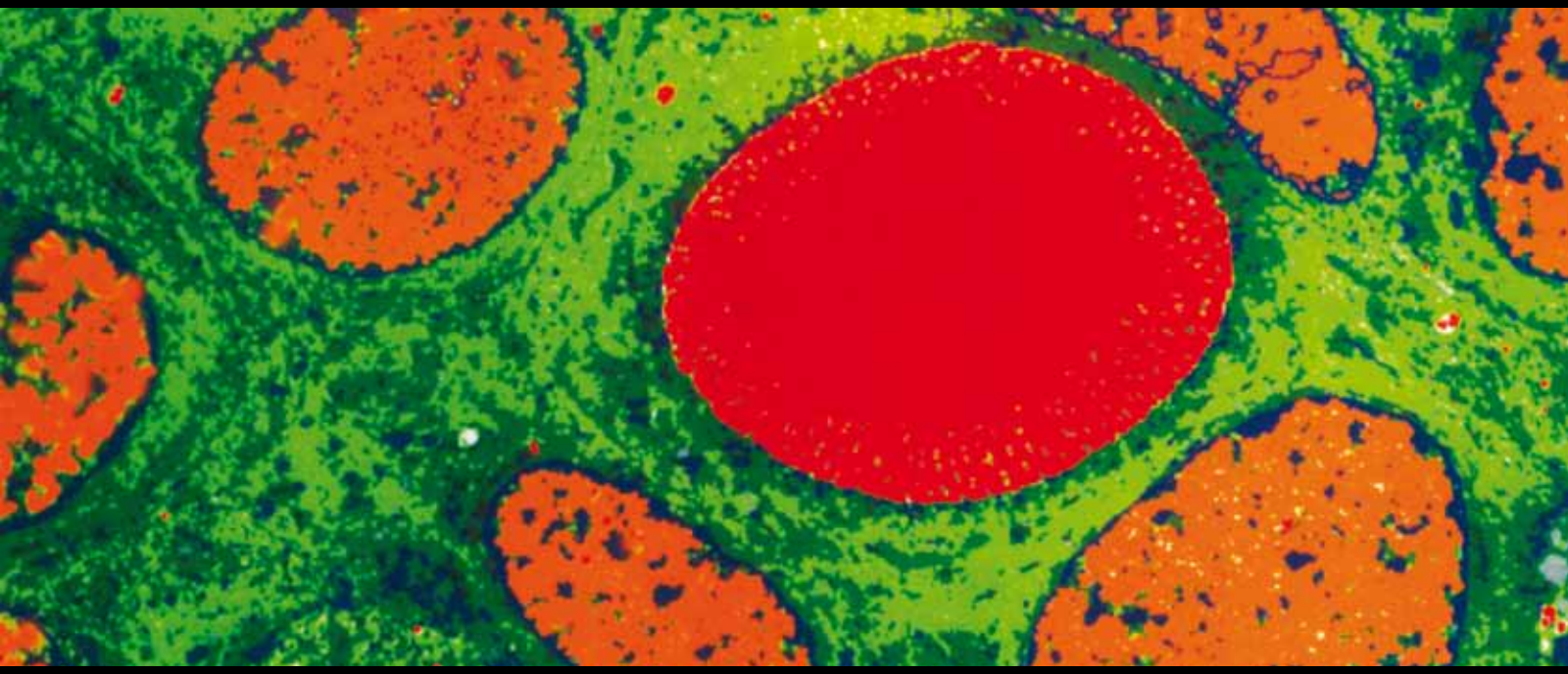
International Journal of Breast Cancer

Large-Format Histology in Diagnosing Breast Carcinoma

Guest Editors: Tibor Tot, Vincenzo Eusebi, and Julio A. Ibarra



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Editorial Board

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Editorial

Large-Format Histology in Diagnosing Breast Carcinoma

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This special issue represents a unique effort to review the experience with this beneficial technique in different pathology laboratories from the United States, Canada, and Europe. Although large-format histology was the topic of a few monographs and has also been discussed in individual reports in a number of journal articles, this special issue is the first to collect knowledge and experience with this technique as used in routine diagnostic histopathology.

The authors of the first paper by L. Tabár et al. underline the necessity of detailed and systematic correlation of the radiological and pathological findings in diagnosing breast carcinoma and also the need for adequate histology techniques for achieving this correlation. They illustrate how thin and thick large-format histology technique can contribute to achieving such correlation. M. P. Foschini et al. widen the indication for using large-format slides from breast pathology to the pathology of other organs in their beautifully illustrated paper. Tot presents a unique series of 1000 consecutive breast cancer cases worked up with detailed and systematic radiological—pathological correlation and documented in large-format histology slides. The study illustrated the complex morphology of breast carcinoma, where the majority of the cases have multifocal or diffuse in situ or invasive components. M. R. Foster et al. calls attention to the unexpected findings of potential clinical significance which may remain occult if conventional histology technique is used instead of large-format histology. They have evidenced such findings in 26% of their case series. J. A. Ibarra reports on his unique experience with combining standard sections and large-format sections to assess surgical specimens removed following neoadjuvant chemotherapy in a community hospital in the USA, which is an increasingly important issue in every pathology laboratory diagnosing breast carcinomas. In a comprehensive contribution to this special issue, F. L. Tucker summarizes his experience with introducing imaging-assisted large-format histopathology in a pathology department. He demonstrates the detailed program of this process, the rationale and the development in a non-profit health system in the United States. He also reports on clear advantages of the large-format technique used in context with magnetic resonance imaging, compared to conventional histology without such guidance. S. Hofmeyer et al., a research team from a laboratory with 30 year experience in using large-format histology in Falun, Sweden, presents interesting results of a scientific study comparing the subgross morphology of invasive ductal and invasive lobular carcinomas of the breast. One of the highlights of the special issue is the paper demonstrating 3D pathology volumetric technique for calculating breast tumor volume from digitalized whole-mount serial large-format sections by G. M. Clarke et al., a research team from Toronto. As similarities between breast cancer and prostate cancer in both morphology and technical requirements for adequate histological analysis are obvious, the special issue also includes the paper of R. Montironi et al. on total embedding of radical prostatectomy specimens and using large-format histology.

Despite the distance between the laboratories and the differences in working conditions, the experience and expert opinions reported in the papers of this special issue is

consistent in evidencing advantages of the large-format histopathology method over the traditional small-block sampling method both as a substitute for standard histopathology but also as an adjunctive tool. The unconcealed ambition of the authors, the publisher, and the editors is to, by providing this evidence, influence the members of the pathology community to accept this method and introduce it in their own everyday practice.

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Research Article

3D Pathology Volumetric Technique: A Method for Calculating Breast Tumour Volume from Whole-Mount Serial Section Images

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Tumour size, most commonly measured by maximum linear extent, remains a strong predictor of survival in breast cancer. Tumour volume, proportional to the number of tumour cells, may be a more accurate surrogate for size. We describe a novel "3D pathology volumetric technique" for lumpectomies and compare it with 2D measurements. Volume renderings and total tumour volume are computed from digitized whole-mount serial sections using custom software tools. Results are presented for two lumpectomy specimens selected for tumour features which may challenge accurate measurement of tumour burden with conventional, sampling-based pathology: (1) an infiltrative pattern admixed with normal breast elements; (2) a localized invasive mass separated from the *in situ* component by benign tissue. Spatial relationships between key features (tumour foci, close or involved margins) are clearly visualized in volume renderings. Invasive tumour burden can be underestimated using conventional pathology, compared to the volumetric technique (infiltrative pattern: 30% underestimation; localized mass: 3% underestimation for invasive tumour, 44% for in situ component). Tumour volume approximated from 2D measurements (i.e., maximum linear extent), assuming elliptical geometry, was seen to overestimate volume compared to the 3D volumetric calculation (by a factor of 7x for the infiltrative pattern; 1.5x for the localized invasive mass).

1. Introduction

Tumour size is a commonly used predictor of survival in breast cancer and correlates strongly with lymph node involvement [1–5]. Tumour size is included in the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) Cancer Staging Manual [6] and is represented by the maximum linear extent of disease.

Here we describe a novel methodology for measuring tumour volume in lumpectomies, referred to as the 3D pathology volumetric technique. For proof of concept, we demonstrate the technique using two lumpectomy specimens, comparing volumes as measured from serial whole-mount sections versus simulated conventional, sampling-based pathology. Serial, whole-mount sections are produced utilizing "3D pathology" techniques and then digitized [7]. Conformational distortion is minimized by first encapsulating the fresh tissue sample in a buoyant, density-matching gel. Automatic microwave processing is employed to accelerate processing of the large tissue samples. Quantitative

analysis is performed on the large image dataset (30–70 GB) using custom software tools to create volume renderings and estimate volumes of both *in situ* and invasive disease.

Tumour volume may provide a more accurate representation of size, because tumour is a 3D entity. Volume can vary significantly among tumours which have the same maximum linear extent. A simple ellipsoid model has been used to approximate volume using maximum linear extent and has been shown to provide a more accurate assessment of the volume of breast tumours compared to modelling the tumour as a sphere; in a retrospective study of 165 tumours measuring 2.5 cm or less, the largest diameters in anterior-posterior (AP), medial-lateral (ML), and superior-inferior (SI) dimensions were distinct in 96.4% of the cases [8]. Biologically, tumour volume is proportional to the number of cells, and theoretical models have shown that metastatic potential depends on the total number of cells and the probability of each to disseminate [9].

To date there are no clear data supporting the superiority of tumour volume over maximum linear dimension as a predictor of outcome in breast cancer, although current pathology guidelines are beginning to incorporate 3D parameters (e.g., eccentricity factor) [10–12]. Some studies (limited to unifocal tumours) fail to demonstrate stronger prediction with tumour volume, when estimated using the ellipsoid approximation, compared to maximum linear extent [13]. However, for lung cancer, a significant association between tumour volume and both overall survival and disease-free survival has been shown [14, 15]. In staging of prostate cancer, tumour volume has been shown to be a strong predictor of lymph node metastasis [16]. Typically, in conventional work for these sites volume is estimated from linear measurements assuming ellipsoid geometry.

One of the impediments to establishing the prognostic value of tumour volume in breast cancer staging stems from inconsistencies in measurement technique, especially for more complex tumour patterns (e.g., diffusely infiltrating or multifocal). Diffusely infiltrating tumours exhibit a morphology in which the cancer cells are interspersed with normal epithelial and stromal elements making precise measurement of the volume of tissue occupied by tumour cells difficult. Multifocality occurs in about 30% of breast cancers [17] and is associated with local recurrence [18] and decreased survival [19, 20]. Multifocality is also an independent prognostic factor for local relapse and distant metastases [21]. AJCC/UICC guidelines are based on the size of largest focus while some studies demonstrate prognostic value for the aggregate diameter instead [22] or show that volume must be controlled to demonstrate the association between multifocality and lymph node involvement [23]. For multifocal prostate cancer, significant overestimation of mean volume has been shown and at least one measurable tumour is missed in approximately 17% of cases, when the ellipsoidal method is used, assuming a gold standard based on serial sectioning [24]. Similar observations have been noted for lung carcinoma, when estimating volume using an ellipsoidal approximation along with maximum extent measured from serial standard-format histological sections [25].

Whole-mount sections have been proposed as a "gold standard" for evaluating multifocality (defined here as two or more foci of either invasive or *in situ* carcinoma where the foci are separated by intervening normal breast tissue) and may permit more accurate assessment of tumour burden where conventional sampling is difficult (e.g., due to lack of desmoplastic reaction or infiltrative growth pattern). Studies using whole-mount or large-section pathology techniques confirm accepted prevalence rates for multifocality (observed in 31.9% of 1–14 mm invasive breast carcinomas, in a study of 301 consecutive cases) and confirm that multifocality is an independent prognostic factor for survival at 10 years [26, 27]. Furthermore, multifocality is associated with a more than twofold increased risk of vascular invasion and lymph node metastasis compared to unifocal cancer [26]. However, the AJCC/UICC guidelines require only measurement of the largest tumour focus [6]. Whole-mount sections would enable the entire tumour burden comprising any secondary foci to be more fully assessed.

In this work, we extend the principle of increasing coverage by incorporating serial sectioning, while supporting the flaccid specimen to reduce conformational distortion, to create a 3D representation of tumour histology. We describe a "3D pathology volumetric" technique which utilizes a set of whole-specimen, whole-mount serial section images to create volume renderings and calculate invasive and *in situ* tumour volumes. The volumetric analysis is demonstrated using two lumpectomies with features that might be associated with underestimation of tumour burden when conventional histological sampling is used. Calculated volumes are compared with those obtained from simulated conventional histological sampling, and also with estimated volumes obtained from linear measurements assuming ellipsoid tumour geometry.

2. Methods

Two lumpectomy cases were selected retrospectively from the whole-mount tumour bank at the Biomarker Imaging Research Laboratory at Sunnybrook Health Sciences Centre. The specimens were initially obtained from the Department of Pathology at Sunnybrook with the approval of the institutional Research Ethics Board, excluding lumpectomies that would be submitted *in toto* when conventional sampling-based techniques would be used. The diagnosis in both cases was reported as infiltrating ductal carcinoma not otherwise specified (IDC NOS). One case (Case A) is an invasive tumour that infiltrates diffusely, mostly without a desmoplastic reaction or destroying intervening benign breast elements, such that a typical tumour section shows invasive carcinoma admixed with benign breast elements. The other case (Case B) is a localized invasive tumour with foci of *in situ* carcinoma away from the invasive tumour and separated from it by intervening benign tissue. Case B is, therefore, representative of the challenge inherent in measuring tumour volume for multifocal disease, using conventional techniques.

Both specimens were prepared and processed using techniques collectively referred to as "3D pathology" [7, 28].

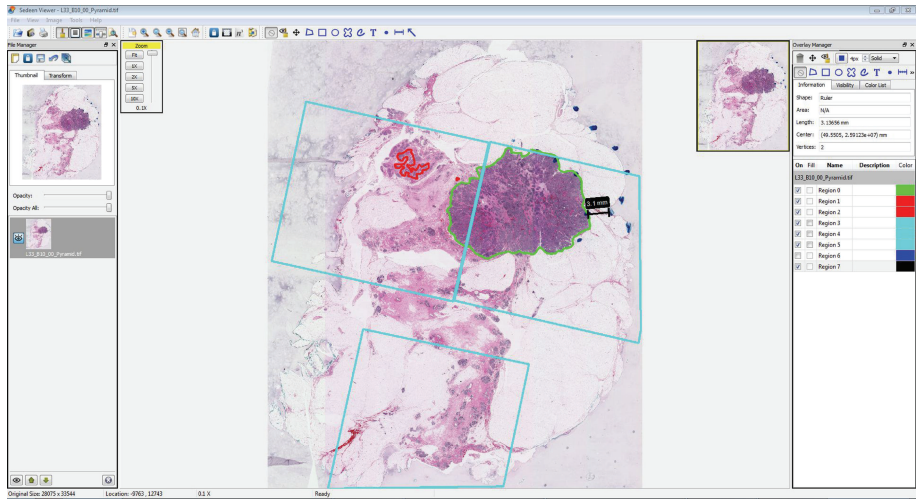

Figure 1: Sedeen viewing program. A whole-mount breast section is annotated, regions of interest are defined, and coordinates are stored for quantitative analysis.

Each fresh, unfixed specimen was first suspended in a buoyant gel (3.5% agar), and after setting, each tissue-gel block was serially sliced (in the ML dimension) into uniform, 4 mm thick slices using a rotary slicer (Berkel Products Co., Ltd.; ITW; Glen Lake, Il, USA). The tissue-gel slices were fixed overnight in 10% neutral buffered formalin and then processed using a 16-hour program developed for whole-mount breast tissues in an automatic tissue processor that uses microwave assistance (Pathos Classic; Milestone Medical srl; Sorisole, Italy). The processed slices were embedded in custom moulds and one 4 μm thick tissue section was obtained from the top of each block using a sliding microtome (SM2500; Leica Microsystems, Germany). The sections were stained with hematoxylin and eosin (H&E) using manual techniques.

The two sets of whole-mount sections, mounted on 7.62 cm $\times$ 10.16 cm glass microscope slides, were produced comprising 14 slides from Case A, and 23 from Case B. The set was digitized (Tissue Scope; Huron Technologies Inc.; Waterloo, ON, Canada) using a pixel spacing of 2 μm as previously determined to optimize tumour detectability and computational feasibility [29]. The size of the total image dataset was 28 GB and 69 GB for Case A and B, respectively (average 0.4–3 GB per section). The images were interpreted by a pathologist (KL) using Sedeen Viewing Program (SElective Decoding and Encoding Engine [30]; developed by Dr. Anne Martel and Danoush Hosseinzadeh at Sunnybrook Research Institute). This software tool was developed to enable interactive display and quantitative work for large image datasets using conventional workstations. Features include panning and zooming, tools for contouring, and annotating and measuring tumours or multiple regions of interest (e.g., invasive tumour and *in situ* tumour) (Figure 1). Using this software, manual, digital contouring of tumour in all of the images was performed and coordinates were stored to file in "extensible markup language" (.xml) format. Using a "virtual sampling" technique described elsewhere [7], a set of images simulating the conventional, sampling-based pathology evaluation (i.e., standard sized slides) was created.

For volume calculations and visualization, the .xml files were imported into MATLAB (MATLAB7.11.0.584 (R2010b); Math Works Inc.; Natick, MA, USA), to generate a volume rendering for each case, and to calculate tumour volumes, for *in situ* and invasive disease separately. For each whole-mount image, three binary image arrays were generated to represent the following three features as defined by the digital contouring: *in situ* disease, invasive disease, and normal tissue, with pixels enclosed in a contour set to intensity values R, G, B = 1, and 0 otherwise. The three binary arrays were stacked and then extruded in the medial-lateral dimensions, by layering duplicate copies of the arrays at every 2 μm, corresponding to the lateral resolution, to fill the 4 mm gap between whole-mount sections. In this way, isotropic 4 mm voxels were preserved in the volume rendering. Tumour volumes were calculated by Riemann summation of all the positive pixels (RGB intensity = 1) in the volume rendering, for *in situ* and invasive disease separately. For the set of images simulating conventional pathology technique, volume was calculated similarly by multiplying the sampled tumour area by the thickness of 4 mm.

Finally, tumour volume was estimated from 2D measurements of maximum linear extent in the ML, AP, and SI dimensions using the following ellipsoidal approximation: $\text{Volume}_{\text{ellipsoid}} = 1/6\pi\ \text{ML}\cdot\text{AP}\cdot\text{SI}$. The maximum linear extent in ML, AP, and SI dimensions was measured from the whole-mount sections.

3. Results

For Case A, the set of serial whole-mount sections which contain tumour is shown in Figure 2. These images also depict the locations where conventional samples would

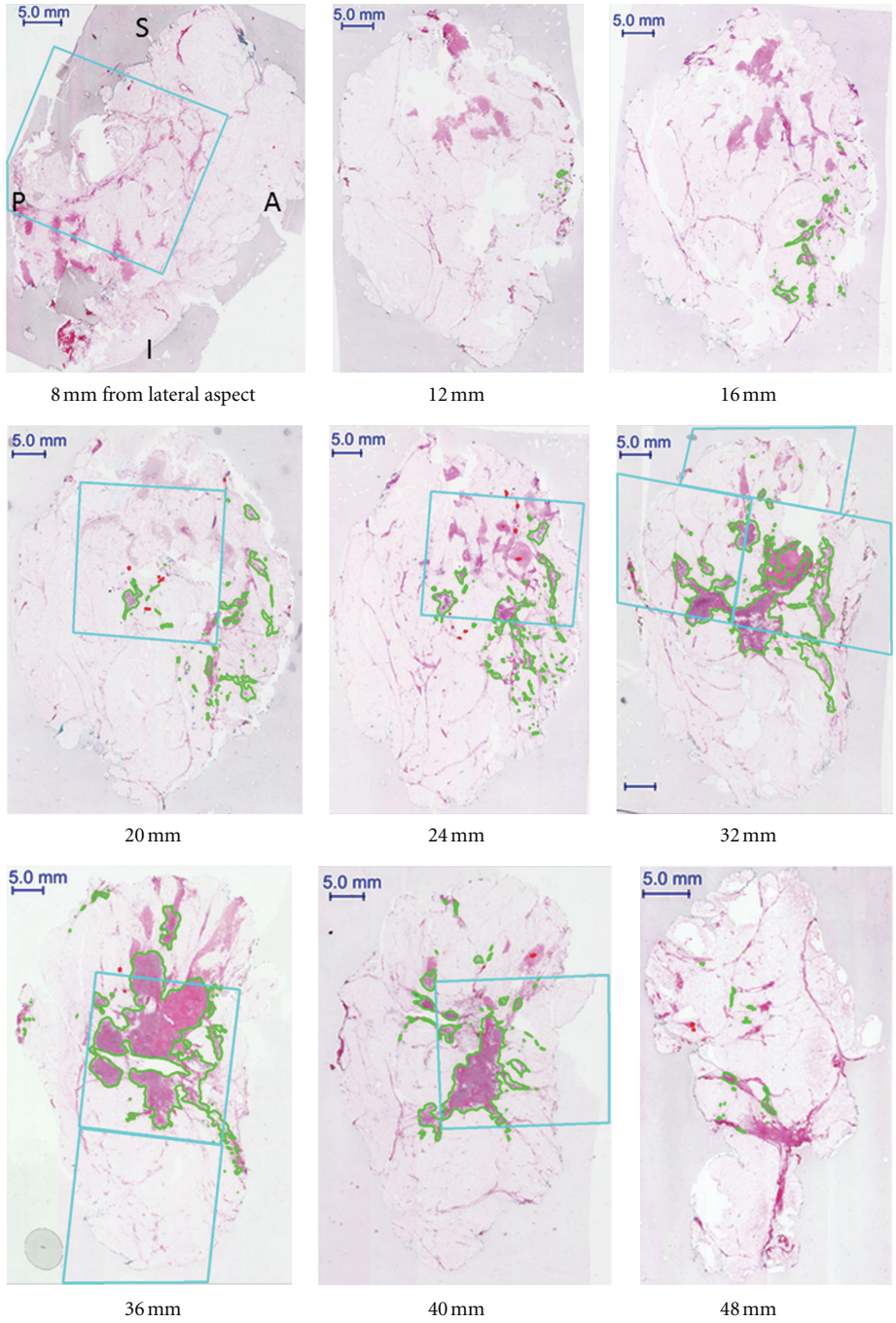

Figure 2: Selected serial whole-mount sections (Case A) in IDC NOS, diffuse pattern. The orientation is consistent for all of the images (A = anterior; P = posterior; S = superior; I = inferior). Invasive tumour is digitally contoured in green. The cyan boxes represent areas which would be sampled in the conventional pathology evaluation, as assessed by the pathology assistant on optical images aided by palpation of tissue slices. There are 14 whole-mount sections in total.

be taken as determined by the pathology assistant [7]. The volume rendering for this case is shown in Figure 3. Figures 4 and 5 present the serial whole-mount sections and volume rendering for Case B.

Tumour volume, calculated from the full renderings as well as from the locations corresponding to conventional sampling, are compared in Table 1, along with measurements of maximum linear extent and the corresponding ellipsoidal volume.

4. Discussion

The 3D pathology volumetric technique facilitates visualization of spatial relationships within lumpectomies and may

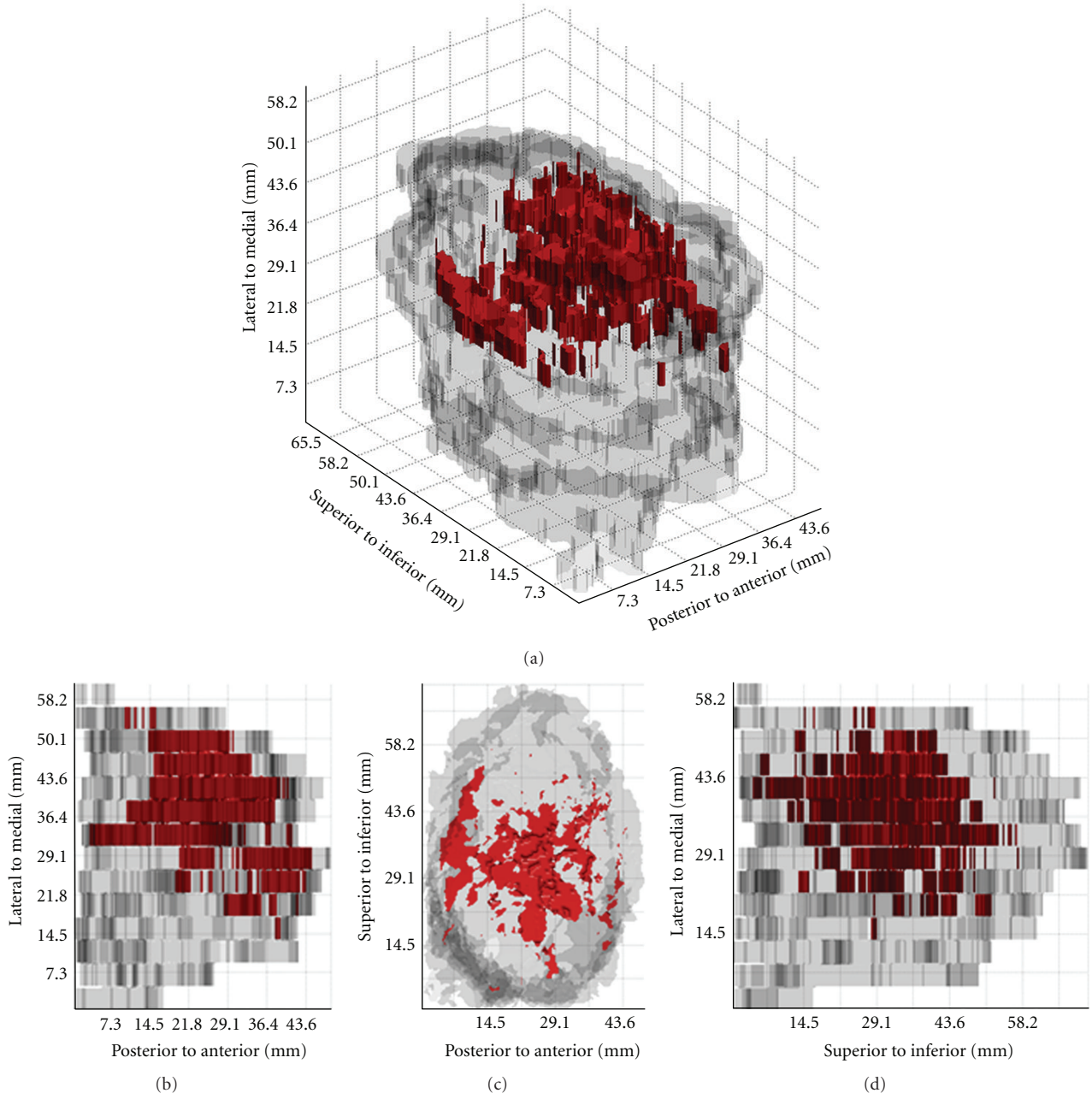

Figure 3: 3D tumour visualization for Case A: (a) volume rendering and projection views through the following dimensions: (a) SI, (b) ML, and (c) AP.

provide a more accurate surrogate for tumour burden. It is seen from Case A that when the tumour infiltrates without eliciting a desmoplastic reaction and without destroying the normal benign breast elements, the conventional 2D measurements using maximum extent in the three dimensions (AP, ML, and SI) may overestimate tumour burden. If the tumour is approximated by an ellipsoid, then using these three measurements the tumour volume is overestimated by a factor of approximately 7 (Table 1). However, when the tumour is localized (Case B), the estimated volume based on measurements of maximum extent assuming ellipsoid tumour distribution more closely approximates the calculated volume, overestimating by a factor of 1.5. Thus, the 3D volumetric technique may remove bias introduced by assumptions of tumour geometry when less precise approximations are used.

From the volume renderings (Figures 3 and 5), the relationship of invasive tumour to the *in situ* component that is present away from the tumour can be readily appreciated. Similarly, the locations of close or involved margins can be easily identified. Taking the close or involved margins seen in the serial whole-mount section images (Figures 2 and 4) as a

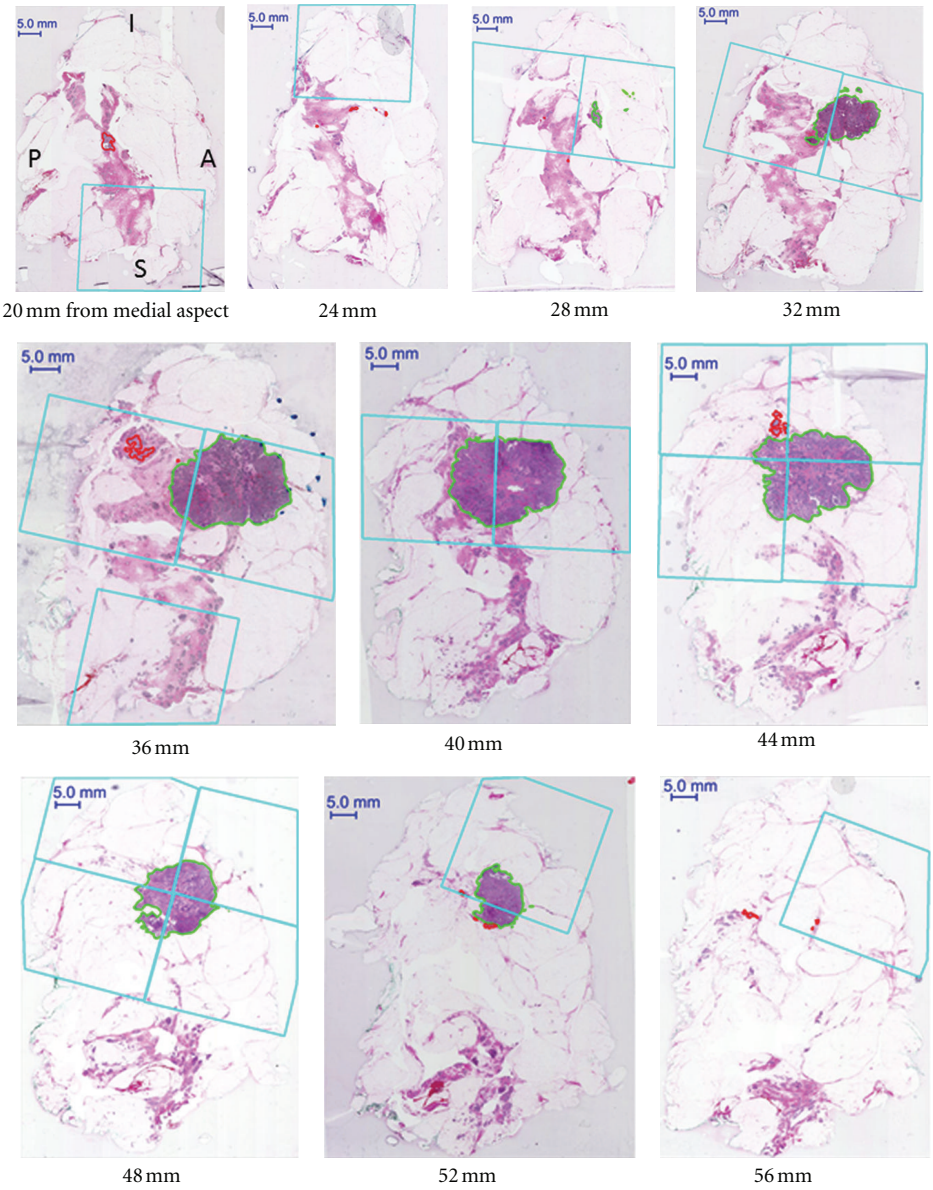

Figure 4: Selected serial whole-mount sections (Case B) in IDC NOS, localized pattern with *in situ* component extending away from the tumour. The orientation is consistent for all of the images (A = anterior; P = posterior; S = superior; I = inferior). Invasive tumour is contoured in green, *in situ* in red. The cyan boxes represent areas which would be sampled in the conventional pathology evaluation, as assessed by the pathology assistant on optical images aided by palpation of tissue slices. There are 23 whole-mount sections in total for this case.

tissue landmark, corresponding areas are seen in the volume renderings (Figures 3 and 5) within the context of the 3D specimen. For example, tumour at the inferior margin in the section 10 mm from the lateral aspect in Case A (Figure 2) is seen in the volume rendering (Figure 3), relative to another close margin near the inferior-posterior midpoint.

Even within the limitations of a 2D presentation, whole-mount serial sections can enable more accurate estimates of tumour burden (Case A, Figures 2 and 4) compared to conventional sampling. Case A indicates the power of the 3D volumetric technique to identify and measure the extent of tumour in those lesions in which the tumour cells extensively infiltrate the normal tissue. Comparing tumour volume to the subset which would be sampled, consequently in this case, only 70% of the total invasive tumour volume would have been captured in the conventional approach. For the localized mass in Case B, however, the representation increases to 97%.

Case B illustrates the ability of the 3D pathology volumetric technique to enhance visualization and resolve tumour foci which are separated by normal tissue. At least two distinct foci of *in situ* disease appear as follows: one at 20–28 mm from the medial aspect and one at 36–44 mm from the medial aspect. In this case, only 56% of the *in situ*

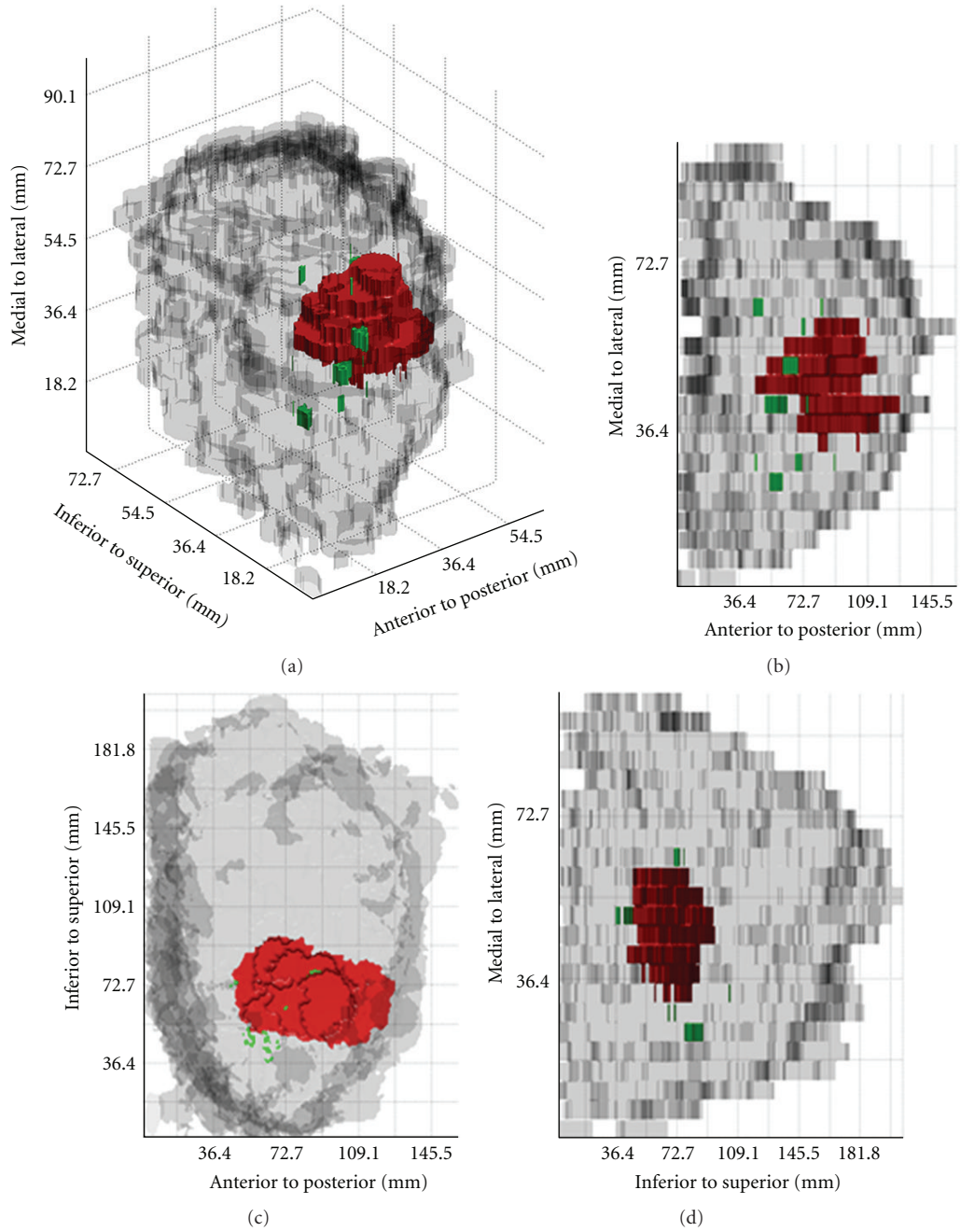

Figure 5: 3D tumour visualization for Case B: (a) volume rendering and projection views through the following dimensions: (a) SI, (b) ML, and (c) AP. Regions of invasive tumour are shown in red, and *in situ* disease in green.

component would be captured using conventional sampling. Whole-mount serial sections may also help to capture DCIS, which might otherwise be difficult to sample adequately. Moreover, the focality of the *in situ* component in the ML dimension is better appreciated from the volume rendering (Figure 5) compared to the serial section presentation (Figure 4) in which continuity between serial sections is more difficult to synthesize.

Studies comparing methodologies for measuring tumour volume in other cancer sites support our observations. For prostatectomies, where the majority of tumours assume a multifocal distribution, five techniques were compared. These include the ellipsoidal approximation and Riemann summation for serial sections 3 mm and 6 mm apart. Taking the calculation for serial sections 3 mm apart as the "gold standard," using the sections 6 mm apart instead would cause the mean tumour volume to be overestimated by 29.5%. Using the ellipsoidal approximation, volume varied widely compared to the gold standard (0.5%–132%) [24]. Similarly for lung tumours, it was shown that Riemann summation

Table 1: Summary of tumour volumes. Tumour volumes were calculated following three methods: from digitally contoured, serial whole mount sections following the 3D pathology volumetric technique; from the regions indicated by the locations of simulated conventional sampling; from three linear measurements of maximum tumour extent (in ML, AP, and SI dimensions) assuming an ellipsoidal tumour shape.

	Method	Case A	Case B
Invasive	3D pathology volumetric	4.179 cm^3	5.105 cm^3
	Conventional samples	2.857 cm^3	4.954 cm^3
	Ellipsoidal approximation	29.489 cm^3 (44 mm × 40 mm × 32 mm)	7.540 cm^3 (25 mm × 24 mm × 24 mm)
In situ	3D pathology volumetric	N/A	132 mm^3
	Conventional samples	N/A	75 mm^3
	Ellipsoidal approximation	N/A	6 mm^3 (2 mm × 2 mm × 3 mm)

(using sections 10 mm apart) is most accurate compared to the ellipsoid approximation and especially compared to a spherical approximation, with the less accurate methods overestimating volume [25].

The 3D pathology volumetric technique is well suited to validation of noninvasive imaging modalities (e.g., magnetic resonance imaging) which are used to estimate 3D tumour descriptors including volume and surface area. Surface area might also serve as a useful reporter for tumour burden in conjunction with volume. Our methodology lends itself to exploration of other quantitative measures including surface area as a simple extension of the volumetric principle. Using the techniques we describe here, we can obtain precise, volumetric measurements of challenging features (e.g., separate foci of disease, infiltrating pattern) and in future studies the prognostic significance of these presentations can be explored using the volumetric technique. Studies on the prognostic significance of tumour volume with relation to biology are scarce. It has been suggested that tumour volume is inversely related with microvessel density, and that metastasis may, therefore, be an early event [31]. The 3D volumetric technique can provide a platform for precise, volumetric measurements of both morphological and biological patterns, which can be correlated to validate prognostically significant relationships.

5. Conclusion

Using the 3D pathology volumetric technique, tumours are visualized in 3D and spatial relationships between key features (e.g., close or involved margins) are readily appreciated. The technique also enables calculation of tumour volume, which may be a more accurate representation of tumour burden or size compared to 2D linear measurements. This technique also reduces the errors associated with undersampling in conventional histopathology, which can result in underestimation of tumour burden. The volumetric technique may be particularly useful in cases where accurate representation using conventional tissue samples may be difficult (e.g., infiltration without desmoplastic reaction and tumour admixed with normal epithelium, or localized invasive mass with multifocal *in situ* carcinoma away from the tumour). We have shown that in such cases tumour burden can be significantly underestimated using conventional methods. Conversely, tumour volume can be markedly overestimated, especially for more complex tumour distribution (e.g., infiltrative), when simple approximations such as an ellipsoid model are used instead of the volumetric approach. Additional studies to evaluate the predictive value of tumour volume on patient outcome are underway.

Conflict of Interests

The authors have no conflict of interests to declare.

Acknowledgments

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Review Article

Imaging-Assisted Large-Format Breast Pathology: Program Rationale and Development in a Nonprofit Health System in the United States

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Modern breast imaging, including magnetic resonance imaging, provides an increasingly clear depiction of breast cancer extent, often with suboptimal pathologic confirmation. Pathologic findings guide management decisions, and small increments in reported tumor characteristics may rationalize significant changes in therapy and staging. Pathologic techniques to grossly examine resected breast tissue have changed little during this era of improved breast imaging and still rely primarily on the techniques of gross inspection and specimen palpation. Only limited imaging information is typically conveyed to pathologists, typically in the form of wire-localization images from breast-conserving procedures. Conventional techniques of specimen dissection and section submission destroy the three-dimensional integrity of the breast anatomy and tumor distribution. These traditional methods of breast specimen examination impose unnecessary limitations on correlation with imaging studies, measurement of cancer extent, multifocality, and margin distance. Improvements in pathologic diagnosis, reporting, and correlation of breast cancer characteristics can be achieved by integrating breast imagers into the specimen examination process and the use of large-format sections which preserve local anatomy. This paper describes the successful creation of a large-format pathology program to routinely serve all patients in a busy interdisciplinary breast center associated with a community-based nonprofit health system in the United States.

1. Introduction

Significant among the advances of the last 3 decades in breast cancer diagnosis and management are the broad access to mammographic service screening by asymptomatic individuals [1–5], diagnosis by minimally invasive needle biopsy techniques, and the widespread acceptance of breast conservation surgery. Surgical management, specifically the complete surgical removal of all detectable breast carcinoma, remains the preferred first therapeutic step for the majority of men and women with Stage 0–III breast cancer [6, 7]. Breast-conserving procedures, as an evidence-based alternative to mastectomy, have gained widespread acceptance since the NSABP B-04 and B-06 trials over 30 years ago [8, 9]. Pathologic examination of resected tissue continues to serve as the definitive means of establishing adequacy of breast conservation surgery (BCS) with the reporting of surgical margin status. As such, margin evaluation remains a key data point in the clinical decision of whether to administer radiation therapy postoperatively [10] or to perform an additional excision to obtain negative margins. Neither the NSABP B-04 or B-06 trials defined margin negativity beyond the simple absence of neoplasm on the margin itself, so although margin negativity was required for accrual into the BCS-radiation therapy arm, proximity of tumor or tumor subtype to surgical margins was neither reported nor controlled.

The last 30 years have yielded confusing and contradictory information regarding the risk of local or systemic recurrence and survival following BCS. Numerous authors have sought to identify variables useful in the prediction of breast cancer recurrence or at least to serve as criteria for surgical reexcision. Many of these variables have been drawn from the characteristics of the neoplasm itself or

the proximity of the neoplasm to surgical margins. Investigators have reported the relationship between recurrence (or the presence of residual cancer in reexcision specimens) and the proximity of invasive cancer to negative BCS margins [11–13], the number of involved margins [14], multifocality, and the presence of an extensive intraductal component (EIC) associated with an invasive cancer [15, 16]. Unfortunately, many reports attempting to correlate recurrence risk with proximity to surgical margins failed to define margin status relative to duct carcinoma *in situ* (DCIS) [17–19], and so it was not known to what extent margin positivity for DCIS may have contributed to observed recurrence rates. Gradually, DCIS was identified as an independent risk factor in breast cancer recurrence after BCS. More recent studies have demonstrated varying degrees of correlation between the overall extent of DCIS [20–22], nuclear grade [23], and proximity to surgical margins [24–27] and risk of breast cancer recurrence. In some series, over 50% of women undergoing breast conservation surgery required additional surgery to achieve satisfactory margin status [28]. Most of these reports have been inspired by one or more persistent clinical questions, relevant to the clinical management and prognosis of a patient diagnosed with breast cancer (Table 1).

It has been difficult to draw a clear consensus from much of the breast cancer recurrence literature published over the last 3 decades; however some general conclusions are reasonable. Positive margins for both DCIS and invasive cancer place a patient at high risk for local recurrence and are to be avoided. Secondly, negative margins are not a guarantee against recurrence, yet increasing margin width crudely correlates with decreasing risk of recurrence by reducing the likelihood of residual carcinoma in the adjacent breast. Greater extent of DCIS [29] and higher nuclear grade are positively correlated with recurrence, even with negative margins (21, 23). Despite these broad generalizations, which in retrospect appear intuitive, a high degree of concordance across these studies is not observed with covariables such as margin width of DCIS and risk of local failure [30] or DCIS extent and risk of recurrence. The lack of consensus in this literature is frustrating to surgeons and oncologists alike who seek reproducible data to support evidence-based management protocols. It seems obvious that margin evaluation is not an exact science, but how much of the conflicting data is related to the biology of breast cancer itself? Some authors have pointed to difficulties in standardizing, optimizing, and reporting breast specimen examinations by pathologists [31, 32]. This general lack of concordance suggests that the pathology data collection itself is not optimized for reproducibility; a reasonable conclusion considering the conventional methods of gross specimen examination used to report breast cancers is essentially unchanged from the preimaging era [33].

The literature addressing tumor attributes and risk of recurrence mostly relies upon data abstracted from pathology reports based on traditional methods of gross and microscopic examination. In the vast majority of these laboratories, surgical specimens are sectioned to conform to industry-standard 25 mm × 175 mm glass microscope slides. Most reports do not describe centralized or expert remeasurement of reported breast cancer metrics. Measurement methodologies for DCIS extent vary from laboratory to laboratory and are often not specified in the publication. Lester et al. [34] describe a protocol sanctioned by the College of American Pathologists for the examination of specimens from patients with DCIS. The protocol is a welcome effort to assist pathologists in the identification and reporting of DCIS in resected tissue. The authors appropriately enumerate the varied methods used by pathologists to measure DCIS extent but no recommendation was made to standardize the process or correlate imaging data into specimen examination. Commonly, pathologists measure the largest single focus on an individual slide or the span of DCIS across a single slide and report the result as DCIS extent. Other pathologists calculate extent by counting the number of slides involved with DCIS. Some pathologists add the gross section width (e.g., 3 mm, 5 mm) of involved slides to estimate cumulative DCIS extent [35], a practice found by Dadmanesh et al. to underestimate extent in 72% of cases [36]. Rarely, a pathologist will attempt to correlate histopathology with imaging studies and measure DCIS extent across images using the locations of slides positive for DCIS. Reconciliation of presurgical imaging characteristics with pathologic measurements of size, extent, or margin status prior to finalizing the pathology report seems to be the exception, rather than the rule.

In clinical practice, margin evaluation is now routinely facilitated by surgical specimen orientation. Reporting of 6-axis BCS margins is standard practice in most centers; however numerous technical limitations plague the process. Breast tissue is inherently pliable, and the dimensions of the specimen and associated neoplasia can change dramatically with changes in patient position from mammogram to MRI, operating suite and pathology work station. Pathologists or their assistants typically apply multicolored inks to the specimen circumference to approximate the orienting clips or sutures placed surgically; however this process typically occurs without surgeon validation of the designated margins. The resulting margin boundaries (e.g., anterior-medial or posterior-lateral) may or may not represent the surgeon's view of the same boundaries. The risk for the patient is that a reexcision of a close or involved margin may not align with the margin as identified by the pathologist essentially leaving a critical margin unresected.

Despite remarkable advances in breast imaging technology since the early screening trials including digital mammography, 3D automated ultrasound, digital tomosynthesis, magnetic resonance imaging (MRI), and breast-specific gamma imaging (BSGI), the techniques employed by pathologists to grossly examine resected breast tissue have changed little, save for the measurement of margins on oriented specimens. Specimen radiographs obtained for wire-localization BCS procedures are usually available for pathologist review; however the contribution of these images to the gross and microscopic examination is only occasionally documented in pathology reports. The degree to which wire-localization radiographs guide the pathologist's determination of cancer size, extent, and proximity to margins is unknown. In a study of 135 BCS procedures

Table 1: Surgical margins: persistent management questions.

(i) Why does breast cancer recur after breast conservation surgery (BCS) with negative margins?
(a) Corollary: why is carcinoma present in re-excisions/mastectomies performed following excisions with negative margins?
(ii) What margin width is necessary to consider surgical management complete for DCIS and invasive carcinoma?
(iii) Why are re-excisions of breast performed following BCS with positive margins often negative for residual cancer?
(iv) Why does breast cancer locally recur following mastectomy when the mastectomy margins are negative?
(v) Can extent or grade of DCIS predict recurrence when BCS margins are negative?

for DCIS, mammography was found to significantly underestimate the extent of DCIS as measured pathologically even when performing complete specimen radiography and extensive tissue sampling [37]. Compared to mammography, presurgical MRI has significantly higher sensitivity in the detection of DCIS [38]; however presurgical MRI studies are typically not available to pathologists at the time of specimen gross examination. Lacking knowledge of the imaging extent and distribution of a neoplasm in three dimensions (as is possible with MRI), gross surgical specimens are sectioned without reference to the axis of greatest cancer extent or mammographically occult encroachment on surgical margins. Pathologists intentionally select 6-axis sections for microscopic study when a grossly palpable or visible area of concern is identified, when wire-localization images suggest margin compromise or when a surgeon identifies margins of concern. Otherwise, sections are randomly selected based on gross inspection to represent 6-axis margins. The specimen is further morcellated until the individual tissue blocks conform to the industry-standard tissue cassette, an area no larger than 25 mm × 35 mm.

Improved characterization of DCIS extent and margin status has resulted from protocols requiring submission of 100% of lumpectomy tissue, notably the serial subgross techniques utilized by Holland et al. [39], MacDonald et al. [21, 23], Cheng et al. [20], Kato et al. [40], and Sigal-Zafrani et al. [41]. Three-dimensional reconstruction techniques to improve margin evaluation were reported by Mai et al. [42] and Ichihara et al. [43]. Techniques referred to as serial sequential sectioning employed by Grin et al. [35] and Dadmanesh et al. [36] approach the goal of optimizing cancer extent measurements. These techniques typically maintain the spatial orientation of the tissue sections for subsequent three-dimensional reconstruction. Unfortunately, these methods require that additional breast tissue is trimmed from the submitted tissue blocks so they conform to standard tissue processing methods. The result is a diminution or loss of local breast and tumor anatomic relationships. In some centers, mammographic examinations are routinely performed on intact tissue slices [22, 23, 35]; in others, radiographic examination of gross specimen sections is either performed on a subset of sections or not at all.

A significant limitation to the accurate reporting of DCIS extent and margin status results from poor information flow among the breast care team members at the time of surgery. Modern imaging techniques provide the breast imager and surgeon with an increasingly lucid presurgical depiction of the extent and distribution of breast neoplasia [43]. Functional imaging, particularly breast MRI, gives insight into the anatomic distribution of neoplasm in three dimensions [44, 45] and can identify disease, particularly DCIS extent beyond the sensitivity and specificity of mammography or ultrasound [46, 47]. Many studies have attempted to evaluate the sensitivity or specificity of mammography, ultrasound, and MRI using reported pathologic tumor characteristics as the reference standard. Most of the published imaging-pathology correlation studies import tumor measurements directly from cancer registry or pathology reports. The data thus obtained is subject to all of the limitations associated with conventional pathologic techniques. As an example, Ichihara et al. [43] found MRI was not only more sensitive than mammography in detection of DCIS (88% versus 27%) but maximum extent of DCIS measured by mammogram (90 mm) or MRI-detection (110 mm) far exceeded maximum pathology extent measurements (25 mm). Schouten van der Velden et al. [45] in comparing reported pathology cancer size measurements of 49 cancers with estimates by mammogram and MRI found agreement within 5 mm in only 27% of mammogram and 38% of MRI studies. Some authors regard the discrepant size and extent measurements as evidence that MRI overestimates the extent of breast carcinoma [44] and raise concerns of unnecessary or additional surgery [47, 48].

Notably absent from the current debate on the sensitivity and specificity of breast imaging technology and the clinical utility of breast MRI as a presurgical planning procedure is a discussion of the limitations of the pathologic techniques used to validate modern imaging studies. The practical limitations of conventional breast pathology reporting techniques [31, 49, 50] are summarized in Table 2.

Prior to the widespread clinical use of breast MRI, breast pathologists using large-format contiguous histologic sections measuring up to 8 × 10 cm visualized breast cancer in three dimensions [33, 51] and improved correlation between imaging and pathologic measurements of cancer size and extent [49, 52]. Subsequent reports have confirmed the clinical advantages of large-format breast specimen processing with improved imaging-pathology correlation of cancer distribution and optimized evaluation of surgical margins [50, 53, 54]. The suitability of these techniques to the community hospital setting and the benefit to both imager and pathologist were described by Biesemier and Alexander [55] and Méchine-Neuville et al. [56].

Table 2: Summary of limitations of conventional pathologic technique in the diagnosis and reporting of breast carcinomas in breast-conserving surgical specimens.

(i) Gross inspection and palpation have insufficient sensitivity to guide section submission of imaging-only detected neoplasia, including DCIS and multifocal invasive carcinoma
(ii) Complete imaging data, including MRI features are not available to pathologists at time of specimen evaluation
(iii) Spatial 3D integrity of the specimen is lost through sectioning, section submission and histopathologic examination
(iv) Margin evaluation is often reliant on gross inspection and palpation, even for imaging-only detected disease
(v) Lack of standardization of methods to measure DCIS extent, multifocality, and margins
(vi) Suboptimal correlation between pathology and pre-surgical imaging studies

Table 3: Required program elements of the image-assisted large-format breast pathology initiative.

(i) Planning-phase knowledge base and budget development
(ii) Specialized large-format/MRI interdisciplinary conference
(iii) Surgical pathology gross specimen protocol
(iv) Breast imaging department specimen radiography protocol
(v) Histopathology laboratory large-format procedures
(vi) Pathologist signout and reporting procedures
(vii) Comprehensive breast cancer database development
(viii) Training of pathologists, histopathology staff, breast imagers, and radiologic technologists

2. Materials and Methods

The image-guided large-format breast pathology (LBP) initiative at Carilion Clinic necessitated preimplementation development of several key program elements (Table 3).

2.1. Planning-Phase Knowledge Base and Budget Development. The specimen processing methods at Carilion Clinic were developed over a six-month period. They were adapted from procedures in place at Falun Central Hospital, Falun, Sweden and Central Virginia Pathology Consultants, Lynchburg, Virginia following site visits to those locations. Capital and operating budgets were prepared, and all laboratory capital and operating expenses were funded exclusively from laboratory operating revenue. A separate cost center was not created within the histology laboratory for the large-format program; however expenses attributed to histology labor and consumables were recorded for analysis of incremental costs in comparison with conventional pathology processing. Specimen radiography and radiograph interpretation were performed in the breast imaging department in a cost center created for the purpose of supporting the large-format pathology program. The technical and professional component billing and revenue capture for specimen radiography and interpretation using CPT codes 76098-TC and 76098-26 were booked to the specimen radiography cost center.

2.2. Specialized LBP/MRI Interdisciplinary Conference. This weekly conference, attended by breast imagers, surgeons, and pathologists, was created to optimize surgical outcomes through the continuous correlation of large-format pathology with clinical findings and all pre- and postimaging data, including MRI. As an adjunct to the existing weekly interdisciplinary breast pretreatment planning conference, this conference was designed to accomplish two primary goals. A detailed postsurgical analysis of tumor size, extent, multifocality, and proximity to surgical margins was performed by correlating all presurgical breast imaging studies with subgross and histopathologic findings. This was accomplished with real-time projection of large-format pathology slides alongside MRI and other projected presurgical imaging studies. Presurgical imaging estimates of cancer extent, geographic distribution, and proximity to anatomic landmarks were reviewed and revised with large-format pathologic validation. Final surgical margin measurements were scrutinized in view of presurgical procedure planning goals and intraoperative surgical findings. Secondly, presurgical planning was conducted with a goal of optimizing the utilization and outcomes of breast-conserving procedures whenever possible. The conference differed from the usual presurgical planning conference by leveraging the team's experience gained from the post-surgical large-format-imaging correlations. Breast MRI was routinely performed on most cases diagnosed and treated in the LBP era and was generally not available in this breast program prior to implementation of LBP.

2.3. Surgical Pathology Gross Specimen Protocol. All mastectomy, lumpectomy, and reexcision specimens are included in the large-format program with specialized processing procedures designed for each specimen type. Mastectomy specimens were phased-in beginning August 2004 in order to gain experience with the technique. Breast-conserving specimens and reexcisions were added to the program in November 2004. Nearly 400 surgical specimens representing between 250 and 275 new breast cancer diagnoses are accessioned and processed annually.

Upon receipt in the surgical pathology suite, all specimens are checked for proper surgical orientation, and any deficiencies identified are promptly corrected by the surgeon. All surgical margins are marked before sectioning with India ink with a discrete color coded to each of six axial margins. Mastectomy specimens are sectioned in the sagittal plane at uniform 5 mm increments with preservation of medial-to-lateral sequence and cephalocaudad orientation. Initially, 100% of each mastectomy specimen was submitted for

specimen radiography. Experience permitted introduction of time and cost conserving measures so that approximately one-half to two-thirds of each specimen was submitted for radiography, focusing on the location of previous biopsy or lumpectomy sites and any additional guidance provided by breast surgeons and imagers. Breast-conserving specimens and reexcisions are inked for margins and sectioned at 5 mm increments. The plane of sectioning varies with the requirements of the case. Occasionally, demonstration of maximum extent of disease or multifocality in one plane is of paramount importance. In these cases, the plane of sectioning is guided by image interpretation of size, extent, and multifocality, and sections are typically made in the coronal (frontal) plane. In cases where the status of chest wall or anterior cutaneous margins (or both) are of particular concern or the sagittal plane shows maximum extent, the sagittal plane is selected for sectioning and large-format section submission. This approach allows convenient correlation of sagittal large histology sections with MRI sagittal-reconstructed images. All BCS and reexcision specimens are entirely submitted for specimen radiography.

Orientation of all specimens is maintained with the use of radioopaque alphabet characters to denote axis margins in each plane as well as the sequence of sections, for example, "superficial to deep" in the coronal plane or "medial to lateral" in the sagittal plane. Tissue slices are placed on previously exposed radiographic film with orienting characters and transported expeditiously to the breast imaging facility for radiography. Following specimen radiography and radiographic interpretation (vide infra), the specimen with accompanying radiographs is returned for pathologic examination with a "Large-Format Specimen Checklist" detailing the clinical and specimen radiographic findings in written narrative and using template diagrams of both breasts. This worksheet communicates the clinical and radiographic findings of concern with specific questions to be addressed pathologically. Imaging abnormalities visible mammographically are marked directly on the films with a wax pencil as a guide for histopathologic section submission (Figure 1). The location and extent of ultrasound or MRI-detected findings, not visible mammographically or with specimen radiography, are marked on the specimen radiographs. Section(s) best demonstrating critical findings such as proximity to margins or maximum extent of neoplasm are encircled for possible large-format submission.

Following traditional pathologic specimen examination with gross inspection and palpation, sections are selected for histologic study to incorporate gross pathologic findings as well as findings of concern communicated by imagers and surgeons (Figure 2). Microscopic evaluation of surgical margins is typically accomplished with a combination of both large-format and conventionally sized tissue blocks. Large sections usually permit margin evaluation along 100% of the circumference of a BCS specimen and are selected to encompass critical margins whenever close proximity to a surgical line of excision is suspected. Margins perpendicular to the large-format plane of section are usually submitted as conventional tissue blocks. Orientation of all conventionally sized margin sections is perpendicular to the specimen surface to allow measurement of margin width to the nearest whole millimeter. The precise location of these perpendicular margins is selected with guidance from both imaging and clinical information and conventional pathologic examination. Reexcision specimens, including surgical shave excisions of biopsy cavities, are sectioned and oriented perpendicular to the surgical margin so that margin distance from the cavity to the new surgical margins remains measureable. The anatomic location of all tissue blocks removed from the specimen is recorded in the gross dictation narrative of the pathology report as well as on the specimen radiographs which are preserved as a permanent record of pathology slide origin. This record is provided to the pathologist at microscopic sign-out as an aid in the reconstruction of the specimen in three dimensions.

2.4. Breast Imaging Department Specimen Radiography Protocol. Upon receipt in the imaging center, specimens are placed in an analog specimen radiography unit (Faxitron Bioptics, LLC). Specimen film images are examined by a breast imager, and all relevant mammograms, core biopsy specimen radiographs, wire-localization images, ultrasound, and MRI studies are reviewed. Mammographically detected lesions of concern are marked on the radiograph films, and the location of mammogram occult abnormalities identified with ultrasonography and MRI is encircled. To facilitate communication of clinical, presurgical imaging and specimen radiography findings to the pathologist, the "Large-format Specimen Checklist" is completed with reference to notations made by the imager on the specimen radiographs. A graphical depiction of the location and extent of clinical and imaging-suspected neoplasia is marked on a template bilateral breast diagram. A written narrative conveys clinical concerns and imager-initiated questions for pathologist reconciliation. This form is returned with the specimen and annotated specimen radiographs to the surgical pathology suite.

2.5. Histopathology Laboratory Large-Format Specimen Procedures. Upon receipt in the histopathology laboratory, conventional tissue blocks are separated from the large-format tissue blocks and separately processed in the routine manner. Large-format blocks are fixed for an additional 24–32 hours in 10% neutral buffered formalin. After fixation, all breast tissue is processed on automated processors (Tissue-Tek VIP, Sakura Finetek USA, Inc.). Paraffin embedding of large tissue sections requires reusable cassette forms fabricated on-site from 14 ga $\times$ 1 $-$ 1/4″ wide aluminum bar stock (Figure 3). Paraffin block dimensions are thereby conformable to the size of the tissue block and measure up to 8×10 cm. Sections are prepared at 3 to 4 microns on a Leica sliding microtome model SM2500 (Leica Microsystems). Slide staining is performed on an automated histology slide stainer with slide baskets customized to hold large-format glass slides (Shandon Varistain 24-4, Thermo Electron Corporation).

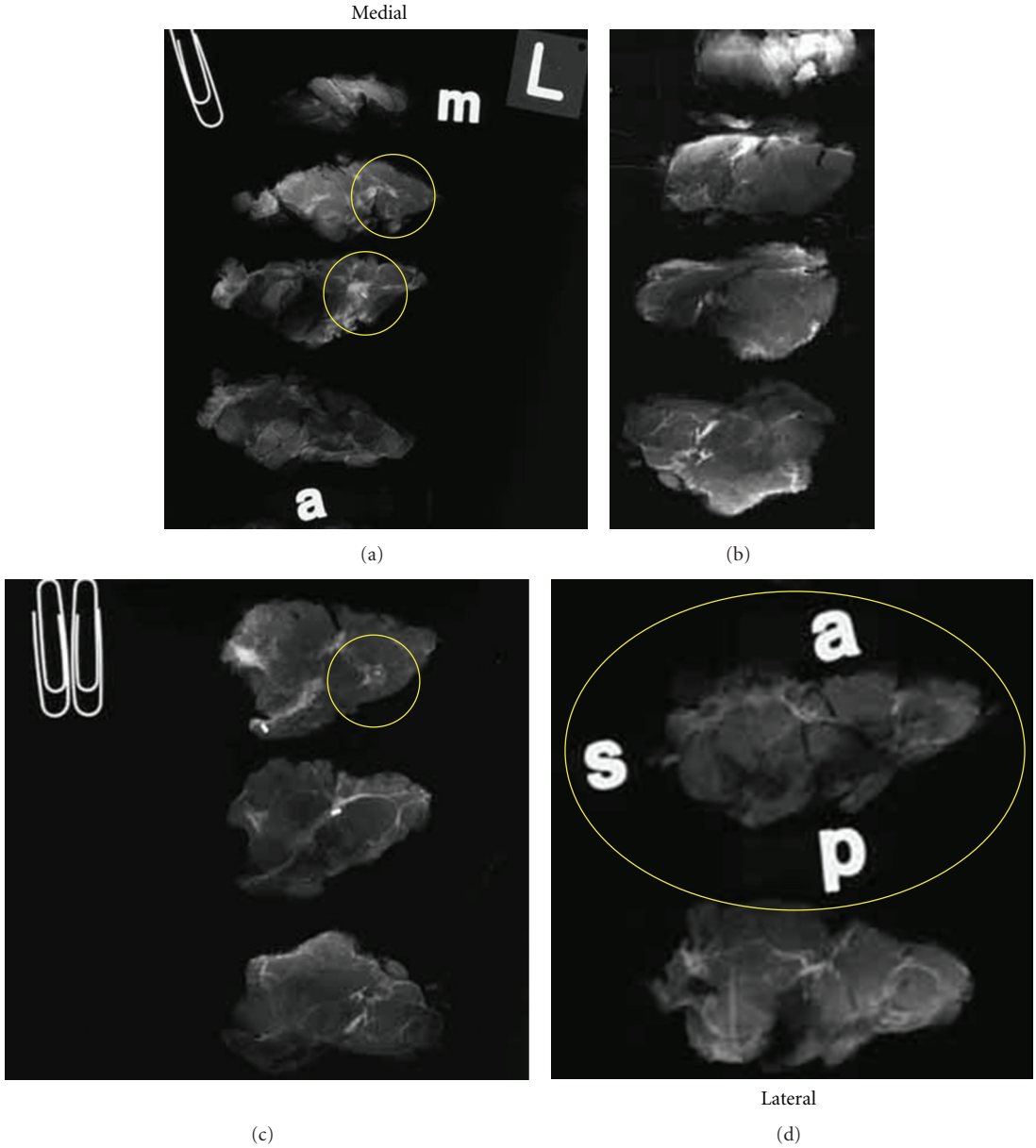


Figure 1: Specimen radiographs of a lumpectomy from a left breast sectioned at 5 mm intervals in the sagittal plane. Sections are sequentially oriented from medial (a) to lateral (d). Yellow circles correspond to marks made by a breast imager following review and correlation of relevant imaging studies to denote significant radiographic findings such as calcification, mass density, architectural distortion, ultrasound findings and enhancement on MRI. The imager selectively marks sections to demonstrate maximum size and extent of neoplasia and close proximity to margins with the expectation that the pathologist will provide histopathologic correlation of identified imaging findings.

2.6. Pathologist Signout and Reporting Procedures. Pathologists are provided with transcribed gross dictation, all slides including large-format slides, annotated specimen radiographs, and the "Large-format Specimen Checklist" completed by the breast imager. With attention to the specific questions communicated by the breast imager, histopathology slides and radiographs are analyzed to complete all relevant data fields following the College of American Pathologists' recommended synoptic reporting protocol. When maximum size of invasive carcinoma or extent of *in situ* carcinoma is represented by the large-format slide, that dimension is directly measured from the slide and reported. When clinical or imaging information indicates a greater size/extent than represented on the large-format slide, a three-dimensional reconstruction of tumor size/extent is performed. Following guidance provided by the breast imager on the large-format specimen worksheet, specimen radiographs are correlated with histopathology slides keyed to individual specimen radiographic images. Size and extent are either directly measured or calculated using the uniform 5 mm section thickness as a guide and reported to the nearest whole millimeter.

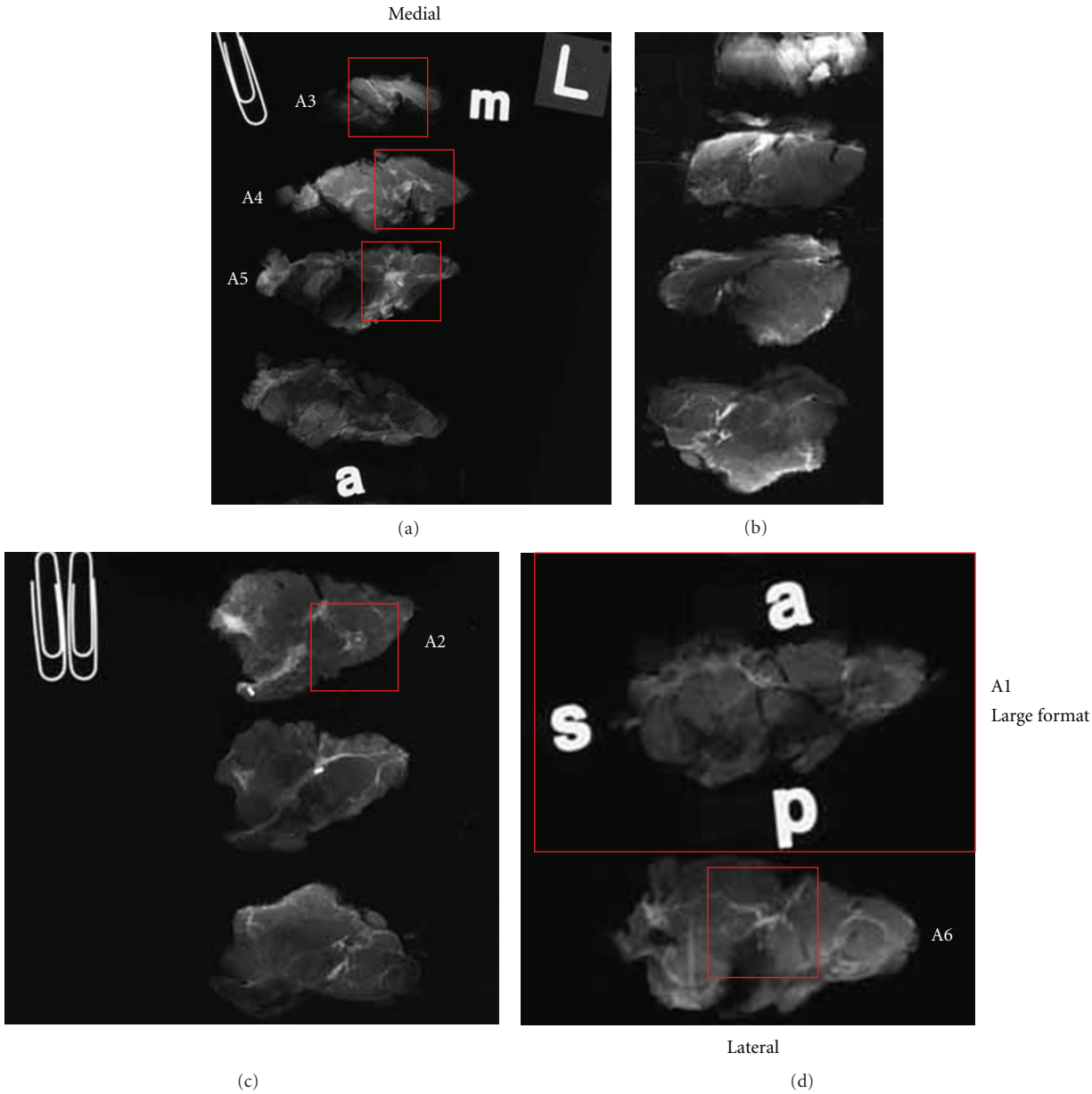


Figure 2: Specimen radiographs from lumpectomy shown in Figure 1 marked after pathologic examination. Red rectangles denote tissue blocks removed for microscopic examination. Block A1 consists of a large-format tissue section with circumferential surgical margins in the sagittal plane. Orientation is maintained on the radiograph with opaque letters "a" (anterior), "s" (superior), and "p" (posterior). The inferior margin is opposite superior (unmarked). Blocks A2–A6 are conventionally sized. Medial and lateral surgical margins are represented by conventional sections A3 and A6, respectively. Blocks A2 and A4 were removed to address specific findings identified by the breast imager.

Margin evaluation of BCS specimens is accomplished with similar reference to imager guidance. In sagittal-plane sections, circumferential microscopic evaluation of margins is correlated between large-format slides and specimen radiographs depicting anterior, inferior, posterior, and superior margins. Medial and lateral margin sections, whether conventional or of the large-format type, are similarly correlated with images. Proximity to each of six axial margins is measured to the nearest whole millimeter and recorded as 0 mm (positive), 1–10 mm in 1 mm increments, or >10 mm. Margin involvement of a boundary between two margins is reported as involvement of both margins at their junction, for example, "anterior-lateral" or "superior-medial." A series of 135 consecutive lumpectomy specimens with a diagnosis of DCIS without invasive carcinoma were analyzed for margin status using the above techniques. Ninety-two specimens processed following the LBP protocol including presurgical MRI were compared to forty-three specimens processed using conventional pathologic techniques without presurgical MRI. The surgical reexcision rate and breast

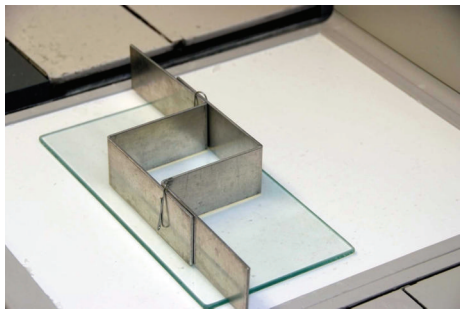


Figure 3: Adjustable embedding forms.

conservation rate in each category were retrieved from cancer registry data, and the volume of each lumpectomy specimen was computed with dimensions obtained from the gross pathology specimen description.

2.7. Technical Expense Analysis. The average number of conventional pathology blocks produced per mastectomy and lumpectomy specimen was tabulated for a total of 100 resections. The block totals were compared between cases submitted in calendar year 2004 with conventional pathology processing and in 2005 with large-format processing. An average of one large-format block per case was submitted in 2005.

Technical expense for labor and materials associated with the processing of breast pathology specimens were calculated for 50 consecutive cases in calendar year 2005. Histotechnologist time devoted to the processing of conventional and large-format breast tissue from the time of receipt to delivery of stained and coverslipped slides was separately recorded for conventional and large-format processed tissue. Total technical time, expressed in fractional hours in each category was multiplied by the average hourly wage, including all benefits, of the histotechnologists directly involved with the project to arrive at total labor expense (TLE) for conventional and large-format processing. Unit labor expense per slide was calculated by dividing TLE by the total number of finished slides produced in each category.

The direct cost of consumables per slide followed a similar methodology. All materials used in processing and slide production were allocated to each category as appropriate. For liquid reagents and stains shared in processing, a *pro-rata* allocation between categories was estimated using the surface area of conventional versus large-format slides multiplied by the number of slides in each category. Total cost of consumables in each category was divided by the number of slides produced to calculate consumable cost per completed slide for each group. Finally, TLE and material cost per finished slide in each category were combined to calculate total technical expense for both conventional and large-format slides. Amortization of capital equipment and indirect costs, such as overhead and staff training, were not included in the calculation of technical expense. Professional time devoted to the examination and reporting of conventional and large-format sections was not compared.

2.8. Comprehensive Breast Cancer Database Development. A searchable breast database was developed at the inception of the program using Microsoft Access (Microsoft Corporation). All data collections and analyses were performed in compliance with Institutional Review Board approved research protocols. Separate pages in the database were created for clinical, mammography and ultrasound, MRI, and pathology data fields. The database was jointly maintained by cancer registry and Information Technology Department staff. Training of cancer registry and breast center staff in the population of data fields and search methodology was conducted by Information Technology staff.

3. Results

3.1. Surgical Margin Evaluation. Conventional histopathologic evaluation of surgical margins is typically accomplished in BCS specimens with six tissue blocks, one selected in each margin axis. In any one plane of section, four conventionally sized tissue blocks are usually removed for microscopic analysis (Figures 4 and 5).

The relationship between histologic section orientation and percent of the surface margin available for microscopic study in a hypothetical 8.0 cm diameter BCS specimen is presented in Table 4. If each of the conventional margin sections spans 1.0 cm of arc, approximately 16% of the circumference in one plane of section is available for microscopic study. Alternatively, if the margin sections each span 2.0 cm of margin circumference, the proportion represented rises to 32%. In contrast, the large-format method permits microscopic examination of 100% of the circumference of the selected plane of section of a BCS specimen. The margin evaluation of a three-dimensional resection specimen is more complex than the evaluation of margins in a single plane, however. En-face margin sampling theoretically permits the examination of a greater percentage of the specimen surface; however this method does not allow for the measurement of margin width. Perpendicular orientation of margin blocks facilitates measurement of margin distance at the expense of proportionate sampling of the specimen surface. As seen in Table 4, routine histologic sections of 4-micron thickness represent a very small percentage of the specimen surface area, even when compared with the amount of tissue theoretically available in a 3 mm thick paraffin block. The representative nature of margin evaluation, as evidenced by the small proportion of surface margin actually examined, illustrates the necessity of obtaining the greatest yield and relevance possible from each margin section removed from a specimen. In the LBP program, large-format section selection was guided by review of imaging and clinical data conveyed at the time of specimen gross examination. In approximately 10% of cases, two or more large-format sections were submitted to gain experience with the technique and to correlate the extent of neoplasia with MRI. With experience however, one optimally selected large-format section supplemented with conventionally sized blocks proved sufficient to evaluate

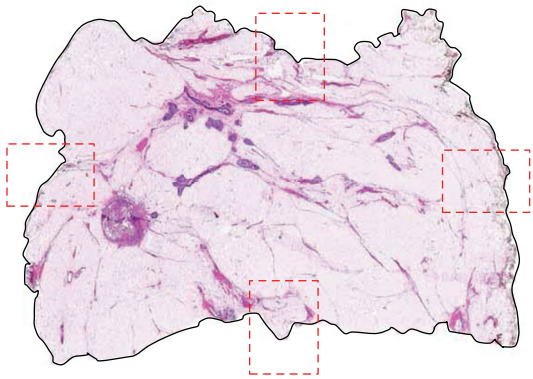


Figure 4: Large-format histologic section of a lumpectomy specimen. The circumferential large-format surgical margin is outlined with solid black line. Conventional margin evaluation would consist of four axis margin sections in one plane represented here by broken red rectangles (H&E).

Table 4: Percent of surface area available for conventional histopathologic evaluation of surgical margin status. Hypothetical 8.0 cm diameter lumpectomy specimen with one 2.0 cm × 2.0 cm tissue block removed for each of six axial margins. Specimen surface area = 201 cm^2.

Examination method	Total surface margin area examined (cm^2)	Percent of specimen surface area
En-face, six 2 × 2 cm sections	24 cm^2	12%
Perpendicular, six sections 2 cm of arc × 0.3 cm thick*	3.6 cm^2	1.8%
Perpendicular, six sections 2 cm of arc × 4 microns thick**	0.048 cm^2	0.02%

*Assumes 6 tissue blocks 2 cm × 2 cm measuring 3 mm thickness each, maximum amount of tissue available for examination in the paraffin block.
**Assumes 6 tissue blocks 2 cm × 2 cm with final section thickness on microscopic slide of 4 microns.

margins, demonstrate maximum extent of neoplasia, and correlate with imaging studies.

The influence of the LBP program on reported surgical margins and reexcision rate for DCIS was compared with preimplementation conventional pathologic reporting. The effect of the LBP initiative was evaluated in the context of a fully integrated interdisciplinary breast program characterized by meticulous presurgical planning, routine use of large-format sections, and correlative postsurgical interdisciplinary analysis of tumor size, extent, multifocality, and proximity to surgical margins. Adequacy of breast conserving surgery was expressed as margin width in millimeters exceeding a selected distance in each of six axes. The integrated LBP program with presurgical MRI is compared with conventional breast pathology without presurgical MRI in Table 5.

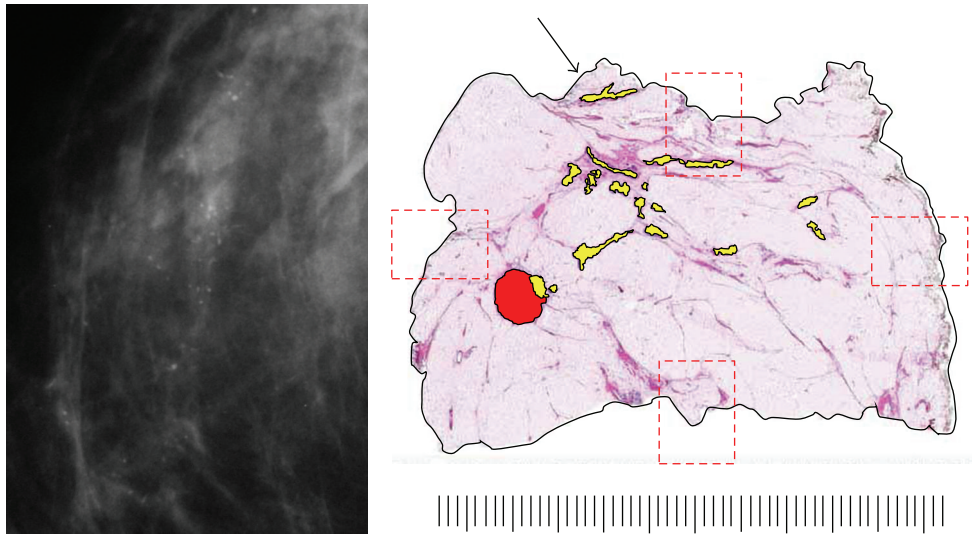
Table 5: Comparison of 135 consecutive breast conserving surgical specimens with DCIS. Conventional pathology without MRI (CP) and large format breast pathology with MRI (LBP).

	CP $n = 43$	LBP $n = 92$
One or more of six margins measuring 0–4 mm	20 (47%)	22 (24%)
One or more of six margins measuring 0–9 mm	23 (54%)	31 (34%)
Re-excised after breast conservation	14 (32%)	11 (12%)
Volume of breast conservation specimen, median (cm^3)	97.1	191.2
Breast conservation rate	65%	63%

3.2. Ink Migration. India ink applied to the surface of a breast specimen to mark the surgical margin may migrate into cracks and clefts in the specimen surface as noted by Campbell et al. [32] (Figure 6). On microscopic examination, the pathologist encountering an inked tissue edge on a conventional pathology slide may regard it as a true inked margin and measure margin width from a focus of cancer to the visible ink, unaware that the true margin is at greater distance (Figure 7).

The migration of India ink into the interior of breast surgical specimens is relatively common and appears to occur along fissures in the specimen exposed or created by the surgical procedure or specimen handling. With conventional histologic sections, tissue blocks removed from a surgical specimen exhibiting an inked tissue edge may be construed as a surgical margin. A potential for misinterpretation of the margin width exists if the tissue block was taken from an area of ink migration. In such a circumstance, the margin measurement would be lower than the true margin width. Preservation of the anatomy of the specimen with large-format sections permits clear identification of the peripheral margin contour so that ink migration into the interior of the tissue block is recognized and not confused with India ink on a true peripheral surgical margin (Figure 8).

3.3. Measurements of DCIS Extent. Imaging studies, particularly specimen radiographs, are correlated with histopathology in the determination of greatest extent of DCIS and maximum size of invasive carcinoma at the time of pathologist signout in all cases. In many but not all instances, large-format histologic sections provide the best depiction of maximum extent of disease. In such cases, extent can be measured directly from the large section slides themselves. Frequently, however, it is necessary to reconstruct the specimen in three dimensions. Three-dimensional reconstructions are made by correlating the annotated specimen radiographs with individual tissue blocks keyed to the radiographs. Reference is made to imaging estimates of tumor size and extent. In these reconstructions, calculations of DCIS extent are based on a combination of direct slide measurement and calculation of



(a) (b)

Figure 5: Analysis of case shown in Figure 4. (a) Asymptomatic 53-year-old woman with linear-casting calcifications on screening mammogram presented with no palpable abnormality. (b) Stereoguided biopsy showed grade 2 DCIS with necrosis. Core biopsy site is highlighted in red. DCIS is highlighted in yellow with maximum extent of 46 mm. Specimen on gross examination was minimally fibrotic and negative for a palpable lesion or gross evidence of DCIS. Specimen radiography guided selection of tissue blocks and large-format section to best demonstrate extent of neoplasm and proximity to superior margin (Arrow). Presurgical MRI was not performed. Conventional margin section selection guided only by gross inspection and palpation could have missed DCIS encroachment on superior margin which measured 1.5 mm. Scale: small divisions = 1.0 mm (H&E).

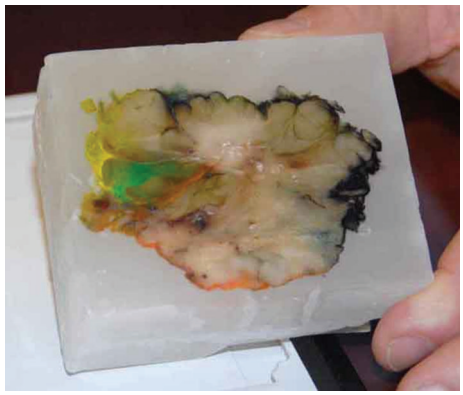


Figure 6: Paraffin block from lumpectomy specimen marked with India ink in multiple colors for orientation of surgical margins. Note migration of ink into interior of specimen.

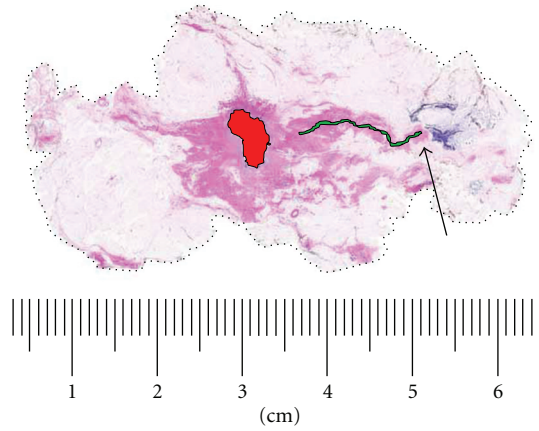


Figure 7: Large-format section of a lumpectomy specimen with circumferential surgical margin (broken line). Biopsy cavity is centrally located (solid red). Grade 2 DCIS (solid green) extends 15 mm from the cavity to within 9 mm of true surgical margin. Arrow marks migration of India ink into specimen to within 1 mm from DCIS. A conventional histologic section taken in this area could misrepresent the margin distance as 1 mm. This pitfall is easily avoided on the large-format slide. Scale: small divisions = 1.0 mm (H&E).

maximum distance of histologically confirmed DCIS across uniform 5 mm tissue slices. A comparison of DCIS extent measurements between conventional pathologic techniques and imaging-guided large-format breast pathology (LBP) is given in Table 6.

A typical case depicting tumor size and extent measurement and margin analysis using the LBP technique is shown in Figures 9 and 10. In this example, the 71-year-old patient presented with a palpable 1-2 cm nodule of the upper-inner quadrant. Mammogram showed a BIRADS 4 stellate density 14 mm in diameter corresponding to the palpable lesion. Ultrasonography disclosed an 15 mm hypoechoic lesion amenable to ultrasound-guided core needle biopsy, which was positive for grade 3 invasive ductal carcinoma. Presurgical MRI obtained for surgical planning purposes confirmed the presence of the biopsied lesion and additionally demonstrated a 60 mm area of enhancement extending

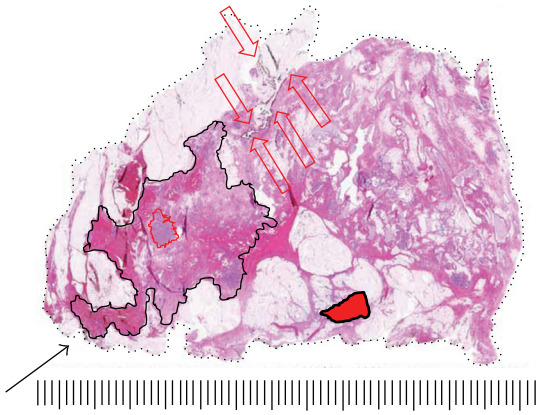


Figure 8: Large-format section of a lumpectomy specimen from a 72 year woman. India Ink migration into the interior of the specimen is marked with open red arrows. The true surgical margin is represented by broken black line. Grade 2 DCIS extending 35 m (solid black line) is present 2 mm from migrating ink. A small grade 1 invasive ductal carcinoma is outlined by a solid red line. A biopsy cavity is located lower-central (solid red fill). The biopsy was positive for DCIS. No residual DCIS was found within 12 mm of the biopsy site. The solid black arrow points to a positive surgical margin suspected on specimen radiograph which led to the submission of this section. Scale: small divisions = 1.0 mm (H&E).

anteriorly and superiorly from the index lesion. The patient was offered and accepted breast conservation surgery. The specimen was sectioned in the sagittal plane to correlate with MRI sagittal-reconstructed images and to best demonstrate extent of MRI enhancement and proximity to anterior, posterior, superior, and inferior margins. Gross examination of the specimen disclosed a firm 15 mm nodule; however, most of the enhancing 60 mm MRI-detected lesion consisted of clinically, surgically, and pathologically impalpable grade 3 DCIS. Final margin analysis reported DCIS present 2.0 mm from the anterior-superior margin. All other margins were >10 mm for DCIS and invasive carcinoma.

3.4. Technical Expense Analysis. The adoption of LBP techniques incurs additional technical expense. The primary capital expense is for a sliding microtome which can be purchased new for $55,000–$65,000 (Leica Microsystems). Existing laboratories may configure their tissue processors and staining equipment to accommodate large-format specimens if excess capacity exists. Otherwise, conventional automated processors (e.g., Sakura Tissue-Tek VIP, $50,000) and stainers (Sakura Tissue-Tek DRS, $30,000) may be acquired for this purpose and are easily adapted to large sections.

The direct labor and materials expense for LBP is moderated by cost savings resulting from a reduction in the number of conventional pathology sections submitted per case (Table 7). The focused, image-guided nature of the LBP technique results in the elimination of low-yield "random" sections typically submitted in mastectomy and lumpectomy cases. The direct technical component labor and materials expense for conventional and large-format slides is given in Table 8. This expense calculation does not include indirect expenses such as allocated overhead and staff training or amortization of capital equipment. Also not included in the expense calculation is an allowance for additional professional time for LBP gross specimen examination by pathologists or pathology assistants. The net incremental technical expense per case is derived by subtracting the savings realized in reducing the number of conventional pathology sections per case from the increased expense associated with large section submission (Table 9).

Table 6: Extent measurements of duct carcinoma *in situ*. Comparison of 462 consecutive breast conserving surgical specimens. Conventional pathology without MRI (CP) versus large format breast pathology with MRI (LBP).

DCIS Extent, mm median (range)	CP $n = 250$	LBP $n = 212$
Histopathology	6.1 (1–24)	29.1 (1–125)
Mammography	11.0 (0–69)	12.0 (1–68)
MRI	Not performed	27.0 (2–113)

4. Discussion

For nearly three decades, numerous reports have analyzed pathologist-derived data such as DCIS extent or distance to surgical margins to predict risk for cancer recurrence or provide an evidence-based rationale for surgical reexcision following breast conservation surgery. The pathologic methods used to generate this data have been poorly standardized and largely bereft of imaging correlation. The reproducibility of the methods typically used by pathologists to examine surgical specimens and report breast cancer attributes has been largely unchallenged until recently.

The analysis and reporting of surgical margins, as it is practiced today, remains a crude science. For example, DCIS may escape gross detection in the dense as well as fat-replaced breast. Without gross findings to provide guidance, margin sections are often randomly submitted without reference to imaging studies. On all but the very smallest of excisions, only a subset of the specimen surface is examined histologically. For these reasons, the typical margin analysis may not accurately and completely document proximity of neoplasm to the surface margin. To its credit, the 2005 International Consensus Committee Panel on Image-Detected Breast Cancer made general recommendations for the examination of surgical specimens including the routine use of correlative specimen radiography or ultrasonography [57]. The panel also recommended "rigorous and documented" specimen sampling to allow for a targeted return for additional sampling or, better still, processing of the specimen "in its entirety." These recommendations, however welcome, have not resolved the barriers to information flow between imaging and pathology departments nor were they intended to address the additional costs associated with complete specimen processing. The panel did not reference large-format techniques as described by Tot and others [50, 56].

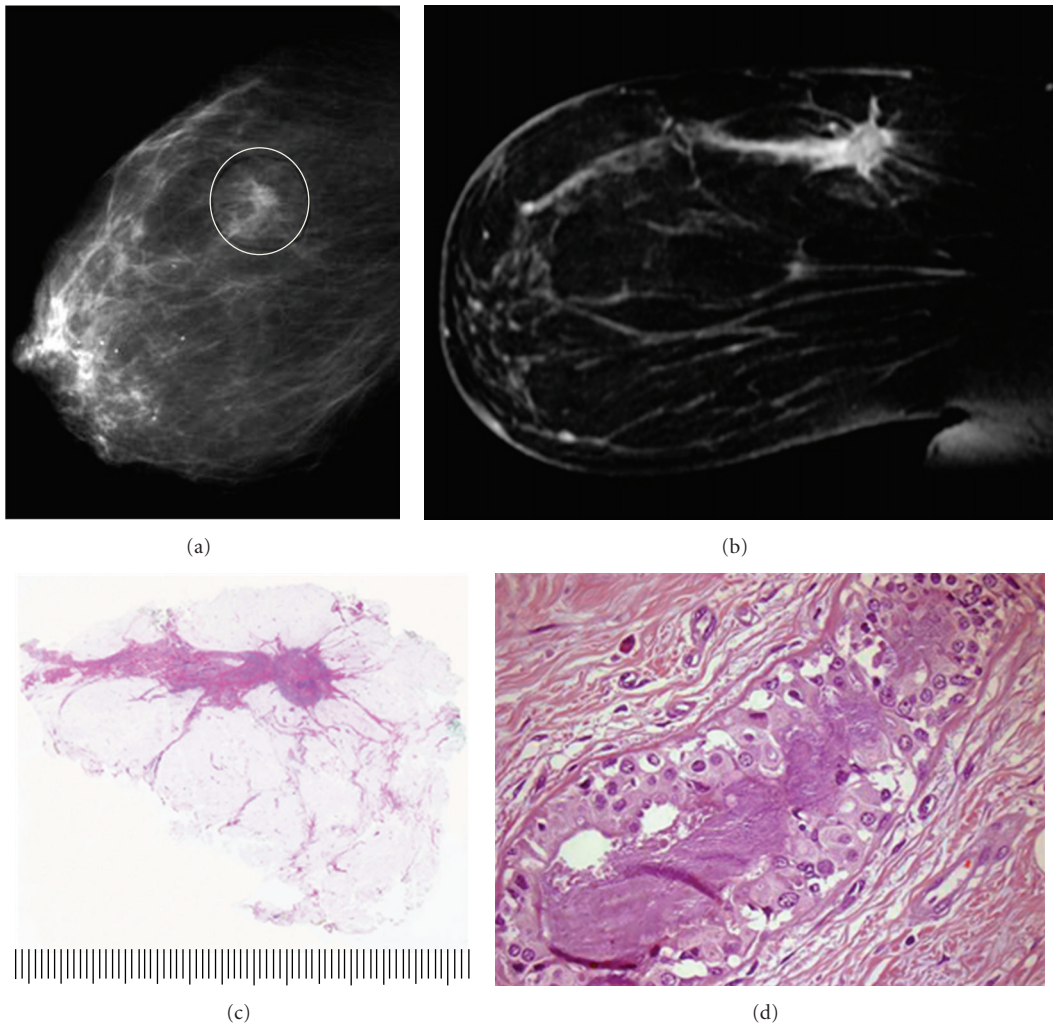


Figure 9: Seventy-one-year-old woman presenting with a palpable nodule of the right breast. (a) Right MLO mammogram with 14 mm stellate density. (b) MRI of right breast, sagittal reconstruction. The estimated overall extent of enhancing lesion 60 mm. (c) Large-format pathology slide of lumpectomy specimen, sectioned in the sagittal plane. Overall extent of DCIS is 60 mm with a superimposed 15 mm invasive ductal carcinoma corresponding to stellate density (H&E). (d) DCIS grade 3 located 2 mm from the anterior-superior surgical margin (H&E, 100x).

Table 7: Average number of conventional tissue blocks per case, year 2004-2005. Comparison between conventional pathology (CP) and LBP Program (LBP). $N = 100$.

CP	LBP
Lumpectomy, mean (range) 14.9 (7–33) $n = 28$	4.9 (3–12) $n = 22$
Mastectomy, mean (range) 13.0 (6–31) $n = 28$	6.9 (5–11) $n = 22$

In clinical practice, less than optimal correlation occurs between imaging and pathologist-reported tumor characteristics. The College of American Pathologists, as part of its voluntary Q-Probes quality assessment program, recently reported a retrospective pathology-imaging correlation study from 48 institutions [58]. This study is an important step in the right direction but was restricted to self-reported pathology departmental processes from core needle biopsies and did not purport to evaluate routines involving surgical excision specimens. Even so, it provides valuable insight into the prevailing attitudes and practices among the subset of pathologists participating in the program. In the CAP Q-Probes study, most pathology departments (65%) did not have a formal mechanism in place to correlate imaging with core biopsy results. The frequency with which pathologists conduct formal imaging correlation with lumpectomy or mastectomy results prior to finalizing the pathology report is unknown and may be even lower. Many breast pathologists currently use specimen radiography to identify calcifications in surgical specimens, needle biopsy cores,

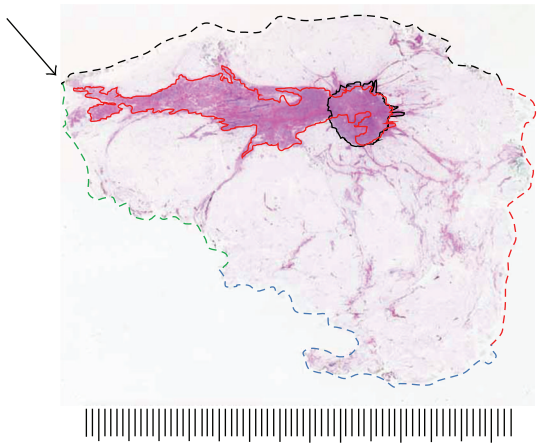


Figure 10: Large-format section of lumpectomy specimen from Figure 9. Analysis of neoplasm size, extent, and margin status. Invasive grade 2 ductal carcinoma is outlined with solid black line, 15 mm greatest dimension. Grade 3 DCIS extent outlined with solid red line, 60 mm overall. Circumferential margins marked with broken line. Specimen was sectioned in the sagittal plane to best evaluate critical anterior-superior margin. DCIS extends to within 2 mm of intersection of anterior and superior margins (arrow). Scale: small divisions = 1.0 mm (H&E).

Table 8: Direct labor and material expense per finished slide excluding amortization, year 2005. Comparison of conventional and large-format slides. $USD. $N = 50$ cases.

Slide type	Labor and materials per slide ($USD)	Approximate maximum surface area (cm^2)	($USD)/$cm^2$
Conventional	3.22	8.0	0.40
Large Format	38.22	80.0	0.48

Table 9: Imaging-guided large-format breast pathology net incremental technical expense per case. $USD, 2005.

Lumpectomy/excision ($n = 20$)	$USD
Increased expense from large-format section	38.22
Savings from reduction in conventional slides/case (mean reduction = 10 slides)	<32.20>
Net expense increase per case	6.02
Mastectomy ($n = 20$)	
Increased expense from large-format section	38.22
Savings from reduction in conventional slides/case (mean reduction = 6.1 slides)	<19.64>
Net expense increase per case	18.58

or paraffin blocks; breast imager involvement with these investigations is variable and largely unknown. The imaging-guided large-format breast pathology techniques described here require imager involvement and eliminate barriers to the communication of relevant clinical and imaging data to the pathologist. These techniques involve the imager prospectively in the identification and reporting of multiple tumor foci, axis of greatest cancer extent, and the proximity of impalpable neoplasm to surgical margins. The addition of imaging guidance to the pathologist's examination is not only relevant but timely. The best opportunity for pathologist identification and anatomic localization of these findings comes before the specimen is morcellated and the 3D relationships are lost. Preservation of the breast anatomy in three dimensions is an essential aspect of this technique. Not only is the pathologist able to reconstruct the extent of the neoplasm in three dimensions, but the breast imager is better able to reconcile true cancer extent with imaging findings, especially MRI enhancing lesions.

The long-recognized discordance between reported pathologic tumor characteristics and imaging estimates of tumor size and extent has persisted despite a better presurgical impression of the complexity of breast cancers in three dimensions. This discordance has been attributed by some to a lack of specificity on the part of the imaging studies themselves [44] without reference to the limitations inherent in the pathology techniques long held as the gold standard for measuring breast cancer characteristics. A formal validation study of breast MRI using benchmark large-format examination techniques is beyond the scope of this paper but could contribute significantly to our understanding of the sensitivity and specificity of presurgical MRI in predicting breast cancer extent and multifocality.

Although it is generally agreed that most breast cancer recurrences after BCS are related to residual cancer in the ipsilateral breast, large-format specimen analysis illustrates how complex the 3D architecture of breast cancer, particularly DCIS, can be. Clearly, any margin sampling method short of including 100% of a specimen surface will be representative by nature. To improve the sensitivity of margin analysis, a strategy to improve the yield and specificity of margin section selection is required. At the present time, imaging guidance combined with large-format histopathology offers the most accessible technique for the routine clinical laboratory. The prevalence of ink migration into the interior of resection specimens and its influence on margin reporting is unknown; it may contribute to the underreporting of margin width in some cases. A fair question is whether a more thorough approach to margin evaluation resulting from LBP results in a higher incidence of close or inadequate surgical margins. In this paper, the opposite was found to be the case (Table 5). As an isolated undertaking, an LBP program would hypothetically result in the reporting of close or positive margins with greater frequency. In contrast, this paper describes an improvement in margin status and reexcision rate. The reported outcomes occurred, not from a large-format program in isolation, but from an LBP program fully integrated into an interdisciplinary breast center. Meticulous interdisciplinary presurgical planning with post-surgical outcome analysis and correlation of pre- and postsurgical imaging studies routinely occurred in a context of large-format pathology mapping of tumor extent and margin proximity. Optimized in this way,

large-format-imaging correlations can influence not only the selection of surgical procedure, but the extent and volume of breast tissue removed to follow the anatomic distribution of cancer in the breast. Imagers become more conversant with the biologic behavior of breast cancer subtypes and their varied appearance in imaging studies, particularly MRI. Surgeons likewise gain confidence in the interpretation and implications of presurgical imaging studies. It seems likely that including a breast surgeon in the enhanced imaging large-format correlation process can improve the cross-disciplinary understanding of case-specific nuances in cancer distribution and optimize utilization of presurgical imaging studies to plan breast-conserving surgical procedures.

The present-day clinical reliance on nonstandardized measurements of DCIS extent and proximity of DCIS to surgical margins to guide management decision making should raise theoretical and practical concerns. Most breast pathologists are aware of the challenges faced in the gross specimen identification of DCIS, the difficulty in measuring overall DCIS extent in resections and the practical necessity of limiting the number of sections taken from surgical specimens. As a result, the resulting data presented in the literature is a confusing blend of methodologies and is neither comparable across studies nor is it applicable to individual practice environments. The implication for future investigative work is that nonstandardized pathologist-generated breast cancer data will continue to obfuscate clinical research. All of these limitations existed well before the advent of breast conservation surgery, but their relevance is greater today. Many clinical decisions, such as whether to offer breast conservation, surgical reexcision, or radiation therapy, are based on pathologic parameters not deemed relevant to report a generation ago. In the present era of screen-detected breast cancer in asymptomatic women, the unnecessary limitations posed by traditional examination methods are a relevant concern not only for the occult component of a symptomatic or palpable lesion (as they undoubtedly were in the past), but also for the entire neoplastic process involving some patients' breasts.

Pathologists are understandably reluctant to intentionally increase costs and effort associated with specimen processing and reporting, especially in an environment of fixed reimbursement. Objectively, the incremental direct costs associated with the LBP program (Table 9) are moderated by a reduction in low-yield, random histopathology sections. Expressed as dollar expense per unit surface area examined, the technical costs of conventional and large section pathology are quite similar (Table 8). With a targeted, imaging-assisted specimen examination protocol, the pathologic reporting of clinically relevant data becomes more focused and efficient. The additional expense associated with an LBP program must be viewed in the broader context of interdisciplinary improvements in clinical efficiency and compassionate care and not as the sole burden of the pathology laboratory or department. The clinical consequences of improved margin status and lower reexcision rates for individual patients are readily apparent; viewed from the perspective of enhanced cost-efficiency on a national or global scale the gains could be substantial.

5. Conclusions

This paper describes the successful incorporation of a large-format breast pathology program into an existing comprehensive breast care center based on a non-profit health system in the United States. From its inception, the program was designed to enhance the bidirectional information flow among breast imagers, surgeons, and pathologists. A goal of the initiative was to provide the breast team members with a more precise characterization of breast cancer attributes relevant to prognosis and clinical management through improved imaging, pathology, and clinical correlation. These measures were deemed appropriate in view of the increasing clinical reliance upon pathology-reported breast cancer attributes, wherein relatively small increments in reported tumor variables can translate into significant changes in clinical management and perceived prognosis. The experience derived from the development of this program indicates it is feasible and desirable for community hospital-based pathology and breast imaging departments to adopt these processes for the benefit of all patients in a comprehensive, interdisciplinary breast center.

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Review Article

The Value of Large Sections in Surgical Pathology

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Large format sections (LS) first have been introduced in breast pathology more than a century ago. Since then, they constituted for longtime a research tool to better understand breast microanatomy and the relationship between radiological images and pathological features. Similarly LS have been used to study neoplastic, inflammatory, and degenerative diseases affecting various organs, as brain, lung, gastrointentinal tract, bone, urinary tract, prostate, and placenta. Currently LS are mostly applied to diagnostic routine to better stage tumours such as prostate and breast carcinomas or to correlate radiologic imaging to gross specimens. The purpose of the present paper is to review the historical background and the basis of the applications of LS in surgical pathology, with special emphasis on breast tumours.

1. Introduction

Large format sections (synonym macrosections) (LS) comprise an entire histological section of the organ under study. LS allow the histological study of a large part of the organ of interest, that include not only the lesions but also the surrounding tissues possibly inclusive of the entire margins at least along a plane of sectioning. The purpose of the present paper is to review the history and the present practical diagnostic applications of LS.

2. Historical Background

LS have been introduced in surgical pathology more than a century ago. The first study based on LS was published in 1906 by Cheatle [1] who realized that most patients were diagnosed to be affected by breast cancer only when the disease was too advanced to be cured. Therefore, as breast was the first organ to be studied, which has generated a large number of papers, this paper will first deal with breast tumours, followed by the application of LS in other organs.

Dr. Cheatle used LS in cases of breasts affected by cancer to better understand the relation between the neoplastic mass and the surrounding normal tissue and the possible existence of premalignant changes [1–3].

The relation between breast cancer and the surrounding tissue was the object of a further study in 1939 by Ingleby and Holly [4]. Subsequently, Marcum and Wellings [5] improved and simplified the method which then was applied to study the early phases of breast cancer development [6, 7]. In 1973, Wellings and Jensen [8] analysed cases of in situ and invasive ductal and lobular in situ and invasive carcinoma leading to the proposal that most breast carcinomas arise in the terminal ductular-lobular unit (TDLU), in spite of the different morphological features.

These papers were paralleled with useful studies on mouse and human breasts to better understand the breast microanatomy [9–11] which was also accurately described by Going and Moffat [12] together with the distribution of the mammary lobes. Tot [13] based his theory of the sick lobe on studies performed on LS.

Although LS, especially at the beginning, were used in a few laboratories only, their impact on the evaluation of multifocality and multicentricity of breast cancer appeared relevant. Egan [14], in a seminal paper, demonstrated that the incidence of cases showing multifocal breast cancer was more frequent than that obtained from studies based on conventional small block slides. This led to the discovery that multifocal breast carcinomas have a worse prognosis in comparison with unifocal lesions. Similar results were

subsequently obtained in 1986 by Sarnelli and Squartini [15] and, more recently, by Tot et al. [16, 17].

Since 1992, Faverly et al. [18] employed LS for a three-dimensional reconstruction of the breast glandular tree, in order to evaluate the extention and type of growth of different types of in situ duct carcinomas (DCIS) [19, 20]. These studies greatly increased the knowledge on the extent and distribution of DCIS, as they demonstrated that DCIS is frequently a multifocal process and that multifocality is a typical feature of low grade rather than high grade DCIS. Along this line were the results obtained on multifocality and multicentricity by Tot et al. [16, 17] and Foschini et al. [21].

In the last decade, LS were demonstrated to be useful to correlate radiological findings and pathology as it is simple to compare radiological images to the large histological sections [22]. Specifically the widespread use of mammographic screening for early detection of breast cancer identifies numerous benign lesions that can be difficult to interpret on the mammogram. This was mostly done by Tot et al. [23] who gave a better definition of several benign breast lesions, among which radial scar, which does not need any further diagnostic investigation. More recently Tot and Tabár [24] correlated the nuclear magnetic resonance (NMR) findings with LS demonstrating that LS can help to better diagnose the different histological lesions and features that characterize all the breast malignant tumours.

3. Methods

In spite of their utility in diagnostic pathology, LS are still used in a relatively few laboratories, mainly as a consequence of the fact that their preparation is perceived as more timeconsuming and expensive than conventional blocks. This difference is only apparent as in LS a large portion of tissue is examined that is far superior to the conventional small blocks. Tot [25] calculated the costs of LS in daily practice and compared them with those obtained from conventional blocks, demonstrating that LS costs do not substantially differ from those of accurate conventional blocking. Tucker [26] calculated the cost of LS in a breast care centre and concluded that the LS costs increase from 6.02 USD in cases of lumpectomies to 18.58 USD in cases of mastectomies which was regarded relatively inexpensive and balanced by a better staging and more accurate evaluation of resection margins [26]. As a consequence additional surgical procedures were lowered in number, which led to a decrease of the overall cost of each single patient's treatment [26].

The method to obtain LS has been previously described in several papers [27], as well as it is described by Tucker [26] in the present issue.

At our institution, the breast specimens (as well as specimens from other organs) are sliced with a large blade, into sections 5 to 10 mm thick, possibly under radiological guidance or following indications given by the surgeon. One to three LS are obtained from each case and in addition the of rest of the surgical specimen is embedded using traditional small blocks. Additional automatic processor is used to work overnight. In cases of small quadrants, when the LS major axis is less than 5 cm, the entire specimen is embedded using the same procedure employed for prostatectomies as illustrated by the specific paper of this issue [28]. This last procedure is less time consuming with a shorter turn-around time to obtain the LS. In addition these "smaller" LS are easy to manage at the microscope and can be easily digitalized into virtual slides with the proper hardware (personal comunication).

Paraffin blocks from paraffin embedded LS [26, 27, 29] are then cut with a dedicated macrotome. Finally haematoxylin-eosin large slide is obtained. Orientation is maintained during the whole embedding process and reported in the final slide.

When immunohistochemistry or molecular studies are needed, areas of interest are selected from the LS and cut to obtain small conventional blocks [30].

LS can be used also for 3-dimensional reconstructions as previously shown [19, 29] and summarized as follows.

LS blocks are deparaffinided by melting paraffin at 60°C from 3 to 4 hours, subsequently tissue is immersed in xylene (four times) for at least 24 h to remove residual paraffin. Tissues are then rehydrated as follows: 50% absolute alcohol and 50% xylene (1 hour), absolute alcohol (2 hours), and 70% alcohol (2 hours). Blocks are washed overnight in distilled water, stained in Harris' hematoxylin for 4-5 min, rinsed in tape water for 10 min, and immersed in four baths of acid alcohol for 8 min each. Finally tissues are dehydrated, through a graded series of alcohol to xylene, and finally immersed in methyl salicylate for one night. 3D examination is performed using a stereomicroscope. The H&E-stained LS slide is used to retrace the lesions to examine on the corresponding cleared tissues.

4. Large Sections in Breast Pathology

LS are useful during the everyday breast routine practice to better evaluate the tumour dimension, the in situ carcinoma extension, and the resection margins. In the series studied by Foster et al. [31], LS gave more information than conventional blocks in 172 cases out of 656, as they evidenced additional findings of potential clinical use, as involved margins, minute multiple foci of invasive or in situ carcinoma, or change in size and extent of the tumour under study.

Correct evaluation of resection margins has become an increasingly important issue especially in cases treated with quadrantectomy. LS are cut and oriented according to the radiological images and the indications given by the surgeon, and orientation is maintained during the paraffin embedding procedures. This allows the exact evaluation of the relationship between invasive or in situ carcinoma and the adjacent surgical margin. By contrast conventional blocking is based on gross inspection at naked eye of the lesions and on palpation of the tissues; therefore, minute cancer foci, immersed within the fatty breast stroma can escape from examination (Figure 1).

In addition having the possibility to visualize the whole section of the breast specimen, it is easier to distinguish the

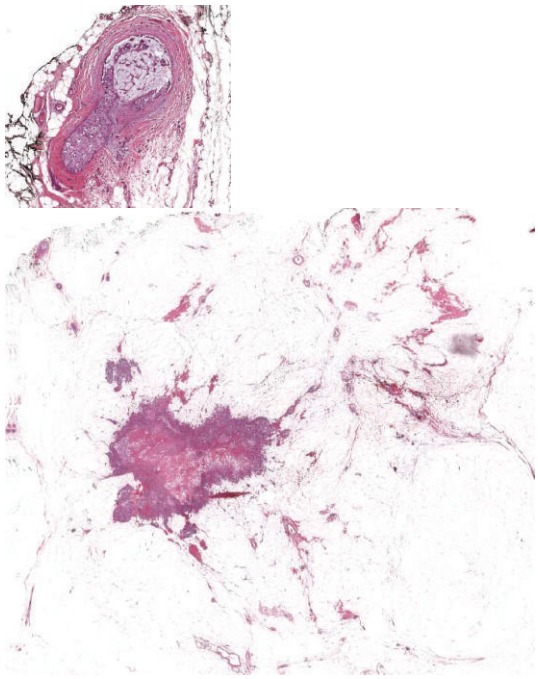

Figure 1: Quadrantectomy: a small focus of DCIS, immersed within the fatty tissue, is present in a surgical margin (inset).

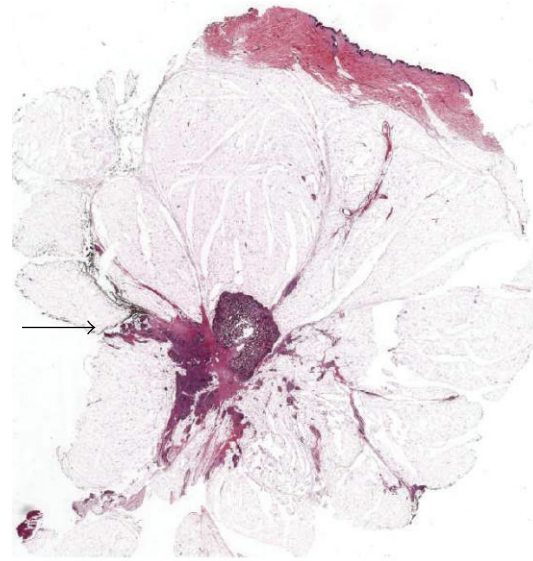

Figure 2: Quadrantectomy: the real inked margin can be easily recognized and distinguished from ink migration into fissures of the fat tissue (arrow).

real inked margin from ink migration through tissue fissures frequently present in breast tissues as also stated by Tucker [26] (Figure 2).

Exact evaluation of tumour dimension is at the basis of a correct staging. Jackson et al. [32] compared two series of breast carcinomas, one studied with conventional histological method and the second studied with LS. Accordingly the correct size of the tumour was assessed in all the cases studied with LS, while it was assessed in 63% only of the cases studied by conventional histology. We compared the tumour size on a series of 102 consecutive quadrantectomies evaluated with both LS and conventional blocks. Accordingly, in 9/102 (8,8%) LS helped to correctly assess the dimension of the tumour better than the small blocks, especially in invasive lobular carcinoma, where the macroscopic borders of the tumours were ill defined and difficult to be measured at macroscopical level only [33].

In addition the widely spread breast cancer screening programs lead to the detection of a high rate of in situ carcinomas and of microscopic foci of invasive carcinomas.

Due to the use of LS, it is becoming evident that breast cancer often presents with multiple foci and unifocal, multifocal and multicentric in situ, or invasive carcinomas [16, 17, 21] appear better demonstrated. Tot et al. [17], on a study performed on 574 consecutive cases studied with LS, found that invasive carcinomas was multifocal in 24% and diffuse in 5% of the cases, percentages that are largely superior to those published in paper based on conventional blocking.

The prognostic value of multifocality in breast cancer has been widely debated in the literature. Nevertheless, studies performed on LS [14, 17] have shown that multifocality has a great impact on survival, as the risk of death for breast cancer is higher in patients with multifocal and/or diffuse carcinomas when compared with those with unifocal carcinomas. Similar results were obtained at our institution, when LS were compared to conventional small blocks in a series of 102 consecutive cases diagnosed during the year 2010. The most consistent additional information that LS have provided, over conventional small blocks, was tumour multifocality (27/102 cases, 26,4%). Patients with multifocal tumours exhibited axillary lymph-node metastases in 71,42% while those with unifocal tumours showed axillary nodal involvement in 40,54% [34]. These data confirm that multifocality can be useful in the evaluation of the risk of axillary involvement by breast cancer metastases and confirm that the detection of multiple breast cancer foci has a great prognostic impact and therefore should be carefully searched in all cases of breast cancer.

In cases of breast cancer diagnosed in advanced stages, surgery is preceded by neoadjuvant chemotherapy with the aim of reducing the tumour mass. In order to correctly stage breast cancers treated by neoadjuvant chemotherapy, it is of vital importance to evaluate the presence of residual tumour and the degree of tumour regression [35]. In cases showing good response to neoadjuvant chemotherapy the tumour mass greatly decreases and sometimes is difficult to evaluate on macroscopy. Therefore, when histology is performed on conventional blocks, small residual tumour foci frequently escape detection. To this purpose the use of LS improves the correct evaluation of the resection specimen (Figure 3). Embedding the whole area previously occupied by the tumour can improve the chance to detect even small residual neoplastic foci.

Finally LS are of use also in the evaluation of other prognostic parameters, such as vascular invasion, which is

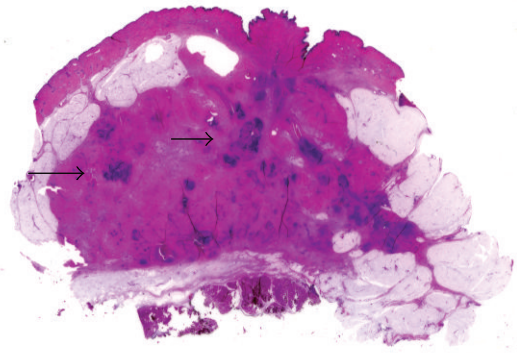

Figure 3: LS performed on a case treated with neoadjuvant chemotherapy evidence small foci of residual invasive breast carcinoma (arrows).

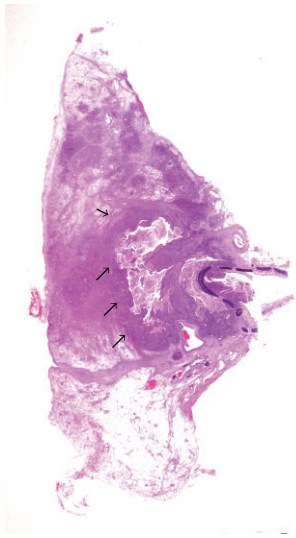

Figure 4: LS clearly visualizes the relationship between lung cancer (indicated by the arrows) and the surrounding obstructive pneumonia.

used to plan chemotherapy. Comparing conventional blocks and LS on 102 consecutive cases treated at this institution, in 14 cases (13,72%) vascular invasion was detected in LS only [34].

5. Large Section in Anatomic and Surgical Pathology

Similarly to breast pathology, during the last century, LS have been used to shed light on different pathological processes of various organs.

Since 1960 LS have been proven to be useful to study the extension and distribution of lung diseases as emphysema [36] and, more recently the size of the tumours, to plan radiotherapy in cases of nonsmall lung cancer [37]. In current practice, LS are useful in staging cases of lung cancer (Figure 4) as the relationship between the tumour mass and the adjacent obstructive pneumonia is difficult to establish at macroscopic level (Figure 5). In addition the assessment of pleural invasion or resection margins that may be problematic on macroscopic examination, are readily evaluated on LS.

LS have been widely used in bone pathology to compare the radiological images to the different pathological aspects of benign and malignant bone tumours [38–40]. Specifically, type of growth and extension of osteosarcoma and chondrosarcoma were elucidated comparing radiological imaging and LS from surgical resection specimens. Bertoni et al., [41] using LS, have demonstrated that paraosteal osteosarcomas with areas of dedifferentiation usually show intralesional radiolucencies on radiological images.

LS have also occasionally been used to study the inner ear anatomy [42], but have been largely used to study normal brain anatomy [43] and degenerative brain diseases. LS of brain tissue are stained using several histochemical methods that help to evidence grey and white matter (Figure 6) and the related lesions and, as recently demonstrated by Howell et al. [44], are useful to perform a detailed mapping of degenerative brain lesions.

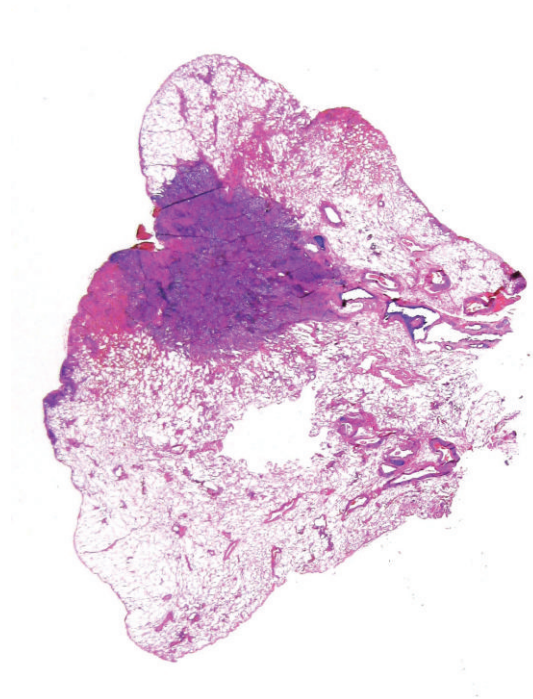

Figure 5: Lung cancer is easily staged using LS that evidence pleural invasion and distance from the bronchial margin.

In practice, in the daily practice, LS are potentially useful to understand, diagnose, and manage the pathological lesions from all organs.

Accordingly, Slootweg and Grot [45] applied LS to stage the neoplastic lesions of the head and neck district, comprising the different areas of the oral cavity and the larynx. Tumours arising in the head and neck region often involve

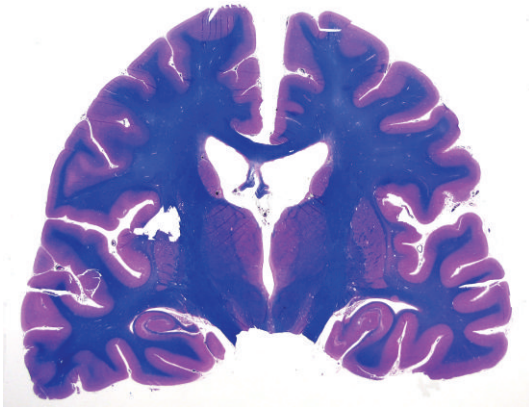

Figure 6: Large format section of brain, stained with the Luxol-fast blue technique, evidence the normal brain anatomical structures.

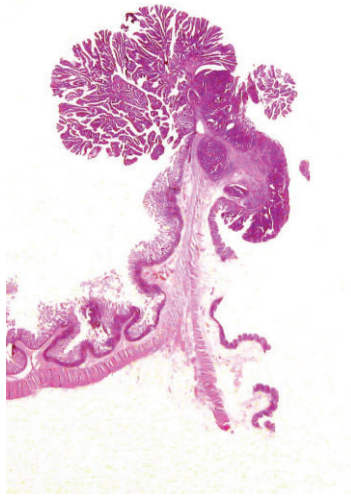

Figure 7: Large format section from an intestinal adenomatous polyp with adenocarcinoma. The level of invasion by the adenocarcinomatous area is easily assessed.

mandibular and maxillary bones and the surrounding soft tissues. Evaluation of the extent of the tumoural growth and tissue involvement, especially in this district, is of crucial importance to perform a correct staging. As tissues from head and neck region have different consistency. Slootweg and Grot [45] proposed to cut the surgical specimens using an engine driven water-cooled diamond saw and to obtain LS inclusive of bone and surrounding tissues. These LS are optimal for all types of neoplastic lesions affecting the head and neck region and can easily and unequivocally demonstrate the type and extension of tumoral growth.

LS have been proven to be useful in visualizing gastrointestinal tumours [46] (Figure 7). During the last two decades, the wider use of colorectal endoscopies and the application of screening programs for colorectal cancer have led to the recognition of early neoplastic lesions that can be of difficult interpretation using conventional blocks histology. LS allow

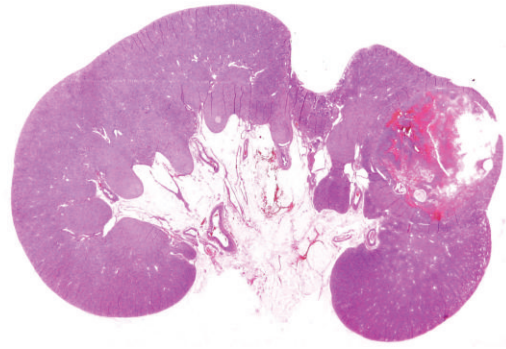

Figure 8: Example of large format section of a case of clear cell carcinoma of the kidney. The relationship with the tumour and the surrounding tissue, capsule, and urinary pelvis are well evident. In addition the renal parenchyma shows an area of pyelonephritis.

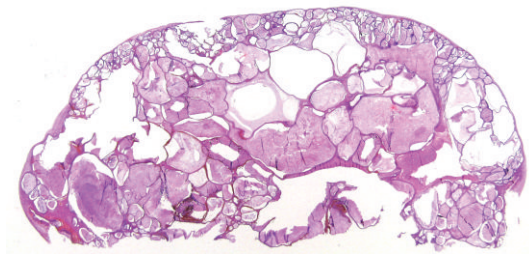

Figure 9: Mucinous cystoadenocarcinoma borderline of the ovary, measuring 11 cm in greatest axis. A whole section of the tumour leads to a more accurate diagnosis.

to visualize the whole lesions and to correlate the histological features with the endoscopic findings.

LS were useful to demonstrate the pathway of placental diffusion of cytomegalovirus infection in twins [47] and more recently to study the amyloid involvement in the heart [48].

For diagnostic purposes LS can be applied to almost organs (Figures 8, 9, and 10).

As shown in Figure 11 in a case of transitional cell carcinoma of the urinary pelvis, LS clearly demonstrated that the tumour under study was composed of two distinct neoplastic foci, separated by uninvolved urinary epithelium.

Currently in prostate pathology, LS constitute a standard of care in the staging of prostate cancer, as carefully explained by Montironi et al. [28] in this issue.

In addition as prostate cancer must be excluded in donor candidates during organ explanation, a method for cyrosectioning the whole prostate has been proposed [49, 50].

6. Conclusions

LS have been applied in pathology for research and diagnostic purposes since the beginning of the 20th century. In spite of this long history that downgrades the LS to the level of an "old" technique its value is still consistently useful in the

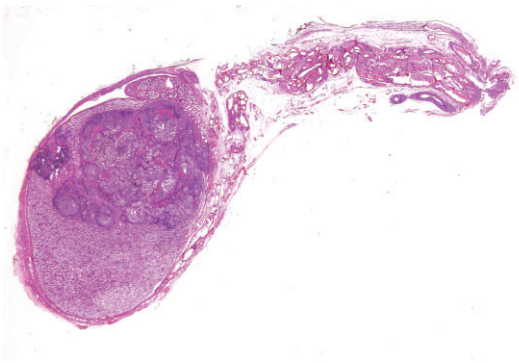

Figure 10: Testicular seminoma: the relationship between the tumour and the tunica albuginea is well evident.

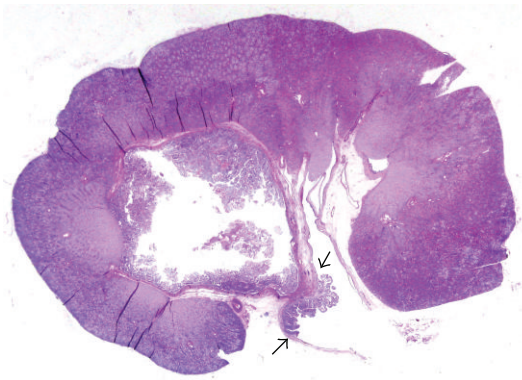

Figure 11: LS on a transitional cell carcinoma of the urinary pelvis demonstrates that the tumour shows two separate foci (arrows).

every day practice, especially in tumour pathology both for breast and most organs.

The criticism that the LS increase the cost and turnaround time of the surgical pathology routine work is not anymore tenable, as it has been demonstrated their cost-effectiveness and the turnaround time not longer than 24 hours. Therefore, an increasing application of LS in the daily pathological practice is auspicial.

Acknowledgments

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Review Article

The Role of Large-Format Histopathology in Assessing Subgross Morphological Prognostic Parameters: A Single Institution Report of 1000 Consecutive Breast Cancer Cases

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Breast cancer subgross morphological parameters (disease extent, lesion distribution, and tumor size) provide significant prognostic information and guide therapeutic decisions. Modern multimodality radiological imaging can determine these parameters with increasing accuracy in most patients. Large-format histopathology preserves the spatial relationship of the tumor components and their relationship to the resection margins and has clear advantages over traditional routine pathology techniques. We report a series of 1000 consecutive breast cancer cases worked up with large-format histology with detailed radiological-pathological correlation. We confirmed that breast carcinomas often exhibit complex subgross morphology in both early and advanced stages. Half of the cases were extensive tumors and occupied a tissue space ≥40 mm in its largest dimension. Because both in situ and invasive tumor components may exhibit unifocal, multifocal, and diffuse lesion distribution, 17 different breast cancer growth patterns can be observed. Combining in situ and invasive tumor components, most cases fall into three aggregate growth patterns: unifocal (36%), multifocal (35%), and diffuse (28%). Large-format histology categories of tumor size and disease extent were concordant with radiological measurements in approximately 80% of the cases. Noncalcified, low-grade in situ foci, and invasive tumor foci <5 mm were the most frequent causes of discrepant findings.

1. Introduction

Breast cancer is a heterogeneous group of diseases which deviate from each other in natural history, morphology, molecular phenotype, clinical and radiological manifestations, and prognosis. Prognostic parameters are essential for predicting the outcome and response to therapy in individual cases. The long list of more or less powerful prognostic parameters that includes patient age, mode of detection, tumor size, histologic grade, lymph node status, and presence or absence of distant metastases was recently widened with molecular tumor phenotypes assessed with either genetic tests or immunohistochemistry. Since the number of therapeutic options is rather limited, the parameters for which assessment is routinely required for therapeutic decisions are also few. Whereas hormone receptor status, HER-2 status, and proliferative activity are the major determinants of oncological therapy, proper characterization of the subgross morphology of breast carcinoma is essential for planning appropriate surgery and radiation therapy [1–4]. The prognostic significance of subgross parameters is also observed [1, 4, 5].

For correct subgross characterization of a case, the following parameters should be assessed: tumor size (defined as the largest diameter of the largest invasive focus), lesion distribution (unifocal, multifocal, or diffuse distribution of the invasive and in situ tumor components), disease extent (corresponding to the tissue volume containing all the malignant structures within the breast), intratumoral or intertumoral heterogeneity, and the position of the tumor within the breast [5]. These parameters can be assessed with radiological and histopathological methods, the most efficient being a combination of these methods in the form of detailed and systematic radiological-pathological correlation [5–10].

An applied histopathology method substantially influences the success rates of documenting and assessing this subgross morphological parameters and correlating them to

radiological findings. The traditional small block sampling method is based on taking 1-2 cm sized samples from breast specimens, often under the control of only the pathologist's naked eye and sometimes using radiological guidance. This way, the specimen is fragmented and the interrelationship of the different tumor components, which are not represented in the same block, is destroyed. Taking large numbers of small blocks, sequential numbering of the blocks, and marking the sample placement on a macrophotograph of the specimen or in the specimen radiograph represent attempts to compensate for the obvious limitations of the sampling method. At the same time, these attempts are proof that such compensation is necessary. Large-format histopathology is based on embedding and processing contiguous tissue slices representing the entire cross section of a segmentectomy specimen, preserving the interrelationships of the components of the tumor, and documenting them together in one plane. This advantage makes this method the best approach in correctly assessing the subgross morphological parameters, which also facilitates the detailed radiological-pathological correlation [5, 6, 8–10]. This technique has been successfully adapted to the needs of busy routine laboratories and the procedure has been repeatedly described in detail [5, 6, 11–13]. The advantages of this method have also been observed in a recent cost-benefit analysis [14].

2. Documenting the Extent of the Disease

Defined as the tumor volume containing all the actual malignant structures within the breast, the extent of disease is the most important subgross parameter influencing the feasibility of breast-conserving surgery in an actual case [15]. This is the volume of breast tissue the surgeon aims to remove within certain margins in order to prevent local recurrences. Disease extent that is ≥40 mm in the greatest dimension is associated with an approximately three-fold risk of ipsilateral local recurrence after breast conserving surgery and irradiation compared with those cases with disease extent limited to a volume of <40 mm [4, 16]. In addition, patients with extensive disease (≥40 mm in the largest dimension) have significantly decreased long-term disease-specific survival compared with those with tumors of limited extent [17]. All this underlines the importance of correctly assessing the subgross morphological prognostic parameter.

In everyday routine, the pathologist should begin the analysis of a case by recapitulating the radiological findings, including the radiological disease extent. The next step should be comparing the uncut specimen with the whole specimen radiograph and keeping the *in vivo* orientation of the specimen by inking it at its margins [11].

Breast cancer is a lobar disease most often involving parts of a single sick lobe [18, 19]. The lobe is a pyramid-like structure, with the lactipherous duct opening in the nipple, branching in the direction of the pectoralis muscle, and ending up in a large number of terminal units. In order to demonstrate the largest cross-section of the involved lobe, the segmentectomy specimen has to be sliced into 3-4 mm slices parallel to the pectoralis fascia, but not perpendicular to it. The perpendicular slicing method leads to a substantial underestimate of the extent of the disease in the vast majority of ductal carcinoma in situ cases [20, 21].

The space the malignant structures occupy in the breast rarely shows the regular shape of a geometric body; it is almost always irregular. This means that the borders of this space are different at different levels of the specimen and in different projections. Consequently, the area representing the cross section of this tissue space in the tissue slices of the specimen also varies. For correct visualization of the real disease extent, the slice with the largest disease area should be chosen (based on the specimen radiograph and macroscopy), embedded, and processed; but additional levels should also be embedded because some components of the disease may not be visible on imaging and macroscopy [11].

The microscopic analysis should begin with determining the disease extent. Approaching from the periphery of the section, the pathologist should mark the most peripheral malignant structures (in situ or invasive) and repeat the process from all directions. The result will be a marked area representing a cross section through the diseased tissue. Summarizing the findings in adjacent tissue slices and/or tissue slices taken at different levels of the specimen is often necessary. Correlating the radiological and histological findings is essential [11]. The realistic aim of the disease extent assessment is an appropriate categorization of the tumor as of extensive (occupying a space ≥40 mm) or limited (occupying a space <40 mm) extent rather than achieving millimetric concordance of the radiological and the histological extent.

In a consecutive series of 1000 newly diagnosed breast cancers in our material (Central Hospital Falun, Sweden, period Dec 2007 to Jun 2012), 495 cases were extensive and occupied a tissue volume of ≥40 mm in the greatest dimension and 505 were nonextensive occupying smaller tissue volumes. Purely in situ carcinomas together with microinvasive (<1 mm, 4 cases) tumors comprised 14% (144/1000) of the series, and half of the cases were extensive (48%, 69/144) and half were nonextensive (52%, 75/144). Early invasive carcinomas (1–14 mm) comprised 35% (349/1000) of the series; 42% (146/349) were extensive, and 58% (203/349) were nonextensive. In more advanced cancers (≥15 mm in size, 50% of the series, 500/1000), 55% (273/500) of the cases were extensive (Table 1).

3. Assessing Lesion Distribution

After the extent of the disease is characterized as described in Table 1, the pathologist should judge whether the lesions within the tissue area are individual (well demarcated and separate from each other) or confluent (inseparable). This judgment is easier if the invasive tumor component(s) and the in situ component(s) are assessed separately. A simple practice of encircling the separable invasive foci with one color and the separable in situ foci with another color is helpful. While characterization of the foci requires microscopic control, the judgment of lesion distribution

Table 1: 1000 consecutive breast cancer cases by focality, disease extent, and stage. Falun, Dec 2007 to Jun 2012.

	Unifocal % (*n*/*N*)		Multifocal % (*n*/*N*)		Diffuse % (*n*/*N*)		Total % (*n*/*N*)
	Extensive	Nonextensive	Extensive	Nonextensive	Extensive	Nonextensive	
In situ	0	31 (44/144)	17 (25/144)	11 (17/144)	3 (44/144)	10 (14/144)	14 (144/1000)
Early Invasive	0	40 (140/349)	20 (68/349)	14 (48/349)	22 (78/349)	4 (15/349)	35 (349/1000)
Advanced	3 (16/500)	33 (166/500)	30 (148/500)	8 (41/500)	22 (109/500)	4 (20/500)	50 (500/1000)
Extent	4 (16/366)	96 (350/366)	69 (241/347)	31 (106/347)	83 (231/280)	17 (49/280)	99 (993/1000)
Total	36% (366/1000)		35 (347/1000)		28 (280/1000)		100* (1000/1000)

* Disease extent was undetermined in 7 cases.

after the individual lesions are marked must be carried out using a naked eye examination of the large-format histology sections, without using a microscope.

On the preoperative tumor board, the pathologist should register the radiological lesion distribution and plan the dissection of the specimen on the basis of this information. Radiologically unifocal lesions are usually properly represented in one or two large-format histology sections, provided that one of these contains the tumor at its largest cross-section [11]. In radiologically multifocal cases, several slices should be embedded to visualize as many tumor foci as possible. In radiologically diffuse cases, the most important task is to visualize the correct extent of the disease and, if the diffuse component is in situ, to catch the radiologically or macroscopically evident invasive component(s).

Our previously published system is the only one that takes into account both the invasive and in situ components of the tumor and defines their distribution both individually and in combination [22]. In addition, our system recognizes the diffuse distribution of both the in situ and invasive tumor components, in contrast with other systems described in publications on breast cancer multifocality [1, 23]. In our system, invasive lesions are considered "unifocal" if only one invasive focus can be observed that is well delineated and may or may not contain an in situ component. "Multifocal" invasive lesions are characterized by the presence of multiple, well-delineated, invasive tumor foci separated from each other by uninvolved breast tissue containing normal tissue, benign lesions, or in situ carcinoma, regardless of the distance between the invasive foci. Tumors dispersed over a large area with no distinct tumor mass, for example, like a spider's web, are classified as "diffuse." In situ carcinomas are regarded as "unifocal" if they appear to involve a single terminal ductal lobular unit (TDLU) or several neighboring TDLUs. In situ carcinomas are designated "multifocal" if they involve several distant TDLUs with uninvolved breast ducts and TDLUs in between and as "diffuse" if they mainly involve the larger ducts [22]. The distribution of the invasive and in situ components is then combined so that a diffuse distribution of either the in situ or the invasive component qualifies the lesion to be categorized as "diffuse." Multifocality of the invasive and/or in situ component indicates a "multifocal" designation. Typical cases with unifocal, multifocal, and diffuse in situ and invasive breast carcinomas are illustrated in Figure 1. As shown in Figure 2, there are 17 different combined distribution patterns in breast carcinomas (unifocal, multifocal, diffuse, or missing in situ component combined with the same invasive categories, plus a mixed category). Although the combined pattern of lesion distribution in breast carcinomas is not always easy to assess, and higher levels of interobserver reproducibility may require substantial experience [24], the combinations reduce the 17 different pattern possibilities to 3 aggregate patterns.

Multifocality is often described in the literature as the presence of satellite tumors around and in the vicinity of a dominant mass [25]. Although this situation is common, the concept is erroneous because there are cases with multiple tumor foci of approximately the same size, without the presence of a dominant mass. These foci may be dispersed over a large area without the tendency to concentrate around one foci. With regard to their evolution, two different types of multifocal invasive cancer may exist: one with multiple individual invasive foci, which develops from in situ lesions at different parts of the same lobe simultaneously or with a time difference, and one in which the individual foci represent "in transit" metastases [26] of a primary focus and are not related to an in situ component.

The cases in our series of 1000 breast carcinomas showed the following combined lesion distribution: unifocal in 36% (366/1000), multifocal in 35% (347/1000), and diffuse in 28% (280/1000), as shown in Table 1. In addition, there were 7 cases with mixed or undetermined lesion distribution. In situ carcinomas, including 4 cases of microinvasive tumors, were unifocal in 31% (44/144), multifocal in 28% (42/144), and diffuse in 41% (58/144) of cases. The majority (68%, 236/349) of the early invasive cancers (<15 mm in size) had a unifocal invasive component, but when the combined morphology of the in situ and invasive components was taken into account, the majority (60%, 209/349) were in fact multifocal or diffuse. Approximately one-third of more advanced (≥15 mm in size) breast carcinomas (36%, 182/500) had unifocal combined (in situ plus invasive) morphology, one-third (38%, 189/500) had multifocal, and the remainder (25%, 129/500) had diffuse-combined lesion distributions, mainly because the diffuse in situ component (Figure 3). Diffuse invasive cancers were rare. These data are in full agreement with our previously published results [22, 27–30] and are similar to the results of other studies based on an analysis of large-format histology slides [31, 32].

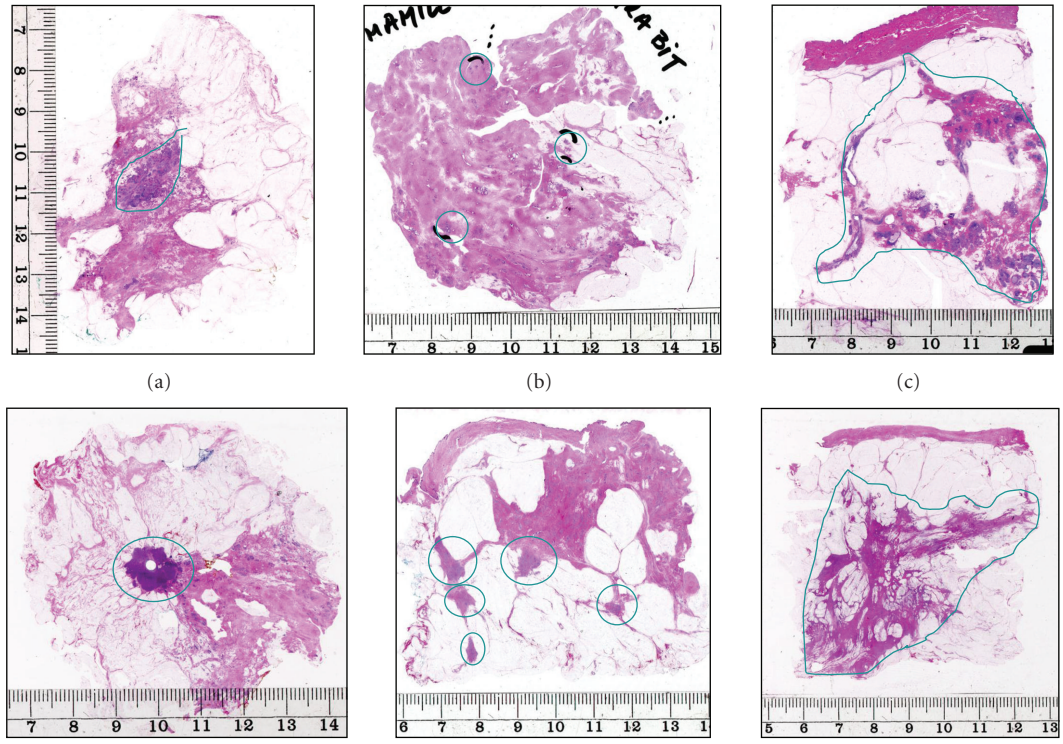

Figure 1: The basic breast cancer growth patterns. (a) Unifocal in situ carcinoma: the tumor involves neighboring terminal ductal-lobular units. (b) Multifocal in situ carcinoma: the tumor involves distant terminal ductal-lobular units. (c) Diffuse in situ carcinoma: the tumor involves large ducts and many terminal ductal-lobular units. (d) Unifocal invasive carcinoma: a single well-delineated invasive focus. (e) Multifocal invasive carcinoma: several well-delineated invasive foci in the same specimen. (f) Diffuse invasive carcinoma: poorly delineated, spider's web-like structure. All the malignant lesions are encircled.

Testing the prognostic significance of the lesion distribution defined above has resulted in clear separation of the unifocal, multifocal, and diffuse tumors with regards to the invasive component, the in situ component, and the combined distribution. Patients with multifocal or diffuse invasive carcinomas have a more than double risk of lymph node metastasis compared with unifocal tumors [22, 28–30, 33, 34], and the differences are related to macrometastatic disease [35]. Differences in disease-specific survival are also evident; patients with diffuse invasive or diffuse combined tumor growth patterns have a worse outcome, those with multifocal disease an intermediate outcome, and those with unifocal tumors have the best long-term outcome [17]. A worse survival of patients with multifocal tumors was also observed in both early [36] and recent studies [23, 37].

By stereomicroscopic examination of large-format thick histological sections, Foschini at al. demonstrated that the distance between the individual foci of some low-grade in situ carcinomas is more than 20 mm indicating the possibility that these foci are located within different lobes [32]. Although some breast lobes are large and widespread, synchronous or asynchronous development of a carcinoma in different lobes of the same breast is a real possibility. These multilobar/multicentric cases are regularly associated with multiplicity of tumor foci and with large disease extent. In practice, the above described rules of assessing the lesion distribution and disease extent are also applicable in the multilobar cases.

4. Documenting Tumor Size

Tumor size is defined as the largest diameter of the largest invasive tumor focus [25] and represents one of the most powerful prognostic parameters, a constituent of the TNM staging system. Many studies document its prognostic significance, and the larger the tumor, the purer the prognosis. This represents the basis for the success of mammography screening by finding tumors at an earlier stage of their natural history when they are still small, improving the overall prognosis of breast cancer patients in the screened population [38]. In addition to purely in situ carcinomas, microinvasive cancers, which have invasive foci <1 mm, and invasive carcinomas <15 mm belong to the category of early breast carcinomas [39, 40]. Patients with these tumors have an excellent, over 90%, 10-year disease-specific survival [40] and, provided that they are detected by mammography screening, the overall survival of these patients does not differ from the survival of age-matched women in the general population [41]. Forty nine percent of cases in our material were classified in this category: 14% (144/100) were in situ and in situ with microinvasive cancers, and 35% (349/1000)

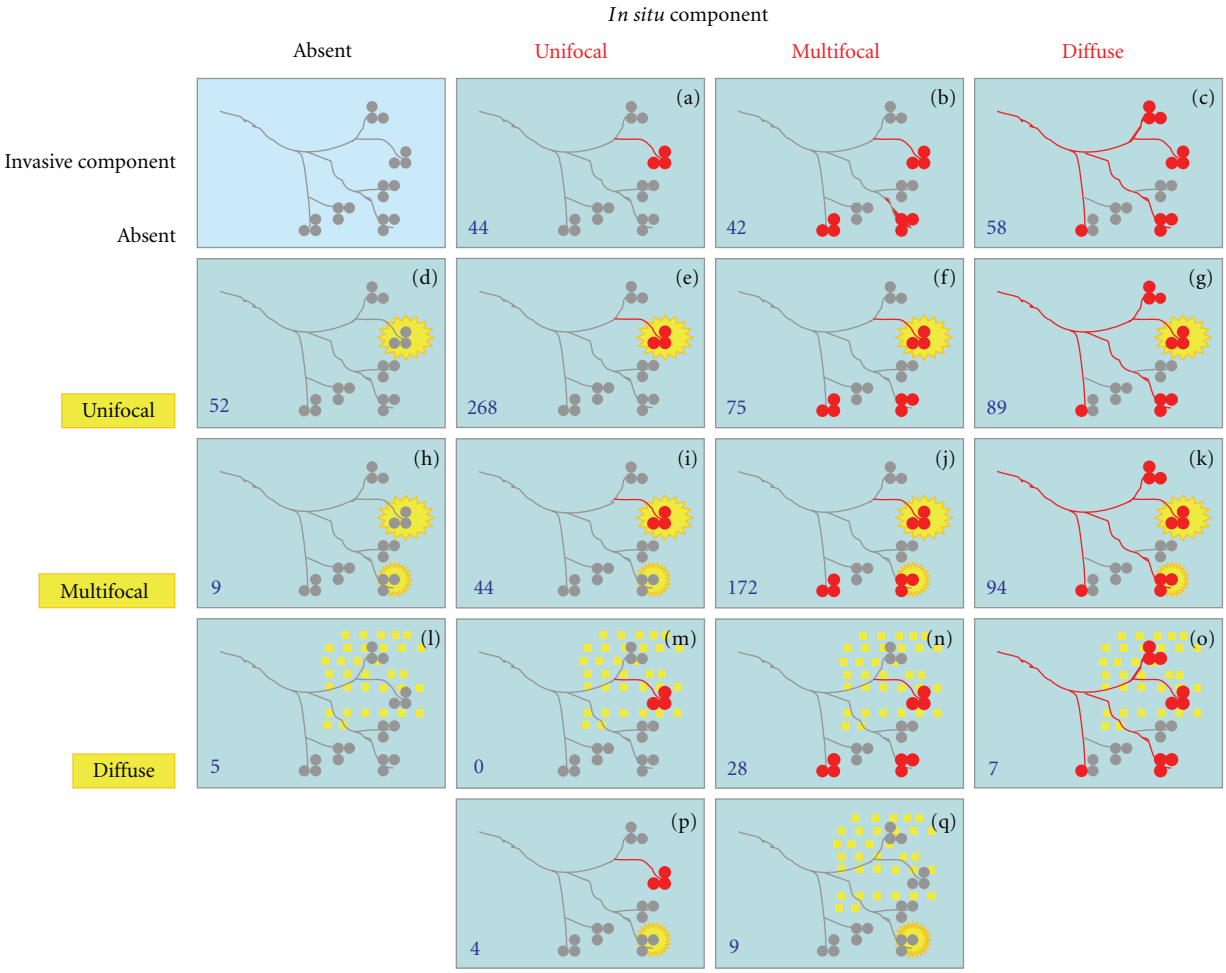

Figure 2: Schematic illustration of the possible combined growth patterns in breast carcinomas. (a) Unifocal in situ component, no invasive component. (b) Multifocal in situ component, no invasive component. (c) Diffuse in situ component, no invasive component. (d) Unifocal invasive component, no in situ component. (e) Unifocal invasive component, unifocal in situ component within the area of the invasive focus, and unifocal combined pattern. (f) Unifocal invasive component, multifocal in situ component, and multifocal combined pattern. (g) Unifocal invasive component, diffuse in situ component, and diffuse combined pattern. (h) Multifocal invasive component, no in situ component. (i) Multifocal invasive component, unifocal in situ component in one of the invasive foci, and multifocal combined pattern. (j) Multifocal invasive component, multifocal in situ component, and multifocal combined pattern. (k) Multifocal invasive component, diffuse in situ component, and diffuse combined pattern. (l) Diffuse invasive component, no in situ component. (m) Diffuse invasive component, unifocal in situ component, and diffuse combined pattern. (n) Diffuse invasive component, multifocal in situ component, and diffuse combined pattern. (o) Diffuse invasive component, diffuse in situ component, and diffuse combined pattern. (p) Unifocal invasive component, unifocal in situ component outside the invasive focus, and multifocal combined pattern. (q) Drawing illustrating one of the possible mixed patterns with both diffusely growing and well-delineated invasive foci, with a diffuse combined pattern. The upper right image illustrates the sick lobe. Numbers in the lower left corner of the drawings indicate the number of cases in the series of 1000 consecutive breast carcinomas belonging to that category.

were invasive carcinomas of <15 mm. More advanced cancers have an invasive component measuring ≥15 mm. Patients with these tumors have less favorable survival outcomes compared with early breast cancer cases [39, 40]. The proportion of cases in our material classified in this category was 51% (500/1000 unifocal, multifocal, or diffuse cases plus 7 cases with mixed growth patterns) (Table 1).

Determining tumor size is a complex task. The pathologist should register the radiologically measured tumor size on the preoperative tumor board. Breast cancers are often irregular in shape, such that the largest diameter of their nongeometric body varies in different projections. During the dissection, the pathologist should attempt to slice the specimen so that the cross section with the largest diameter of the tumor can be visualized (see Figure 4) and to document it in its entirety in a large section, without fragmenting the tumor. Embedding slices at different levels of the specimen and summarizing the findings in different slides are as important as in determining the extent of the disease [11].

Radiological methods, especially modern ultrasound and magnetic resonance imaging, provide an accurate measure

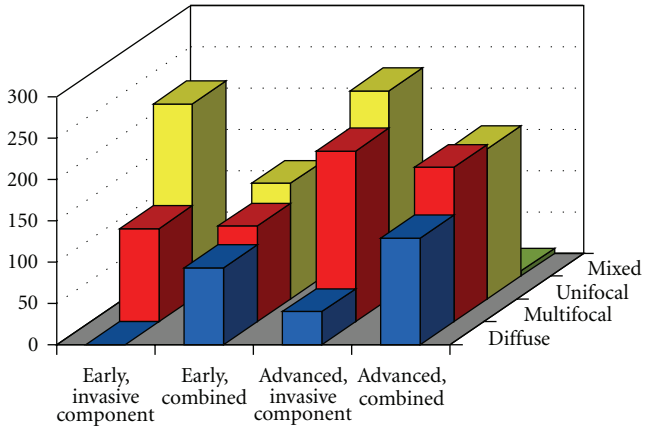

Figure 3: Distribution of the invasive component and combined (in situ plus invasive) lesion distribution in 855 consecutive invasive breast carcinoma cases documented in large-format histology slides. Falun, Dec 2007 to Jun 2012.

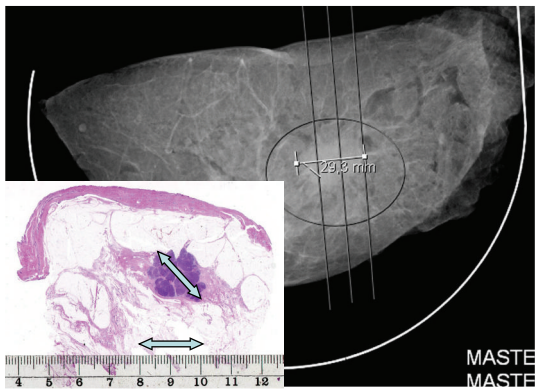

Figure 4: Mastectomy specimen-large-format histopathology correlation: unifocal invasive cancer. The plane of slicing the mastectomy specimen was erroneously chosen, which resulted in a discrepantly smaller tumor size in the histology slide compared with the mammographic size of the specimen. The specimen mammography image is courtesy of Dr. Mats Ingvarsson.

of the size of the tumor in several projections. The main shortcoming of these otherwise very accurate measurement methods is that they do not always distinguish in situ and invasive parts of the same tumor; because of this, the histologically verified tumor size may deviate from the radiological one. Obvious additional discrepancies between the radiological and histological tumor size may be the result of preoperative neoadjuvant therapy, but can also be caused by an erroneous choice of the embedded slice during the dissection or result from a failure in the radiological-pathological correlation.

There is no international consensus about measuring tumor size; for example, as a size restricted to measuring the tumor body or including the invasive extensions (spiculations). Because the spiculations may be long but are usually thin, they contain invasive cancer representing only a minor part of the tumor burden. Including such extensions when measuring tumor size may lead to an overestimate of the tumor burden. The aim of the tumor size measurement should be to categorize the case as early (<15 mm in size) or more advanced, rather than to expect a millimetric concordance of radiological and histopathological findings.

5. Radiological-Pathological Correlation in the Multimodality Imaging Era

Radiological-pathological correlation is essential for diagnosing breast carcinoma and in assessing the subgross morphological prognostic parameters listed above. A pathologist who is not familiar with the radiological findings when processing a preoperative biopsy or an operative specimen is more likely to make mistakes. Testing the concordance between the radiological and histological findings is not a matter of just comparing the values provided by these methods. Deviating data may result from technical/natural factors. The breast is hanging during the magnetic resonance imaging examination and the antero-posterior axis of the breast becomes transiently longer than when the patient is in an upright position. During mammography, the breast is compressed to a certain level, and the cranio-caudal axis becomes shorter. The breast tissue is much softer than the tumor itself and is easily deformed when placed on the firm surface of a transport plate or the bottom of a formalin-filled dish. Formalin fixation will cause shrinkage of the specimen, but deformation of the specimen during fixation in a dish of inadequate size may cause much more obvious discrepancies. The most common cause of discrepancies is, however, failure in the radiological-pathological correlation.

Modern multimodality breast radiology is very accurate in determining the subgross morphological prognostic parameters [7]. It uses different imaging modalities for the same lesion, which when combined can compensate for the limitations of the results of the individual methods. Tables 2 and 3 show our preliminary results regarding tumor size measurement with the imaging methods of mammography plus ultrasound versus magnetic resonance imaging as compared with the findings in large-format histological sections. As mentioned previously, it is not realistic to expect a perfect millimetric concordance of the radiological and the histological values; rather, the findings should be categorized in clinically important groups, like early versus more advanced breast cancer, or nonextensive versus extensive tumors. The concordance analysis only means comparing the results without naming a gold standard method; histopathology is as likely to underestimate or overestimate the subgross parameters as the radiological methods. Concordant results were reached in at least 80% of our cases when the cases were categorized by tumor size into early and more advanced categories (Table 2). Similar levels of concordance were reached when diagnosing extensive tumors. However, a substantial proportion of cases characterized radiologically as nonextensive turned out to actually be extensive in the histological examination (Table 3). These discrepant cases corresponded to radiologically occult, most often noncalcified, low-grade multifocal or diffuse in situ carcinomas (72/162 cases) or to radiologically occult, most

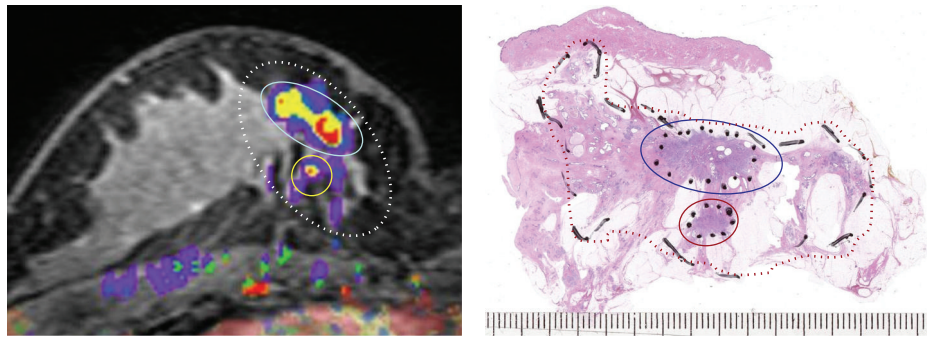

Figure 5: Magnetic resonance imaging-large-format histopathology correlation in a tumor with two invasive foci and a diffuse in situ component (combined pattern diffuse). Dotted lines indicate the extent of the disease, 57 × 30 mm. Tumor size (the largest dimension of the largest invasive focus) is 16 mm. The magnetic resonance image is courtesy of Dr. Mats Ingvarsson.

Table 2: Concordance of radiological and pathological size categories in 647 consecutive breast cancer cases, Falun, 2008–2011.

Tumor size category	Large-format histopathology versus magnetic resonance imaging concordance % (*n*/*N*)	Large-format histopathology versus mammography + ultrasound concordance % (*n*/*N*)	Size distribution of the cases in the same period % (*n*/*N*)
Early invasive cancer (<15 mm)	79 (87/110)	74 (172/231)	39 (255/647)
More advanced (≥15 mm)	80 (213/264)	92 (254/276)	61 (392/647)
All histologically verified	80 (300/374)	84 (426/507)	100 (647/647)

Table 3: Concordance of radiological and pathological extent categories in 675 consecutive breast cancer cases, Falun, 2008–2011.

Radiological extent category	Large-format histopathology extent categories
Nonextensive (<40 mm) 72% (486/675)	Nonextensive 66% (321/486)
	Extensive 33% (162/486)
	3 cases not assessable
Extensive (≥40 mm) 28% (189/675)	Non-extensive 13% (24/189)
	Extensive 84% (159/189)
	6 cases not assessable
Overall concordance	71% (480/675)

often <5 mm in size, invasive tumor foci (78/162 cases). Very rarely, large diffuse invasive breast carcinomas were radiologically occult or manifested with nonspecific signs. The magnetic resonance imaging-large-format histopathology correlation of a case of breast carcinoma with multifocal invasive and diffuse in situ components is shown in Figure 5.

6. Conclusions

Most breast carcinomas exhibit both in situ and invasive components. Although up to 70% of invasive tumors have only an unifocal invasive component, most breast carcinomas have a complex morphology when the distribution of the in situ and invasive components are combined. This complexity is evident both at early and more advanced stages of the disease. Half of breast cancer cases are extensive and occupy a tissue volume measuring ≥40 mm in the greatest dimension. Tumor size, disease extent, and lesion distribution are essential parameters for planning appropriate therapy and also have very significant prognostic power. Proper assessment of these parameters requires additional effort from the pathologists, including a detailed and systematic radiological-pathological correlation in every case of breast cancer. The method of large-format histopathology is a prerequisite for such correlations.

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Clinical Study

The Value of Combined Large Format Histopathology Technique to Assess the Surgically Removed Breast Tissue following Neoadjuvant Chemotherapy: A Single Institution Study of 40 Cases

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Historically, neoadjuvant chemotherapy has been used to treat patients with advanced breast disease in an attempt to convert them into candidates for breast conservation surgery. The ultimate goal of histopathologic examination of the specimens removed after neoadjuvant chemotherapy is the identification of either residual disease or positive identification of the tumor bed. We report a series of 40 patients treated with neoadjuvant chemotherapy and evaluation of the surgical specimens by a combination of standard histopathology and the use of large format histopathology techniques.

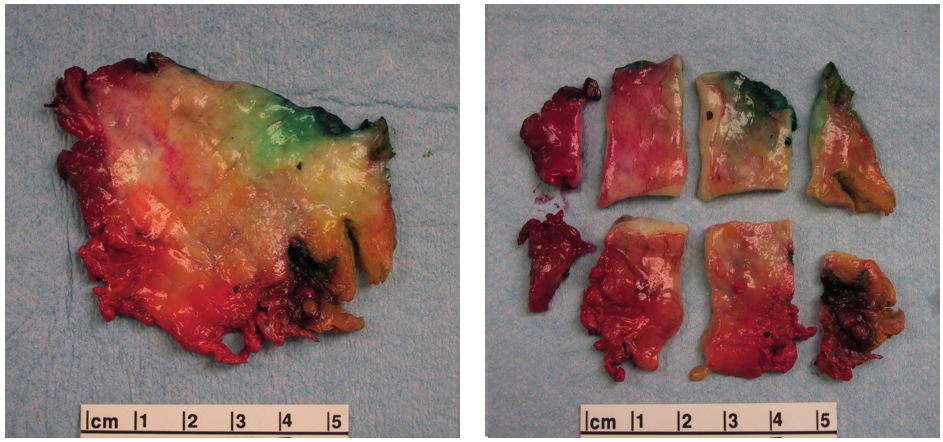
1. Introduction

The use of preoperative systemic therapy has increased in the last several years. Originally this therapy was used predominantly for patients with locally advanced breast cancer without systemic disease; the purpose was to convert these inoperable patients into candidates for breast conservation surgery [1–3]. However, neoadjuvant chemotherapy has also been extended to patients without locally advanced breast cancer that traditionally were subjected to surgery as the primary treatment modality [4–7]. The definition of pathologic complete response (pCR) was proposed in the NSABP B18 and B27 protocols; it is defined as the complete absence of invasive carcinoma both in the breast and in the axillary lymph nodes. The presence of residual duct carcinoma in situ (DCIS) was acceptable for the definition of pCR in these original studies. This definition has been challenged by others, some of which include small areas of residual tumor [8] or noninvasive disease in the pathologic complete response group [9].

Regardless of the definition used, the role of the pathologist in the evaluation of the resected specimens, whether it is a mastectomy or a partial mastectomy, is the identification of residual viable tumor or documenting the presence of the tumor bed and the absence of residual tumor in cases with pathologic complete response. In order to accomplish this task, the pathologist has to work in close cooperation with the radiologist in order to determine whether there was a residual "mass" or the fiducial clip placed by the radiologist before the start of therapy. A comprehensive review on the evaluation of pathology specimens after neoadjuvant therapy was published by Sneige and Page [10]. They indicate the importance of radiology and the fact that "extensive" sampling is required for complete pathologic evaluation. There are, however, no strict guidelines regarding the volume of tissue recommended for investigation as long as the tumor bed or residual tumor are found.

2. Materials and Methods

The standard processing of the tissue is done by obtaining and processing blocks that measure no more than 2.5×2 cm from the areas of most interest according to the macroscopic



(a) (b)

Figure 1: Each tissue slice will generate anywhere between 6 and 10 standard sections depending on the size. In this example of a slice measuring 6 cm in the largest dimension we created 8 generous standard sections.

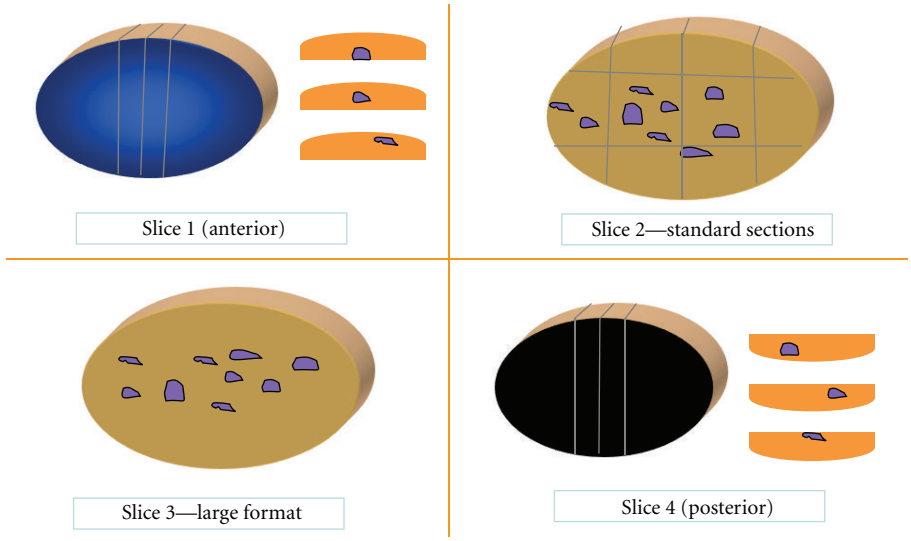


Figure 2: Partial mastectomy cut into four slices. Slice one (top) is anterior and slice 4 (bottom) is posterior. These two are sectioned in the perpendicular plane to evaluate those two margins microscopically. Slice 2 is cut into small pieces and entirely submitted for standard histologic sections. Slice 3 is processed intact for large format histopathology.

(gross) evaluation by the pathologist. This works relatively well when there is a "mass" or an abnormality that is either visible or palpable by the pathologist. Many cases of neoadjuvant chemotherapy, however, will not have these changes and therefore it becomes extremely difficult to determine where to sample a breast specimen without the aid of the radiologist.

The tissue is inked with 6 colors for partial mastectomies and with 3 colors for mastectomies; the tissue is then sliced at 5–10 mm thick intervals with a sharp knife and the slices placed on separate paper towels. The radiology information is used to localize the residual tumor or clip that was placed preoperatively. This is accomplished with X-rays of the intact resected specimen and/or X-rays of the slices of tissue. Once the area (tumor bed) is located, it is extensively sampled. The slices that are determined to contain the residual tumor or the tumor bed are used one for large format and one for standard sections. We obtain an average of 1 large format histopathology slide per case that can measure up to 7×9 cm and process it with the techniques described elsewhere [11–14]. Then we submit the mirror image for standard sections; usually 8–10 standard sections are equivalent to one large format slide (Figure 1). For partial mastectomy cases, the large format and the mirror image standard sections allow us to evaluate 100% of four out of six radial margins. The other two surgical margins (top and bottom slices) are sampled by cutting perpendicular sections of those slices (Figure 2). For mastectomies we have submitted one to three large format sections and several standard sections from the tissue adjacent to the large format (average 16 per case compared

to 10 in partial mastectomies); the margins are sampled as needed depending on the location of the "tumor bed" and the radiologic findings.

The cases were histologically graded using the modified Bloom Richardson score system (MBRS).

3. Results

We have evaluated the surgical specimen of 40 cases (18 partial mastectomies and 22 mastectomies) following neoadjuvant chemotherapy. The technique used resulted in a total of 530 standard sections slides (average: 13 per case) and 52 large format sections (average: 1.3 per case).

Among these 40 cases there were 31 invasive ductal carcinomas, 8 invasive lobular carcinomas, and 1 mixed ductal and lobular carcinoma.

The cases were histologically graded using the modified Bloom Richardson score system (MBRS). The prechemo grades were low (5/9 MBRS) in two cases, intermediate (6-7/9) in eighteen cases, and high (8-9/9 MBRS) in twenty cases. The histopathologic grade of the tumors was an average of 7.4 prechemo and 4.8 post-chemotherapy.

The goal was to identify either residual viable cancer or the tumor bed in cases with complete response. Of the 40 cases, we have observed complete histopathologic response in 11 (27.5%) cases; near complete response was identified in 2 cases (defined as only rare clusters of residual invasive tumor cells involving an area equal or smaller than 1 mm). 27 (67.5%) cases had partial response; one of the cases with partial response had residual tumor cells only within lymphatic vessels without residual "infiltrating carcinoma"; another case had only residual disease in the axillary lymph nodes.

In these 40 cases, the average pretreatment tumor size was 3.5 cm by imaging studies. Inflammatory carcinoma and four quadrant disease were arbitrarily given a 10 cm measurement for purposes of pretreatment size estimation.

There was no significant difference between standard and large format slides in the identification of the tumor bed or the residual tumor, however in the large format is easier to see the spatial relationship and easier to be confident that a tissue edge in fact represents the margin and not an artifact created by the sectioning of the tissue.

Using standard sections the average post-treatment size was 1.8 cm; using large format, the average post-treatment size was 1.6 cm with a range of 0 to 10 cm for both. The post-treatment size reflects the overall area with tumor, either made up by scattered foci or by a single nodule of residual disease. This is easily measured in the large format slides by simply using a ruler and measuring 2 dimensions; the larger of the two is recorded as the final size of the tumor. For standard sections it is a combination of either measuring residual tumor when there are nodules smaller than 15 mm that can be measured on one slide or by adding the number of sequential slices with tumor multiplied by the thickness of the slices. An example would be a case where tumor is found in 3 slices and each slice measures 0.5 cm in thickness; this results in a residual tumor size of 1.5 cm.

Tumor regression has been described as "scatter" or "concentric" in type. Scatter cases are characterized by residual tumor cells, either singly or in clusters, identified within an area of the breast similar to the original tumor size. In Figure 3 , an example of concentric regression, the tumor is composed of a dense 1.4 cm nodule of residual viable tumor. An example of the scatter pattern is seen in Figure 4 where the original tumor size was 5 cm and after treatment the residual scattered viable cells were present involving an area of 4.7 cm. These measurements are very difficult to obtain using standard sections. Figure 5 had originally a 2.5 cm tumor; after neoadjuvant therapy, the patient had complete imaging and clinical response. A large partial mastectomy with skin was performed and histopathologic examination showed complete response with proper identification of the tumor bed. Figures 6 and 7 demonstrate how simple it is to measure residual disease using the large format histopathology. In Figure 6 the patient had a 6 mm nodule of residual viable tumor. Likewise in Figure 7 , the tumor size can be determined by using the caliper on the large section. Trying to measure the residual tumoral area by standard sections would be quite difficult because of the elongated nature of this lesion.

4. Discussion

The use of neoadjuvant chemotherapy has increased and is no longer limited to patients with locally advanced breast cancers; it is being used in patients who have relatively small tumors. The role of the pathologist is to assess the impact of chemotherapy on the primary breast cancer and/or its metastases to the axillary lymph nodes. The pathologist has to identify the location where the regressed tumor used to be (tumor bed) and identify the presence or absence of residual disease. This is accomplished by a close working relationship with the radiologist who usually inserts a metallic marker (fiducial marker) in the area of the tumor before the initiation of chemotherapy. Radiologic-pathologic correlation is critical and provides the most accurate results in the evaluation of cases after neoadjuvant chemotherapy [15]. After the patient has been treated, the tumor may be extremely difficult to see by the radiologist and no longer palpable by the clinician; therefore the surgeon has to rely on the radiologist to localize the "tumor bed" by placing a metal wire in the location of the fiducial marker. This way the surgeon knows with relative accuracy the area that needs to be removed. The volume of tissue that needs to be removed will depend on whether the tumor was a unifocal/multifocal or diffuse lesion. In cases of complete response the surgeon will be guided by localizing wires placed preoperatively by the radiologist. It will be the pathologist's responsibility to determine if the tumor bed has in fact been removed and whether the margins of resection are clear. Marchio and Sappino [16] reported that the use of large format histopathology was valuable in cases of neoadjuvant chemotherapy, particularly in the evaluation of the residual tumor burden and the status of the margins of resection. The margins are negative by definition in cases

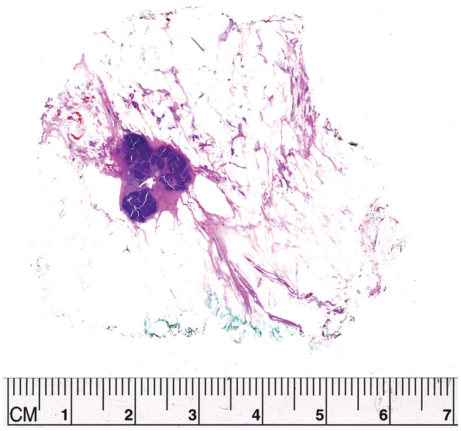


Figure 3: An example of "concentric regression" of tumor after neoadjuvant chemotherapy. She started with a 3 cm high grade (9/9 MBRS) invasive ductal carcinoma. At the end of treatment the tumor measured 1.4 cm.

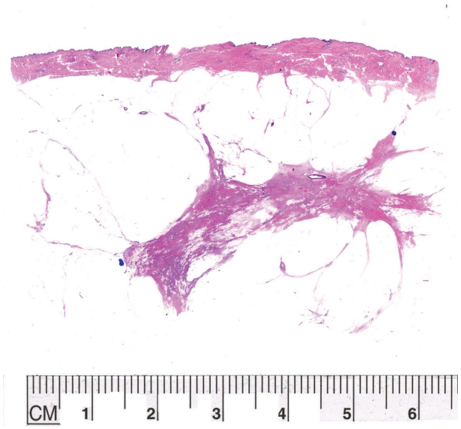


Figure 4: An example of "scatter regression" of tumor after neoadjuvant chemotherapy. She started with a 5 cm intermediate grade (6/9 MBRS) invasive ductal carcinoma. At the end of treatment the "tumor bed" with scattered foci of viable tumor cells involved an area of 4.7 cm represented by the irregular scar (density).

with complete pathologic response; however in cases where there is incomplete response, the disease may be microscopic and scattered over an area similar in size to the original area occupied by the intact tumor. It is in these cases when using the large section helps.

The comprehensive sampling of the circumferential margins performed in our cases is not the standard across the United States. Most laboratories submit random standard sections instead of the entire tissue slice. In a report by Tucker [17], he estimates that the average pathology practice examines 16% of the margins. Based on this incomplete information clinicians are making decisions every day regarding reexcisions and radiotherapy use.

Our collection of cases is not consecutive. The specific workup of the cases is quite unique with having the ability

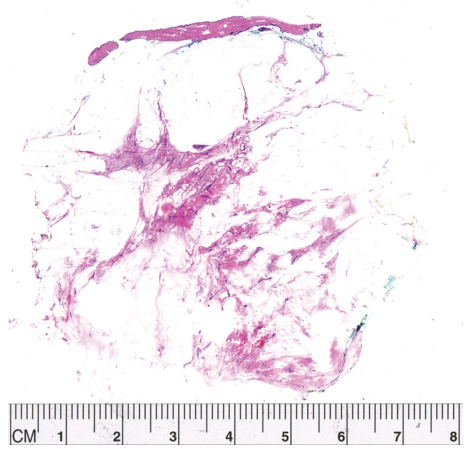


Figure 5: In this case, the patient had pathologic complete response (pCR). The entire specimen was examined microscopically. Slices one and three were cut in the perpendicular plane and slice 2 submitted for large format. A 100% of this 7.5 cm lumpectomy was examined microscopically with 12 standard sections and one large section.

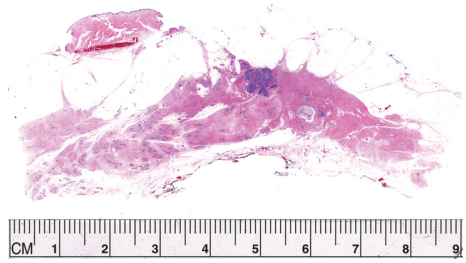


Figure 6: Example of "concentric regression" of tumor after neoadjuvant chemotherapy. She started with a 1.5 cm high grade (8/9 MBRS) invasive ductal carcinoma. After neoadjuvant chemotherapy she has a 0.6 cm focus of residual tumor.

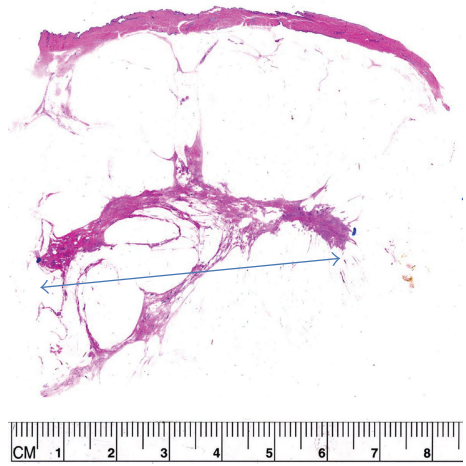


Figure 7: In this case, there is an area of 6 cm of residual invasive and in situ carcinoma. This would be difficult to measure in standard sections because of the difficulty in the orientation of the cut pieces.

to compare the same area within a single slide (large format histopathology) versus 8–10 separate slides (standard histopathology). This type of comparison has not been done as far as we know.

Our cost estimation showed that the preparation of the 13 standard slides per case cost approximately $130.00 ($10.00 per slide) and for the 1.3 large format slides per case cost approximately $104.00 ($80.00 per slide); if we were to submit more of the tissue for large format and only the top and bottom margins for standard sections we would end up with small but real cost saving. For example, in a partial mastectomy with 4 tissue slices such as that depicted in Figure 2, we could submit 3 standard sections from the top and bottom slices (×6 slides = $60.00) and the two center pieces for large format slides (×2 slides = ($160.00) for a total of $220.00 per case.

5. Conclusion

We found that the combination of large format histopathology and standard sections provides accurate information in the identification of residual disease and margins width is easy to measure. For both, mastectomies and partial resections, we found no significant difference between large format and standard sections in the margin width or the size of the residual tumor or in the identification of the tumor bed in cases with complete histopathologic response. This is only true because of the extensive sampling utilized in these cases by standard sections and the fact that the large format slides are the mirror images of the standard sections. We recommend extensive sampling, either by large format or standard sections to accurately report the size of the residual tumor and the margin measurements.

One major advantage of the large format slides is the fact that we do not have to reassemble the "puzzle" using the standard sections. Finally, our cost analysis suggests that using primarily large format for our cases results in a slight cost savings ($208.00 versus $234.00) when compared with standard sections.

The correlation with imaging studies will be published in a separate paper but there is no doubt that it is much easier when large format histopathology is used.

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Research Article

Comparison of the Subgross Distribution of the Lesions in Invasive Ductal and Lobular Carcinomas of the Breast: A Large-Format Histology Study

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To compare the lesion distribution and the extent of the disease in ductal and lobular carcinomas of the breast, we studied 586 ductal and 133 lobular consecutive cancers. All cases were documented on large-format histology slides. The invasive component of ductal carcinomas was unifocal in 63.3% (371/586), multifocal in 35.5% (208/586), and diffuse in 1.2% (7/586) of the cases. The corresponding figures in the lobular group were 27.8% (37/133), 45.9% (61/586), and 26.3% (35/133), respectively. When the distribution of the in situ and invasive component in the same tumors was combined to give an aggregate pattern, the ductal carcinomas were unifocal in 41.6% (244/586), multifocal in 31.6% (185/586), and diffuse in 26.8% (157/586) of the cases. The corresponding figures in the lobular category were 15.0% (20/133), 54.2% (72/133), and 30.8% (41/133), respectively. Ductal cancers were extensive in 45.7% (268/586), lobular in 65.4% (87/133) of the cases. All these differences were statistically highly significant ($P < 0.0001$). While the histological tumor type itself (ductal versus lobular) did not influence the lymph node status, multifocal and diffuse distribution of the lesions were associated with significantly increased risk of lymph node metastases in both ductal and lobular cancers.

1. Introduction

Breast cancer is a heterogeneous disease in which the individual cases deviate from each other in morphology, protein expression, molecular phenotype, genetic characteristics, and prognosis. Breast carcinomas of "special-types" have been delineated based on their microscopical characteristics, but the vast majority of tumors belongs to the category of not otherwise specified (NOS) ductal carcinomas. Invasive lobular carcinomas represent the most frequent "special-type" breast carcinoma and comprise 5–15% of all breast cancer cases [1]. In addition both the ductal and the lobular tumors also represent heterogeneous groups of diseases and can be prognostically stratified with grading or delineating distinct histological subtypes.

Numerous studies have compared ductal and lobular breast carcinomas using different criteria, and reported more [2, 3] or less favourable [4, 5] outcome in lobular compared to ductal carcinomas, or no significant differences in outcome [6, 7]. On the other hand, studies on subgross morphology (lesion distribution and disease extent) of these tumors are very rare. Tot has previously described the diffuse variant of invasive lobular carcinoma and reported a poorer prognosis when compared to unifocal and multifocal lobular cancers [8]. Foschini et al. [9, 10] studied in detail the subgross morphology of both in situ and invasive lobular carcinomas and observed that these tumors tended to be multifocal and extensive. A growing body of evidence exists regarding the biological and prognostic significance of tumor multifocality, diffuse lesion distribution, and

extensive tumoral growth in breast cancer in general [11–13], but few studies have addressed this topic specifically in the ductal and lobular subgroups [14].

The present study was designed to compare the distribution of the in situ and invasive lesions and the extent of the disease in ductal and lobular carcinomas of the breast, and to evaluate the influence of these subgross morphological parameters on lymph node status in both histological categories.

2. Methods

2.1. Study Population. This study is a retrospective analysis of a consecutive series of breast carcinoma cases diagnosed at the Department of Pathology and Clinical Cytology of the County Hospital in Falun, Sweden, from January 2008 to July 2012. Patients with recurrent breast carcinomas that were initially diagnosed before the study period were excluded. In situ carcinomas with no invasive component, microinvasive (<1 mm) carcinomas, and patients who received preoperative neoadjuvant therapy were excluded. The remaining study population comprised 586 ductal, 133 lobular, and 102 invasive carcinomas of other histological types. Only the ductal and the lobular cancers were analyzed in the present study. The study was approved by the The Regional Ethical Review Board of Uppsala University.

2.2. Large-Section Histopathology. All specimens were prepared using the large-format histopathology method that has been performed routinely in our laboratory since 1982. The method has been described in detail elsewhere [15]. Briefly, all cases are discussed at a preoperative tumor board, and the radiological (mammography, ultrasound, and magnetic resonance imaging) appearance are recorded, including the radiological extent and distribution. This information, together with the whole-specimen radiograph received with the surgical specimen, guides the pathologist during the work up. The sector-resection specimens are sliced into 3-4 mm thick tissue slices parallel to the pectoralis fascia and are also radiographed. One to five of the most representative slices (measuring up to 9×8 cm) are selected and embedded in large paraffin blocks. Larger slices are bisected and embedded into separate blocks. Mastectomy specimens are sliced perpendicular to the pectoralis fascia to visualize the surgical margin in one histological level. All of the cases are further discussed at the postoperative tumor board to check the concordance of the radiological and histological findings. Most cases that are discrepant in favor of radiological findings can be resolved with additional specimen sampling for histological analysis.

3. Diagnostic Criteria

3.1. Histological Tumor Type. Invasive lobular carcinomas were defined by their cellular characteristics, growth pattern and E-cadherin expression, following the WHO criteria [1]. E-cadherin (DAKO, clone M3612) staining was performed routinely in all invasive carcinoma cases during the study period; the largest tumor focus was stained. Typical cases of invasive lobular cancer were built up of small uniform cells exhibiting a small, regular, darkly stained round, or oval nucleus. These cells often contained an intracytoplasmic vacuole of mucin pressing aside the small nucleus of the cells. The cells in invasive lobular carcinoma typically grew in cell files (one or two cell thick) or were haphazardly (diffusely) dispersed. Glandular lumina were absent in most cases. The two criteria, that is, typical cytological characteristics and typical histological growth pattern were used as alternative criteria, that is, tumors exhibiting both of these basic morphologic criteria (typical cells and typical histological growth pattern) were categorized as "classical" type of invasive lobular carcinoma, while tumors exhibiting only one of the criteria comprised the variants of it. This means that tumors consisting of typical cells were categorized as invasive lobular carcinomas irrespective of variations in histological growth patterns (tumors with solid, alveolar, and tubulolobular growth patterns were also included into lobular category). Tumors with typical histological growth pattern were also classified as lobular even if they consisted of different tumor cells. The vast majority of invasive lobular carcinomas exhibited complete loss of E-cadherin expression as demonstrated by immunohistochemistry. Cases with typical morphology and partial E-cadherin expression were, however, included into the lobular category. Cancers not showing any histological characteristics of special types tumors (including lobular cancers) in at least 90% of their cross-section surface were categorized as ductal carcinomas not otherwise specified. The vast majority of these tumors expressed E-cadherin.

The distributions of the invasive and the in situ components of the same lesion were determined separately using the previously published criteria by Tot [14]. The invasive component of the tumors was classified as follows: (1) unifocal tumors: one invasive focus observed in the large sections, with the tumor focus containing or not containing an in situ component. (2) Multifocal invasive lesions: multiple, well-delineated, invasive tumor foci separated from each other by uninvolved breast tissue, regardless of the distance between the foci. We did not analyze cases of "multicentricity" (defined as the presence of malignant structures in different quadrants of the same breast) separately, because it represents a clinical and/or radiological parameter; these cases were regarded as multifocal. (3) Diffuse tumors: tumors that are dispersed over a large area of the section, similar to a spider's web, with no distinct tumor mass. The in situ component of the tumors were regarded as "unifocal" if they seemed to involve a single terminal ductal lobular unit or several neighbouring terminal units without uninvolved breast tissue in between; they were regarded as "multifocal" if they involved several distant terminal ductal lobular units with uninvolved breast tissue in between, and as "diffuse" if they involved mainly the larger ducts. The distribution of the in situ and invasive components was combined with each other. Diffuse distribution of either in situ or invasive component qualified the lesion to be "diffuse." Tumors without evidence of diffuse growth were classified as "multifocal" if either the in situ or the invasive component or both were multifocal.

When the distribution of the lesions was assessed, an attempt was made in each case to summarize the findings in different tissue levels of the large sections to reconstruct the in vivo situation before surgery. Detailed correlations between radiological and pathological findings were essential. If a complete surgical intervention was performed in addition to the primary sector resection, an attempt was made to summarize the findings of the entire excised tissue. However, sector resection specimens (average size of 9 × 6 cm) were sufficient for categorizing the findings in most cases. Typical cases of unifocal, multifocal, and diffuse breast carcinomas are illustrated in Figures 1, 2 and 3.

Disease extent was defined as the tissue area in the large-format histology sections containing all the in situ and invasive malignant structures. Cases in which the tumor structures occupied an area 40 mm or larger in its largest dimension were categorised as extensive tumors [16] while the others were categorised as nonextensive.

3.2. Assessment of the Lymph Nodes. The axillary lymph nodes (both sentinel and nonsentinel nodes) were measured and sliced parallel to the longitudinal axis. Lymph nodes with a thickness of <5 mm were bisected, and thicker nodes were sliced to yield approximately 2 mm thick slices. Sentinel lymph nodes were examined during surgery with imprint cytology from all cut surfaces and frozen sections of 1-2 slices. The frozen sections were routinely stained and also stained intraoperatively with a cytokeratin 8/18 antibody (1 : 50, clone Cam 5.2; BD Biosciences). All slices were embedded in paraffin blocks; at least two sections from each of the blocks were stained with hematoxylin and eosin, and those from sentinel nodes were stained with the cytokeratin 8/18 antibody. Lymph node metastasis was assessed according to the sixth edition of the TNM staging system [17], which defines macrometastasis as at least one metastatic deposit >2 mm within a lymph node, micrometastasis as deposit(s) 0.2–2 mm, and isolated tumor cells as <0.2 mm deposits. For the purposes of the present study, cases with metastatic deposits 0.2 mm or greater in at least one of the examined lymph nodes were characterized as lymph node positive.

3.3. Study Execution. All of the large histological sections in this series and the slides from the lymph nodes were reviewed by two of the authors (TT, GP) for the purposes of the postoperative tumor board. Histological data, including the distribution of lesions, disease extent, tumor type, and lymph node status, were determined according to the diagnostic criteria described above and registered in a database. Statistical analyses (relative risk (RR) and comparison of proportions using the chi-square test) were carried out using commercially available software (MedCalc statistics for biomedical research; MedCalc Software, Belgium), with P values <0.05 regarded as significant.

4. Results

Among the 719 cases of the present series of newly diagnosed breast carcinomas, 586 were diagnosed as ductal and 133 as lobular applying the above-described criteria. The invasive component was unifocal in 408, multifocal in 269, and diffuse in 42 cases. Ductal carcinomas were unifocal in 63.3% (371/586), multifocal in 35.5% (208/586), and diffuse in 1.2% (7/586) of the cases. The corresponding figures in the lobular group were 27.8% (37/133), 45.9% (61/586), and 26.3% (35/133), respectively. These differences were highly significant indicating that the invasive component of lobular carcinomas is more often multifocal and much more often diffuse compared to ductal tumors (Table 1).

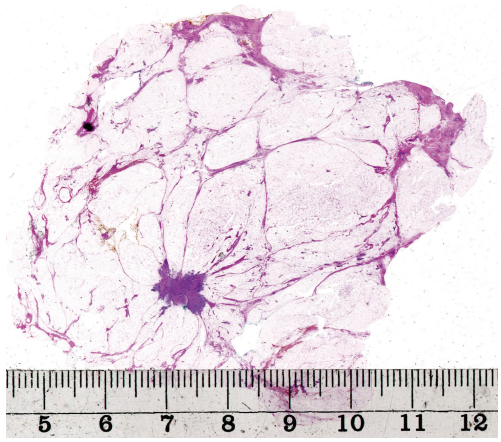

Figure 1: Large-format histology section showing a unifocal breast carcinoma.

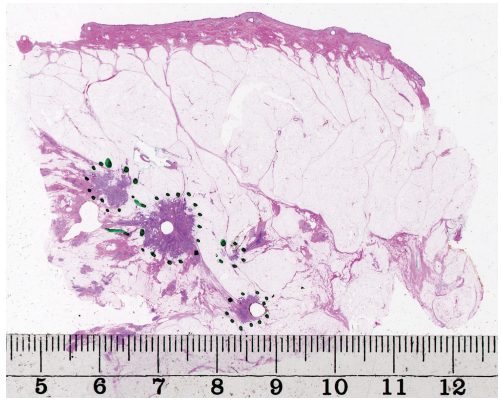

Figure 2: Large-format histology section showing a multifocal breast carcinoma.

In situ component was not found in 5.3% (31/586) of the ductal and 5.2% (7/133) of the lobular cases. The in situ component of the other ductal tumors was unifocal in 42.8% (251/586), multifocal in 25.1% (147/586), and diffuse in 28.8% (157/586) of the cases. The corresponding figures in lobular tumors were 18.8% (25/133), 69.2% (92/133), and 6.8% (9/133), respectively. These differences were statistically also highly significant indicating that the invasive component of lobular carcinomas is more often multifocal compared to the ductal cases, and much less often diffuse.

When the distribution of the in situ and invasive components of the same tumors were combined, giving an aggregate pattern, the ductal carcinomas were unifocal in 41.6% (244/586), multifocal in 31.6% (185/586), and diffuse in

Table 1: Lesion distribution and disease extent in ductal and lobular breast carcinomas, Dalarna, Jan 2008–Jul 2012.

			Ductal	Lobular	χ^2 test
Lesion distribution	Invasive component	Unifocal	63.3% (371/586)	27.8% (37/133)	$P < 0.0001$
		Multifocal	35.5% (208/586)	45.9% (61/586)	
		Diffuse	1.2% (7/586)	26.3% (35/133)	
	In situ component*	Unifocal	42.8% (251/586)	18.8% (25/133)	$P < 0.0001$
		Multifocal	25.1% (147/586)	69.2% (92/133)	
		Diffuse	28.8% (157/586)	6.8% (9/133)	
	Combined in situ + invasive components	Unifocal	41.6% (244/586)	15.0% (20/133)	$P < 0.0001$
		Multifocal	31.6% (185/586)	54.2% (72/133)	
		Diffuse	26.8% (157/586)	30.8% (41/133)	
Disease extent**	Nonextensive		54.1% (317/586)	33.8% (45/133)	$P < 0.0001$
	Extensive		45.7% (268/586)	65.4% (87/133)	
Total			586	133	719

*5.3% (31/586) of the ductal and 5.2% (7/133) of the lobular cases had no demonstrable in situ component.
**The extent of the disease was not assessable in 0.2% (1/586) of the ductal and 0.8% (1/133) of the lobular cancers.

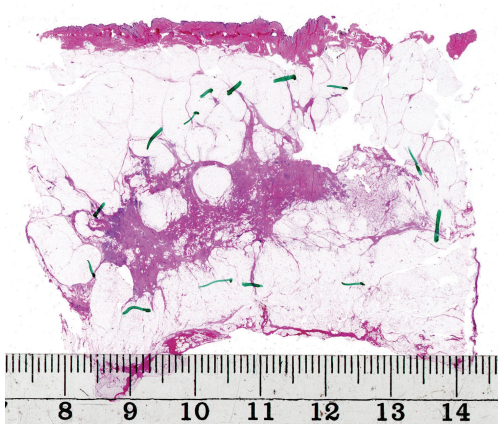

Figure 3: Large-format histology section showing a diffuse invasive carcinoma of the breast.

26.8% (157/586) of the cases. The corresponding figures in the lobular category were 15.0% (20/133), 54.2% (72/133), and 30.8% (41/133), respectively. These differences were also statistically significant, indicating that lobular carcinomas are more often multifocal than their ductal counterparts. The proportions of cases with diffuse combined lesion distribution were similar because of the frequent diffuse distribution of the in situ component in ductal carcinomas and the frequent diffuse distribution of the invasive component in lobular carcinomas.

The extent of the disease was assessable in all cases with exception of a single case of ductal and a single case of lobular cancer. Ductal cancers were extensive in 45.7% (268/586) of the cases compared to 65.4% (87/133) in lobular tumors ($P < 0.0001$).

There were no significant differences in the proportion of lymph nodes positive cases between ductal and lobular cancers in any lesion distribution category or in the extent categories. However, numerically, the proportions in almost all the categories were higher in ductal carcinomas than in lobular cases (Table 2).

In Table 3, the relative risk of having lymph node metastasis in ductal and lobular breast carcinomas by lesion distribution is given. Taking into account only the invasive component of the tumors in the entire study material, 26.7% (109/408) of the patients with unifocal tumors and 49.4% (133/269) of the patients with multifocal tumors had lymph node metastases giving a relative risk of 1.85 (95% CI 1.5136–2.2629, $P < 0.0001$). The relative risk of having lymph node metastasis in diffuse invasive tumors compared to unifocal ones was 2.05 (95% CI 1.4908–2.8184, $P < 0.0001$). In the group of ductal carcinomas, multifocality of the invasive component carried a relative risk of 1.96 (95% CI 1.5912–2.4106, $P < 0.0001$) of having lymph node metastasis compared to unifocal cases; the corresponding relative risk with diffuse distribution was 3.12 (95% CI 2.2088–4.4005, $P < 0.0001$). Multifocality of the invasive component of the lobular cases carried similar relative risk (RR = 1.82) which did not reach statistical significance ($P = 0.1185$). The relative risk (2.57) in diffuse versus unifocal invasive lobular carcinomas was statistically significant ($P = 0.0136$). As shown in Table 3, multifocality and diffuse distribution of the in situ component of the tumors carried lower relative risk of having lymph node metastases than multifocality or diffuse distribution of the invasive component in both the ductal and lobular categories. This was also observed regarding the aggregate (in situ + invasive components) lesion distribution.

5. Discussion

The proportions of multifocal breast cancer cases vary substantially in the literature, depending on definition, methodology of assessment, and criteria [18]. Most publications on this topic focused on the invasive component of the tumors, did not include the in situ component, and did not take diffuse distribution of the lesion into account. Tot published a system that included all components of the tumors and also the possibility of diffuse growth of both the in situ and invasive components. The criteria were

Table 2: Proportion of cases with lymph node metastases in ductal and lobular carcinomas by lesion distribution and disease extent, Dalarna, Jan 2008–Jul 2012.

			Ductal	Lobular	Comparison of proportions
Lesion distribution	Invasive component	Unifocal	27.5% (102/371)	18.9% (7/37)	$P = 0.9375$ (−35.3%–29.4%)
		Multifocal	53.8% (112/208)	34.4% (21/61)	$P = 0.1486$ (−5.5%–41.2%)
		Diffuse	85.7% (6/7)	48.6% (17/35)	$P = 0.2713$ (−16.7%–65.1%)
	In situ* component	Unifocal	33.1% (83/251)	16.0% (4/25)	$P = 0.8706$ (−42.2%–36.5%)
		Multifocal	43.5% (64/147)	40.2% (37/92)	$P = 0.8549$ (−17.3%–24.4%)
		Diffuse	43.3% (68/157)	33.3% (3/9)	$P = 0.7997$ (−48.7%–44.6%)
	Combined in situ + invasive components	Unifocal	29.9% (73/244)	20.0% (4/20)	$P = 0.8918$ (−48.1%–33.1%)
		Multifocal	42.2% (78/185)	31.9% (23/72)	$P = 0.5360$ (−15.1%–31.2%)
		Diffuse	43.9% (69/157)	43.9% (18/41)	$P = 0.7898$ (−26.0%–27.6%)
Disease extent**	Nonextensive		27.1% (86/317)	26.7% (12/45)	$P = 0.7285$ (−23.1%–33.4%)
	Extensive		50.0% (134/268)	37.9% (33/87)	$P = 0.2975$ (−8.5%–30.5%)
Total			586	133	

*5.3% (31/586) of the ductal and 5.2% (7/133) of the lobular cases had no demonstrable in situ component.
**The extent of the disease was not assessable in 0.2% (1/586) of the ductal and 0.8% (1/133) of the lobular cancers.

tested on a consecutive series of 500 breast carcinoma cases documented on large-format histology sections [14] and are also described in detail in another report on the findings in 1000 consecutive cases in this special issue of the journal [19]. In the study published in 2007 [14], 34% (170 of 500 cases) of the cases were unifocal, 36% (180 of 500 cases) were multifocal, and 28% (138 of 500 cases) were diffuse when the in situ and invasive components of the tumor were combined into an aggregate pattern. These results are almost identical to the results in the study on 1000 cases [19] in which 36% (366/1000) unifocal, 35% (347/1000) multifocal and 28% (280/1000) diffuse cases were found. The corresponding figures in the present study are very similar (36.7% unifocal, 35.8% multifocal, and 27.5%, diffuse tumor growth). In our previous studies, we found about 45% of the breast cancer cases being extensive (occupying a tissue are ≥40 mm in the largest dimension) which was confirmed in the present series [16].

Foschini et al. studied the distribution of the lesions (both in situ and invasive) in lobular carcinomas of the breast through three-dimensional stereomicroscopy of thick large-format histological sections in fifteen cases and demonstrated multiple tumor foci in most cases [9]. The in situ component was multicentric in nine of their cases and the average maximum distance among the in situ foci was 37.9 mm, while the average maximum distance among the invasive foci was 58.2 mm. Although this study was performed on a limited number of cases, the results clearly indicated frequent multifocality of the lesions and extensive tumoral growth in lobular carcinomas.

In another seminal paper by Foschini and coworkers [10], the advantages of large-format histology were used to assess the distribution of the lesions in in situ and invasive ductal breast carcinomas. They found that the in situ component was multifocal in 42 of their 45 cases, but the invasive component was multifocal in only four of their cases. They also frequently found low-grade carcinoma in situ to be widely spread in the breast.

In 2003, Tot described the diffuse variant of the invasive lobular carcinoma [8] and reported the distribution of the lesions in 130 consecutive cases of invasive lobular carcinomas diagnosed at the Department of Pathology in Falun, Sweden during the period 1991–1997. All these tumors were documented on large-format histological sections and the patients were followed up for on average 78 months (range 4–131 months). In that study, 39% of the cases were unifocal, 12% multifocal, 28% diffuse while 19% were combined (the term "combined" designated those cases which contained only minor areas of diffuse growth of the tumor cells and being otherwise multifocal). The distribution of the in situ component was not analysed in that series of cases. In the present paper we report novel results on a series of consecutive cases diagnosed at the same department during the period Jan 2008–Jul 2012 and worked up with the same

Table 3: Relative risk of having lymph node metastasis in ductal and lobular breast carcinomas by lesion distribution, Dalarna, Jan 2008–Jul 2012.

		Unifocal	Multifocal	Diffuse	Relative risk multifocal versus unifocal	Relative risk diffuse versus unifocal
Invasive component	Ductal	27.5% (102/371)	53.8% (112/208)	86.7% (6/7)	RR = 1.9585 $P < 0.0001$ (1.5912–2.4106)	RR = 3.1176 $P < 0.0001$ (2.2088–4.4005)
	Lobular	18.9% (7/37)	34.4% (21/61)	48.6% (17/35)	RR = 1.8197 $P = 0.1185$ (0.8582–3.8585)	RR = 2.5673 $P = 0.0136$ (1.2138–5.4303)
	Total	26.7% (109/408)	49.4% (133/269)	54.8% (23/42)	RR = 1.8507 $P < 0.0001$ (1.5136–2.2629)	RR = 2.0498 $P < 0.0001$ (1.4908–2.8184)
In situ* component	Ductal	33.1% (83/251)	43.5% (64/147)	43.3% (68/157)	RR = 1.3534 $P = 0.0192$ (1.0506–1.7435)	RR = 1.3098 $P = 0.0351$ (1.109–1.6835)
	Lobular	16.0% (4/25)	40.2% (37/92)	33.3% (3/9)	RR = 2.5153 $P = 0.0526$ (0.0989–6.3841)	RR = 2.0833 $P = 0.2642$ (0.5743–7.5575)
	Total	31.5% (87/276)	42.3% (101/239)	42.8% (71/166)	RR = 1.3406 $P = 0.0119$ (1.0668–1.6848)	RR = 1.3569 $P = 0.0156$ (1.0595–1.7377)
Combined in situ + invasive components	Ductal	29.9% (73/244)	42.2% (78/185)	43.9% (69/157)	RR = 1.4093 $P = 0.0085$ (1.0913–1.8198)	RR = 1.4690 $P = 0.0039$ (1.1316–1.9069)
	Lobular	20.0% (4/20)	31.9% (23/72)	43.9% (18/41)	RR = 1.5972 $P = 0.3284$ (0.6245–4.0854)	RR = 2.1951 $P = 0.1020$ (0.8555–5.6328)
	Total	29.2% (77/264)	39.3% (101/257)	43.9% (87/198)	RR = 1.3474 $P = 0.0156$ (1.0581–1.7158)	RR = 1.5065 $P = 0.0011$ (1.1790–1.9250)

* 5.3% (31/586) of the ductal and 5.2% (7/133) of the lobular cases had no demonstrable in situ component.

method of large-format histology. Compared to the previous study, the design was extended to include also analyses of the in situ component, not only in lobular but also in ductal tumors, and analyzing the extent of the disease and the lymph-node status. In the present study we avoided the "combined" category of the distribution of the invasive component and classified the cases based on the dominant pattern of invasion. This explains the differences in the proportions of unifocal and multifocal cases between these two studies.

Our present study showed that there are statistically highly significant differences in lesion distribution between invasive lobular and invasive ductal carcinomas in all analyzed scenarios (only invasive component, only in situ component, and in situ and invasive components combined). The differences clearly indicate that the invasive component of lobular carcinomas compared to ductal carcinoma tends to be multifocal more often (45.9% versus 35.5%) and much more often diffuse (26.3% versus 1.2%). On the contrary, the in situ component of the ductal carcinomas as compared to lobular carcinoma was much more often diffuse (28.8% versus 6.8%) and much less often multifocal (25.1% versus 69.2%). This also resulted in significant differences in combined lesion distribution between ductal and lobular carcinomas. We also observed that lobular carcinomas were significantly more frequently extensive than their ductal counterparts.

The majority of the publications related to metastatic potential of multifocal breast carcinomas reported higher risk of lymph node involvement in multifocal than in unifocal cases [20–23]. In our recently published series [23], 25.6% of the unifocal, 53.4% of the multifocal and 35.7% of the diffuse cases had lymph node metastases (tumor deposits >0.2 mm). In another a previous study, we found that the odds ratio of having lymph node metastasis was 2.33 for diffuse carcinomas compared to unifocal tumors [14]. Similar results were generated in the present study. Although the differences between the proportions of the lymph node positive cases were significant between unifocal, multifocal and diffuse cases, no such differences could be related to the histological tumor type (ductal versus lobular) in the present series.

The present study reported several histopathological significant differences between ductal and lobular tumors. In conclusion, the invasive component of the lobular carcinomas was more often multifocal and diffuse than the invasive component of the ductal tumors. The in situ component was more often multifocal but less often diffuse in lobular carcinomas. Lobular carcinomas were more often extensive than the ductal tumors. The histological tumor type (ductal versus lobular) was not directly related to the metastatic

potential of the tumors, but multifocal and diffuse lesion distribution (especially that of the invasive component) significantly increased the relative risk of having lymph node metastasis in both ductal and lobular breast carcinomas.

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Review Article

The Ongoing Revolution in Breast Imaging Calls for a Similar Revolution in Breast Pathology

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Communication between pathologists and radiologists suffers from a lack of common ground: the pathologists examine cells in ultrathin tissue slices having the area of a postage stamp, while the radiologists examine images of an entire organ, but without seeing the cellular details. The current practice of examining breast cancer specimens is analogous to scrutinizing individual pieces of a jigsaw puzzle, without examining all of them and never putting all the pieces into place. The routine use of large section histopathology technique could help to alleviate much of this problem, especially with nonpalpable, screen-detected breast cancers. The study of three-dimensional (3D) images of subgross, thick section pathology specimens by both radiologists and pathologists could greatly assist in the communication of findings.

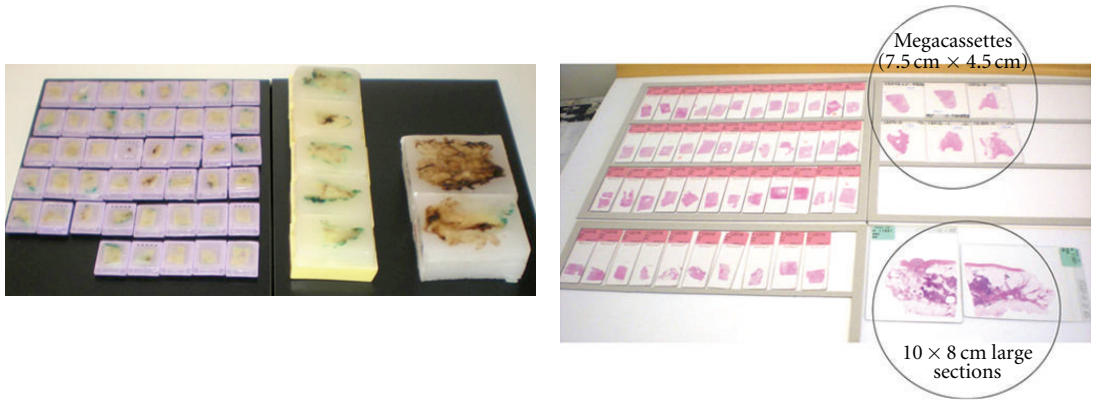
1. Introduction

The introduction of large-scale, population-based mammography screening of asymptomatic women at regular intervals—the prerequisite for a significant decrease in mortality from breast cancer—has added a new dimension to the traditional interaction between pathologists and radiologists. The demands upon the screening radiologists are to find the pathologic lesions, including the 5–7 breast cancers among 1,000 asymptomatic women (high sensitivity) and also to rule out the presence of breast cancer in those women who do not have the disease (high specificity). The radiologists perform these demanding tasks by analyzing images of the structural elements of the breast, while the pathologists are primarily concerned with cellular details not seen by the radiologists.

2. The Need to Narrow the Communication Gap between Radiologists and Pathologists

The currently used small section histology technique examines samples of the surgically removed breast tissue which are only 2.5×2.0 cm in area, while the radiologist images the entire organ. The 4-micron histology slides are viewed at a very high spatial resolution, which reveals cellular details, but the resolution of the breast imaging methods is in the order of 80–100 microns, which is not sufficient to demonstrate cells, but is adequate for visualizing the most important breast structural elements, the ducts, and lobules. To approach a common ground for visualizing breast anatomy and pathology, the small section histopathology technique should be upgraded to the currently available large section (8×10 cm) technique [1–4]. The small section histology technique in current use is inadequate for the correlation of imaging findings in most multifocal and diffusely infiltrating breast cancer cases as the piecemeal reconstruction of the tumor foci and surgical margins is unlikely to have a realistic 1 : 1 correlation with modern imaging methods. Large section histology enables the examination of about 65 cm^2 of contiguous tissue, while the standard glass slides greatly limit the reliable assessment of tumor size and disease extent (Figures 1(a) and 1(b)).

Whereas the mammogram is a projection image of all the 3-dimensional breast anatomic structures superimposed, the thin (4-micron) section histopathology slides provide a highly detailed 2-dimensional image of a slice through



(a) (b)

Figure 1: Comparison of the relative number of paraffin blocks (a) and glass slides (b) needed to cover an equivalent area from the same surgical specimen: 45 small, conventional paraffin blocks/slides, five megacassettes/megaslides (7.5 × 4.5 cm), and two 10 × 8 cm paraffin blocks/slides.

the ducts and lobules. To view these structural elements in their entirety, the histopathology slice needs to be on the order of 1,500 microns, called the large section subgross (3D) histology technique. This technique can serve to bridge the gap that separates the viewpoints of the pathologist and radiologist (Figures 2(a) and 2(b)).

Combining the large thin section (10 × 8 cm) histology technique with the subgross, 3D method facilitates a better understanding of normal and pathologic breast tissue and makes precise correlation with the radiologic imaging methods a reality. "Progress in histologic-mammographic correlation can only be made by examining a histologic specimen of greater length, width and depth" [1]. The current practice of histopathology of breast specimens has serious limitations. "Complete specimen examination is rarely performed in clinical practice" and "In a typical 8-cm diameter lumpectomy specimen, assuming four conventional pathology margin sections are removed in a single plane, only 16% of the circumference is examined microscopically" [5]. In contrast, because the large section histology technique covers a contiguous area which is 10–20 times larger than the small section, it facilitates a more comprehensive evaluation of the lesion(s) relative to the surrounding tissue, a more accurate assessment of the true extent of the disease (including the number of tumor foci) and more complete evaluation of the surgical margin [6]. All these benefits culminate in more accurate diagnoses, leading to more appropriate treatment, fewer recurrences, fewer reoperations, and improved patient care.

Special training in mammographic-histologic correlation, using the techniques of large thin/thick section histology, can enable the radiologist to account for every detail on the normal mammogram (Figures 3(a) and 3(b)).

Trained in this manner, the radiologist will be able to maximize the benefits of screening, while minimizing over/underdiagnosis.

3. Establishing the Diagnosis and Determining the Extent of the Disease

Having found an abnormality on the mammogram, the multimodality approach is used to establish the preoperative diagnosis. This includes a combination of imaging methods and image-guided percutaneous biopsy. Determination of disease extent is an integral part of the preoperative diagnostic workup in order to provide better treatment planning. In particular, it is important to distinguish between unifocal, multifocal, and diffusely infiltrating cancers preoperatively, as the therapeutic approach must necessarily be different for each to avoid under/overtreatment. Determination of disease extent can be accomplished with modern, high-quality imaging tools, in particular preoperative magnetic resonance imaging (MRI) of the breast. Correlating the findings of modern imaging techniques with large section histology on a routine basis will lead to a considerably improved mapping of disease extent, which helps to prevent incomplete resection of breast cancer at primary surgery. Incomplete resection of invasive cancer foci is associated with a poor outcome: "For patients who underwent second surgery, the finding of a residual invasive carcinoma was associated with increased risk for distant recurrence (22.8% versus 6.6%; HR 3.5; 95% confidence interval, 1.8–7.4; $P < .0001$)" [7]. The fatality ratio for multifocal and diffusely infiltrating tumors is 2 and 3 times greater, respectively, compared to unifocal tumors of the same TNM size range [6]. This greater fatality ratio emphasizes the importance of determining the full extent of the disease preoperatively. As breast imaging tools, particularly breast MRI, continue to improve in finding more and smaller tumor foci, radiologists all over the world experience a disturbing scenario, in which there may be a considerable discrepancy between the number of tumor foci and disease extent found at MRI and the corresponding number of tumor foci and disease extent described in the pathology report. In these cases the radiologist may be accused of overdiagnosis. "People blame MRI instead of the

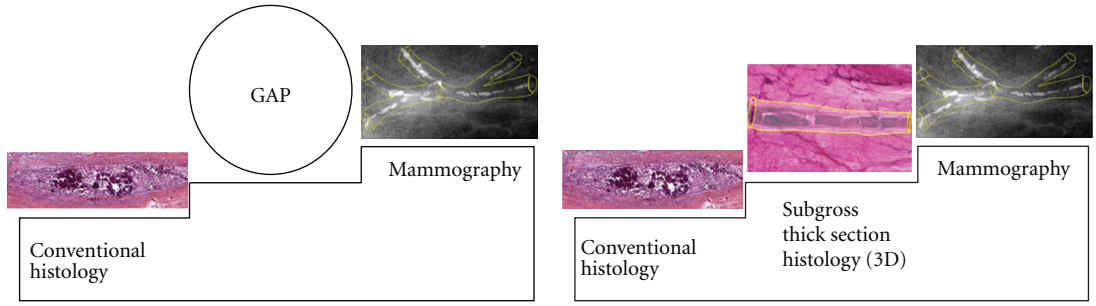


Figure 2: (a) A stepwedge expressing the discrepancy between the low-resolution mammographic image and high-resolution histologic image. The mammogram shows calcifications localized in a well-defined *structure* of the breast (ducts), while the interpretation of the histopathologic finding emphasizes the *cellular details* not seen by the radiologists. There is a communication gap since the radiologist expects a detailed description of tissue structure, in addition to the description of cellular features. (b) The communication gap can be overcome with the help of subgross (3D) histology. In this case the location of fragmented casting type calcifications and the intraductal pathologic process producing them can be clearly seen on the subgross (3D) image, providing excellent radiologic-pathologic correlation.

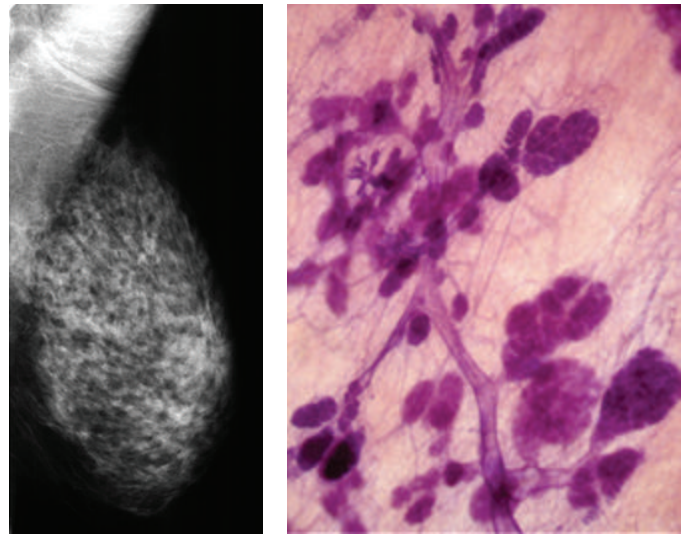


Figure 3: Mammogram of the left breast in the mediolateral oblique projection (a) and large section, subgross (3D) histology of normal breast tissue. (b) On the mammogram the nodular/oval shaped densities (TDLUs) and the linear densities (larger milk ducts) are surrounded by radiolucent adipose tissue, making it possible to account for each nodular and linear density. The nodular densities are the radiologic images of the lobules of varying sizes, and the linear densities are ducts as seen on the 3D histology.

limitations of conventional pathology *and* a failure of small section pathology to correlate with MRI and mammography" (Lee Tucker, M.D., F.C.A.P., personal communication 2012). The following is a typical example. An asymptomatic 50-year-old woman was called back from screening for assessment of a subtle parenchymal contour change in the axillary tail of the left breast, seen on the mediolateral oblique projection (Figure 4(b)). At preoperative breast MRI fifteen separate tumor foci were identified. Several foci were biopsied under ultrasound guidance. Histology of the 14 g core biopsy specimens confirmed the malignant nature of these foci, and mastectomy was performed.

The histology report of the mastectomy specimen described only three invasive tumor foci. As is customary, the pathology report was regarded as the gold standard and the radiologist was accused of overcalling, which, allegedly, led to an unnecessary mastectomy. At the instigation of the radiologist, a thorough examination of the remaining mastectomy specimen was performed, and 12 additional invasive cancer foci were found (Figures 4(j)–4(o)). In this case, as in many other cases, MRI was blamed for overcalling, when in reality, it is the histopathologic examination that was undercalling. In other situations, such as in extensive *in situ* carcinomas or diffusely infiltrating invasive cancers,

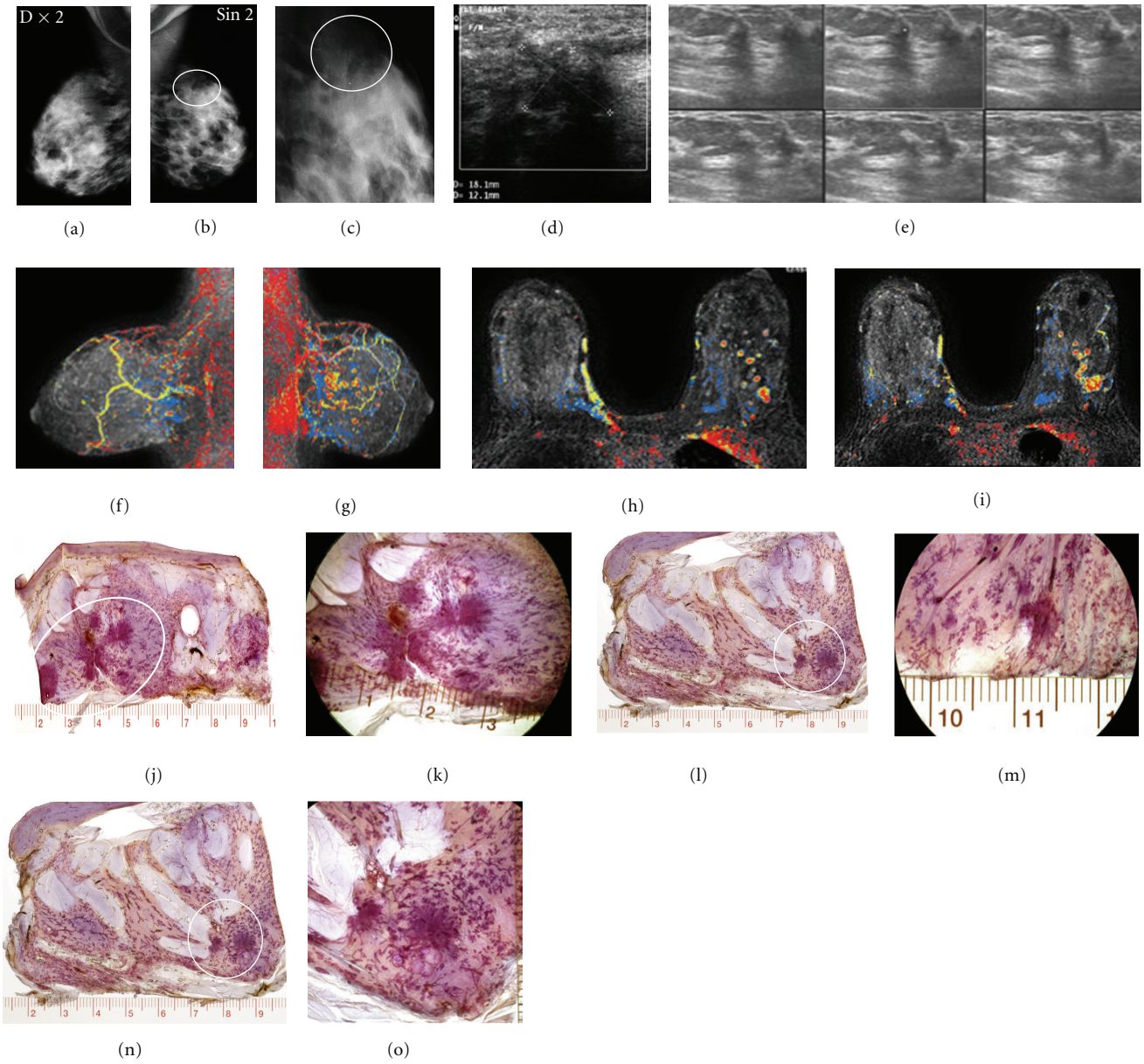


Figure 4: Right (a) and left (b) mammograms, mediolateral oblique projections. Subtle architectural distortion and parenchymal contour change are confirmed on the microfocus mammographic image (c) of the left breast (encircled). Hand-held ultrasound (d) and automated, 3D ultrasound (e) images confirm the presence of malignancy and suggest multifocality. Magnetic resonance examination in the sagittal (f, g) and axial (h, i) planes demonstrates fifteen separate malignant tumor foci. Since only 3 invasive tumor foci were described on the postsurgical histopathology report, the remainder of the mastectomy specimen was also prepared for microscopic examination. Three subgross (3D) histology slices from the reexamination of the mastectomy specimen (j, l, n) and photomicrographic magnification of the areas containing the 12 additional individual invasive cancer foci (k, m, o). This reexamination completes the radiologic-pathologic correlation.

the specimen should be sliced according to the location and extent of the disease as demonstrated with preoperative imaging (Figures 5(a)–5(e)).

Exclusive use of the "breadloafing" technique may seriously underestimate the true extent of diffuse breast cancers (diffusely infiltrating classic invasive lobular carcinomas and extensive microcalcification cases), because in the flattened mastectomy specimen the distance between the skin and the chest wall will be minimized. For example, if the mammograms show malignant type calcifications extending from the nipple to the chest wall over a distance of 8 cm, and the breast MRI also shows a similarly large extent of the disease, then one might expect that the same or larger extent of the disease will be found at histopathologic examination. In such cases slicing of the mastectomy specimens should take the geometry of the malignancy into account, which

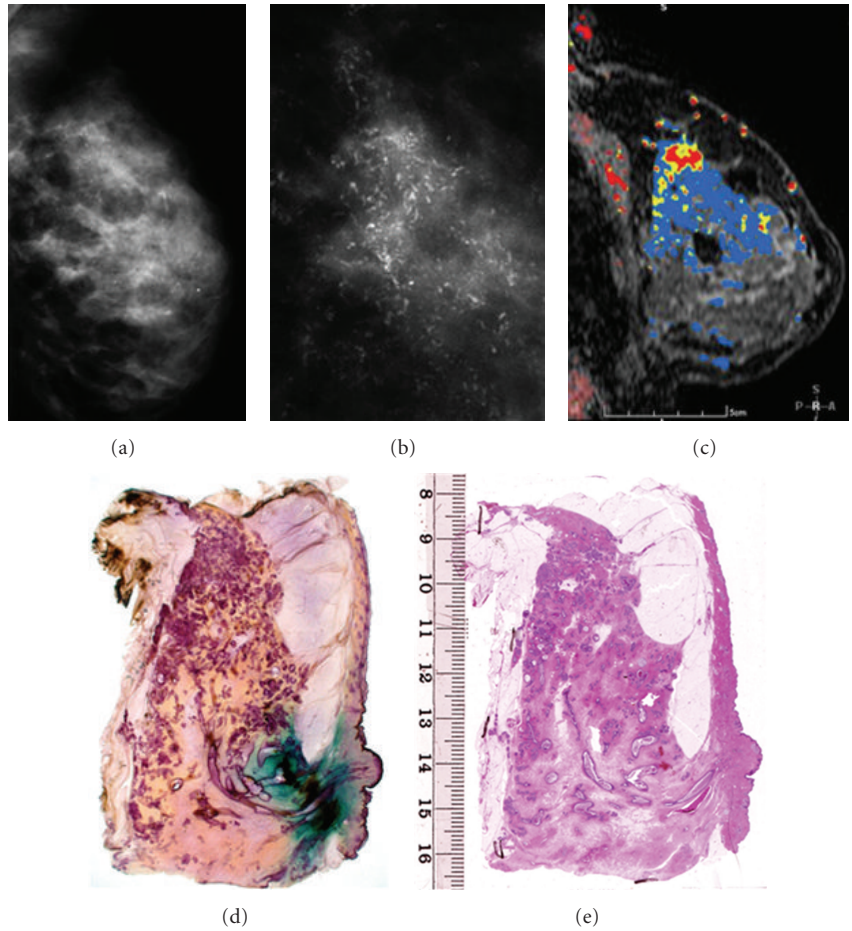


Figure 5: Left breast, mediolateral oblique projection (a) and microfocus magnification (b). Casting type calcifications are seen in the entire upper portion of the left breast, indicating an extensive malignant process within the ducts. The breast MRI examination (c) confirms the extensive nature of the disease and detects an invasive focus in the axillary tail. Large thick section (subgross, 3D) (d) and large thin section (e) histology provide excellent correlation with the imaging findings by demonstrating a 12×12 mm invasive breast cancer and a large number of cancer-filled, distended, duct-like structures occupying the entire upper portion of the left breast.

may require slicing the specimen parallel to the table, instead of using the breadloafing approach. Cooperation between the radiologist and pathologist should prevent discrepancies in determination of true disease extent.

We recommend that *large section histopathology should be standard* for all breast cancer surgical specimens, as it also provides better correlation with breast imaging.

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Clinical Study

Large Format Histology May Aid in the Detection of Unsuspected Pathologic Findings of Potential Clinical Significance: A Prospective Multiyear Single Institution Study

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Large format histology offers several unique advantages over traditional tissue processing. Over 12 years of experience with this technique provide insight into its limitations and benefits. We conducted a prospective multiyear analysis of the potential advantages of large format histology. 656 cases were examined prospectively over an eight-year period. In 172 cases the sign-out pathologist documented an unexpected finding of potential clinical significance as present only on the large format sections and not present on the accompanying standard format slides. These include closer margins, a change in size or extent of disease, and previously undocumented invasive and/or in situ carcinoma. Based on over a decade of experience and eight years of data, our results demonstrate that a quarter of cases had an unexpected finding of potential clinical significance that may not have been fully realized without the use of the large format technique.

1. Background

Large format histology (LFH), a form of tissue processing utilizing large paraffin blocks, a large format microtome, and a large glass slide accommodate a large contiguous portion of breast tissue. The methods and history of the large format technique have been documented elsewhere [1–3]. Our laboratory has utilized this technique for over 12 years and have realized an advantage afforded by the large format process of examination of a larger continuous intact portion of breast tissue. In our laboratory, the typical large format tissue sample measures up to $6.0 \times 8.0 \times 0.5$ cm with a glass slide measuring 12.0×8.5 cm as compared to a standard tissue size of $2.0 \times 2.5 \times 0.3$ cm and standard slide measurement of 2.5×7.5 cm. Most previously recognized benefits result from the obvious size difference. As reported elsewhere large format histology facilitates examination of large, diffuse, or multifocal processes in addition to facilitating evaluation of the adequacy of excision, locating and quantifying residual disease in the neoadjuvant setting, and enhancing pathologic-imaging correlation [4, 5]. Our ongoing experience continues to prove that the large format technique can be successfully incorporated into a community-based practice without significant increases in cost, staffing, or time.

However, to our knowledge, no prospective data exist about the potential diagnostic and therapeutic benefits of large format histology versus standard format tissue processing in a community hospital setting. In an attempt to quantify what observable differences or advantages may be realized both pathologically and clinically from the large format technique, we designed a prospective multiyear analysis of large format histology compared with standard format histology.

2. Methods

From January 2004 to May 2012, we conducted a prospective analysis of the potential benefit of large format histology.

Using an assessment sheet provided to the sign-out pathologist at the time of the original evaluation, we compared large format histology to standard format histology. We relied on the sign-out pathologist to record the findings on a standardized data collection sheet, in particular asking them to note any significant findings which were not anticipated preoperatively and were not present on the accompanying standard format slides. We did not provide a definition of a significant finding during the study and instead relied on the pathologist's judgement to note specific findings as appropriate in order to allow for a more broad scope of what might be considered significant. However, in evaluating the data at the conclusion of the study we defined a significant finding as one which had the potential to alter clinical management. A total of seven pathologists all in the same community practice, including two of the authors of this paper (M. R. Foster and K. W. Biesemier), participated in the study. Six of the seven pathologists participated for the entire eight-year duration of this study. Review of the submitted data sheets revealed commonly reported unexpected findings. These include unanticipated invasive carcinoma or ductal carcinoma in situ (DCIS), an unanticipated change in size or extent of the lesion when compared to the preoperative clinical and/or radiographic impression, and an unanticipated closer surgical margin.

The number of large format blocks varied from case to case, dependent on the size of the lesion, the diagnosis (DCIS versus invasive), and the nature of the specimen (e.g., a reexcision with a visible biopsy cavity or a specimen resected after neoadjuvant therapy). The selection of which portion of the specimen, if any, submitted for LFH was made at the time of gross examination by the sign-out pathologist and assistant (PA), if applicable. In general these ranged from one to three blocks. Traditional small blocks were submitted on most cases for directed sampling, comparison with the large format blocks, and for potential supplemental prognostic/predictive biomarker studies (ER, PR, Her2-neu, Oncotype DX, etc.). When the lesion of interest was included only in the large format blocks, conventional small slides from directed areas were prepared from the large format blocks for special studies. Rare cases, typically small lumpectomies with a reported large region of DCIS which was not completely identified on gross examination, may have been entirely submitted for large format histology. This pathologist directed balanced approach to the examination of breast specimens utilizing a combination of LFH and conventional small block histology that has been enthusiastically endorsed by our laboratory for over a decade, providing enhanced pathologic mammographic correlation as previously described [4].

3. Results

From 2004 to 2012 a total of 656 large format cases were analyzed prospectively. Both standard format and large format slides were utilized in most cases. In 593/656 (90%) the original sign-out pathologist felt large format histology was helpful in establishing the pathologic diagnosis and allowing for accurate assessment of currently accepted parameters for the examination of specimens from in situ and invasive breast cancer as outlined in the CAP guidelines for examining breast carcinoma [6]. In 172/656 (26%) of cases an unexpected finding was present on the large format slides which was not seen on the accompanying standard format slides from the same case. As reflected in Figure 1, these include 78 (12%) with unexpected DCIS, 54 (8%) with unexpected change in size or extent of the DCIS or invasive carcinoma, 29 (4%) with closer margin than expected based on initial examination and imaging, and 11 (2%) with unexpected invasive disease. Unexpected invasive disease was generally microinvasion associated with DCIS or very small tumor foci not previously detected by imaging.

No unexpected findings	484	74%
DCIS	78	12%
Larger tumor size	54	8%
Closer margin	29	4%
Invasive carcinoma	11	2%
Subtotal	172	26%
Total	656	

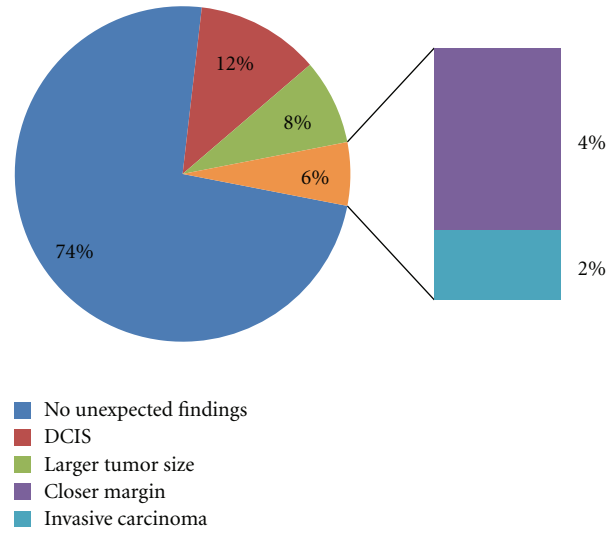

Figure 1: Distribution of potentially clinically significant unexpected findings in 656 large format cases from 2004 to May 2012. Tumor is defined as DCIS or invasive.

4. Discussion

One obvious limitation of this study remains how many of the unexpected findings would have been identified on standard format sections without the use of large format slides. It certainly remains plausible that some if not all observed findings may have been identified if a similar specimen area were submitted via standard format sections. However, others have shown this to be an inefficient means of examining breast tissue [7, 8]. Our experience is similar. We continue to believe that the use of large format histology allows for a timely and efficient means to assess a broad

contiguous region of breast tissue. Further, as documented elsewhere, the large format technique can allow for better correlation with imaging studies [4, 9]. Our data collection forms did not specify the degree to which LFH findings may have altered TNM classification or specific histologic features of any newly discovered invasive disease. These details may be further evaluated by retrospective review of individual cases with significant findings.

We did not exclude from analysis cases in which only large format sections were submitted. However, we estimate this to be a small subset, representing less than 10 total cases, and we believe inclusion of this small number does not significantly alter our results or conclusions.

Our data show that the large format technique enabled the sign-out pathologist to identify potentially significant previously unsuspected findings and allowed for a more complete and accurate assessment of the extent to which in situ and invasive cancer impacts the de novo breast architecture. We continue to realize several unique advantages which large format histology offers over traditional tissue processing. Over 12 years of experience with large format histology provide insight into its limitations and benefits. Based on over a decade of experience and eight years of data, our results demonstrate that over a quarter of our cases had an unexpected finding that may have not been fully realized without the use of the large format technique.

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Review Article

Handling of Radical Prostatectomy Specimens: Total Embedding with Large-Format Histology

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A problem when handling radical prostatectomy specimens (RPS) is that cancer is often not visible at gross examination, and the tumor extent is always underestimated by the naked eye. The challenge is increased further by the fact that prostate cancer is a notoriously multifocal and heterogeneous tumor. For the pathologist, the safest method to avoid undersampling of cancer is evidently that the entire prostate is submitted. Even though whole mounts of sections from RPS appear not to be superior to sections from standard blocks in detecting adverse pathological features, their use has the great advantage of displaying the architecture of the prostate and the identification and location of tumour nodules more clearly, with particular reference to the index tumour; further, it is easier to compare the pathological findings with those obtained from digital rectal examination (DRE), transrectal ultrasound (TRUS), and prostate biopsies. We are in favour of complete sampling of the RPS examined with the whole mount technique. There are reasons in favour and a few drawbacks. Its implementation does not require an additional amount of work from the technicians' side. It gives further clinical significance to our work of uropathologists.

1. Introduction

Handling of radical prostatectomy specimens is a challenging task for the pathologist. The prostate undergoes faster autolysis than most other organs, prostate cancer is notoriously difficult to identify with the naked eye, the tumors are smaller but yet more multifocal than most other clinically diagnosed cancers and prostate cancer is very heterogeneous, both morphologically and genetically. Thus, these specimens need to be handled with great care and according to standardized protocols to enable accurate assessment of grade and stage [1].

The aim of this contribution is to briefly review the current literature on complete versus partial sampling of radical prostatectomy specimens and on whole-mount versus standard sections. Special reference is made to the International Society of Urological Pathology (ISUP) consensus conference on handling and staging of radical prostatectomy specimens [2]. A final section of this paper is dedicated to the Ancona protocol based on the complete sampling of the surgical specimens with whole-mount sections [3].

2. Total versus Partial Embedding

A problem when handling radical prostatectomy specimens is that cancer is often not visible at gross examination, and the tumor extent is always underestimated by the naked eye. The challenge is increased further by the fact that prostate cancer is a notoriously multifocal and heterogeneous tumor. For the pathologist, the safest method to avoid undersampling of cancer is evidently that the entire prostate is submitted. In some institutions, partial sampling is practiced. This requires that the pathologist adheres to a strict protocol, which may be somewhat cumbersome [2, 4, 5].

In 1994, a report on how prostate specimens were examined by American pathologists showed that only 12% of pathologists embedded the entire prostate [6]. Since then the proportion of laboratories that use partial embedding has decreased. In a recent ENUP survey among 217 European pathologists from 15 countries, only 10.8% used partial embedding routinely [7]. In some European countries total embedding is even mandatory, according to national guidelines.

The recent study by Dr. Vainer et al. analyzes 238 radical prostatectomy specimens (RPS) to determine whether significant prognostic information is lost when a partial sampling approach with standard cassettes is adopted, compared with total embedding [8]. In their study, upon arriving at the Pathology Department, the prostate is partly divided by a cut in the mid-sagittal plane through the anterior surface, separating the two lobes for optimal fixation. The gland is then fixed for an additional 20 hours in formic acid and 24 hours in 4% buffered formalin. The gross examination includes measurement in three dimensions, weighing the prostate after removal of the seminal vesicles, and separating the left from the right lobe after inking the anterior and the posterior halves with two different colours. Apical and basal slices of 5–10 mm, depending on the total size of the RPS, are cut horizontally, subsequently sliced parasagittally, and placed in cassettes with often more than one section per cassette. The remaining part of the prostate is cut horizontally in approximately 3-mm thick slices and placed in standard cassettes, ensuring laterality. Large slices are divided to fit standard cassettes. Finally, sections from the seminal vesicles (as a minimum the apex and a cross-section) are embedded. Postfixation in 4% formalin and embedding in paraffin are followed by 4-μm sectioning and staining with haematoxylin and eosin (no. of cassettes/total slides: 18 to 76). For the purpose of the study, glass slides from every second horizontal slice are withheld (no. of slides initially removed: 3 to 26, i.e., 29.9%). The remaining slides are evaluated microscopically.

According to this group of researchers, such an approach decreases the laboratory workload by 30%, and at the same time little information is lost with this procedure, overlooking features significant for the postoperative treatment in only 1.2%. They conclude that partial embedding is acceptable for valid histopathological assessment.

The findings reported by Dr. Vainer et al. [8]are slightly better than those reported by others. Hall et al. [4] showed that by submitting only gross stage B cancer along with standard sections of the proximal and distal margins, the base of seminal vesicles, and the most apical section (next to distal margin), 96% of positive surgical margins and 91% of instances of extraprostatic extension were detected, as compared with identification by complete microscopic examination. In the study by Cohen et al. [9] involving patients with clinical stage B carcinoma, each gland was serially sectioned with sections mounted whole on oversized glass slides. Using only alternate sections, there was a 15% false-negative rate for extraprostatic extension. In a study by Sehdev et al. [5], cT1c tumours with one or more adverse pathological findings, such as Gleason score 7 or more, positive, margins and extraprostatic extension, were compared using ten different sampling techniques. The optimal method consisted of embedding every posterior section and one mid-anterior section from the right and left sides of the gland. If either of the anterior sections had sizable tumour, all anterior slices were blocked in a second step. This method detected 98% of tumours with Gleason score 7 or more, 100% of positive margins, and 96% of cases with extraprostatic extension, through examination of a mean number of 27 slides. It was also shown that sampling of sections ipsilateral to a previously positive needle biopsy detected 92% of Gleason score 7 or greater cancers, 93% of positive margins and 85% instances of extraprostatic extension, from a mean number of 17 slides.

3. Whole-Mount versus Standard Sections

Radical prostatectomy specimens may be processed as either whole-mount or standard sections. Disadvantages with whole-mount sections that include recuts are more difficult to make and it is more expensive and difficult to perform immunohistochemistry. Tissue microarrays can be constructed from whole-mounts for immunohistochemistry, but this technique damages the paraffin blocks and it is a time-consuming process to set up a tissue microarray experiment on prostate cancer. Moreover, whole-mount sections do not fit into standard slide holders for slide collections and standard slide archives. However, whole-mount sections give the pathologist a better overview and the identification of multiple separate tumor foci is facilitated. Laboratory technicians who are trained to cut whole-mounts may find them less time-consuming than cutting multiple small blocks. Thus, the choice between whole-mounts versus standard sections is entirely up to the individual laboratory and should not be standardized [1].

4. 2009 International Society of Urological Pathology Survey and Consensus Conference

In order to identify the methods most commonly employed by urological pathologists worldwide, a web-based survey on handling and reporting of radical prostatectomy specimens was distributed to 255 members of the International Society of Urological Pathology. The International Society of Urological Pathology survey was followed up with a consensus conference held in conjunction with the 2009 Annual Scientific Meeting of the United States and Canadian Academy of Pathology held in Boston, Massachusetts. The aim was to obtain consensus relating to the handling and reporting of radical prostatectomy specimens. Those who completed the electronic survey were invited to attend the consensus conference, which was held on 8 March [2].

Many recommendations of this consensus conference have already been incorporated into international guidelines, including the recent College of American Pathologists protocol and checklist for reporting adenocarcinoma of the prostate and the structured reporting protocol for prostatic carcinoma from the Royal College of Pathologists of Australasia [10, 11].

In response to the question relating to how much of the prostate should be blocked, >60% of conference participants supported complete embedding, whereas >60% also supported partial embedding. This apparent contradiction arose as several respondents selected both options depending on the situation. In view of this, it was concluded that both methods were considered acceptable. Pathologists have to balance the extra expense and time involved in processing entire specimens against the risk of missing important prognostic parameters, and decide whether partial or complete embedding should be performed. There was consensus that if partial embedding is performed, a specific protocol should be followed and the methodology should be documented in the pathology report [2].

From the survey, a majority of respondents reported using standard blocks and only 16% reported the use of whole-mounts, for at least some slices. A minority reported using both methods. On discussion at the consensus conference it was considered that both standard blocks and whole-mounts were acceptable for examination of radical prostatectomy specimens, although no ballot was taken on this point [2].

5. Ancona Experience

In the last few years, 3,000 RPS have been totally embedded and examined with the whole-mount technique by one of our group (RM) at the Section of Pathological Anatomy of the Polytechnic University of the Marche Region and United Hospitals, Ancona, Italy (Figure 1).

The prostate is received fresh from the operating room. Its weight without the seminal vesicles and all three dimensions (apical to basal (vertical), left to right (transverse), and anterior to posterior (sagittal)) are recorded, the latter used for prostate volume calculation. To enhance fixation, 20 mL 4% buffered formalin is introduced into the prostate at multiple sites using a 23G needle. To ensure homogenous fixation the needle is inserted deeply and the solution injected while the needle is retracted slowly. The specimen is then covered with India ink and fixed for 24 hours in 4% neutral buffered formalin. After fixation, the apex and base (3 mm thick slices) are removed from each specimen and examined by the cone method. The prostate body is step-sectioned at 3 mm intervals perpendicular to the long axis (apical-basal) of the gland. For orientation a cut with a surgical blade is made in the right part of each prostate slice. The seminal vesicles are cut into two halves (sandwich method) and processed *in toto*. The cut specimens are postfixed for an additional 24 hours in 4% neutral buffered formalin and then dehydrated in graded alcohols, cleared in xylene, embedded in paraffin (the material is processed together with regular cassettes), and examined histologically as 5 μm-thick whole-mount haematoxylin and eosin (H&E) stained sections [12].

The body of each prostate is represented with 3 to 6 whole-mount slides, whereas the apex, base, and seminal vesicles with 6 to 8 regular slides, totalling between 9–14 slides (in Dr. Vainer et al.'s study [8], up to 76 regular slides are needed to examine the whole prostate). The time needed to section each specimen with an ordinary delicatessen meat slicer is 15–20 minutes. The time taken by a technician to cut all the blocks of an individual case is 30–40 minutes. The time needed by the pathologist to report a case ranges from 40 to 60 minutes. Since the slides do not fit into the current staining machines, the slides are manually stained. The paraffin blocks and glass slides are stored in dedicated containers because of their large size. The comparison between Dr. Vainer et al.'s and our approach is presented in Table 1 [8].

Slides with substandard sections, however with cancer still evaluable, were observed in 7 cases (0,23% of RPS). Only in one case (0,03%) the quality was so poor that the features could not be evaluated. An individual block had to be serially sectioned to visualize the entire inked surface in 15 cases (0,5%). Immunohistochemistry (mainly the basal cell marker p63, racemase and chromogranin A) was done, always successfully, in 30 cases (1%), cutting from the whole-mount section the part to be evaluated in 28, and using the whole-mount section in the remaining two. A procedure was developed to search for residual cancer prostate cancer on pT0 radical prostatectomy after positive biopsy [13, 14]. When applied to 10 cases, a minute focus of cancer was successfully found in 8.

The complete set of slides of each case is examined macroscopically and then microscopically and information on morphological items with diagnostic and prognostic importance are gathered and interpreted in conjunction with clinical information and the macroscopic description of the specimen, including the following:

(1) quality indicators of the surgical procedure: specimen integrity, including missing parts, capsular incision into tumour, and benign glands at the surgical margins;

(2) type of surgical procedure applied, that is, nerve sparing, and previous surgical procedure, such as transurethral resection of the prostate;

(3) presence of tissues other than prostate, that is, rectal wall;

(4) morphologic prognostic and predictive features, such as Gleason score, stage, surgical margin status, and tumour volume;

(5) comparison of pathological findings with digital rectal examination (DRE), transrectal ultrasound (TRUS), and prostate biopsies findings.

Even though whole-mounts of sections from RPS appear not to be superior to sections from standard blocks in detecting adverse pathological features [9], their use has the great advantage of displaying the architecture of the prostate and the identification and location of tumour nodules more clearly, with particular reference to the index tumour; further, it is easier to compare the pathological findings with those obtained from DRE, TRUS, and prostate biopsies.

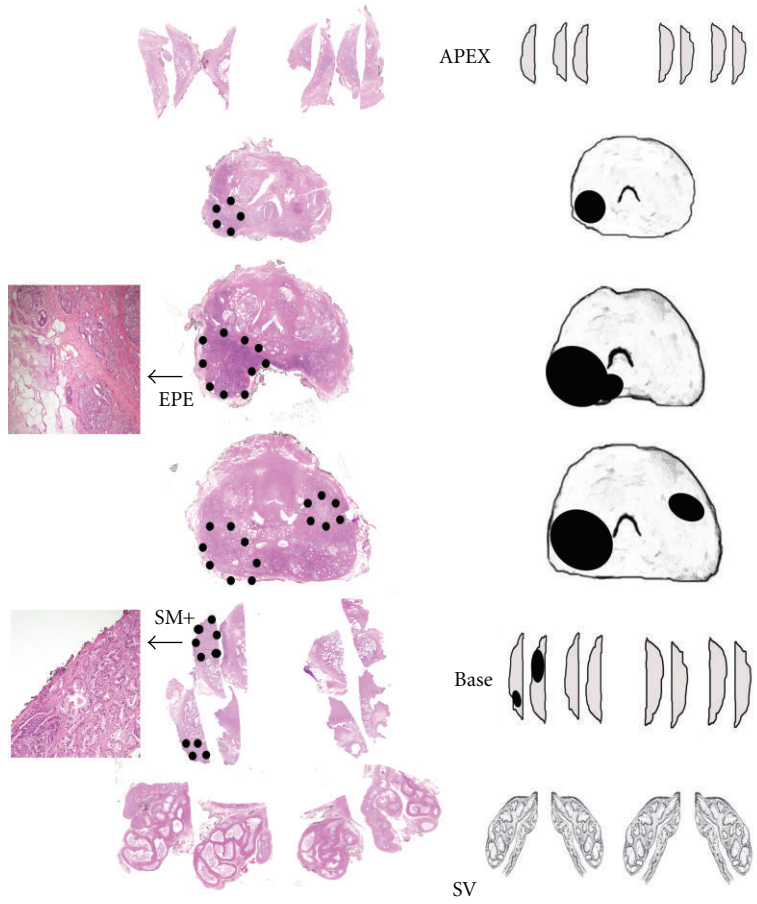
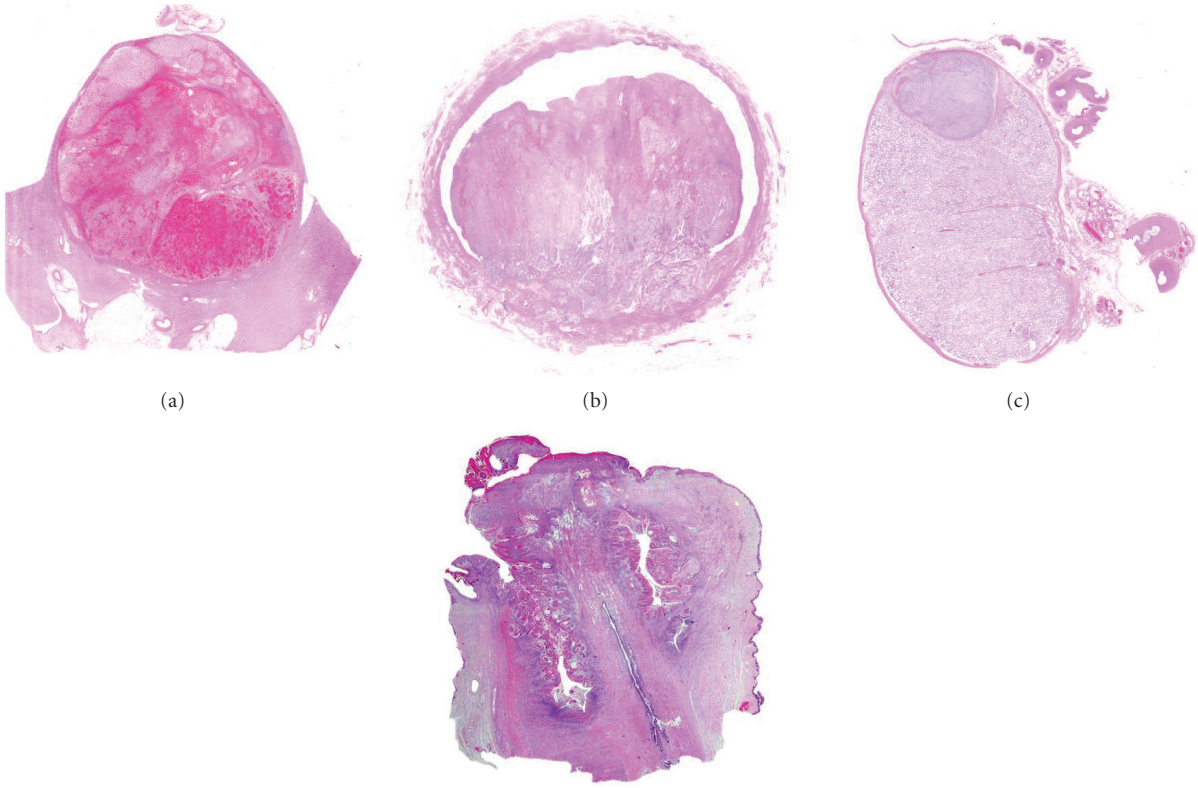


Figure 1: Complete sampling with the whole-mount technique of a prostate specimen. Hematoxylin and eosin-stained sections of prostate specimen are shown on the left and the corresponding mapping on the right. The dotted areas on the slides and the black areas of the map represent two prostatic cancer foci, the index tumour being present on the left of the slides. Extraprostatic extension (EPE) and positive surgical margin (SM+) are present in the posterolateral aspect of the body of the prostate and in one of the slides of the base (see details in the separate images) (SV: seminal vesicles).

Table 1: Comparison between Dr. Vainer et al.'s study [8] and Ancona experience [3].

Features	Dr. Vainer et al.'s study	Ancona experience
Prostate weight and size (and volume)	Yes (not mentioned)	Yes (yes)
Fixation enhancement	Separating the two lobes	Formalin injection
Inking of the surface	Two colours, anterior, and posterior halves	One colour; orientation with a cut on the right
Presectioning fixation (time)	Acid formic (20 h) and 4% buffered formalin (24 h)	4% buffered formalin (24 h)
Sectioning interval	Approximately 3 mm (Apex and base: 5–10 mm)	3 mm (Apex and base: 3 mm)
Subdivision of the slices of the prostate body	Yes, to fit standard cassettes	No (whole mounts)
Seminal vesicles	As a minimum the apex and a cross-section	Sandwich method (all included)
Postsectioning fixation (time)	4% buffered formalin (not mentioned)	4% buffered formalin (24 h)
No. of cassettes/total slides (% examined)	18–76 (70%)	9–14 (100%)
Processing	Not mentioned	As for regular size cassettes
Slide size (section thickness)	7.5 cm by 2.5 cm (4 μm)	7.5 cm by 5.0 cm (5 μm)
Slide staining procedure	Not mentioned	Manual



(a) (b) (c) (d)

Figure 2: Examples of large-format histology of a kidney with clear cell renal cell carcinoma (a), of a urinary bladder with urothelial carcinoma (b), of a testis with seminoma (c) and a penis with squamous cell carcinoma (d).

6. Conclusions

At the 2009 International Society of Urological Pathology consensus conference on handling and staging of radical prostatectomy specimens it was recommended that pathologists balance the expense and time involved in processing entire specimens against the risk of missing important prognostic parameters, and decide whether partial or complete embedding should be performed. A majority of respondents reported using standard blocks and only 16% reported the use of whole-mounts, for at least some slices.

We are in favour of complete sampling of the RPS examined with the whole-mount technique. There are reasons in favour and a few drawbacks. Its implementation does not require an additional amount of work from the technicians' side. It gives further clinical significance to our work of uropathologists [15]. In particular it gives us important pieces of information with paramount importance in relation to the definition of insignificant versus significant prostate cancer as well as to contemporary approaches in prostate cancer treatment, including active surveillance and focal therapy [16].

Appendix

At the Section of Pathological Anatomy of the Polytechnic University of the Marche Region and United Hospitals, Ancona, Italy, a large-format histology is also used to evaluate tumors of the kidney, urinary bladder, testis, and penis. Figure 2 shows examples of large-format histology of kidney with clear cell renal cell carcinoma (a), of urinary bladder with urothelial carcinoma (b), of testis with seminoma (c) and penis with squamous cell carcinoma (d).

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