Protocol for the Examination of Biopsy Specimens From Patients With Invasive Carcinoma of the Breast

Version: Breast Invasive Biopsy 1.0.0.0  Protocol Posting Date: February 2019

Accreditation Requirements
The use of this protocol is recommended for clinical care purposes but is not required for accreditation purposes.

This protocol may be used for the following procedures AND tumor types:

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy</td>
<td>Includes specimens designated needle biopsy, fine needle aspiration and others (for excisional biopsy, see below)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive breast carcinoma of any type, with or without ductal carcinoma in situ (DCIS)</td>
<td>Includes microinvasive carcinoma and carcinoma with neuroendocrine features</td>
</tr>
</tbody>
</table>

The following should NOT be reported using this protocol:

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resection (consider Breast Invasive Carcinoma Resection protocol)</td>
<td></td>
</tr>
<tr>
<td>Excisional biopsy (consider Breast Invasive Carcinoma Resection protocol)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tumor Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ductal carcinoma in situ (DCIS) without invasive carcinoma (consider the DCIS Biopsy protocol)</td>
</tr>
<tr>
<td>Paget disease of the nipple without invasive carcinoma (consider the DCIS Biopsy protocol)</td>
</tr>
<tr>
<td>Encapsulated or solid papillary carcinoma without invasion (consider the Breast DCIS Biopsy protocol)</td>
</tr>
<tr>
<td>Phyllodes tumor</td>
</tr>
<tr>
<td>Lymphoma (consider the Hodgkin or non-Hodgkin Lymphoma protocols)</td>
</tr>
<tr>
<td>Sarcoma (consider the Soft Tissue protocol)</td>
</tr>
</tbody>
</table>

Authors
Patrick L. Fitzgibbons, MD*; James L. Connolly*, MD; Mary Edgerton, MD, PhD; Ross Simpson, MD.

* Denotes primary author. All other contributing authors are listed alphabetically.

Accreditation Requirements
The use of this biopsy case summary is recommended for clinical care purposes, but is not required for accreditation purposes. The core and conditional data elements are routinely reported for biopsy specimens. Non-core data elements are included to allow for reporting information that may be of clinical value.

Summary of Changes
1.0.0.0 – New Breast Invasive Carcinoma Biopsy protocol
Surgical Pathology Cancer Case Summary

Protocol posting date: February 2019

INVASIVE CARCINOMA OF THE BREAST: Biopsy

Notes:
This case summary is recommended for reporting biopsy specimens but is NOT REQUIRED for accreditation purposes. Core data elements are bolded to help identify routinely reported elements.

Select a single response unless otherwise indicated.

Procedure, Laterality, and Site may be listed separately or on 1 line.

Procedure
___ Needle biopsy
___ Fine needle aspiration
___ Other (specify): ____________________________
___ Not specified

Specimen Laterality
___ Right
___ Left
___ Not specified

Tumor Site (select all that apply)
___ Upper outer quadrant
___ Lower outer quadrant
___ Upper inner quadrant
___ Lower inner quadrant
___ Central
___ Nipple
___ Clock position (specify): _____ o’clock
___ Distance from nipple (centimeters): ______ cm
___ Other (specify): ____________________________
___ Not specified

Histologic Type (Note A)
___ Invasive carcinoma of no special type (invasive ductal carcinoma, not otherwise specified)
___ Micro-invasive carcinoma
___ Invasive lobular carcinoma
___ Invasive carcinoma with lobular features
___ Invasive carcinoma with ductal and lobular features (“mixed type carcinoma”)
___ Mucinous carcinoma
___ Tubular carcinoma
___ Invasive carcinoma, tubulo-lobular variant
___ Invasive cribriform carcinoma
___ Invasive micropapillary carcinoma
___ Invasive papillary carcinoma
___ Invasive carcinoma with medullary features
___ Metaplastic carcinoma
___ Low-grade adenosquamous carcinoma
___ Fibromatosis-like metaplastic carcinoma
___ Metaplastic carcinoma, spindle cell type
Metaplastic carcinoma, mixed epithelial and mesenchymal type
Invasive carcinoma with metaplastic features
Squamous cell carcinoma
Adenoid cystic carcinoma
Invasive carcinoma with apocrine features
Invasive carcinoma with clear cell (glycogen rich) features
Invasive carcinoma with neuroendocrine features
Invasive carcinoma, with signet-ring cell features
Secretory carcinoma
Invasive carcinoma, type cannot be determined
Other histologic type not listed (specify): ____________________________

Histologic Grade (Nottingham Histologic Score) (Note B)
Not applicable (microinvasion only)

Glandular (Acinar)/Tubular Differentiation
Score 1 (>75% of tumor area forming glandular/tubular structures)
Score 2 (10% to 75% of tumor area forming glandular/tubular structures)
Score 3 (<10% of tumor area forming glandular/tubular structures)
Score cannot be determined

Nuclear Pleomorphism
Score 1 (nuclei small with little increase in size in comparison with normal breast epithelial cells, regular outlines, uniform nuclear chromatin, little variation in size)
Score 2 (cells larger than normal with open vesicular nuclei, visible nucleoli, and moderate variability in both size and shape)
Score 3 (vesicular nuclei, often with prominent nucleoli, exhibiting marked variation in size and shape, occasionally with very large and bizarre forms)
Score cannot be determined

Mitotic Rate (see Table 1)
Score 1
Score 2
Score 3
Score cannot be determined

Overall Grade
Grade 1 (scores of 3, 4, or 5)
Grade 2 (scores of 6 or 7)
Grade 3 (scores of 8 or 9)
Score cannot be determined (explain: ___________________)

Ductal Carcinoma In Situ (DCIS) (Note C)
Not identified
Present
Cannot be excluded

Architectural Patterns (if DCIS is present select all that apply)
Comedo
Paget disease (DCIS involving nipple skin)
Cribriform
Micropapillary
Papillary
Solid
Other (specify): ____________________________
Nuclear Grade (if DCIS is present)
___ Grade I (low)
___ Grade II (intermediate)
___ Grade III (high)

Necrosis (if DCIS is present)
___ Not identified
___ Present, focal (small foci or single cell necrosis)
___ Present, central (expansive "comedo" necrosis)

Lymphovascular Invasion
___ Not identified
___ Present
___ Cannot be determined

Additional Pathologic Findings (Note D)
Specify: ____________________________

Microcalcifications (select all that apply) (Note E)
___ Not identified
___ Present in DCIS
___ Present in invasive carcinoma
___ Present in non-neoplastic tissue
___ Other (specify): ________________________________

Ancillary Studies
Note: For hormone receptor and HER2 reporting, the CAP Breast Biomarker Template should be used.
www.cap.org/cancerprotocols.

Biomarker Studies
___ Pending

Comment(s)
**A. Histologic Type**

This protocol applies to all invasive carcinomas of the breast. The World Health Organization (WHO) classification of breast carcinoma is presented below, although the protocol does not preclude the use of other classifications or histologic types. Carcinomas may be classified based on the H&E appearance without the use of immunohistochemical studies.

A modified list is presented in the protocol, based on the most frequent types of invasive carcinomas and terminology that is in widespread usage. The modified list is intended to capture the majority of tumors and reduce the classification of tumors being reported as “other.” The WHO classification is presented for completeness.

**WHO Classification of Invasive Carcinoma of the Breast**

1. Microinvasive carcinoma
2. Invasive carcinoma of no special type (NST)
   - Pleomorphic carcinoma
   - Carcinoma with osteoclast-like stromal giant cells
   - Carcinoma with choriocarcinomatous features
   - Carcinoma with melanotic features
3. Invasive lobular carcinoma
   - Classic lobular carcinoma
   - Solid lobular carcinoma
   - Alveolar lobular carcinoma
   - Pleomorphic lobular carcinoma
   - Tubulolobular carcinoma
   - Mixed lobular carcinoma
4. Tubular carcinoma
5. Cribriform carcinoma
6. Mucinous carcinoma
7. Carcinoma with medullary features
   - Medullary carcinoma
   - Atypical medullary carcinoma
   - Invasive carcinoma NST with medullary features
8. Carcinoma with apocrine differentiation
9. Carcinoma with signet-ring-cell differentiation
10. Invasive micropapillary carcinoma
11. Metaplastic carcinoma of no special type
    - Low-grade adenosquamous carcinoma
    - Fibromatosis-like metaplastic carcinoma
    - Squamous cell carcinoma
    - Spindle cell carcinoma
    - Metaplastic carcinoma with mesenchymal differentiation
      - Chondroid differentiation
      - Osseous differentiation
      - Other types of mesenchymal differentiation
    - Mixed metaplastic carcinoma
    - Myoepithelial carcinoma
12. Papillary carcinoma
    - Encapsulated papillary carcinoma with invasion
    - Solid papillary carcinoma, invasive
13. Epithelial-Myoepithelial tumors
14. Adenomyoepithelioma with carcinoma
15. Adenoid cystic carcinoma
16. Rare types
17. Carcinoma with neuroendocrine features
Neuroendocrine tumor, well-differentiated
Neuroendocrine carcinoma poorly differentiated (small cell carcinoma)
Carcinoma with neuroendocrine differentiation
Secretory carcinoma
Invasive papillary carcinoma
Acinic cell carcinoma
Mucoepidermoid carcinoma
Polymorphous carcinoma
Oncocytic carcinoma
Lipid-rich carcinoma
Glycogen-rich clear cell carcinoma
Sebaceous carcinoma

References

B. Histologic Grade
All invasive breast carcinomas should be graded. The Nottingham combined histologic grade (Elston-Ellis modification of Scarff-Bloom-Richardson grading system) should be used for reporting. Within each stage grouping there is a relation between histologic grade and outcome.

The Nottingham combined histologic grade evaluates the amount of tubule formation, the extent of nuclear pleomorphism, and the mitotic count (or mitotic rate). Each variable is given a score of 1, 2, or 3, and the scores are added to produce a grade. The mitotic score is determined by the number of mitotic figures found in 10 consecutive high-power fields (HPF) in the most mitotically active part of the tumor. Only clearly identifiable mitotic figures should be counted; hyperchromatic, karyorrhectic, or apoptotic nuclei are excluded. Because of variations in field size, the HPF size must be determined for each microscope and the appropriate point score determined accordingly. It is recommended that the size be measured by using a micrometer. However, the diameter of an HPF can also be calculated by using the method below.

Measuring the Size of a High-Power Field (HPF) With a Ruler

Use a clear ruler to measure the diameter of a low-power field. This number can be used to calculate a constant based on the following formula:

\[
\text{Eyepiece Magnification} \times \text{Objective Magnification} \times \text{Microscopic Field Diameter} = \text{A Constant}
\]

When the value of the constant is known, the diameter of an HPF can be calculated for other objectives by using the following formula:

\[
\text{Unknown Field Diameter} = \frac{\text{Constant}}{\text{Eyepiece Magnification} \times \text{Objective Magnification}}
\]

Half of the field diameter is the radius of the field \((r)\), which can then be used to calculate the area of the HPF:

\[
3.1415 \times r^2 = \text{Area of Microscopic Field}
\]

If the microscopic field diameter or the area of the field is known, Table 1 can be used to determine the number of mitoses corresponding to different scores.
### Table 1. Score Categories According to Field Diameter and Mitotic Count

<table>
<thead>
<tr>
<th>Field diameter (mm)</th>
<th>Area (mm²)</th>
<th>Number of mitoses per 10 fields corresponding to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Score 1</td>
</tr>
<tr>
<td>0.40</td>
<td>0.125</td>
<td>≤ 4</td>
</tr>
<tr>
<td>0.41</td>
<td>0.132</td>
<td>≤ 4</td>
</tr>
<tr>
<td>0.42</td>
<td>0.139</td>
<td>≤ 5</td>
</tr>
<tr>
<td>0.43</td>
<td>0.145</td>
<td>≤ 5</td>
</tr>
<tr>
<td>0.44</td>
<td>0.152</td>
<td>≤ 5</td>
</tr>
<tr>
<td>0.45</td>
<td>0.159</td>
<td>≤ 5</td>
</tr>
<tr>
<td>0.46</td>
<td>0.166</td>
<td>≤ 6</td>
</tr>
<tr>
<td>0.47</td>
<td>0.173</td>
<td>≤ 6</td>
</tr>
<tr>
<td>0.48</td>
<td>0.181</td>
<td>≤ 6</td>
</tr>
<tr>
<td>0.49</td>
<td>0.189</td>
<td>≤ 6</td>
</tr>
<tr>
<td>0.50</td>
<td>0.196</td>
<td>≤ 7</td>
</tr>
<tr>
<td>0.51</td>
<td>0.204</td>
<td>≤ 7</td>
</tr>
<tr>
<td>0.52</td>
<td>0.212</td>
<td>≤ 7</td>
</tr>
<tr>
<td>0.53</td>
<td>0.221</td>
<td>≤ 8</td>
</tr>
<tr>
<td>0.54</td>
<td>0.229</td>
<td>≤ 8</td>
</tr>
<tr>
<td>0.55</td>
<td>0.238</td>
<td>≤ 8</td>
</tr>
<tr>
<td>0.56</td>
<td>0.246</td>
<td>≤ 8</td>
</tr>
<tr>
<td>0.57</td>
<td>0.255</td>
<td>≤ 9</td>
</tr>
<tr>
<td>0.58</td>
<td>0.264</td>
<td>≤ 9</td>
</tr>
<tr>
<td>0.59</td>
<td>0.273</td>
<td>≤ 9</td>
</tr>
<tr>
<td>0.60</td>
<td>0.283</td>
<td>≤ 10</td>
</tr>
<tr>
<td>0.61</td>
<td>0.292</td>
<td>≤ 10</td>
</tr>
<tr>
<td>0.62</td>
<td>0.302</td>
<td>≤ 11</td>
</tr>
<tr>
<td>0.63</td>
<td>0.312</td>
<td>≤ 11</td>
</tr>
<tr>
<td>0.64</td>
<td>0.322</td>
<td>≤ 11</td>
</tr>
<tr>
<td>0.65</td>
<td>0.332</td>
<td>≤ 12</td>
</tr>
<tr>
<td>0.66</td>
<td>0.342</td>
<td>≤ 12</td>
</tr>
<tr>
<td>0.67</td>
<td>0.353</td>
<td>≤ 12</td>
</tr>
<tr>
<td>0.68</td>
<td>0.363</td>
<td>≤ 13</td>
</tr>
<tr>
<td>0.69</td>
<td>0.374</td>
<td>≤ 13</td>
</tr>
</tbody>
</table>

*From Pathology Reporting of Breast Disease.*

2 Copyright 2005 National Health Service Cancer Screening Programme and The Royal College of Pathologists. Adapted with permission.
References


C. Ductal Carcinoma In Situ

Ductal carcinoma in situ associated with invasive carcinoma increases the risk of local recurrence for women undergoing breast-conserving surgery. It is more important to report the features of DCIS when in situ disease is predominant (eg, cases of DCIS with microinvasion or extensive DCIS associated with T1a carcinoma). If DCIS is a minimal component of the invasive carcinoma, the features of the DCIS have less clinical relevance. Therefore, most of the reporting elements for DCIS are optional and should be used at the discretion of the pathologist.

The pathology report should specify whether extensive DCIS is present. Extensive intraductal component (EIC)-positive carcinomas are defined in 2 ways (Figure 4, A through D):

![Figure 4](image)

Figure 4. Extensive Intraductal Component (EIC). A. Extensive intraductal component (EIC)-positive carcinomas are defined by the following criteria: (1) ≥25% of the area within the invasive carcinoma is ductal carcinoma in situ (DCIS) and (2) DCIS is also present outside the area of invasive carcinoma. B. EIC-positive carcinomas also include carcinomas in which DCIS is associated with a “small” (approximately 10 mm or less) invasive carcinoma or carcinomas. C. EIC-negative carcinomas do not fulfill the criteria for being positive for EIC. D. Some carcinomas do not strictly fulfill the criteria for EIC but are associated with extensive DCIS in the surrounding tissue. In such cases it is helpful to provide some measure of the extent of DCIS in the specimen.
1. Ductal carcinoma in situ is a major component within the area of invasive carcinoma (approximately 25%) and DCIS is also present in the surrounding breast parenchyma.

2. There is extensive DCIS associated with a small (~10 mm or less) invasive carcinoma (i.e., the invasive carcinoma is too small for DCIS to comprise 25% of the area).

Extensive intraductal component-positive carcinomas are associated with an increased risk of local recurrence when the surgical margins are not evaluated or focally involved. The finding of EIC positivity has less significance when DCIS does not extend close to margins.

In some cases, extensive DCIS can be present outside the area of invasive carcinoma although the carcinoma does not technically fulfill the criteria for EIC positivity. In such cases, quantification of the amount of DCIS present is helpful for planning radiation therapy.

The extent of DCIS will be most relevant for cases of extensive DCIS with microinvasion and least relevant for large EIC-negative invasive carcinomas. Methods for estimating the extent of DCIS include directly measuring the lesion when confined to a single histologic slide, determining size by submitting the entire specimen in sequence and in sections of uniform thickness, or counting the number of blocks with DCIS. The College of American Pathologists (CAP) DCIS protocol\(^2\) provides additional information on determining the extent of DCIS.

**Architectural Pattern of DCIS**
The architectural pattern has traditionally been reported for DCIS. However, nuclear grade and the presence of necrosis are more predictive of clinical outcome.

**Nuclear Grade of DCIS**
The nuclear grade of DCIS is determined using 6 morphologic features (Table 1).\(^3\)

**Table 2. Nuclear Grade of Ductal Carcinoma in Situ**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Grade I (Low)</th>
<th>Grade II (Intermediate)</th>
<th>Grade III (High)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleomorphism</td>
<td>Monotonous (monomorphic)</td>
<td>Intermediate</td>
<td>Markedly pleomorphic</td>
</tr>
<tr>
<td>Size</td>
<td>1.5 to 2 x the size of a normal red blood cell or a normal duct epithelial cell nucleus</td>
<td>Intermediate</td>
<td>&gt;2.5 x the size of a normal red blood cell or a normal duct epithelial cell nucleus</td>
</tr>
<tr>
<td>Chromatin</td>
<td>Usually diffuse, finely dispersed chromatin</td>
<td>Intermediate</td>
<td>Usually vesicular with irregular chromatin distribution</td>
</tr>
<tr>
<td>Nucleoli</td>
<td>Only occasional</td>
<td>Intermediate</td>
<td>Prominent, often multiple</td>
</tr>
<tr>
<td>Mitoses</td>
<td>Only occasional</td>
<td>Intermediate</td>
<td>May be frequent</td>
</tr>
<tr>
<td>Orientation</td>
<td>Polarized toward luminal spaces</td>
<td>Intermediate</td>
<td>Usually not polarized toward the luminal space</td>
</tr>
</tbody>
</table>

**Necrosis**
The presence of necrosis is correlated with the finding of mammographic calcifications (i.e., most areas of necrosis will calcify). Ductal carcinoma in situ that presents as mammographic calcifications often recurs as calcifications. Necrosis can be classified as follows:

- **Central ("comedo"):** The central portion of an involved ductal space is replaced by an area of expansive necrosis that is easily detected at low magnification. Ghost cells and karyorrhectic debris are generally present. Although central necrosis is generally associated with high-grade nuclei (i.e., comedo DCIS), it can also occur with DCIS of low or intermediate nuclear grade.

- **Focal:** Small foci, indistinct at low magnification, or single cell necrosis.
Necrosis should be distinguished from secretory material, which can also be associated with calcifications, but does not include nuclear debris.

References

D. Additional Pathologic Findings
In some cases, additional pathologic findings are important for the clinical management of patients. If multiple invasive carcinomas are present and differ in histologic type, grade, or the expression of ER, PgR, or HER2, this information should be included as text in this section.

E. Microcalcifications
Cancer found in biopsies performed for microcalcifications will almost always be at the site of the calcifications or in close proximity. The presence of the targeted calcifications in the specimen should be confirmed by specimen radiography. The pathologist must be satisfied that the specimen has been sampled in such a way that the lesion responsible for the calcifications has been examined microscopically. The relationship of the radiologic calcifications to the invasive carcinoma and the DCIS should be indicated.

If calcifications can be seen in the specimen radiograph but not in the initial histologic sections, deeper levels should be examined. If needed, radiographs of the paraffin block(s) may be obtained to detect calcifications remaining in the block(s). If microcalcifications cannot be confirmed by routine microscopic evaluation, polarized light may be helpful, since calcium oxalate crystals are refractile and polarizable but usually clear or tinged yellow in H&E sections. On rare occasions, calcifications do not survive tissue processing or prolonged fixation in formalin. Foreign material can sometimes simulate calcifications (eg, metallic fragments after surgery or trauma).