



# Diagnosis and Treatment of Patients with Primary and Metastatic Breast Cancer



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## Pathology

# Pathology

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- **Versions 2004–2017:**  
**Blohmer / Costa / Fehm / Friedrichs / Huober /  
Kreipe / Lück / Schneeweis / Sinn / Thomssen**
  
- **Version 2018:**  
**Kreipe / Schmidt**

# General Principles for Histopathologic Examination of Breast Cancer Specimens

- **Any statement in the histological report should reflect its clinical significance**
- **The terminology used is chosen according to current national guidelines and international classifications**
- **Quality control measures are required in all areas of diagnostic pathology**

# Preamalytics: Fixation

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- **Minimize time to fixation (cold ischemia time)**
- **Minimal fixation time of 6 hours for optimal antigen preservation**
- **Optimal fixation time 6 - 72 h for core biopsies**
- **Optimal fixation time for resection specimens: 12 - 72 h**
- **Use of neutral buffered formalin**

| Oxford |    |     |
|--------|----|-----|
| LoE    | GR | AGO |
| 5      | D  | ++  |
| 5      | D  | ++  |
| 5      | D  | ++  |
| 5      | D  | ++  |
| 5      | D  | ++  |

# Use of Fine Needle Aspiration Cytology\*

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- **Nipple secretion**
- **Tumor**
- **Cyst**
- **Lymph node**

| Oxford |    |     |
|--------|----|-----|
| LoE    | GR | AGO |
| 5      | D  | +   |
| 5      | D  | -   |
| 5      | D  | +/- |
| 5      | D  | +/- |

# Workup: Macroscopy and Specimen Radiography

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- **Consideration of preoperative imaging results (e.g. multifocality, intraductal component, adjacent structures) for sampling and documentation**
- **Routine documentation of macroscopic findings by using diagrams or macro image, with relation to topography**
- **Specimen radiography for non-palpable lesions and microcalcifications**

# Workup: Core Needle Biopsies (US-guided or stereotactic)

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- **Routine workup in step sections  
(14G: 3 sections / 11G, 8G: 6–8 sections)**
- **Correlation with imaging (density, calcifications),  
use of B-classification**
- **Frozen section diagnosis on core biopsies**
- **Routine evaluation of ER/PgR and HER2 status**
- **Turn-around time < 24 h (histology)**

| Oxford |    |     |
|--------|----|-----|
| LoE    | GR | AGO |
| 5      | D  | ++  |
| 1b     | B  | ++  |
| 5      | D  | --  |
| 3b     | C  | ++  |
| 5      | D  | +   |

# Workup: Breast-Conserving Specimens

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- **Slicing perpendicular to the longitudinal axis (or perpendicular to the nipple-peripheral axis in case of spherical specimens)**
- **Systematic sampling, at least 1 tissue block every 1 cm**
- **Inking of resection margins. Sampling of resection margins in all dimensions**
- **Documentation after slicing using specimen radiography, photo documentation or diagram**

| Oxford |    |     |
|--------|----|-----|
| LoE    | GR | AGO |
| 5      | D  | ++  |
| 5      | D  | ++  |
| 5      | D  | ++  |
| 5      | D  | +   |



# Workup: Mastectomy Specimens

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- **Margins always to be sampled**
  - Skin close to tumor, at least 2 directions
  - Deep margin
  - Other margins, if close (< 1 cm)
- **Attention to soft tissue margins in skin sparing mastectomy**
- **Routine sampling of uninvolved quadrants, skin above tumor, and retroareolar region**
- **More extensive sampling in prophylactic mastectomies (BRCA-1/2 pos. patients)**

| Oxford |    |     |
|--------|----|-----|
| LoE    | GR | AGO |
| 5      | D  | ++  |
| 5      | D  | ++  |
| 5      | D  | ++  |
| 5      | D  | ++  |

# Workup: Sentinel Node Biopsy

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- **Full workup using step sections of  $\leq 500 \mu\text{m}$  on paraffin embedded tissue**
- **Cytokeratin immunohistochemistry**
  - When suspicious, to detect micrometastases
  - For micrometastasis detection after preoperative therapy
  - As a routine procedure
- **Frozen section (compromises paraffin histomorphology)**
  - If clinical consequence
  - If no clinical consequence from frozen section (e.g. cT1 or cT2 and cN0 and BCT)
- **Imprint cytology instead of, or in addition to frozen section**
- **RT-PCR for epithelial genes**
  - OSNA

| Oxford |    |     |
|--------|----|-----|
| LoE    | GR | AGO |
| 5      | D  | ++  |
| 2b     | B  | +   |
| 2b     | B  | +   |
| 5      | D  | +/- |
| 5      | D  | +/- |
| 5      | D  | -   |
| 3b     | D  | +/- |
| 4      | D  | -   |
| 3b     | B  | -   |

# Workup: Sentinel Node Biopsy

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|  | Oxford |    |     |
|--|--------|----|-----|
|  | LoE    | GR | AGO |
| <ul style="list-style-type: none"> <li>■ <b>Sentinel node biopsy for invasive cancer (compromises final paraffin histomorphology)</b> <ul style="list-style-type: none"> <li>■ If clinical consequence</li> <li>■ If clinical consequence</li> </ul> </li> </ul> | 5      | D  | +   |
|  | 5      | D  | -   |
| <ul style="list-style-type: none"> <li>■ <b>Closest margin of resection</b> <ul style="list-style-type: none"> <li>■ If macroscopically &lt; 1 cm</li> <li>■ If macroscopically &gt; 1 cm</li> </ul> </li> </ul>   | 5      | D  | +   |
|  | 5      | D  | -   |
| ■ <b>Lesions ≥ 1 cm, without core biopsy</b>   | 5      | D  | +   |
| ■ <b>Non-palpable lesions or lesions &lt; 1 cm</b>   | 5      | D  | --  |
| ■ <b>Conservation of fresh tissue (tumor banking)</b>  | 5      | D  | +   |

# Reporting: Histologic Tumor Type

| Oxford |    |     |
|--------|----|-----|
| LoE    | GR | AGO |
| 3a     | C  | ++  |

- **Histologic tumor typing according to WHO-Classification, (4th ed., 2012)**
  - **Partial special differentiation:**  
> 50% NST component  
and < 50% special tumor type (minor component)
  - **Mixed differentiation:**  
> 50% special tumor type  
and < 50% NST component  
**Example: mucinous breast cancer, mixed type**
  - **Pure types:**  
> 90% special tumor type  
**Examples: tubular or cribriform Ca.**

# Reporting: Grade of Malignancy

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- **Use of Nottingham grading system (Elston & Ellis 1991) for all types of invasive breast cancer**
- **In case of very little tumor tissue, pure nuclear grading or additional criteria, such as Ki-67 proliferation fraction, may be used**
- **Grading of DCIS according to WHO-Classification, (4th ed., 2012)**
- **Reporting of tumor grading in numeric form (e.g. G3)**

|   | Oxford |    |     |
|---|--------|----|-----|
|   | LoE    | GR | AGO |
| ■ Use of Nottingham grading system (Elston & Ellis 1991) for all types of invasive breast cancer                                      | 5      | D  | ++  |
| ■ In case of very little tumor tissue, pure nuclear grading or additional criteria, such as Ki-67 proliferation fraction, may be used | 5      | D  | ++  |
| ■ Grading of DCIS according to WHO-Classification, (4th ed., 2012)  | 5      | D  | ++  |
| ■ Reporting of tumor grading in numeric form (e.g. G3)  | 5      | D  | ++  |

# Reporting: Tumor Size and Total Extent of Tumor

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- Reporting of invasive tumor size taking into account macroscopic and histologic findings and clinical imaging results
- Additional reporting of total extent of invasive carcinoma in case of satellite nodules or multifocality
- Reporting of size of noninvasive component (DCIS or LCIS) when DCIS or LCIS component is extensive (more than 2x invasive Ca)

|   | Oxford |    |     |
|---|--------|----|-----|
|   | LoE    | GR | AGO |
| Reporting of invasive tumor size taking into account macroscopic and histologic findings and clinical imaging results         | 5      | D  | ++  |
| Additional reporting of total extent of invasive carcinoma in case of satellite nodules or multifocality                      | 5      | D  | ++  |
| Reporting of size of noninvasive component (DCIS or LCIS) when DCIS or LCIS component is extensive (more than 2x invasive Ca) | 5      | D  | ++  |

# Reporting: pTNM

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| Oxford |    |     |
|--------|----|-----|
| LoE    | GR | AGO |
| 5      | D  | ++  |

- Use of current UICC classification (7th ed.)

**pT 1-3:** Invasive tumor size (largest focus in case of multiplicity)

**pT4:** Invasion of dermis alone does not qualify as pT4. Criteria for pT4a/b/c/d must be met.

**pT4d:** Negative skin biopsy does not rule out pT4d (inflammatory carcinoma).

**pM:** pM1 indicates any non-regional disease, except 2<sup>nd</sup> primary contralateral.  
Use of MX is not recommended.

# Reporting: Margins of Resection and R-Classification

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- Evaluation of distance to all resection margins macro-scopically and close margins histologically (< 1 cm)
- Reporting of minimal distance to resection margin and topography thereof
- R-Classification

**R0:** No residual tumor

**R1:** Microscopic invasive or noninvasive Carcinoma involving resection margin

**RX:** Presence of residual tumor cannot be assessed (e.g. tumor in multiple specimens)

|  | Oxford |    |     |
|--|--------|----|-----|
|  | LoE    | GR | AGO |
| Evaluation of distance to all resection margins macro-scopically and close margins histologically (< 1 cm) | 5      | D  | ++  |
| Reporting of minimal distance to resection margin and topography thereof                                   | 5      | D  | ++  |
| R-Classification   | 5      | D  | ++  |



# Reporting: Lymphovascular Invasion

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- **L1: Lymphovascular invasion**  
**L0: No lymphovascular invasion**
- **IHC for evaluation of lymphovascular invasion**
- **Differentiation of peritumoral and extensive lymphovascular invasion**
- **Reporting of venous invasion (V0/V1) optional, prognostic significance not established**

| Oxford |    |     |
|--------|----|-----|
| LoE    | GR | AGO |
| 5      | D  | ++  |
| 3b     | C  | -   |
| 3b     | C  | ++  |
| 5      | D  | +   |

# Reporting: Evaluation of Tumor-Infiltrating Lymphocytes (TIL)



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- **Identification of tumors with predominant lymphocytic infiltrate (> 50%) in tumor stroma (according to Salgado et al.\*)**

**Consider only lymphocytic infiltrate in tumor stroma and not at the invasion front**

**Do not consider central fibrosis and necrotic areas**

**Report average of lymphocytic infiltrate as percentage**

| Oxford |    |     |
|--------|----|-----|
| LoE    | GR | AGO |
| 5      | D  | +/- |

\* Salgado, R., Denkert, C., Demaria, S., Sirtaine, N., Klauschen, F., Pruneri, G., et al. (2014). The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. *Annals of Oncology*

# Reporting: Evaluation after Neoadjuvant Chemotherapy

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|   | Oxford |    |     |
|---|--------|----|-----|
|   | LoE    | GR | AGO |
| ■ Identification of tumor bed, otherwise ypTX   | 4      | D  | ++  |
| ■ Reporting of tumor size as total extent of tumor bed area involved by infiltrates of residual vital invasive carcinoma  | 4      | D  | ++  |
| ■ pCR when absence of invasive Ca. and absence of angioinvasion or LN metastases.<br>Presence of ypTis should be recorded | 2b     | D  | +   |
| ■ Use of IHC to identify tumor residues   | 4      | D  | +/- |
| ■ Reporting of ypTN after therapy   | 5      | D  | ++  |
| ■ Repeat IHC for ER, PgR, and HER2  | 5      | D  | +/- |

# Special Studies: ER-Testing by IHC

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- Immunohistochemical detection on paraffin embedded (FFPE) tissue
- Reporting percentage of pos. tumor nuclei (pos. if  $\geq 10\%$ , low pos. if  $\geq 1\% - 9\%$ )
- Staining intensity of pos. tumor nuclei (0 - 3)
- Allred Score (0 - 8), Remmele Score (0 - 12)
- Re-evaluation on excision specimen if uncertain or triple-negative on core biopsy

| Oxford |    |     |
|--------|----|-----|
| LoE    | GR | AGO |
| 1a     | A  | ++  |
| 1a     | A  | ++  |
| 4      | D  | +   |
| 4      | D  | +   |
| 5      | D  | +   |

# Special Studies: PgR-Testing by IHC

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- Immunohistochemical detection on paraffin embedded (FFPE) tissue
- Reporting percentage of pos. tumor nuclei (pos. if  $\geq 10\%$ )
- Staining intensity of pos. tumor nuclei (0 - 3)
- Allred Score (0 - 8), Remmele Score (0 - 12)

|  | Oxford |    |     |
|--|--------|----|-----|
|  | LoE    | GR | AGO |
|  | 1a     | A  | ++  |
|  | 1a     | A  | ++  |
|  | 4      | D  | +   |
|  | 4      | D  | +   |

# Additional Special Studies: Molecular Analysis of ER/PgR Status

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- Evaluation of hormone receptors using validated gene expression test kits
- Evaluation of hormone receptor by RNA-quantification
- Use of molecular receptor analysis for subtyping

| Oxford |    |     |
|--------|----|-----|
| LoE    | GR | AGO |
| 3b     | A  | +/- |
| 5      | D  | -   |
| 3b     | A  | +   |

# Additional Special Studies: Molecular Analysis of ER/PgR Status

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|  | Oxford |    |     |
|--|--------|----|-----|
|  | LoE    | GR | AGO |
| <ul style="list-style-type: none"> <li>■ <b>Reporting of immunohistochemistry (IHC):</b> <ul style="list-style-type: none"> <li>■ HER2+ if strong complete circular membrane staining of &gt; 10% invasive cells (3+ staining pattern)</li> <li>■ if &gt; 10% circular but moderate/weak membrane staining or ≤ 10% strong staining, U-shaped staining in micropapillary carcinoma (2+ staining pattern): ISH required (CISH, SISH, FISH)</li> </ul> </li> </ul> | 1a     | A  | ++  |
| <ul style="list-style-type: none"> <li>■ <b>Reporting of single-color In-Situ-Hybridisation (ISH):</b> <ul style="list-style-type: none"> <li>■ HER2+ if signal counts ≥ 6 in at least 20 cohesive cells, negative if signal counts &lt; 4 signals/nucleus</li> </ul> </li> </ul>  | 3a     | C  | ++  |
| <ul style="list-style-type: none"> <li>■ <b>Reporting of dual-color ISH:</b> <ul style="list-style-type: none"> <li>■ positive if signal ratio HER2:CEP17 ≥ 2,0 and/or HER2-signals ≥ 6</li> </ul> </li> </ul>   | 3a     | C  | ++  |
| <ul style="list-style-type: none"> <li>■ <b>Equivocal results (2+ IHC, ≥ 4 - &lt; 6 HER2 signals ISH):</b><br/>Retest using other method and/or tissue block</li> </ul>  | 3a     | C  | ++  |
| <ul style="list-style-type: none"> <li>■ <b>Validation of immunohistochemistry on core biopsies</b></li> </ul>   | 5      | D  | ++  |

# HER2 Testing on Core Biopsies

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**False positive immunohistochemical labeling may occur in core biopsies. Therefore, methods of individual laboratories should be validated by comparison of core biopsies and resection specimens. Background staining should be evaluated by comparison with normal duct epithelium.**

**Alternatively, all G1 and G2 cases with HER2 3+ in core biopsies may be analyzed by ISH or may be re-evaluated in the resection specimen.**

**False positivity is likely when HER+ was reported in G1 tumors of the following types:**

**Infiltrating ductal or lobular carcinoma, ER and PgR positive, Tubular (at least 90% pure), Mucinous (at least 90% pure) Cribriform (at least 90% pure), Adenoid cystic carcinoma (90% pure).**

**In case of discrepancy between core biopsy and specimen, the HER2 overexpressing sample should be re-evaluated by a different method. If still discrepancy – anti-HER2-treatment if amplified in one of both samples.**

**Expected rate of HER2-overexpression: 15% HER2 positive**



# Additional Special Studies: Molecular Analysis of HER2 Status

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- Therapy decisions should be based on IHC and ISH only
- Evaluation of HER2 using validated gene expression test kits
- Evaluation of HER2-amplification by RNA-sequencing
- Use of molecular HER2-testing for subtyping

|  | Oxford |    |     |
|--|--------|----|-----|
|  | LoE    | GR | AGO |
|  | 1a     | A  | ++  |
|  | 3b     | B  | +/- |
|  | 5      | D  | -   |
|  | 3b     | B  | +/- |

# Special Studies: Evaluation of Ki-67 Score

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|   | Oxford |    |     |
|---|--------|----|-----|
|   | LoE    | GR | AGO |
| ■ Counting of tumor nuclei at the invasion front                                    | 5      | D  | ++  |
| ■ Semiquantitative eyeballing or counting of labelled cells in core needle biopsies | 2      | A  | ++  |
| ■ Consideration of weakly stained tumor nuclei                                      | 5      | D  | ++  |
| ■ Reporting of Ki-67 positive nuclei as percentage                                  | 5      | D  | ++  |
| ■ Establishing of laboratory standards and cut-off values                           | 5      | D  | ++  |
| ■ Use of image analysis for objective Ki-67 evaluation                              | 5      | D  | +   |

# Intrinsic Breast Cancer Types (Molecular and Immunohistochemical Definitions)

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- **Currently there are no generally accepted and proven translation of molecularly defined types (basal, luminal A/B-Typ, HER2) into immunohistochemical counterparts neither with regard to markers nor to thresholds**
- **In terms of practical consequences, re-labeling of clinically established and immunohistochemically defined subgroups might be useful (ER/PR+ for luminal, HER2+ for HER2-type, triple negative for basal type)**
- **The basal type shows an 80% overlap with the triple negative subgroup of ductal invasive breast cancer (ER < 1% & PgR < 1% & HER2 0/1+2+ (non-amplified, ratio < 2))**
- **None of the available markers (Ki-67, grading, recurrence score etc.) can reliably discriminate between luminal A and luminal B type**
- **Although derived from RNA expression studies, RNA measurements are not suited for the definition of intrinsic types for purposes of therapy**

# Quality Assurance: Immunohistochemistry

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- **Use of automated staining platform**
- **Participation in ring trials**
- **Strict adherence and monitoring of requirements of preanalytics (fixation)**
- **Use of on-slide controls**
- **Plausibility controls (e.g. tumor type, grading)**

# Quality Assurance: HER2-Status

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- **Continuous documentation of HER2 tests**
- **Quality goal: Rate of HER2-positivity: 15%  $\pm$  5%**
- **Use of standardised and validated HER2 test kits**
- **Participation in ring trials**

# Quality Assurance: Reporting

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- **Responsibility of one or two pathologists with special expertise in breast pathology**
- **Regular interdisciplinary conferences with radiologic-pathologic correlation**
- **Participation in quality circles**