma essentially, 3D imaging must be introduced for well-rounded representation of the actual specimen. Two ideas derived from this are digital breast tomosynthesis (DBT) and micro-computed tomography (micro-CT). Both techniques found their way of scanning freshly excised breast tissue at different depths and reconstructing 2D cross-sections based on algorithm, much like CT. Eventually, the margin assessment would be performed using pseudo-3D image. A great deal of research has verified the effectiveness of DBT while research on micro-CT used for intraoperative margin assessment are mostly feasibility test. Urano *et al.* conducted a clinical trial in 2015 comparing DBT with conventional 2D specimen mammography on their diagnostic ability of DCIS and EIC respectively. It turned out that DBT outperformed digital mammography in both anterior-posterior (AP) views and LL views. In AP views DBT won by a narrow margin that was statistically insignificant (EICs 65% *vs.* 55%, DCISs 38% *vs.* 31%); in LL view, DBT could detect significantly more EICs and DCISs (EICs 42% *vs.* 10%, DCISs 42% *vs.* 8%). Therefore the study concluded that DBT could detect breast cancer more accurately than DM in LL views, indicating its potential to more precisely diagnose vertical invasion (46). Park *et al.* performed a similar study on a larger scale of 98 histologically diverse BCS patients. DBT images eventually yielded 74% sensitivity and 91% specificity which were superior to that of digital mammography (47). Pilot studies on micro-CT revealed unresolved issues including low sensitivity, lack of contrast at tumor-glandular inter-surface and absence of training and guidelines given the preclinical nature of the technology (48-50).

MRI was previously used for diagnosis of breast cancer

and preoperative planning for BCS, despite long-standing dispute revolving its efficacy (51). Some doctors even included breast MRI as part of their routine workup for newly-diagnosed patients since they believed that breast MRI could give accurate estimation of lumpectomy volume, spot multi-centric disease in advance and identify occult contralateral tumors (52). Accommodating MRI to operating room setting seems promising, albeit highly impractical which probably accounts for the reason why there was surprisingly few articles on this matter. Intraoperative MRI often comes with tailored operating rooms, a handful of trained personnel standing by which are costly. Not to mention the risks of infections and other complications which haven't been proper addressed (53). But even so, one team of researchers managed to fit MRI

in the Advanced Multimodal Image Guided Operating (AMIGO). Ensuing standard BCS, lumpectomy cavity was filled with normal saline to improve post-excisional MRI image-quality. Radiologist reviewed preoperative breast MRI to provide comparison for traces of residual tumor. The ultimate reoperation rate was 16.7% and mean operative duration of this method was 36 min (54). Some researchers took on another route by shrinking the size of MRI machine. They succeeded in their attempt to build a portable size magnetic resonance diffusion weighted imaging (MRDWI) machine who works by the principle of measuring the rate of water diffusion to reflect on structural mutations. Initially, the technology assisted in diagnosing acute cerebral infarction. In recent years, its use was further exploited in the field of breast cancer, rectal cancer, prostate cancer as well as glioma (55). The ClearSight^{TM} system (prototype of the ClearSight^{TM} system; Clear-Cut Medical Ltd., Rehovot, Israel) was a novel device which utilized DWI. When freshly excised specimens were scanned by the ClearSight^{TM} system, apparent diffusion coefficient (ADC) values were calculated as a quantifiable measurement of water diffusion to distinguish malignancy. This system is compact, easily transportable and each specimen measurement takes up 1–2 s, which are all qualities of great value in clinical practice (56). The results mentioned above seem optimistic but frankly most are rather experimental. Larger cohort is required to verify its usefulness and make further adjustments.

Unconventional imaging techniques

With the booming of imaging technologies, unconventional imaging techniques for intraoperative margin assessment in BCS have proliferated. Radiofrequency (RF) spectroscopy, bio-impedance spectroscopy, optical coherence tomography (OCT), photoaccoustic microscopy, nonlinear microscopy, Raman spectroscopy (RS), hyperspectral imaging and confocal microscopy are all examples of technology based on optical and non-optical characteristics of tissue, which have demonstrated superiority in detecting minute alterations of micro-structure. Among them, the RF spectroscopy-based the MarginProbe (Dune Medical Devices Ltd, Caesarea, Isarel) was approved by FDA in 2013 as an aid to margin assessment and it has in turn accumulated more clinical data. It was acknowledged that the tissue's overall electrical properties were codetermined by the electrical properties of cells and extracellular matrix. If breast cancer cells are present at margins, a certain electrical reading will differ from that of tumor-free margins. The MarginProbe is a handheld self-contained detector which automatically picks up signs of residual tumor within 7mm diameter range and a few millimeters deep and reports back to surgeons in the form of positive/negative. In 2014 Schnabel *et al.* published the result of a multicenter randomized trial enrolling 596 impalpable breast malignancies divided into two arms. A total of 25.8% of patients underwent re-excision in the control arm to whom standard margin assessment was applied whereas the percentage for the device arm was only 19.8% to whom the MarginProbe was used in addition to standard methods (57). The drop in reoperation rate did not affect the resection volume (58). Sebastian *et al.* provided the first report on routine use of MarginProbe in the USA. The post-operative re-excision rate plummeted from 25.8% to 9.7% with the adoption of the MarginProbe. The report emphasized that even though the majority (67%) of tumors included an intra-ductal component and DCIS patients amounted to 20% in this particular study, the MarginProbe's performance was still outstanding (59). Another research published in 2020 reported otherwise that there was no difference in reoperation rates between the MarginProbe and specimen mammography along with gross inspection (60). Even so, the competitive edge of the device is that it's operable by surgeons with no additional training and each measurement of specimen costs less than 5 min (59). This new technique of equivalent diagnostic efficacy could be a blessing for medical institutions who don't own their pathology lab or simply can't afford the time and human resources to perform intraoperative pathological margin assessment. The cost-effectiveness of the MarginProbe's console and disposable cap do require serious contemplation before launching it into Chinese

market. Another portable, rapid-scanning device was the ClearEdge (CE) based on bio-impedance spectroscopy which tells apart normal fatty tissue, normal fibrous tissue and abnormal tissue by dielectric properties. A unique feature of the CE is that a baseline measurement is made on each patient's normal breast tissue. If base-lined appropriately, the reoperation rate would have plunged to 8% (61).

OCT is viewed as the optical counterpart of ultrasound because both of them analyzes reflection and diffraction to form image of subsurface. OCT takes near-infrared light as an alternative to ultrasonic wave and it possesses surface penetration of 1–2 mm. The interpretation of OCT image is challenging and questions arises as to whether the identification of OCT image can be grasped within reasonable training time. Ha *et al.* conducted a multi-reader study stating that the average training time for radiologists, pathologists and surgeons is 3.4 h to achieve 87% accuracy on distinguishing suspicious from non-suspicious *ex vivo* breast specimen margin by OCT. As a result, the author concluded that training time for physicians from different subspecialties is relatively short and acceptable with the best candidate being radiologists (62). What's worthy of attention is that Cohen kappa coefficients was only 0.4 for the two surgeons, rendering their judgment relatively unreliable. So a couple of research teams have set their eyes on the combination of OCT and deep learning in hopes of using convolutional neural networks (CNNs) to cut back on learning cost and overcome inter-observer variance (63). Their intention was to design a system that could extract and classify certain features from OCT image and make automated assessment of margins. Studies regarding OCT combined with deep learning have attained 90.2–96% sensitivity, 91.7–92% specificity, 90–94% accuracy and 1–2 s assessment time which surpassed the achievements of human doctors (64,65). In retrospect, this also implies that larger database, which will reflect on its development costs, is required to train a more advanced system. Optical coherence elastography (OCE) has made some improvements on the basis of OCT. By measuring tissue deformation in response to mechanical loading reflecting mechanical properties, the contrast between tumor and normal stroma is further enhanced (66). Photoacoustic microscopy utilized multi-wavelength illumination to image different cellular and biological components. Accompanied by ultraviolet illumination which highlights the nuclei as is done in conventional histology, this method ultimately gives a histology-like imaging for margin assessment. The highlight of this modality is that only minimal processing of specimen and no contrasting agent is involved (67). Nonlinear microscopy relies on two-photon excited fluorescence (TPEF) imaging for cellular details and second harmonic generation (SHG) for extracellular matrix. Likewise, the imaging was performed on intact specimens to generate outcomes resembling histopathology. Three pathologists with no previous experience dealing with nonlinear microscopy completed the margin assessment with 93.3% specificity and 95.4% sensitivity (68). Raman spectroscopy deduces molecular composition of the specimen by inelastic scattering of light. Not only can it discriminate malignant tumor from normal tissue, it can also distinguish basal and luminal breast cancer using deep learning methods. The preliminary results using the combination of RS and deep learning is 88.8% sensitivity, 90.8% specificity and 90% accuracy (69). Thomas *et al.* came up with an automated 3D-scanner using RS to shorten the scanning time of all margins within clinically feasible range (70). Hyperspectral imaging of diffusely reflected light served as a unique optical signature of tissue since the light has undergone innumerable times of absorption and scattering. Therefore this fingerprint could be used for assessment (71). Spectrally encoded confocal microscopy (SECM) is a high-speed reflectance confocal microscopy technology that has a potential to rapidly image the entire surgical margin at sub-cellular resolution and accurately determine margin status intra-operatively (72).

Novel imaging agents

The revolution of novel imaging techniques places emphasis on modifying the imaging tool itself rather than the specimen. In fact, most of the optical tools mentioned can work with freshly cut, raw specimen. Working from another angle, labeling the tissue with imaging agents to enhance contrast may provide another solution to intraoperative margin assessment. Firstly, researchers attempted to mark well-known tumor markers such as folate-receptor alpha (FRα) and HER2. Studies at elementary stage include EC17 and Trastuzumab. EC17 was a FRα targeting contrast agent which produced fluorescent at 500 nm wavelength. Since FRα over-expression was observed in a proportion of breast cancers, researchers thought that EC17 was a potential imaging agent for detection of breast cancer. Progress has been made in tumor-specific imaging for FRα positive ovarian cancer using EC17. Notwithstanding, the success couldn't be duplicated on breast cancer due to

the serious interference of auto-fluorescence at 500 nm wavelength. The auto-fluorescence was so interfering to the point that cancer cells were barely discernable. Therefore, the experiment was brought to a temporary stop (73). Trastuzumab binds specifically to HER2. Accordingly, Trastuzumab double-labeled with BHQ3 and fluorescein can visualize HER2 expression with the assistance of dual *in vivo* photoacoustic and fluorescence imaging. This could potentially apply to identifying HER2 positive breast tumors at surgical margins (74). The application is somewhat limited for HER2 overexpression only appears on 25–30% of breast cancer. To ascertain the patients' HER2 status, preoperative biopsies are basically mandatory (75).

Studies have also devoted attention to repurposing common contrasting medium such as indocyanine green (ICG) originally used for liver reserve function assessment and 18F-FDG used for PET-CT. The safety of this these agents are guaranteed by years of clinical experience, however its ability to visualize breast cancer is yet to be seen. Grootendorst *et al.* injected patients with ^{18}F-FDG intravenously 45–60 min prior to surgery. Then they observed the resected tissue by Cerenkov luminescence imaging (CLI) system. The agreement between histological margin distance and margin distance estimated by two surgeons based on CLI is 0.76 and 0.86 respectively (76). ICG coupled with near-infrared (NIR) imaging was shown to detect canine mammary tumors >2 cm with 30% specificity and 93.3% sensitivity (77). Both methods were insensitive to small tumors and DCIS because the fluorescence intensity is dependent on the flurophore intake which is low in small tumors. Wojtynek *et al.* synthesized an ICG-loaded hyaluronic acid nanoparticle in order to improve ICG uptake into tumor cells. They hypothesized that by enhancing tumor signal-to-noise ratio and quality image, visual guidance of tumor removal would be bettered, yet the real impact remained unclear (78). The dual contrast agent of anti-B7-H3 antibody and ICG could be another solution. B7-H3 has been shown to be upregulated in DCIS and breast cancer compared to both normal breast tissue and benign lesions. Even foci of DCIS showed substantially high expression of B7-H3 which makes it a favorable tumor marker. Conjugating the anti-B7-H3 antibody and ICG not only improved its overall specificity but also showed great potential at identifying early-stage DCIS (79).

Other innovations involve enzyme labeling coupled with imaging system. Ueo *et al.* developed γ-glutamyl hydroxymethyl rhodamine green(gGlu-HMRG) based on the fact that the enzyme γ-glutamyltranspeptidase (GGT) is overexpressed on membranes of cancer cells, but is not expressed in normal tissue. gGlu-HMRG is a fluorescent probe activated by GGT (80). The binding of the two substances produces intermediate which transforms into flurophores (81). When gGlu-HMRG was applied to freshly cut specimen, viable cancer cells generated green fluorescence with time-dependent increase which distinctly stood out from the auto-fluorescence at different wavelength with constant intensity generated by normal surrounding tissues. The gGlu-HMRG fluorescence method took 5 min to complete margin assessment and lesions as small as 1 mm could be visualized. The sensitivity was 92% and specificity 94%. If further adjustments can be made to address the false positive caused by benign mastopathy and hyperplasia, this method will have better potential to advance into the clinic (80). Smith *et al.* proposed the use of LUM015, a novel PEGylated protease-activated far-red fluorescent imaging agent. The Lumicell (LUM) Imaging System (Lumicell, Wellesley, MA, USA) which provides detection of residual tumor during surgery contains a hand-held probe for real-time fluorescent recordings, software for image analysis and LUM015 as imaging agent. The Lumicell (LUM) Imaging System can scan the cavity wall *in vivo* as well as the specimen *ex vivo* which was a strength. Also, the auto-fluorescence in the background didn't pose any threat and each scan of 2.6 cm diameter field took 1 s (82).

Limitations

Since all articles were reviewed and selected manually, the number of articles reviewed is limited. A meta-analysis would have provided a more objective evaluation on the methods' precision, sensitivity and specificity, but due to the narrative nature of this review, a meta-analysis was not performed. Furthermore, the definition of negative margin varies among medical centers, undermining the effectiveness of a direct comparison between the efficacy of assessment method. Last but not the least, there is a lack of a universal guidance for intraoperative margin assessment methods, this could render the comparative results invalid.

Conclusions

Ensuring negative margins is crucial to patients' diagnosis. The 2014 SSO-ASTRO Consensus Guideline has defined negative margin as "no ink on tumor", partially settling the debate revolving safe margin distance that's been going on for decades and thus redirecting the center of

attention towards practical ways of achieving negative margins. Pathological intraoperative margin assessment methods are accredited after years of clinical experiments but they are also notoriously strained and strict on the pathologists. Conventional imaging techniques are well-established as part of the preoperative routine checkup for BCS patient. Apparently, some researchers have taken a fancy to its time-efficiency and they attempt to convert it into intraoperative margin assessment methods. Two major problems faced by conventional imaging methods are poor visibility and tremendous workload for radiologists. As for the poor visibility at the junction of tumor and normal breast tissue, one solution is to improve resolution in order to provide a clearer and more concise view and the other solution is enhancing contrast by labeling the tissue with imaging agents. Recently, mainstream seemed to choose the first solution over the latter which was compatible with clinical need. Most unconventional imaging techniques are evolving towards a portable, self-contained and simple form. But most of these techniques were only tested on the feasibility level, pending clinical trials. Also, the cost of these novel devices should also be taken into consideration. Another emerging trend is to have artificial intelligence (AI) participate during the image identification. OCT and RS's attempt at incorporating AI system proved quite successful with preliminary results demonstrating higher accuracy and speed than human doctors.

Besides, BCS for DCISs and BCS after neo-adjuvant chemotherapy remain major obstacles to obtaining negative margins. Despite the numerous researches dedicated to intraoperative margin assessment method on DCIS, most attempt proved unfruitful. The MarginProbe is certainly a promising tool based on the current study outcomes, but more investigations are necessary before leaping to conclusion. Neo-adjuvant chemotherapy is gaining popularity among breast cancer patients. Yet this group of patients is generally excluded from research regarding intraoperative margin assessment. Future study is warranted to fill the gap.

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Footnote

Reporting Checklist: The authors have completed the Narrative Review reporting checklist. Available at https://gs.amegroups.com/article/view/10.21037/gs-21-652/rc

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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