Diagnostik und Therapie primärer und metastasierter Mammakarzinome

Pathology
Pathology

- **Versionen 2004–2014:**
  Costa / Fehm / Friedrichs / Huober / Kreipe / Lück / Sinn / Thomssen

- **Version 2015:**
  Sinn / Friedrichs
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
Preanalytics: Fixation

- 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
Use of Fine Needle Aspiration Cytology*

- Nipple secretion
- Tumor
- Cyst
- Lymph node

* Ultrasound-guided core biopsy recommended
Workup: Macroscopy and Specimen Radiography

- 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)

- **Routine workup in step sections** (14G: 3 sections / 11G, 8G: 6–8 sections)  
  - Oxford / AGO LoE / GR: 5 D ++

- **Correlation with imaging (density, calcifications), use of B-classification**  
  - Oxford / AGO LoE / GR: 1b B ++

- **Frozen section diagnosis on core biopsies**  
  - Oxford / AGO LoE / GR: 5 D --

- **Routine evaluation of ER/PgR and HER2 status**  
  - Oxford / AGO LoE / GR: 3b C ++

- **Turn-around time < 24 h (histology)**  
  - Oxford / AGO LoE / GR: 5 D +
### Workup: Breast-Conserving Specimens

<table>
<thead>
<tr>
<th>Oxford / AGO LoE / GR</th>
<th>5</th>
<th>D</th>
<th>++</th>
</tr>
</thead>
</table>

- **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, photodocumentation or diagram**
Workup: Mastectomy Specimens

- **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)**
## Workup: Sentinel Node Biopsy

<table>
<thead>
<tr>
<th>Workup Method</th>
<th>Oxford</th>
<th>AGO</th>
<th>LoE</th>
<th>GR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full workup using step sections of ≤ 500 μm on paraffin embedded tissue</td>
<td>5</td>
<td>D</td>
<td>++</td>
<td></td>
</tr>
</tbody>
</table>
| Cytokeratin immunohistochemistry  
- When suspicious, to detect micromet.  
- As a routine procedure | 2b | B | ++ |
| Frozen section (invasive Ca.)  
- If clinical consequence  
- If no clinical consequence from frozen section (e.g. cT1 or cT2 and cN0 and BCT) | 5 | D | +/− |
| Imprint cytology instead of, or in addition to frozen section | 3b | C | +/− |
| RT-PCR for epithelial genes  
- OSNA | 4 | D | - |
|                      | 3b | B | - |
### Indications for Immediate Pathological Analysis Including Frozen Sections

<table>
<thead>
<tr>
<th>Condition</th>
<th>Oxford</th>
<th>AGO LoE</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sentinel node biopsy for invasive cancer</td>
<td>5</td>
<td>D</td>
<td>+</td>
</tr>
<tr>
<td>- If clinical consequence</td>
<td>5</td>
<td>D</td>
<td>+</td>
</tr>
<tr>
<td>- If no clinical consequence from frozen section (e.g. cT1 or cT2 and cN0 and BET)</td>
<td></td>
<td></td>
<td>+/-</td>
</tr>
<tr>
<td>Closest margin of resection</td>
<td>5</td>
<td>D</td>
<td>-</td>
</tr>
<tr>
<td>- If macroscopically &lt; 1 cm</td>
<td>5</td>
<td>D</td>
<td>+</td>
</tr>
<tr>
<td>- If macroscopically &gt; 1 cm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesions ≥ 1 cm, without core biopsy</td>
<td>5</td>
<td>D</td>
<td>+</td>
</tr>
<tr>
<td>Non-palpable lesions or lesions &lt; 1 cm</td>
<td>5</td>
<td>D</td>
<td>--</td>
</tr>
<tr>
<td>Asservation of fresh tissue (tumor banking)</td>
<td>5</td>
<td>D</td>
<td>+</td>
</tr>
</tbody>
</table>
Reporting: Histologic Tumor Type

- 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.

Oxford LoE / AGO LoE
3b C ++
## Reporting: Grade of Malignancy

<table>
<thead>
<tr>
<th>Oxford LoE / AGO LoE / GR</th>
<th>Use of Nottingham grading system (Elston &amp; Ellis 1991) for all types of invasive breast cancer</th>
<th>5 D ++</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In case of very little tumor tissue, pure nuclear grading or additional criteria, such as Ki-67 proliferation fraction, may be used</td>
<td>5 D ++</td>
</tr>
<tr>
<td></td>
<td>Grading of DCIS according to WHO-Classification, (4th ed., 2012)</td>
<td>5 D ++</td>
</tr>
<tr>
<td></td>
<td>Reporting of tumor grading in numeric form (e.g. G3)</td>
<td>5 D ++</td>
</tr>
</tbody>
</table>
Reporting: Tumor Size and Total Extent of Tumor

- 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)

<table>
<thead>
<tr>
<th>Oxford LoE / AGO LoE</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>D</td>
</tr>
<tr>
<td>++</td>
<td></td>
</tr>
</tbody>
</table>
Reporting: pTNM

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

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


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

  pM: pM1 indicates any non-regional disease, except 2nd primary contralaterally. Use of MX is not recommended.
Reporting: Margins of Resection and R-Classification

- Evaluation of distance to all resection margins macroscopically and close margins histologically (< 1 cm)
  - Oxford LoE / AGO LoE / GR
  - 5 D ++

- Reporting of minimal distance to resection margin and topography thereof
  - 5 D ++

- R-Classification
  - 5 D ++

  R0: No residual tumor

  R1: Microscopic invasive or noninvasive Carcinoma involving resection margin

  RX: Presence of residual tumour cannot be assessed (e.g. tumor in multiple specimens)
Reporting: Lymphovascular invasion

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

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

Consider only lymphocytic infiltrate in tumor stroma and at the invasion front

Do not consider central fibrosis and necrotic areas

Report average of lymphocytic infiltrate as percentage

### Reporting: Evaluation after Neoadjuvant Chemotherapy

<table>
<thead>
<tr>
<th>Recommendation</th>
<th>Oxford / LoE</th>
<th>AGO GR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification of tumor bed, otherwise ypTX</td>
<td>4</td>
<td>D</td>
</tr>
<tr>
<td>Reporting of tumor size as total extent of tumor bed area involved by infiltrates of residual vital invasive carcinoma</td>
<td>4</td>
<td>D</td>
</tr>
<tr>
<td>pCR when absence of invasive Ca. and absence of angioinvasion or LN metastases. Presence of ypTis should be recorded</td>
<td>2b</td>
<td>D</td>
</tr>
<tr>
<td>Use of IHC to identify tumor residues</td>
<td>4</td>
<td>D</td>
</tr>
<tr>
<td>Reporting of ypTN after therapy</td>
<td>5</td>
<td>D</td>
</tr>
</tbody>
</table>
Special studies: ER-Testing by IHC

- Immunohistochemical detection on paraffin embedded (FFPE) tissue
  
<table>
<thead>
<tr>
<th>Oxford</th>
<th>AGO LoE</th>
<th>AGO GR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>A</td>
<td>++</td>
</tr>
</tbody>
</table>

- Reporting percentage of pos. tumor nuclei (pos. if ≥ 1%)

| 1a     | A       | ++     |

- Staining intensity of pos. tumor nuclei (0 - 3)

| 4      | D       | +      |

- Allred Score (0 - 8), Remmele Score (0 - 12)

| 4      | D       | +      |

- Re-evaluation on excision specimen if uncertain or triple-negative on core biopsy

| 5      | D       | +      |
Special studies: PgR-Testing by IHC

<table>
<thead>
<tr>
<th>Oxford / AGO LoE / GR</th>
<th>1a</th>
<th>A</th>
<th>++</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunohistochemical detection on paraffin embedded (FFPE) tissue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reporting percentage of pos. tumor nuclei (pos. if ≥ 10%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staining intensity of pos. tumor nuclei (0 - 3)</td>
<td>4</td>
<td>D</td>
<td>+</td>
</tr>
<tr>
<td>Allred Score (0 - 8), Remmele Score (0 - 12)</td>
<td>4</td>
<td>D</td>
<td>+</td>
</tr>
</tbody>
</table>
### Additional special studies: Molecular analysis of ER/PgR status

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Oxford / AGO</th>
<th>LoE / GR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluation of hormone receptors using validated gene expression test kits</td>
<td>3b A +/-</td>
<td></td>
</tr>
<tr>
<td>Evaluation of hormone receptor by RNA-sequencing</td>
<td>5 D -</td>
<td></td>
</tr>
<tr>
<td>Use of molecular receptor analysis for subtyping</td>
<td>3b A +</td>
<td></td>
</tr>
</tbody>
</table>
Special studies: HER2 Testing

- Reporting of immunohistochemistry (IHC):
  - HER2+ if strong complete circular membrane staining of > 10% invasive cells (3+ staining pattern)
  - if > 10% circular but moderate/weak membrane staining or ≤ 10% strong staining (2+ staining pattern): ISH required (CISH, SISH, FISH)

- Reporting of single-color In-Situ-Hybridisation (ISH):
  - HER2+ if signal counts ≥6 in at least 20 cohesive cells, negative if signal counts < 4 signals/nucleus

- Reporting of dual-color ISH:
  - positive if signal ratio HER2:CEP17 ≥ 2,0 and/or HER2 signals ≥6

- Equivocal results (2+ IHC, ≥4 - <6 HER2 signals ISH):
  - Retest using other method and/or tissue block

- Validation of immunohistochemistry on core biopsies

Oxford / AGO LoE / GR

1a A ++
3a C ++
3a C ++
3a C ++
5 D ++
HER2 Testing on Core Biopsies

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

<table>
<thead>
<tr>
<th>Oxford / AGO</th>
<th>LoE / GR</th>
<th>1a</th>
<th>A</th>
<th>++</th>
</tr>
</thead>
<tbody>
<tr>
<td>3b</td>
<td>B</td>
<td>+/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>D</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- 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
Special studies: Evaluation of Ki-67 Score

- Counting of tumor nuclei at the invasion front
- Consideration of weakly stained tumor nuclei
- Reporting of Ki-67 positive nuclei as percentage
- Establishing of laboratory standards and cut-off values
- Use of image analysis for objective Ki-67 evaluation
Intrinsic Breast Cancer Types
(Molecular and Immunohistochemical Definitions)

- Currently there is 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-labelling 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

- 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

- Continuous documentation of HER2 tests
- Quality goal: Rate of HER2-positivity: 15% - 20%
- Use of standardized and validated HER2 test kits
- Participation in ring trials
Quality Assurance: Reporting

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

This chapter contains basic recommendations for routine procedures in pathology. It is not intended to replace detailed protocols for the evaluation of operative specimens or for special studies. It is highly recommended to adhere to national quality assurance protocols concerning all aspects of working up and reporting of pathology specimens removed from women with breast cancer. Further information can be found in the following reports:


Guidelines screened:

- Interdisziplinäre S3-Leitlinie für die Diagnostik, Therapie und Nachsorge des Mammakarzinoms. Aktualisierung 2012
- NCCN Breast cancer V.I.2014Cochrane: Decision aids for risk communication update 2009
- EUSOMA position paper: Diagnosis of breast disease
- Royal College of Pathologists & NHS Breast Screening Programme, Pathology Reporting of Breast Disease, January 2005
- European guidelines for quality assurance in breast cancer screening and diagnosis 4th Edition

References:

General principles for Histopathologic Examination of Breast Cancer Specimens (3/30))

No further information

References

Preanalytics: Fixation (4/30)

*No further information*

**References:**

**Antigen preservation**


Retraction artifacts
Use of Fine Needle Aspiration Cytology (5/30)

No further information

References:

Workup: Macroscopy and Specimen Radiography (6/30)

No further information

References:

Clinical-pathological correlation diagnostics

Image documentation

Specimen radiography


Workup: Core Needle Biopsies (US-guided or stereotactic) (7/30)

No further information

References:

Statement: Routine workup in step sections


Statement: Correlation with imaging


Statement: Frozen section diagnosis on core biopsies

Statement: Routine evaluation of ER/PgR and HER-2 status


Statement: Turn-around time < 24h

Workup of Breast-Conserving Specimens (8/30)

No further information

References:


Workup of Mastectomy Specimens (9/30)

No further information

References:

Evaluation of Sentinel Node Biopsy (10/30)

No further information

References:

Statement: Evaluation of sentinel node biopsy:


Statement: Full workup using step sections of ≥ 500 µm on paraffin embedded tissue


Statement: Frozen section


Statement: Imprint cytology instead or in addition of frozen section

Statement: RT-PCR for epithelial genes

Indications for Immediate Pathological Analysis Including Frozen Sections (11/30)

No further information

References:

Statement: Sentinel node biopsy for invasive cancer


Statement: Closest margin of resection

Statement: Lesions $\geq$ 1 cm, without core biopsy


Statement: Non-palpable lesions or lesions $<$ 1 cm

Reporting: Histologic Tumor Type (12/30)

No further information

References:

WHO-Classifikation
2. Lakhani SR, Ellis I, Schnitt S et al. (2012) WHO Classification of Tumours of the Breast. IARC Press, Lyon
**Reporting: Grade of Malignancy (13/30)**

*No further information*

**References:**

**Grading**

2. Lakhani SR, Ellis I, Schnitt S et al. (2012) WHO Classification of Tumours of the Breast. IARC Press, Lyon

**Grading of invasive lobular carcinoma**

Reporting: Tumor Size and Total Extent of Tumor (14/30)

No further information

References:

Determination of tumor size

Multifocality
**Extensive intraductal component (EIC)**


Reporting: pTNM (15/30)

No further information

References:

TNM staging (7th ed.) according to UICC und AJCC

pT4b category: Involvement of the skin

pT4d category: Inflammatory breast cancer
Reporting: Margins of Resection and R-Classification (16/30)

No further information

References:

Pathological margin assessment

**R-Classifikation**

**Reporting: Lymphovascular invasion (17/30)**

*No further information*

**References:**

**Definition of L- and V-Classification**

**Detection of angioinvasion**
Prognostic significance of lymphovascular invasion
Reporting: Evaluation of Tumor-Infiltating Lymphocytes (TIL) (18/30)

No further information

References:

Definition and impact of predominant lymphocytic infiltration


Reporting: Evaluation after Neoadjuvant Chemotherapy (19/30)

No further information

References:

Specimen processing after neoadjuvant chemotherapy

RCB-Score
Special studies: ER-Testing by IHC (20/30)

No further information

References:

IHC-testing for ER-positivity

**IHC Scores**

**Monoclonal Antibodies for ER-Testing**
1. Cheang MC, Treaba DO, Speers CH, Olivotto IA, Bajdik CD, Chia SK, Goldstein LC, Gelmon KA, Huntsman D, Gilks CB, Nielsen TO, Gown AM.
2. Immunohistochemical detection using the new rabbit monoclonal antibody SP1 of estrogen receptor in breast cancer is superior to mouse monoclonal antibody 1D5 in predicting survival.

**Low ER+ Group**
Special studies: PgR-Testing by IHC (21/30)

No further information

References:

IHC-testing for PR-positivity

Prognostic signifikance

Aberrant Expression of ER in triple negative breast cancer

IHC Scores
Additional special studies: Molecular analysis of ER/PgR status (22/30)

No further information

References:

Clinical significance of mRNA expression of ESR-alpha, PgR and concordance with IHC results

Special studies: HER2 Testing (23/30)

No further information

References:

2. Chivukula M, Bhargava R, Brufsky A et al. (2008) Clinical importance of HER2 immunohistologic heterogeneous expression in core-needle biopsies vs resection specimens for equivocal (immunohistochemical score 2+) cases. Mod Pathol 21:363-368
HER2 Testing on Core Biopsies (24/30)

No further information

No references
Additional special studies: Molecular analysis of HER2 Status (25/30)

No further information

References:

Clinical significance of mRNA expression of HER2 and concordance with IHC results


a Hellenic Cooperative Oncology Group (HeCOG) study. British Journal of Cancer, 99(11), 1775–1785. doi:10.1038/sj.bjc.6604769


**Special studies: Evaluation of Ki-67 Score (26/30)**

*No further information*

**References:**

**Ki-67 Methods and Reproducibility**


**Impact of Ki-67 staining**


**Ki-67 Image Analysis**


Intrinsic Breast Cancer Types (27/30)

No further information

No references
Quality assurance: Immunohistochemistry (28/30)

No further information

References:

Quality assurance: HER2-Status (29/30)

No further information

No references
Quality assurance: Immunhistochemistry (30/30)

No further information

No references