Adequate Core Biopsy Samples from Stereotactic Biopsies Needed for Today's Breast Pathology

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Abstract

Background: There is a paradigm shift in breast biopsy philosophy. In the past radiologists and clinicians used to collect as little tissue as possible for pathologists to render a diagnosis on conventional histologic H&E sections. Precision medicine has changed this philosophy in such a way that more optimal core biopsy specimens are now required to provide more data for personalizing the therapy.

Methods: Two cases are presented in this study to illustrate the importance of adequate stereotactic breast biopsy samples. In each case digital mammography revealed grouped heterogeneous calcifications in a postmenopausal woman. A core biopsy was performed using stereotactic 8 gauge vacuum-assisted biopsy equipment. Cores were collected in touch-free collection chambers; and the specimen radiograph confirmed the targeted calcifications. Core biopsy samples were completely submitted for paraffin embedding into two cassettes. Cores bearing microcalcifications on specimen radiography were submitted for paraffin embedding in one cassette; the remainder cores were submitted in another cassette.

Results: In one case, microscopic examination revealed ductal carcinoma in situ grade 1 (DCIS grade 1) measuring more than 2 mm in linear extent involving multiple ducts on a single core with microcalcifications. In another case, microscopic examination revealed ductal carcinoma in situ grade 2 with microcalcifications (DCIS grade 2) mixed with moderately-differentiated invasive ductal carcinoma. P63 and calponin immunostains delineated the size of invasive carcinoma (2.5 mm, pT1a) and DCIS (> 6 mm) on a single core.

Conclusions: An important diagnostic criterion for classification of low nuclear grade atypical ductal proliferations is the size/extent of the lesion. Larger, intact, unfragmented biopsy samples enable atypical ductal hyperplasia (ADH) versus DCIS grade 1 distinction possible as seen in first case. The distinction between these two diagnoses often mean a difference in how the patient proceeds with treatment, reinforcing the need to acquire high quality and quantity of biopsy samples. Adequate, unfragmented samples provide high quality tissue for accurate diagnosis and further ancillary studies as seen in the second case.

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1. INTRODUCTION:

Histopathologic examination of adequate, minimally-invasive breast core biopsy samples corresponding to an abnormal radiological finding is the standard of care prior to any therapeutic intervention or mammographic surveillance decision.

Excision of breast (breast conserving surgery, mastectomy) without prior minimally-invasive core biopsy diagnosis is almost an obsolete clinical practice.

It is imperative to have a quantitatively and qualitatively adequate sample in order for a pathologist to formulate an ironclad diagnosis. Majority of patients with imaging abnormality who have undergone minimally-invasive breast core biopsy with benign diagnosis are spared further excision and excision-related complications. Many of these patients are managed with radiological surveillance only.

Quality of the individual tissue sample should be optimal to provide a confident radiological-pathological correlation. Undersampling of clinically significant lesions (invasive / in situ carcinoma, atypical ductal hyperplasia, complex radial scars, papillary neoplasms with or without atypia) and fragmented inadequate samples could easily lead to a false negative "benign breast tissue" histologic diagnosis. According to a recent article published in the Journal of Clinical Oncology, insufficient cancer tissue for biomarker testing occurred across 4 out of 5 cancer types reported on. The majority of tissue samples in which insufficient tissue was present were acquired through core biopsies (67% of all cases) or FNAs (22% of all cases).[1]

Quantity of the tissue of interest should be optimal to perform ancillary diagnostic studies (P63, calponin, CK903, E-cadherin, CK5/6, CK 7, MNF 116, S100, reticulin stains etc.) or prognostic predictive markers (ER, PR, AR, Ki67, HER2, various commercial predictive-prognostic test batteries), and comprehensive genomic profiling for personalized medicine, customizing the treatment options based on tumor's genetic profile. Each immunostain would require at least a 4 micron-thick tissue section from the paraffin block to prepare a slide. Up to 1050 slides could be theoretically prepared for additional studies from an adequate sample obtained with an 8 gauge equipment which provides a 4.19 mm-thick (i.e. 4190 microns) core biopsy tissue fragment. For invasive cancers more than 1mm (>1mm) linear extent of carcinoma is needed on the glass slide for diagnosis, while microinvasive carcinomas require more than 0 mm but less than or equal to 1mm (>0mm-≥1mm) invasive carcinoma for diagnosis. A core biopsy fragment bearing a (4 mm x 4 mm x 4 mm) tumor mass could have about 8 million cells available for any additional tests. For example HER2-FISH testing requires at least 20 non-overlapping cancer cells. But given the tumor heterogeneity 20 cells may not adequately represent the the tumor; the more cancer cells in a sample the better the adequacy. Microarray assays detect expression patterns by hybridizing labeled mRNA isolated from tissue to microarray chips. Numerous gene products can be examined simultaneously. RT-PCR assays amplify RNA from a few specific genes and can therefore be performed on formalin-fixed tissue. Examples of commercially available assays include but not limited to Oncotype DX® Breast Cancer Assay (Genomic Health Inc, Redwood City, California) and Breast Cancer Gene Expression Ratio Assay (or H:I Ratio Test) (Quest Diagnostics, Madison, New Jersey). The Oncotype DX® test is significant because it predicts the likelihood of the patient benefiting from chemotherapy and can further forecast the likelihood of recurrence. Knowing these results can give physicians more information to determine appropriate treatment. The Oncotype DX® assay for example requires one tumor block and an H&E slide from the same block. When blocks are submitted, typically up to 65 micron thickness of the tumor tissue will be used. When the biopsy sample is inadequate performing these microarrays is challenging.

There is a paradigm shift in biopsy philosophy: In the past radiologists/clinicians used to collect as little tissue as possible for pathologists to provide a diagnosis on conventional H&E sections. Precision medicine has changed this philosophy in such a way that nowadays quantitatively and qualitatively more optimal core biopsy specimens are required to provide more data for personalizing the therapy. Pathologists have started using the core biopsy samples not only for establishing a diagnosis but also providing variety of actionable data to personalize the treatment. One such personalized precision medicine tool is FoundationOne® genomic testing, which includes testing for all classes of alterations in each of the entire coding sequence of 315 cancer-related genes plus select introns from 28 genes often rearranged or altered in cancer. These genes are known to be somatically altered in human solid cancers based on recent scientific and clinical literature. Many genomic alterations among those 315 genes, include (but not limited to) PTEN, PI3K, AKT, mTOR, EGFR, MLL2, CDKN2A/B, CCNE1, and KDM6A, AKT3, CCND1, CCND2, CCND3, CDK4, FBXW7, FGFR/FGF, and SRC in various types of breast cancer. Foundation One® genomic testing requires at least 16 unstained slides (with 5 micron thickness) from formalin fixed paraffin embedded tissue block, with an optimum 25 mm^2 surface area. These assays assist oncologists with matching patients with relevant targeted therapies and immunotherapies. This could be easily achieved with larger gauge core biopsy samples.

Larger gauge core biopsy equipment provides more adequate breast tissue for evaluation. Quantity (volume) of the tissue samples is directly proportional to the length and thickness of the sample. Two core biopsies of same length (h) of tissue with 8 gauge (2r =8 gauge= 4.19 mm) equipment theoretically provides 12% more rectangular cross sectional area on the glass slide (based on flat orientation in the paraffin block) for microscopic examination than a 9 gauge tissue sample (2r =9 gauge= 3.75 mm.). An 8 gauge sample would provide approximately 25% more tumor tissue for ancillary testing than a 9 gauge core biopsy sample **(Figure 1 A-B).**

Figure 1

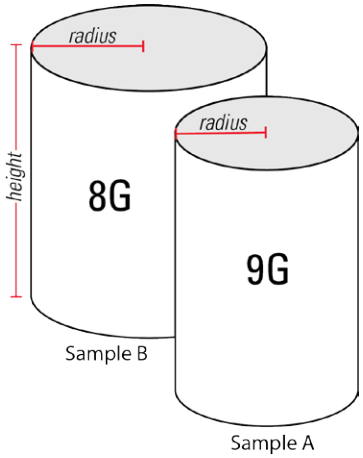


Figure 1. Large bore core biopsy breast samples provide more optimal (larger) cross sectional area for diagnostic evaluation and more tumor tissue for ancillary diagnostic, prognostic-predictive studies. An 8 gauge sample **(B)** would provide approximately 12% more cross sectional area for microscopic examination and 25% more tumor tissue for ancillary testing than a 9 gauge core biopsy sample **(A)**.

Arrangement of cores on a tissue cassette used for tissue processing (fixation, dehydration, paraffin embedding) is critical for optimum microscopic evaluation. Visual scanning of the orderly-aligned cores across the glass slide is easier and less time-consuming for the pathologist compared to randomly (haphazardly) aligned cores **(Figure 2 A-E)**.

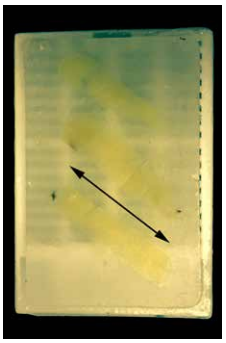


Figure 2A

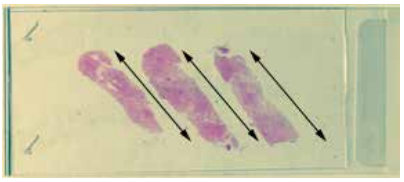


Figure 2B

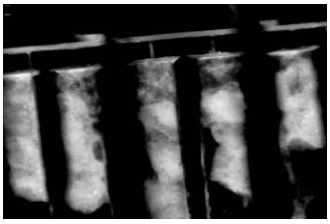


Figure 2C

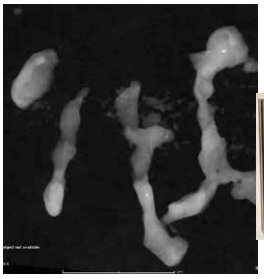


Figure 2D

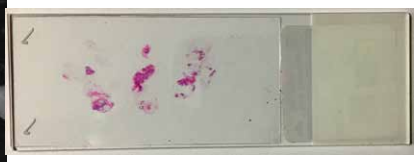


Figure 2E

Figure 2. Minimally invasive breast core biopsy obtained using Mammotome revolve 8 gauge samples (A, B, C). Three of 6 unfragmented cores are regularly aligned flat in one paraffin block **(A)**, from which an H&E slide was prepared **(B). Figure C** shows the specimen radiograph following collection in a touch–free Mammotome revolve collection chambers. Orientation and order of the cores in the glass slides are matching with the orientation and order of the cores in the tissue collection chambers. Visual scanning of the orderly-aligned cores across the glass slide is easier for the pathologist to review and diagnose **(B)**. Pathological-radiological correlation is easier with contiguous (unfragmented), adequate cores to estimate the extent of a given radiologic abnormality. Radiograph and H&E slides of another specimen (obtained using Hologic Eviva, 9 gauge biopsy equipment) are also shown for comparison, please note the haphazard distribution of cores on specimen X-ray **(D)** and fragmented cores on H&E slide **(E)**.

2. CASE STUDIES

CASE 1

Clinical Situation

Digital mammography revealed grouped heterogeneous calcifications in the right breast in a 62-year-old female. A stereotactic biopsy was recommended.

Core Biopsy Procedure

A core biopsy was performed by using stereotactic 8-gauge vacuum-assisted Mammotome revolve equipment. Six cores were collected. Specimen radiograph revealed the targeted calcifications in three of the 6 core samples **(Figure 3A)**.

Microscopic Findings

The specimen was submitted for paraffin embedding in two cassettes, each containing 3 cores. Cores bearing microcalcifications on specimen radiography are submitted for paraffin embedding in one cassette, the remainder 3 cores are submitted in another cassette. Microscopic examination revealed ductal carcinoma in situ grade 1 (DCIS grade 1) with microcalcifications **(Figure 3C arrows).** Surrounding breast tissue also reveals sclerosing adenosis with microcalcifications **(Figure 3B arrowhead)**. An excision with breast conserving surgery (lumpectomy /partial mastectomy) was recommended.

Discussion

An important diagnostic criterion for classification of low nuclear grade atypical ductal proliferations is the size of the lesion. Such lesions larger than 2 mm in a histologically-contiguous linear extent on a core biopsy, completely involving multiple ducts, are classified as DCIS grade 1, while the smaller lesions are classified as ADH. This distinction is often difficult to make according to a study published March 2016 in the Annals of Internal Medicine that included 6900 total interpretations of breast pathology based on a single slide from 240 distinct cases involving 115 pathologists, atypia was overinterpreted 53.% percent of the time and underinterpreted 8.6%. As for ductal carcinoma in situ (DCIS), 18.5% of the cases were overinterpreted and 11.8% underinterpreted.[2] Larger, intact, unfragmented biopsy samples enable ADH versus DCIS grade 1 distinction easier. The distinction between these two diagnoses often mean a difference in how the patient proceeds with treatment, reinforcing the need to acquire high quality and quantity of biopsy samples.

The management and required testing varies depending on a diagnosis of ADH versus DCIS. When and if a DCIS grade 1 diagnosis is rendered, pathologists are obligated to perform ER and PR testing; therefore, an adequate amount of quality tissue must be available. Any other ancillary studies and predictive-prognostic panels such as 12-gene Oncotype DX® Breast DCIS Score™ panel will require adequate additional tissue.

Case 1 underscores the importance of quantitatively and qualitatively adequate samples for pathologic classification, for ancillary studies, and radiologic-pathologic correlation prior to surgical excision.

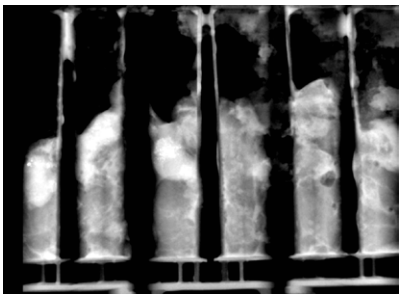


Figure 3A

Figure 3B

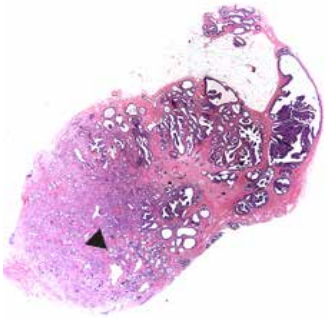


Figure 3C

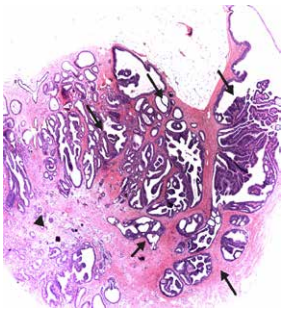
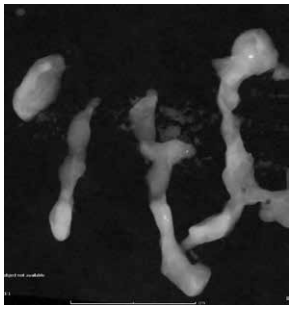


Figure 3D



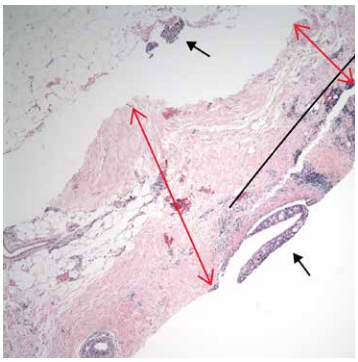


Figure 3E

Figure 3. Radiograph of the samples collected in touch-free collection chambers using Mammotome revolve 8 gauge biopsy system from Case 1 reveals numerous microcalcifications **(Figure A)** corresponding to the targeted calcifications in the mammography. H&E sections reveal DCIS grade 1 (>2mm involving numerous ducts, indicated by arrows) with microcalcifications **(Figure C)**. Sclerosing adenosis (indicated by triangular arrowhead) with microcalcifications is also noted

in the surrounding breast tissue, another source of mammographic calcifications **(Figure B).**

Radiograph and H&E slide of another DCIS specimen of another patient (obtained using Hologic Eviva, 9 gauge biopsy equipment) are also shown for comparison **(Figures D-E)**, please note the haphazard distribution of cores on specimen X-ray **(D)** and fragmentation of core (curved solid line showing the wedge shaped defect) and fragments of DCIS detached from the core on H&E slide (small black arrows). Also noted is the variation of diameter along the core obtained using Hologic Eviva, 9 gauge biopsy equipment (red arrows) **(E)**.

CASE 2

Clinical Situation

Digital mammography revealed grouped heterogeneous calcifications in the right breast in a 67 year-old female. A stereotactic core biopsy was recommended.

Core Biopsy Procedure

A core biopsy was performed by using stereotactic 8 gauge vacuum-assisted

Mammotome revolve biopsy system. Four cores were collected. Specimen radiograph revealed targeted calcifications in 2 of the 4 cores **(Figure 4A, two cores on the right indicated with arrows)**.

Microscopic Findings

The specimen was submitted for paraffin embedding in two cassettes, each containing 2 cores. Two cores bearing microcalcifications on specimen radiography **(Figure 4B)** are submitted in one cassette, the remainder two cores are submitted in another cassette. Microscopic examination revealed ductal carcinoma in situ grade 2 (DCIS grade 2) with focal necrosis and abundant microcalcifications in two cores. Also identified is moderately-differentiated invasive ductal carcinoma (2.5 mm, stage T1a) emanating from the DCIS (> 6 mm) in one core **(Figure 4B-C)**. P63 and calponin immunostains delineated DCIS from invasive carcinoma. Adequate core biopsy sample provided ample invasive and insitu carcinoma tissue for diagnostic P63 and calponin immunostains as well as prognostic/predictive factor testing for ER, PR, AR, Ki67, HER2 (IHC), HER2(FISH) **(Figure 4D** depicting ER immunoprofile). Excision (partial mastectomy) and sentinel lymph biopsy was recommended. Subsequent needle-localized partial mastectomy revealed invasive ductal carcinoma (5 mm, Stage T1a) and DCIS, with negative sentinel lymph node [Stage N0 (sn;i-)].

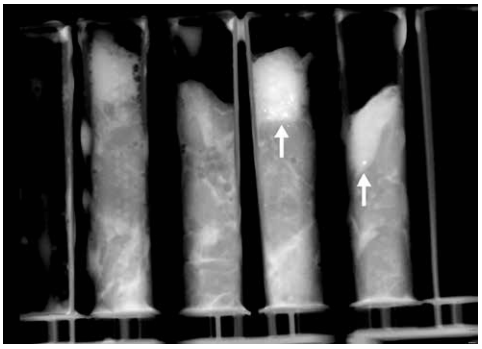


Figure 4A

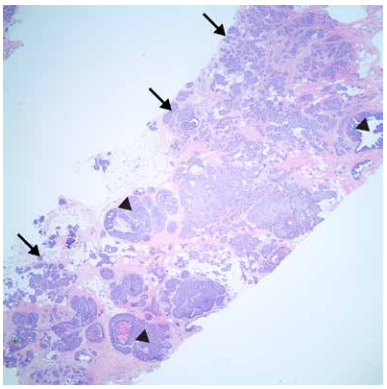


Figure 4B

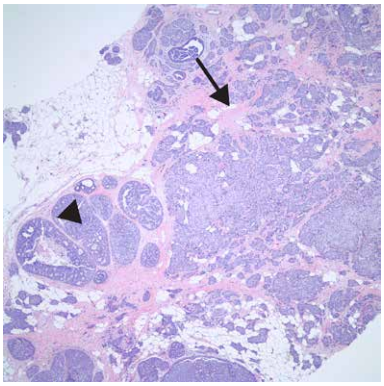


Figure 4C

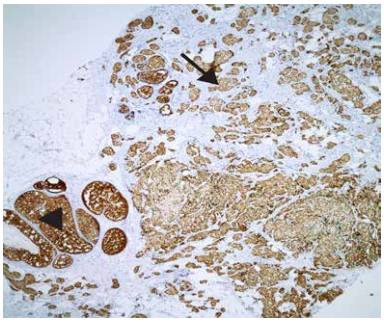


Figure 4D

Figure 4. Radiograph of the samples collected in touch-free collection chambers using Mammotome revolve 8 gauge biopsy system from Case 2 reveals numerous microcalcifications in 2 of 4 cores (**A**, indicated by arrows depicting two cores on the right). Invasive carcinoma and DCIS are identified on one core, and DCIS alone is identified on another core. **Figure B and C** display invasive and in situ carcinoma on one core. **Figure D** displays strong (3+) nuclear ER expression in more than 95% invasive and in situ ductal carcinoma cells. Invasive carcinoma is indicated by arrows while DCIS is indicated by solid triangular arrowheads **(B-D)**.

Discussion

My observation is that large core biopsy samples obtained with Mammotome revolve 8 gauge biopsy system are less vulnerable to crush artifact and fragmentation during tissue processing. Touch-free collection chambers in Mammotome revolve system eliminates/minimizes such artifacts. Precise assessment of the size of invasive/microinvasive cancers and DCIS is possible with large, unfragmented core biopsies.

Adequate (large, unfragmented) core biopsy samples are more representative of the invasive carcinoma and DCIS. Adequate samples mitigate misinterpretation of hormone receptor status if the invasive and/or in situ carcinoma has heterogeneity. Adequate samples provide more neoplastic cells for ER, PR, HER2 (IHC), HER2-FISH, Ki67, AR, and microarray panels to tailor treatment and predict recurrence. Utilization of these ancillary tests on adequate samples should result in reducing both overtreatment of lower-risk patients and undertreatment of higher-risk patients. Larger core biopsy samples are better for tumor genomic profiling for personalized therapies (precision medicine) and tumor banking. Underfixed excision samples may lead to false negative hormone receptor results. Some potential causes of false negative hormone receptor profile such as prolonged cold ischemic time, under or overfixation, and cautery artifacts are unlikely with core biopsy samples. CAP/ASCO guidelines suggest that hormone receptors and HER2 testing are performed on core biopsy samples. If hormone receptors and HER2 are both negative on a core biopsy, repeat testing on a subsequent excision specimen could be considered, particularly when the results are discordant with the histopathologic findings.

3. Conclusions

Stereotactic core biopsies obtained using Mammotome revolve are clinically superior in that it provides high quality, adequate, unfragmented samples to make DCIS and ADH distinction easier. If a DCIS diagnosis is made, additional prognostic/predictive studies are easily performed on well-fixed, undistorted, adequate core biopsy samples.

Touch-free specimen collection system, and specimen radiography of the core biopsy chambers with this biopsy system makes radiological-pathological correlation easier for calcifications. Another advantage of this biopsy system is that core biopsy fragments are aligned and embedded flat in the paraffin blocks in the same order as they are collected from the patient. If an invasive carcinoma (>1mm) is present associated with DCIS, the unfragmented cores enable the pathologist to identify and measure invasive carcinoma so that subsequent partial mastectomy planning includes sentinel lymph node biopsy for appropriate tumor staging. This means one less surgery for the patient.

Routine ancillary studies (ER, PR, HER2, including Ki67, AR), prognostic panels, and advanced comprehensive genetic testing are performed with ease on these samples. Quantitatively and qualitatively more optimal specimens such as those obtained with Mammotome revolve provide more data for personalizing the therapy since precision medicine has changed the core biopsy philosophy from small, fragmented, inadequate samples to unfragmented, undistorted, adequate samples.

FOOTNOTES

[1]Pieter De Richter, Jackie Ilacqua; Ipsos Healthcare, New York, NY; Ipsos Healthcare, Mahwah, NJ. “Correlation between biopsy type and insufficient tissue availability for biomarker testing in five solid cancer types.” J Clin Oncol 31, 2013 (suppl; abstr e22136).

[2]Elmore JG, Nelson HD, Pepe MS Longton GM, Tosteson AN, Geller B, Onega T, Carney PA, Jackson SL, Allison KH, Weaver DL, Ann Intern Med. 2016 May 17;164(10):649-55. Variability in Pathologists’ Interpretations of Individual Breast Biopsy Slides: A Population Perspective.