Diagnosis and Treatment of Patients with early and advanced Breast Cancer

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Pathology

Pathology

- **Versions 2004–2020:**
  Blohmer / Costa / Fehm / Friedrichs / Harbeck / Huober / Kreipe / Lück / Maass/Schneeweiss/ Sinn / Thomssen / Schmidt

- **Version 2021:**
  Kreipe / Sinn / Kühn

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Preanalytics: Fixation

	Oxford		
	LoE	GR	AGO
▪ Minimize time to fixation (cold ischemia time)	5	D	++
▪ Minimal fixation time of 6 hours for optimal antigen preservation	5	D	++
▪ Optimal fixation time 6 - 72 h for core biopsies	5	D	++
▪ Optimal fixation time for resection specimens: 12 - 72 h	5	D	++
▪ Use of neutral buffered formalin	5	D	++

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Use of Breast Cytology*

	Oxford		
	LoE	GR	AGO
▪ Nipple secretion	5	D	+
▪ Tumor	5	D	-
▪ Cyst	5	D	+/-
▪ Lymph node	5	D	+/-

* Ultrasound-guided core biopsy recommended

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

	Oxford		
	LoE	GR	AGO
▪ Routine workup in step sections (14G: 1–3 step sections / 11G, 8G: 6–8 step sections)	5	D	++
▪ Correlation with imaging (density, calcifications), use of B-classification	1b	B	++
▪ Frozen section diagnosis on core biopsies	5	D	--
▪ Routine evaluation of ER/PR and HER2 status	3b	C	++
▪ Turn-around time < 24 h (histology)	5	D	+

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Workup: Breast-Conserving Specimens

	Oxford		
	LoE	GR	AGO
▪ Slicing perpendicular to the longitudinal axis (or perpendicular to the nipple-peripheral axis in case of spherical specimens)	5	D	++
▪ Systematic sampling, at least 1 tissue block every 1 cm	5	D	++
▪ Inking of resection margins. Sampling of resection margins	5	D	++
▪ Documentation after slicing using specimen radiography, photo documentation or diagram	5	D	+

Workup: Mastectomy Specimens

	Oxford		
	LoE	GR	AGO
▪ Margins always to be sampled	5	D	++
▪ Skin close to tumor			
▪ Deep margin			
▪ Other margins, if close (< 1 cm)			
▪ Attention to soft tissue margins in skin sparing mastectomy	5	D	++
▪ Routine sampling of uninvolved quadrants, skin above tumor, and retroareolar region	5	D	++
▪ Systematic sampling in prophylactic mastectomies (patients with BRCA-1/2 mutation)	5	D	++

Workup: Sentinel Node Biopsy

	Oxford		
	LoE	GR	AGO
▪ Full workup using step sections of ≤ 500 µm on paraffin embedded tissue	5	D	++
▪ Cytokeratin immunohistochemistry			
▪ If suspicious, to detect micrometastases	2b	B	+
▪ For micrometastasis detection after NACT	2b	B	+
▪ As a routine procedure	5	D	+/-
▪ Frozen section (compromises paraffin histomorphology)			
▪ If clinical consequences	5	D	+
▪ If no clinical consequences 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	4	D	-
▪ OSNA	3b	B	-

Workup: Intraoperative pathological evaluation and frozen sections

	Oxford		
	LoE	GR	AGO
▪ **Sentinel node biopsy for invasive cancer (compromises final paraffin histomorphology)**			
▪ If clinical consequences	5	D	+
▪ No clinical consequences	5	D	-
▪ **Closest margin of resection**			
▪ If macroscopically < 1 cm	5	D	+
▪ If macroscopically > 1 cm	5	D	-
▪ **Lesions ≥ 1 cm, without core biopsy**	5	D	+
▪ **Non-palpable lesions or lesions < 1 cm**	5	D	--
▪ **Conservation of fresh tissue (tumor banking)**	5	D	+

Reporting: Histologic Tumor Type

Oxford		
LoE	GR	AGO
3b	C	++

- **Histologic tumor typing according to WHO-Classification, (5th ed., 2019)**
  - **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.

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Reporting: Grade of Malignancy

	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, e.g. according to WHO-Classification, (5th ed., 2019)	5	D	++
▪ Reporting of tumor grade in numeric form (e.g. G3)	5	D	++

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Reporting: Tumor Size and Total Extent of Tumor

	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 non-invasive component (DCIS or LCIS) when DCIS or LCIS component is extensive (more than 2x invasive Ca)	5	D	++

Reporting: pTNM

Oxford		
LoE	GR	AGO
5	D	++

- **Use of current UICC classification (8th ed.)**

  pT 1-3: Invasive tumor size (largest focus in case of multifocality or multicentricity)

  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 2nd primary contralateral. Use of MX is not recommended.

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Reporting: Margins of Resection and R-Classification

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

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)

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Reporting: Lymphovascular Invasion

	Oxford		
	LoE	GR	AGO
▪ L1: Lymphovascular invasion L0: No lymphovascular invasion	5	D	++
▪ IHC for evaluation of lymphovascular invasion	3b	C	-
▪ Differentiation of peritumoral and extensive lymphovascular invasion	3b	C	++
▪ Reporting of venous invasion (V0/V1) optional, prognostic significance not established	5	D	+

Reporting: Evaluation of Tumor-Infiltrating Lymphocytes (TIL)

Oxford		
LoE	GR	AGO
5	D	+/-

- **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

* 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

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Reporting: Evaluation after Neoadjuvant Chemotherapy

	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 if absence of invasive Ca. and absence of angioinvasion or LN metastases. Presence of ypTis should be recorded	2b	D	+
▪ Use of IHC to identify tumor residues (lymphnodes)	2b	B	+/-
▪ Reporting of ypTN after therapy	5	D	++
▪ Repeat IHC for ER, PR, and HER2	5	D	+/-
▪ Intraoperative frozen section (reduced sensitivity)	5	D	-
▪ Tumorregression-Scores: RCB-Score or Sataloff-Score	4	D	+/-

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Special Studies: ER-Testing by IHC

	Oxford		
	LoE	GR	AGO
▪ Immunohistochemical detection on paraffin embedded (FFPE) tissue	1a	A	++
▪ Reporting percentage of pos. tumor nuclei (pos. if ≥ 10%, low pos. if ≥ 1%–10%)	1a	A	++
▪ Staining intensity	4	D	+
▪ Only Allred Score (0–8) or Remmele Score (0–12)	4	D	-
▪ Re-evaluation on excision specimen if uncertain or triple-negative on core biopsy	5	D	+

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Low ER+ (1–10%)

Sanford AS et al. Cancer 2015	High Incidence of Germline BRCA Mutation in Patients with ER Low-Positive/PR Low-Positive/HER-2 neu Negative Tumors	314 Pat. 1–9% ER, Anteil BRCA mutierter Fälle wie bei ER -
Deyarmin B et al. Ann Surg Oncol (2013) 20:87–93	Effect of ASCO/CAP Guidelines for Determining ER Status on Molecular Subtype	26 Pat. 1–10% ER, Genexpression eher wie TN oder HER2 enr
Prabhu YS et al. 2014; J Cancer 5(2): 156–165.	A Majority of Low (1–10%) ER Positive Breast Cancers Behave Like Hormone Receptor Negative Tumors	21 Pat. 1–10% ER, Genexpression wie ER-, Überleben < ER+
Yi et al. Annals Oncol. 2014	Which threshold for ER positivity? a retrospective study based on 9639 patients	251 Pat. 1–9% ER Überleben = ER-

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Special Studies: PR-Testing by IHC

	Oxford		
	LoE	GR	AGO
▪ Immunohistochemical detection on paraffin embedded (FFPE) tissue	1a	A	++
▪ Reporting percentage of pos. tumor nuclei (pos. if ≥ 10%)	1a	A	++
▪ Only Allred Score (0 - 8) or Remmele Score (0 - 12)	4	D	-

Additional Special Studies: Molecular Analysis of ER/PR Status

	Oxford		
	LoE	GR	AGO
▪ Evaluation of hormone receptors using validated gene expression test kits	3b	A	+/-
▪ Exclusive evaluation of hormone receptors by RNA-quantification	5	D	-
▪ Use of molecular receptor analysis for subtyping	3b	A	+/-

HER2-Analysis by IHC

	Oxford		
	LoE	GR	AGO
▪ **Reporting of immunohistochemistry (IHC):**	1a	A	++
▪ **3+ staining pattern: HER2+ if strong complete circular membrane staining of > 10% invasive cells**			
▪ **2+ staining pattern: If > 10% circular but moderate/weak membrane staining or ≤ 10% strong staining, U-shaped staining in micropapillary carcinoma: ISH required (CISH, SISH, FISH)**	1a	A	++

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HER2-Analysis by ISH when IHC 2+

	Oxford		
	LoE	GR	AGO
▪ **Single-Color In-Situ-Hybridisation (ISH):**	3a	C	++
▪ HER2+ if signal counts ≥ 6 in at least 20 cohesive cells			
▪ negative if signal counts < 4 signals/nucleus			
▪ 2-Color ISH recommended for ≥ 4 and < 6 signals/nucleus			
▪ **Two-Color In-Situ-Hybridisation (ISH):**	3a	D	++
▪ Group 1: Ratio ≥ 2.0 and signals/nucleus ≥ 4.0 -> HER2+			
▪ Group 2: Ratio ≥ 2.0 and signals/nucleus < 4.0 -> HER2- (no benefit of anti-HER2 therapy)			
▪ Group 3: Ratio < 2.0 and signals/nucleus ≥ 6.0 -> HER2+ (but benefit of anti-HER2 therapy not certain)			
▪ Group 4: Ratio < 2.0 and signals/nucleus ≥ 4.0 und < 6 -> HER2- (no benefit of anti-HER2 therapy)			
▪ Group 5: Ratio < 2.0 und signals/nucleus < 4.0 -> HER2-			

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HER2 testing by validated dual-probe ISH assay when IHC = 2+

Batch controls and on-slide controls show appropriate hybridization

HER2/CEP17 ratio ≥ 2.0		HER2/CEP17 ratio < 2.0		
Group 1 Average *HER2* copy number ≥ 4.0 signals/cell	Group 2 Average *HER2* copy number < 4.0 signals/cell	Group 3 Average *HER2* copy number ≥ 6.0 signals/cell	Group 4 Average *HER2* copy number ≥ 4.0 - < 6.0 signals/cell	Group 5 Average *HER2* copy number <4.0 signals/cell
	mostly	mostly	mostly	
HER2 positive	HER2 negative	HER2 positive	HER2 negative	HER2 negative

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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 PR 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

	Oxford		
	LoE	GR	AGO
▪ Therapy decisions should only be based on IHC and ISH	1a	A	++
▪ Evaluation of HER2 using validated gene expression test kits	3b	B	-
▪ Evaluation of HER2-amplification by RNA-sequencing	5	D	-
▪ Use of molecular HER2-testing for subtyping	3b	B	+/-

Special Studies: Evaluation of Ki-67 Score

	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	+
▪ Determination of Ki-67 dynamics after short term (2-4 weeks) endocrine therapy*	1b	B	+

* See chapter Neoadjuvant Systemic Therapy

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Predictive PD-L1 Assay

Immunohistochemical assay	Oxford LoE	Oxford GR	Oxford AGO
Immune Score (IC): Cytoplasmic staining of at least 1% of the leucocyte stromal infiltrate (lymphocytes, macrophages, plasma cells, granulocytes outside of abscesses) for prediction of atezolizumab efficacy in triple-negative metastatic breast cancer	2	A	++
Metastatic or primary tumor tissue	2	A	++
Detection with antibodies equivalent to Impassion 130 study reagents	3	B	+
Combined positive score (CPS): positive cells (tumor cells, lymphocytes, and macrophages) divided by the total number of tumor cells multiplied by 100; ≥10 = positive) for prediction of pembrolizumab efficacy in metastatic triple-negative breast cancer (FDA approval, EMA pending)	3	B	+/-

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Mutational studies in mBC: „Precision medicine“ for targeted therapies

Gene	Therapeutic Relevance	Gene region	Source	Oxford		
				LoE	GR	AGO
BRCA1, BRCA2	PARP Inhibitor	all exons	Germ line: blood cells Somatic: tissue	1b 2b	A B	++ +/-
PIK3CA	Alpelisib	exons 7,9 and 20	Primary tumor, metastases, plasma	1b	A	++
HER2-mutation (irrespective of HER2-status)	Neratinib, Lapatinib	kinase and extracellular domain; S310, L755, V777, Y772_A775dup	Primary tumor, metastases, plasma	4	C	+/-
ESR1	Resistance vs aromatase inhibit.	exons 4,7 und 8	metastases, plasma	2b	B	+/-
NTRK gene fusion	Larotrectinib, Entrectinib	Gene fusions and splice variants	Tumor tissue, in particular secretory breast caner	2a	B	+
MSI	Pembrolizumab	Mikrosatellite instability	tissue	2a	B	+