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A DOCUMENT SHARED WITHIN GIPAM MEMBERS
(GRUPPO ITALIANO DI STUDIO DI PATOLOGIA MAMMARIA)

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- Request form for cytological and microhistological specimens
- Request form for surgical specimens
- Submission procedures for surgical specimens (from the operating theatre to the pathology laboratory)
- Macroscopical examination and sampling of breast surgical specimens
  - Appendix 1: Marking of surgical margins and specimen sectioning
  - Appendix 2: Sampling and evaluation of surgical resection margins
  - Appendix 3: Sampling of axillary lymph nodes
  - Appendix 4: Diagnostic protocol for sentinel lymph node biopsy
  - Appendix 5: Neoadjuvant chemotherapy
- Check list for the reporting of microscopical diagnosis of invasive breast carcinoma
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- Reporting of prognostic/predictive factors
- Sentinel lymph node and axillary lymph nodes
- Reporting of microscopical diagnosis after neoadjuvant therapy or primary systemic therapy
- *In situ* hibridyzation
  - FISH: interpretation of results
  - CISH/SISH: interpretation of results

Request form for cytological and microhistological specimens

GENERAL DATA

1. Patient identifiers
   - Name
   - Date and place of Birth
   - Gender
   - Unique identifier (i.e. health record, master index number, national insurance number)
   - Address and phone number

2. Identifiers of the authorized person requesting the exam (name, operative unit, phone number)

3. Date and time of specimen collection

RELEVANT CLINICAL DATA ON EXTRA-MAMMARY PATHOLOGIES:

…….....

SENOLOGICAL ANAMNESIS:

❒ prior pathologies ❒ prior surgery
❒ recent/on-going pregnancy ❒ on-going breastfeeding ❒ menopause
❒ use of hormone replacement therapy (HRT) or oral contraceptive (OC)

CLINICAL DATA ON THE PRESENT BREAST LESION:

FEATURES OF THE LESION: ❒ Palpable ❒ Not palpable ❒ Ulceration ❒ Other .....

SITE OF THE LESION/S: ❒ Right breast ❒ Left breast
❒ UOQ (Q1) - UIQ (Q2) - LOQ (Q3) - LIQ (Q4) – Central-reatroareolar (Q5) ❒ Nipple
❒ Intermediate QQ: superior – equatorial internal - inferior – equatorial external
❒ Axillary tail of the breast ❒ Axillary lymph node

GUIDANCE USED FOR SAMPLING:

❒Palpation ❒ Mammography (stereotactic-guidance) ❒ Ultrasound ❒ MRI

TYPE OF CYTOLOGICAL SAMPLE:

❒ Fine needle aspiration cytology (FNAC) ❒ Secretion ❒ Scraping
❒ Smear (specify the number of slides) ..... ❒ Liquid-based cytology (cytolit)

TYPE OF HISTOLOGICAL SAMPLE:

❒ Tru-cut (NCB) ❒ Vacuum-assisted (VANCB)
❒ Needle diameter G..... ❒ Number of specimens.....

PRESENCE OF MICROCALCIFICATIONS AT RX EXAMINATION OF THE BIOPSY: ❒ Yes ❒ No

REQUISITION TO ASSESS PROGNOSTIC/PREDICTIVE FACTORS ON BIOPSY FOR NEOADJUVANT THERAPY: ❒ Yes ❒ No

INSTRUMENTAL DIAGNOSTICS PERFORMED ON THE PRESENT BREAST LESIONS
(enclose a copy of the reports and/or fill in the following form):

MAMMOGRAPHY: (exam date.....)
❒ N° of lesions: ❒ Dimensions (mm): ❒ Radiological category: R..... or BIRADS.....

❒ TYPE OF LESION: ❒ Opacity with spiculated margins ❒ Opacity with well defined margins
❒ Parenchymal distortion ❒ Asymmetrical density

❒ MICROCALCIFICATIONS: ❒ Absent ❒ Morphology: ❒ Distribution: ❒ Extent:

❒ Diagnostic hypothesis.....

ULTRASONOGRAPHY: (exam date.....)
❒ N° of lesions ❒ Dimensions (mm): ❒ Ultrasound category: U……

❒ TYPE OF LESION (solid or cystic) and echogenicity: ……

❒ Diagnostic hypothesis.....

MRI: (exam date.....)
❒ N° of lesions: ❒ Location: ❒ Dimension (mm): ❒ MRI category: .....

❒ Diagnostic hypothesis.....

OTHER INSTRUMENTAL DIAGNOSTICS (specify which): .....

PRIOR CYTOLOGICAL/HISTOLOGICAL EXAMS

CYTOLOGICAL EXAM: ❒ Category (C): C1 (inadequate) C2(benign) C3 (atypical, probably benign) C4 (suspicious, probably malignant) C5 (malignant) (specify the identification serial number C/……. of the Department of pathology of …..…… date of execution……..)

MICROHISTOLOGICAL EXAM: ❒ Category (B): B1 (normal tissue/uninterpretable) B2 (benign) B3 (lesion of uncertain malignant potential) B4 (suspicious of malignancy) B5a (*in situ* carcinoma) B5b (invasive carcinoma) (specify the identification serial number B/……. of the Department of Pathology of …..…… date of execution……..)

HISTOLOGICAL EXAM: (specify the identification serial number B/……. of the Department of Pathology of …..…… date of execution……..) Type of sample: ❒ Punch ❒ Incisional biopsy/lumpectomy
❒ Cuneiform resection of ducts ❒ Excisional biopsy/lumpectomy❒ Quadrantectomy
❒ Breast scar revision for local recurrence ❒ Other .....

Enclose a copy of prior cyto/histological exams performed in a different Institution.

Signature (clear script/legible) of the person requesting the exam

Request form for surgical specimens

GENERAL DATA

1. Patient identifiers
   - Name
   - Date and place of Birth
   - Gender
   - Unique identifier (i.e. health record, master index number, national insurance number)
- Address and phone number

2. Identifiers of the authorized person requesting the exam (name, operative unit, phone number)

3. Date of surgery

RELEVANT CLINICAL DATA ON EXTRA-MAMMARY PATHOLOGIES:

…….....

SENOLOGICAL ANAMNESIS:

❒ prior pathologies ❒ prior surgery ❒ familiarity for breast neoplasms ❒*BRCA1/BRCA2* gene mutations

CLINICAL DATA ON THE PRESENT BREAST LESION:

FEATURES OF THE LESION: ❒ Palpable ❒ Not palpable ❒ Ulceration ❒ Other .....

NEOADJUVANT THERAPY: ❒ No
❒ Yes: **Prior typization:** ❒ ER ❒ PgR ❒ Ki-67 ❒ HER2

Clinical-instrumental response: ❒ Complete ❒ Partial ❒ Absent

SITE OF THE LESION/S: ❒ Right breast ❒ Left breast
❒ UOQ (Q1) - UIQ (Q2) - LOQ (Q3) - LIQ (Q4) – Central-reatroareolar (Q5) ❒ Nipple
❒ Intermediate QQ: superior – equatorial internal - inferior – equatorial external
❒ Axillary tail of breast ❒ Axillary lymph node

TYPE OF SAMPLE:

- Incisional biopsy
- Excisional biopsy (lumpectomy)
- Duct excision/Microdochectomy
- Large resection: ❒ with pectoral fascia ❒ without pectoral fascia
  ❒ with skin ❒ without skin
- Quadrantectomy ❒ with pectoral fascia ❒ without pectoral fascia
  ❒ with skin ❒ without skin
- Simple mastectomy
- Skin sparing mastectomy
- Nipple sparing mastectomy
- Radical mastectomy
- Axillary lymph nodes dissection
- Sentinel lymph node
- Axillary lymph nodes dissection (after sentinel lymph node biopsy)
- Second/additional surgery (specify the orientation/new margin: ……..)
- Breast parenchyma from reduction mammoplasty (specify if it is an aesthetic mammoplasty, or it has been performed to match with the other breast/oncoplastic breast reduction)

• Radicalization mastectomy (specify the histological number of the report of the prior surgery and provide a copy, if surgery was carried out in another institution)
• Retro-areolar disc
• Other (specify)…….

MARGIN MARKERS ON THE SURGICAL SPECIMEN (specify the type of marker):
• Superior-medial margin: .....
• Inferior-lateral margin .....
• Deep margin/toward the fascia: .....
• Superficial margin/toward the skin: .....
• Retro-areolar margin/toward the nipple.....
Note: If the skin is not present, use at least three markers

INSTRUMENTAL DIAGNOSTICS PERFORMED ON THE PRESENT BREAST LESION (enclose a copy of the reports and/or fill in the following form):

MAMMOGRAPHY: (exam date.....)
❒ N° of lesions: ❒ Dimensions (mm): ❒ Radiological category: R..... or BIRADS.....

❒ TYPE OF LESION: ❒ Opacity with spiculated margins ❒ Opacity with well-defined margins
❒ Parenchymal distortion ❒ Asymmetrical density

❒ MICROCALCIFICATIONS: ❒ Absent ❒ Morphology: : ❒ Distribution: ❒ Extent:
❒ Diagnostic hypothesis.....

ULTRASONOGRAPHY: (exam date.....)
❒ N° of lesions ❒ Dimensions (mm): ❒ Ultrasound category: U……

❒ TYPE OF LESION (solid or cystic) and echogenicity: ……
❒ Diagnostic hypothesis.....

MRI: (exam date.....)
❒ N° of lesions: ❒ Location: ❒ Dimensions (mm): ❒ MRI category: .....
❒ Diagnostic hypothesis.....

OTHER INSTRUMENTAL DIAGNOSTICS (specify which): .....

PRIOR CYTOLOGICAL/HISTOLOGICAL EXAMS

CYTOLOCIGAL EXAM: ❒ Category (C): C1 (inadequate) C2(benign) C3 (atypical, probably benign) C4 (suspicious, probably malignant) C5 (malignant) (specify the identification serial number C/……. of the Department of Pathology of …..…… date of execution……..)

MICROHISTOLOGICAL EXAM: ❒ Category (B): B1 (normal tissue/uninterpretable) B2 (benign) B3 (lesion of uncertain malignant potential) B4 (suspicious of malignancy) B5a (*in situ* carcinoma) B5b (invasive carcinoma) (specify the identification serial number B/……. of the Department of pathology of …..…… date of execution……..)

HISTOLOGICAL EXAM: (specify the identification serial number B/……. of the Department of Pathology of …..…… date of execution……..) Type of sample: ❒ Punch ❒ Incisional biopsy/lumpectomy
❒ Cuneiform resection of ducts ❒ Excisional biopsy/lumpectomy ❒ Quadrantectomy
❒ Breast scar revision for local recurrence ❒ Other .....

Enclose a copy of prior cyto/histological exams performed in a different institution.

Surgery (clamping of arterial vessels: beginning of warm ischemia) started at …….

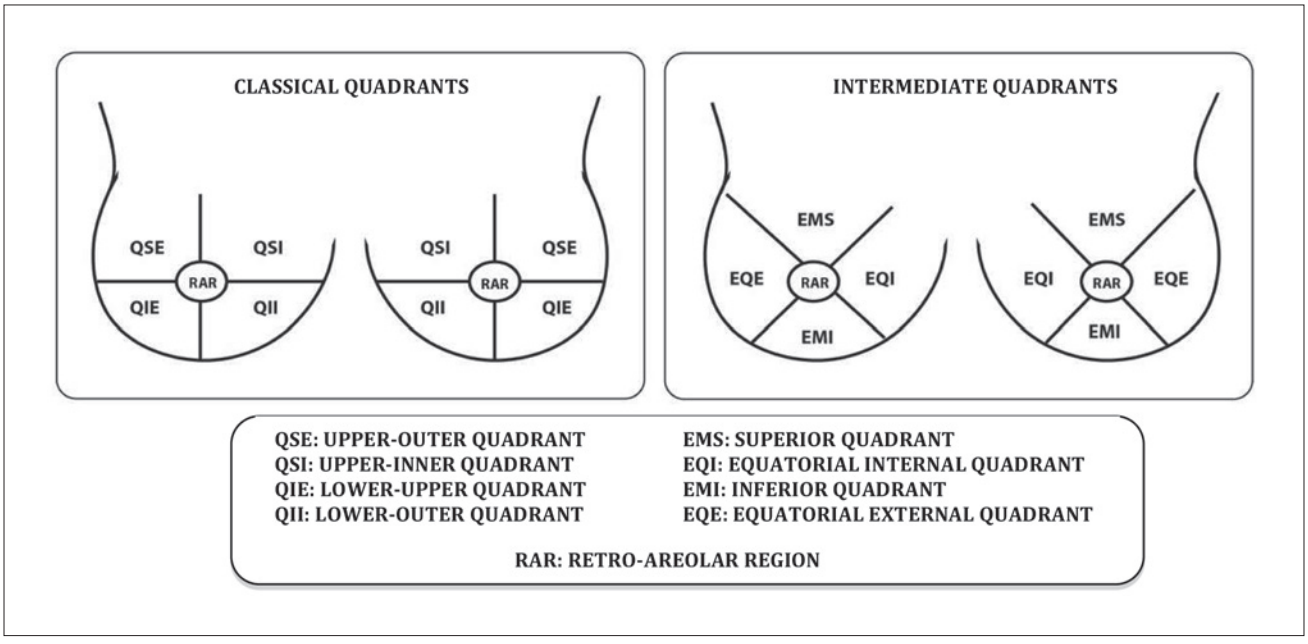
The complete removal of the surgical specimen (end of warm ischemia/beginning of cold ischemia) ended at ……..

After removal, the surgical specimen was
❒ immediately fixed in 10% neutral buffered formalin (end of cold ischemia)
❒ submitted as "fresh" tissue to the pathology laboratory, where it arrived at …….

Signature (clear script/legible) of surgeon

Figure to be used for schematic representation of the **site of the lesion**, the **type of surgical intervention** and the **site of markers** (if any)

For a better localization of the lesion, please specify:
The hour on the face of a clock corresponding to the site of the lesion: hour .....
Distance from the nipple: cm .....
Depth from the skin (distance between the skin and the surface of the lesion): mm .....



How to submit fresh tissue samples to the Pathology Laboratory

In order to allow a correct morphological, immunophenotypical and molecular evaluation (which are necessary to define the therapeutical protocol), surgical samples must be **immediately, accurately and completely fixed**.
The best way to obtain it is to transfer **as quickly as possible (maximum 30 min after the sampling)** the fresh-tissue samples, or the formalin-fixed samples, to the pathology laboratory.
Operators involved in the management of neoplastic samples must reach an agreement on how to guarantee a correct and immediate fixation, also through possible variation of the time of execution of biopsies and surgical sampling.
Surgical samples must be intact and well oriented (with sutures, clips, etc). In absence of cutis, the presence of at least three markers, one of which for the nipple, is required.
When a surgical sample is incidentally or necessarily torn, its reconstruction with a suture is mandatory, and the problem should be reported to the pathologist, in order to allow the optimal evaluation of surgical margin.
When the immediate delivery of the samples to the pathology laboratory is not possible, these **must be quickly (in maximum 30 minutes)** fixed in 10% neutral buffered formalin. Samples must be totally absorbed by formalin, in order to obtain an optimal fixation, also performing parallel cuts from the deep margin to the skin (maximum thickness of 0,5 cm), which may involve neoplastic nodes too.
Alternatively to formalin, samples can be vacuum-packed and maintained this way for a maximum time span of 24-48 hours at the temperature of 4°C. This system is available for samples of any dimension, especially when the diameter is larger than 2 cm. This procedure manages to preserve original tissue features and allows samples for tissue-banking and other scientific purposes.
Remember to enclose radiological reports (MX, US, MRI), cytological and histological reports and, when possible, the RX of the surgical sample, including the evaluation of resection margins. In case of not palpable lesions, always enclose RX of the surgical samples, in order to facilitate a correct sampling.

References

[1] Wells CA. *Quality assurance guidelines for pathology. In: European guidelines for quality assurance in breast cancer screening and diagnosis.* 4th ed. European Communities 2006, pp. 221-311.

[2] *Linee guida refertazione citologia mammaria proposte da SIAPEC-IAP*. Pathol 2012;104:172.

[3] Lester SC, Bose S, Chen YY, et al. *Protocol for the examination of specimens from patients with ductal carcinoma in situ (DCIS) of the breast. Arch* Pathol Lab Med 2009;133:15-25.

[4] Lester SC, Bose S, Chen YY, et al. *Protocol for the examination of specimens from patients with invasive carcinoma of the breast.* Arch Pathol Lab Med 2009;133:1515-38.

[5] *Documento di consenso del gruppo regionale patologi screening mammografico.* Registro Tumori del Veneto, 2012.

[6] *Protocollo diagnostico terapeutico dello screening per la diagnosi precoce dei tumori della mammella*. Regione Emilia-Romagna 4ª Ed, 2012.

[7] *Requisiti minimi e standard di refertazione per carcinoma della mammella*. SIAPEC Piemonte Rete Oncologica, 2009.

[8] Ministero del Lavoro, della Salute e delle Politiche Sociali. *Manuale per la sicurezza in sala operatoria: raccomandazioni e checklist.* Ottobre 2009.

[9] Yildiz-Aktas IZ, Dabbs DJ, Bhargava R. *The effect of cold ischemic time on the immunohistochemical evaluation of estrogen receptor, progesterone receptor, and HER2 expression in invasive breast carcinoma.* Mod Pathol 2012;25:1098-105.

[10] Hammond ME, Hayes DF, Dowsett M, et al. *American society of clinical oncology/college of american pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer.* J Clin Oncol 2010;28:2784-95.

[11] Di Novi C, Minniti D, Barbaro S, et al. *Vacuum-based based preservation of surgical specimens: an environmentally-safe step towards a formalin-free hospital.* Sci Total Environ 2010;408:3092-5.

[12] Bussolati G, Chiusa L, Cimino A, et al. *Tissue transfer to pathology labs: under vacuum is the safe alternative to formalin.* Virchows Arch 2008;452:229-31.

Macroscopical examination and sampling of surgical breast specimens

The target of macroscopical examination and sampling of surgical specimens is the identification of the pathological lesions and their histological evaluation.
An incorrect macroscopical analysis and wrong or incomplete sampling can crucially condition the final diagnosis, with unavoidable and potentially serious prognostic, therapeutic and legal repercussions.
In these phases, the presence of medical staff with proven expertise in breast surgical management is highly recommended.

Macroscopical evaluation

The macroscopical evaluation may comprise a detailed description of surgical sample and its sampling.
It is composed by:

A - External evaluation
Description should include:
the three dimensions of surgical sample;
the two dimensions of cutis (if present) and the possible presence of the nipple;
weight (optional);
the presence of surgical markers for orientation;
the consistency, i.e. the presence of palpable nodules.

B - Inking of surgical margins
Surgical margins in breast-conservative surgery must be inked with Indian Ink or acrylic in different colours for their correct identification and hystological evaluation (see Appendix 1).
For mastectomy samples, inking can be useful in case of macroscopic lesion close to the deep margin (e.g.

pectoralis fascia, pectoralis muscle) or skin (in cases of mastectomy without removal of the skin overlying the lesion).

C - Dissection

It should be performed as early as possible after arrival in Pathology Laboratory (whether specimens arrive fresh, or formalin-fixed or vacuum-packed).

Samples are dissected and immediately absorbed in 10% neutral buffered formalin (pH around 7) to allow a correct penetration of the fixative, without altering the shape or the orientation (see Appendix 1). Fixation must be carried out in formalin for no less than 24 hours for all surgical samples.

A pathologist will then examine macroscopically each section to evaluate the presence of lesions and tissutal characteristics. It is mandatory to note:

- Number, size and location (quadrant/quadrants in case of mastectomy; distance from margins) of identified lesions.
- Distance of the lesion from the skin and deep margin (pectoralis fascia in case of mastectomy) or from the closest margin of excision (in case of breast-conservative surgery) (*optional*).
- Appearance of the lesion: consistence, color, margins, macroscopic presence of necrosis, hemorrhage and calcifications.
- Identification and description of bioptic site, in case of previous agobioptic procedures, if possible.
- Photo of each section and related lesions, if possible.

D - X-ray examination of the surgical material

If the sample size doesn't allow a complete inclusion of the specimen, the execution of radiograms of each macrosections is recommended (in particular, for non-palpable lesions and calcifications), in order to select those areas corresponding to radiological abnormalities. Alternatively, for non-palpable lesions and calcifications, the sample should be positioned on a radiological grid, in order to take samples in areas of radiological interest.

The sampling for histological examination can be performed on fresh material or after formalin-fixation.

The sampling technique and the number of required samples and inclusions vary for each case according to:

- Size of surgical sample.
- Dimension and clinical-radiological features of nodes: palpable lesions (opacity, thickening, distortion) *versus* non-palpable tumours/calcifications.

Regardless of the intrinsic variables in each sampling procedure, the minimal absolute goals are:

- an accurate measurement of the maximum diameter of the lesion;
- a detailed examination of the state of the margins of resection and, when possible, the microscopic distance of the lesion from them.

A prerequisite for achieving these objectives is the entirety of the surgical sample and its correct orientation through the use of markers. It is clear that, in presence of multiple samples or single sample dissected and not reconstructable, the evaluation of the margins may be inaccurate or even impossible, and the pathological dimensions (pT) or the unifocality/multifocality (in case of invasive carcinoma) could not be accurately defined (e.g. for tumour present in more than one sample).

A - General considerations

- For samples of limited size (approximately up to 5-7 cm on major axis) or in the presence of not-palpable/not macroscopically detectable lesions (e.g. calcifications) the total inclusion in blocks through ordinary sequential sampling, or alternatively in "large blocks" (macro-sections) is preferable (see Appendix 2).

- For larger samples, see paragraph "Specific recommendations".

- Surgical margins can be sampled and evaluated in various ways (see **Appendix 2**):
  - Perpendicular sampling in ordinary blocks;
  - Perpendicular sampling in "large blocks" (macrosections);
  - "Shaved" sampling (peeling);
  - Separate biopsies from surgical cavity/surgical bed.

- In case of multiple and macroscopically suspicious lesions, each of them should be sampled; as a general rule, normal interposed tissue should be sampled as well, in order to check whether the lesions are truly separate.

- When present, the nipple should be entirely included in two or more fragments (through longitudinal cuts, perpendicular to the skin) together with the sampling of a retroaerolar disk (through two sections parallel to the skin), which will enable the analysis of the region of the lactiferous ducts.

- Sampling of axillary lymph nodes: all nodes should be collected and included *in toto* for histological examination. Their optimal sampling is described in **Appendix 3**.

B - Specific recommendations related to different types of surgical specimens:

- Nodulectomies / surgical "diagnostic" biopsies;

- Therapeutic excisions / quadrantectomies (breast-conservative surgery)
  - As already indicated (see above), when dimensions allow it, or in presence of not-palpable/not macroscopically visible lesions (e.g. calcifications), **the optimal procedure for processing the samples is their entire inclusion** in sequential blocks or in "large blocks" (macrosections). In case of sequential

sampling in ordinary blocks, the extension of the lesion is defined by multiplying the number of consecutive levels that include the lesion with the thickness of a slice.

When complete inclusion is not possible, it is highly recommended to produce **a radiogram of the sample** and/or of the macro-slices obtained after cutting and **to sample selectively the areas corresponding to mammographic abnormalities.** For a correct spatial reconstruction of the lesion and evaluation of its extension, it is advisable that the sampling of selected areas is made with ordinary sequential technique in blocks or "large blocks" (macrosections).

- In case of preoperative diagnosis of DCIS or suspected DCIS (calcifications) - in which radiological dimensions often "underestimate" the real extension of the lesion - sampling should include the borders (e.g. proximal and distal) of the radiological target and the apparently healthy surrounding tissue; for a useful radiological-histological correlation, sampling sites can be found on the RX using colour markers, or other markers. For the assessment of resection margins, sampling will necessarily include the tissue corresponding to the shorter distance between mammographic abnormality and resection margin. Additional targeted sampling of the remaining surface of resection is highly recommended. When microcalcifications cannot be recognized at microscopic examination, the paraffin blocks can be X-rayed to confirm the presence of the radiological target and to cut further deeper sections.
- In case of not-palpable / not-macroscopically detectable lesions (in particular, DCIS), when sampling takes place with **non-sequential** technique or without macrosection the extension of the tumour can only be approximated by taking into account the number of blocks and inclusions involved.
- In case of macroscopically detectable or palpable lesions, sampling can be targeted and made through sampling in "radial blocks" according to orthogonal planes of space (superior-inferior, medial-lateral, surface-deep). In case of small excisions it is possible to include the lesion and the margins of resection in one inclusion only; for larger samples, the use of more inclusions is necessary, with separate sampling of the various margins. For each breast cancer, when the size allows it, at least 3 inclusions should be obtained, including in some sampling the interface with non-neoplastic parenchyma.
- When more surgical samples are separately sent, the real size of the lesion(s) will be rebuilt only if the pieces are mutually oriented to each other. In any case, it is a good practice to measure the lesion/s in each separate sample.
- In case of conservative interventions, after diagnosis through needle biopsy or surgical biopsy, if total inclusion is not possible, it is essential to sample in a targeted manner (preferably using ordinary sequential samples or macro-blocks) the site of the previous biopsy and the surrounding tissue. Additional samples will be carried out on the parenchyma at a distance from the neoplasia and on the margins of excision.

Second surgery (re-excisions)

- The surgical specimen of an additional surgery or a re-excision for positive margins must be oriented to allow the analysis of the margin corresponding to the margin previously found positive, and to study the new margins of re-excision.

- If tumoral tissue is found in the additional surgical specimen as well, the reconstruction of the real size of the lesion is difficult. However, the separate sampling of tissue adjacent to the previous surgical excision and at a distance is a good practice. This will enable at least a partial reconstruction of the size of the lesion and a proper recognition of unifocal *versus* multifocal lesion.

Mastectomy

- Each neoplastic lesion, when its size allows it, should be sampled with at least 3 inclusions, comprising in some sampling the interface with non-neoplastic parenchyma. Any relationship of the tumour with skin, nipple and fascia/pectoralis muscle should be evaluated with targeted sampling. All macroscopically suspicious lesions must also be sampled, specifying their topography. Random sampling of each quadrant, even in case of apparently normal tissue, is a good practice.

- As for conservative surgery of large dimension (see above), in case of mastectomies performed for not-palpable/not-detectable lesions (usually DCIS) a selective sampling of the areas corresponding to the mammographic abnormalities (usually calcifications), identified by radiograms of the slices from macroscopic sectioning, is recommended. When this is not possible, it is necessary to have at least the reports of previous radiological investigations, on the basis of which topographical areas can be selected to target the sampling.

- In case of a macroscopically evident lesion that is close to the deep margin (e.g. pectoralis fascia/ muscle) or the surface (towards the skin in cases of skin-sparing mastectomy, without removal of the skin overlying the lesion) it is advisable to mark and sample these margins.

Areolar margin in "nipple sparing" mastectomy

For histological (intraoperative and/or definitive) examination of retroareolar parenchyma, the surgeon should send separately a retroareolar disc sampled directly from

the surgical specimen. This fragment (0,5-1 cm thick) should be marked on the side towards the nipple ("true margin") with surgical thread or metal clip. The examination of the retroareolar parenchyma can be performed during surgery as intraoperative examination (intraoperative frozen section) or after surgery as a definitive exam.
The retroareolar disk, usually single, is measured (diameter and thickness) and the side towards the nipple ("true margin") **must be inked**.
Histopathological examination may be performed through:

- Coronal sections (perpendicular to the nipple axis) obtained by sectioning the discoid fragment from the breast as "a dish" (with recovery of the "true margin" through additional sections, almost until the exhaustion of the fragment).
- Sagittal sections (parallel to the nipple axis) from the nipple to the breast: the entire disk is sectioned into slices, 3-5 mm thick, which should be totally included.

The areolar disc analysis may provide 3 sections during intraoperative examination at levels of 200-300 microns and an additional section during definitive exam, or in alternative 4 sections cutted every 200-300 microns for the definitive exam.

Examination of the breast

The breast (without the retroareolar disk, if it was already sent for intraoperative examination by frozen section), is sent to the pathology laboratory with markers (suture threads) to allow the orientation: 1 suture thread marks the site where the retroareolar disc was removed, 2 suture threads mark the axilla; one marker is in the site of the lesion, in its projection on the surface of the gland, to allow the assessment of the relationship of the lesion with the overlying subcutaneous tissue, the deep plan and the remaining retroareolar parenchyma after removal of the disk. It is important that the request of histological examination exactly specifies the site (quadrant) of the lesion, or the sites in case of multiple lesions (possibly by attaching a diagram/draw).

* Taken from "Consensus Document on Nipple Sparing Mastectomy"
http://www.senologia.it/rivista/pdf/59/59forum.pdf

References

[1] Wells CA. *Quality assurance guidelines for pathology. In: European guidelines for quality assurance in breast cancer screening and diagnosis.* 4th ed. European Communities 2006, pp. 221-311.

[2] *Documento di consenso del gruppo regionale patologi screening mammografico.* Registro Tumori del Veneto, 2012.

[3] *Protocollo diagnostico terapeutico dello screening per la diagnosi precoce dei tumori della mammella.* Regione Emilia-Romagna 4ª ed, 2012.

[4] *Requisiti minimi e standard di refertazione per carcinoma della mammella.* SIAPEC Piemonte Rete Oncologica, 2009.

[5] Lester SC, Bose S, Chen YY, et al. *Protocol for the examination of specimens from patients with ductal carcinoma in situ (DCIS) of the breast.* Arch Pathol Lab Med 2009;133:15-25.

[6] Lester SC, Bose S, Chen YY, et al. *Protocol for the examination of specimens from patients with invasive carcinoma of the breast.* Arch Pathol Lab Med 2009;13:1515-38.

APPENDIX 1: Marking of surgical margins and specimen sectioning

MARKING OF SURGICAL MARGINS

Before the ink is applied, the surface of the specimen should be thoroughly dried with blotting paper/tissue paper, so that the ink better adheres to the surface of the sample. Subsequently, the ink is distributed with a brush on the surface and the specimen is plunged (or covered) into Bouin's fixative, or alternately in 10% acetic acid solution or in absolute ethyl alcohol, in order to fix definitely the ink. Finally, the specimen should be dried again with blotting paper to ensure that the ink remains coated on the surface.

In the technique of inking margins with acrylic or tempera paints, different colours can be used to mark the different margins. Standardization in the choice of colours is suggested, so that the same colour always corresponds to the same margin. The colours are applied and lied with a brush covering the areas as indicated by the anatomical markers designated by the surgeon, and taking care to keep net margins between adjacent colours.

SPECIMEN SECTIONING

Cutting and sectioning methods can vary in relation to the various methodologies adopted for sampling. As a general rule, for samples from small to medium size (diagnostic biopsy or conservative therapeutic surgery) the specimen should be dissected with serial sections at 3 to 5 mm intervals.
Sections should be conducted perpendicular to one of the spatial axes (e.g. medial-lateral; cranial-caudal) or in parallel to the direction nipple – pectoral fascia. The cuts can be partial (thus maintaining the orientation of the specimen) or complete at full thickness (in this case the sections obtained should be fixed on a support with labels affixed on it, with the orientation mark written in pencil).
For mastectomy specimens, the breast should be placed on a cutting board with the skin surface down and the deep fascial plane (pectoral fascia) facing upward. Parallel sections with a medial-lateral direction, perpendicular to the skin, should be produced. These cuts should not go all the way through the specimen, but should

leave the sections attached together by a rim of unsectioned breast or skin; the cuts should possibly be directed along the major axis of the lesion as shown in the reports of instrumental exams (ultrasound or mammography). By adopting this procedure, it will be possible to fix the entire breast in a single box; attention should be paid in putting between one slide and the other a few sheets of gauze that will help the formaldehyde to penetrate and will prevent the tissue slices from collapsing.

APPENDIX 2: Sampling and evaluation of surgical resection margins

Sampling may be performed in **perpendicular tissue blocks, obtained on separate and distinct sequential sections (levels**): each section/layer may comprise one or more blocks in relation to the size of the sample. This method enables a precise topographical location of the single block and the reconstruction (at least in two dimensions) of the sampled lesion. Alternatively, it is possible to use the technique in "**large blocks**" (macro-sections), which allows the examination of larger areas of the sample by keeping the topographical relationships between the lesion and the surrounding structures, or between different anatomical lesions.

Concerning the resection margins, both procedures allow a correct evaluation, by providing an accurate microscopic measurement of the distance between the surface of excision and the lesion. By using the technique in "large blocks", the advantages are the possibility to investigate the margins in their entirety (at least for the plan taken into exam) and to define more easily the size of the lesion (in particular for DCIS), and the lower number of inclusions needed.

A further possible method for the study of the margins is the "**shaved margins**" technique (peeling): after inking the margins, the pathologist performs sections parallel/tangential to the margins; sections thus obtained are included by the same side of the margin (side marked with ink). This technique allows the examination of the entire surface of the margins with a smaller number of inclusions than with ordinary blocks; nevertheless, part of the true margin is lost during the process of trimming the paraffin blocks to an optimal cutting surface. Moreover, and most importantly, by using this method it is not possible to measure the distance between the margin and the lesion.

APPENDIX 3: Sampling of axillary lymph nodes

During macroscopic examination of the surgical specimen, attention should be paid to the search of lymph nodes, which can be isolated both from fresh tissue or after fixation (in this case, the search is usually easier). It is important to eliminate as much fat tissue surrounding the lymph node as possible, in order to facilitate fixation and processing.

Each macroscopically "negative" node must be included in its entirety. Sampling must ensure recognition of all macrometastases (> 2 mm). When the size allows it, each node must therefore be dissected with thin cuts (approximately 2 mm) along the major axis. Small lymph nodes can be included into a single block. The inclusion in a single block of more than one lymph node, or of parts of a single node, should be described in order to assess the effective number of lymph nodes by microscopic examination.

In macroscopically positive (metastatic) lymph nodes, it is recommended the sampling of areas suspicious for extra-nodal infiltration.

APPENDIX 4: Diagnostic protocol for sentinel lymph node biopsy

INTRODUCTION AND OBJECTIVES

The intraoperative examination of sentinel lymph node (SLN) should not be requested when the surgical treatment is provided in two times.

The probability to diagnose micro-metastases and isolated tumour cells (ITC) increases with the increase of the number of sections examined (*Weaver et al. Am J Surg Pathol 2009;33:1583-1589*), and the use of immunohistochemistry (IHC) for cytokeratins.

The "European guidelines for quality assurance in breast cancer screening and diagnosis" provide the following information:

a. *Minimal processing method*: identification of metastases > 2 mm.
b. *Optimal processing method*: detection of micrometastases
c. *Processing method for the identification of ITC*: "multistep sectioning" and immunohistochemistry.

SENDING METHOD

Every SLN should be placed in a suitable container, labelled and immediately sent to the Pathology Laboratory. If it is not possible, the sample should be stored in an appropriate amount of 10% neutral buffered formalin.

MACROSCOPIC GROSSING

Lymph nodes with a diameter larger than 5 mm should

be sectioned along the minor axis, at intervals of about 1-2 mm, in order to obtain a more comprehensive evaluation of the capsule and the marginal sinus (preferential site for ITC localization), and included totally, in a single tissue-block, if possible; sections thus obtained should be placed in the block always in the same direction (with the aid of sponges for embedding cassettes) and included by technicians following the orientation provided by the pathologist (*Weaver et al., Modern Pathology 2010;23:S26-S32*).
The lymph nodes with a diameter smaller than 5 mm should be cut in half along the major axis and entirely included. In order to make the cutting of the lymph node easier, we suggest letting the lymph node fix for about 2 hours after the elimination of the peripheral fat tissue (taking care not to tear the capsule away). We recommend to place the sections of the lymph node on sponges for embedding cassettes.

METHODS FOR EXAMINATION

There are four methods for the examination of the lymph nodes:

1. *Examination in paraffin only*: the SLN is fixed in formalin and embedded in paraffin.
2. *Examination exclusively with criostatic sections*: the SLN is exhausted during the intraoperative examination.
3. *Hybrid examination*: part of the lymph node is examined with criostatic sections, and part is fixed in formalin and embedded in paraffin.
4. *Molecular analysis.*

1. EXAMINATION IN PARAFFIN ONLY

Each SLN must be sampled separately for microscopic examination.
In case of metastatic SLN at macroscopic examination, a single section stained with H&E is sufficient, without the need of "multistep sectioning" and immunohistochemistry (IHC).
Immunohistochemistry for cytokeratin is optional, as suggested by the European Guidelines.
If no metastatic lesion is identified using H&E, it is advisable to examine at least 3 sections, cut at different levels, using IHC with broad-spectrum cytokeratin antibodies.
The immunohistochemistry for cytokeratins, rarely needed for the identification of macro-metastases, is useful for the diagnosis of micro-metastases and ITC; in case of metastases from lobular carcinomas, it can also assist in the identification of macro-metastases.
If a concomitant different disease possibly affecting the lymph node is suspected, the above-mentioned protocol should be abandoned.

2. EXAMINATION EXCLUSIVELY WITH CRIOSTATIC SECTIONS

SLN must be evaluated macroscopically first (visually and by palpation). The firm consistency and the variation of colour on the cut surface may be the result of a non-metastatic process (e.g. fibrosis or lymphoma). Adipose tissue in excess should be removed carefully and safeguarding the capsule.
Intraoperative examination of SLN with diameter < 3 mm should be discouraged.
In case of intraoperative examination, the lymph node should be dissected with the same protocols adopted for samples embedded in paraffin.
The intraoperative examination, using cryostatic sections and/or cytology obtained from imprinting, has a low risk of false positive results but a high risk of false negatives, with a sensitivity varying from 66% to 100% for the cryostatic examination and from 65% to 94% for the cytological examination with imprint. The choice between the two methods depends on the preferences and the experience of each centre.
Sensitivity and positive predictive value of intraoperative examination of SLN may be enhanced by special techniques, such as the rapid immunohistochemistry for cytokeratin.
Rapid immunohistochemistry is useful mainly in cases of invasive lobular carcinoma, providing a better and more accurate assessment of the size of the metastatic deposit and the identification and sub-classification of ITC, micro-metastases or macro-metastases.

3. HYBRID EXAMINATION

Part of the lymph node is examined with cryostatic sections and part is fixed in formalin and embedded in paraffin. The SLN, received at the Pathology Laboratory immediately after the removal and not fixed, is dissected following the above-mentioned procedures and frozen *in toto*. After the intraoperative exam, the remaining material is fixed in 10% neutral buffered formalin, embedded in paraffin and analysed according to the above-mentioned procedure.
It should also be clearly stated that a final and definitive diagnosis would follow after the completion of the procedure.
If the SLN is found to be metastatic during intraoperative examination, a single H&E section would suffice for the definitive diagnosis.

4. MOLECULAR ANALYSIS

Centres equipped to perform this type of examination should strictly follow the procedural recommendations of the manufacturers.
One-step nucleic acid amplification (OSNA®) is considered the technique of choice, because it has a high level of accuracy and can be performed intraoperatively.

The evaluation of the lymph node with molecular techniques excludes the comparative histological evaluation; for this reason, it is recommended the execution of an imprint cytological slide in order to exclude potential pathologies (other than metastasis) in the lymph node.

APPENDIX 5: Neoadjuvant chemotherapy

MACROSCOPIC ASSESSMENT AND SAMPLING

The identification of a tumour after neoadjuvant chemotherapy can be very difficult in case of complete clinical and instrumental response to treatment. Therefore, it is highly recommended to mark the lesion (with metal clip or skin tattoo) before therapy starts, in order to allow the localizationn of the lesion after treatment. Without a marker, radiological data (mainly MR) are essential.
When taking into account the type of response to therapy, the macroscopic examination and sampling procedures do not differ in substance from those used for the usual quadrantectomy/wide excision or mastectomy (see above).
If the tumour is still detectable (*absent pathological response*), sampling follows the usual indications for malignant lesions.
In cases of *clinical and instrumental partial response* the residual disease can appear nodular, partially sclerotic, or composed by multiple foci that surround an oedematous and/or sclerotic area. On palpation the residual tumour has a soft consistency.
All macroscopically evident lesions should be described, measured and sampled: if the residual lesion is less than 3 cm in the maximum diameter, it should be included entirely; if it is greater than 3 cm an extensive sampling is recommended (with complete inclusion of the suspect area, if possible). It is always necessary to report the distance from the surgical margins of resection.
In case of *clinical and instrumental complete response*, the identification of the tumoral bed can be difficult. Nodules are usually not present, while it is often present an area with ill-defined contours and a centrally oedematous and/or fibrous appearance. It is therefore necessary the sampling of the whole area with contiguous sections of 3-5 mm, after its measurement in two dimensions.
Similarly, if a marker is present, all of the adjacent area should be systematically sampled. It is useful to remember that microcalcifications associated with the neoplasm do not disappear after chemotherapy; therefore, radiography exam can facilitate the recognition of the area to be sampled. Without a marker it is highly recommended to sample the specimen with the aid of radiographs (in case of calcifications) or on the basis of pre- and post-therapy MR data.
For multifocal lesions the procedure of sampling must be performed on all the identified areas. In all cases, margins between residual lesion and adjacent parenchyma must be sampled.
If present, the skin overlying the tumoral bed (in case of complete clinical and instrumental response) or overlying the tumour (in case of absent pathological response or partial clinical and instrumental response) should be sampled, performing sections in continuity with the tumour, if possible.

Check list for the reporting of microscopical diagnosis of invasive breast carcinoma

- **Histotype** (according to WHO 2012):
- **Histological grade** (according to Elston et al. 1991):
  grade #, # differentiated
  - Mitosis: #/10 HPF score # (field diameter #)
  - Nuclear pleomorphism: score #
  - Formation of tubules: score #
- **Peritumoral vascular invasion** (not evident, present):
- **Massive peritumoral vascular invasion** (OPTIONAL):
- **Peritumoral perineural invasion** (OPTIONAL):
- **Multiple foci of invasive carcinoma** (distinct foci separated by healthy parenchyma):
- **Peritumoral carcinoma *in situ****: (%, histological type, nuclear grade):
  * indicate the presence of extensive intraductal component (when DCIS is > 25%)
- **Presence or absence of necrosis** (OPTIONAL) (absent; present central necrosis "comedo type"; present focal necrosis):
- **Intratumoral carcinoma *in situ*** (OPTIONAL):
- **Size of the microscopic invasive component**:
- **Overall dimensions** (invasive component plus *in situ* component):
- **Location**:
- **Nipple, retroareolar parenchyma, skin and chest wall**:
- **Microcalcifications** (stromal / endoluminal):
- Evaluation of microscopic resection margins using the following definitions:

1. POSITIVE MARGIN (presence of ink on the lesion), specifying:
a) which edge/s is/are involved; b) if it is a single focus or multiple foci of invasion; c) the size of the linear extent of the involvement of the margin expressed in mm; d) the presence of *in situ* component on the margin.
2. FREE FROM INVASION MARGIN/S (there is no ink on the lesion), specifying the extent of the distance from the margins of the sampled lesion, if it is less than 1 cm (including the distance from eventual *in situ* component).

- Parenchyma free from neoplasm:
- **Staging** (pT according to AJCC 2010, seventh edition)

Check list for the reporting of microscopical diagnosis of *in situ* breast carcinoma

- Type:
- **DIN-classification according to Tavassoli** (OPTIONAL)**:**
- Site:
- Main histotype:
- Microscopically-evaluated calcifications:
- **Necrosis** (absent; present central necrosis "comedotype"; present focal necrosis)**:**
- **Maximum extension (microscopically measured):** ..... mm
- Evaluation of microscopic resection margins with the following definitions:

  1. POSITIVE MARGIN (presence of ink on the lesion), specifying: a) which edge/s is/are involved; b) if there is a single focus, or multiple foci of invasion; c) the size of the linear extent of the involvement of the margin expressed in mm; d) the presence of *in situ* component on the margin.

  2. FREE FROM INVASION MARGIN/S (there is no ink on the lesion), specifying the extent of the distance from the margins of the sampled lesion, if it is less than 1 cm.

- **Microinvasion (< or = 1 mm):** absent/present, single or multiple foci
- Nuclear grading (according to European Guidelines 2006):
- Other lesions:

Reporting of prognostic/predictive factors determined by immunohistochemistry

Estrogen receptor (ER) (clone #, company #): #% positive neoplastic cells

Progesterone receptors (PgR) (clone #, company #): #% positive neoplastic cells

Ki-67 (clone #, company #): #% positive neoplastic cells

c-erbB2 oncoprotein (clone #, company #):

1. POSITIVE (score 3+)

Complete and intense staining of the cell membrane, circumferential in > 10% of invasive carcinoma cells (specify the percentage).

2. EQUIVOCAL (score 2+; FISH evaluation will follow)

- Complete staining of the cell membrane, with weak or moderate intensity, circumferential in >10% of invasive carcinoma cells.
- Incomplete (basal-lateral or lateral) staining of the cell membrane, with moderate/intense staining in > 10% of invasive carcinoma cells (rare, usually on micropapillary invasive carcinoma)*.
- Complete and intense staining of the cell membrane, circumferential in 10% or less of invasive carcinoma cells (rare)*.

* These kinds of immunohistochemical results are rare and have to be prudentially considered as score 2+, and tested with ISH analyses.

3. NEGATIVE (score 1+)

Incomplete staining of the cell membrane, with low intensity, in >10 % of invasive carcinoma cells (specify the percentage).

4. NEGATIVE (score 0)

Absence of cell membrane staining of invasive carcinoma cells, or incomplete and low intensity membrane staining in 10% or less of invasive carcinoma cells.

5. INDETERMINATE

[ASCO/CAP 2013 recommendations; AIOM-SIAPEC 2014 Consensus; Best possible care in Breast Cancer (BICE); GIPaM 2014].

Specify if the immunohistochemical evaluation is performed using:
- Image analysis techniques.
- Controls (of high intensity, low intensity and negative) of the proteins' expression on the slides.

The evaluation of hormone receptors (ER and PgR) in ductal carcinoma *in situ* is optional.

References

[1] Wolff AC, Hammond ME, Hicks DG, et al.; American Society of Clinical Oncology; College of American Pathologists. *Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update.* J Clin Oncol 2013;31:3997-4013.

[2] Rakha EA, Pigera M, Shaaban A, et al. *National guidelines and level of evidence: comments on some of the new recommendations in the American Society of Clinical Oncology and the College of American Pathologists human epidermal growth factor receptor 2 guidelines for breast cancer.* J Clin Oncol 2015;33:1301-2.

[3] Wolff AC, Hammond ME, Hicks DG, et al. *Reply to E.A. Rakha et al.* J Clin Oncol 2015;33:1302-4.

[4] Hammond ME, Hicks DG. *American Society of Clinical Oncology/College of American Pathologists Human Epidermal Growth Factor Receptor 2 Testing Clinical Practice Guideline upcoming modifications: proof that clinical practice guidelines are living documents.* Arch Pathol Lab Med 2015 Apr 17.

SENTINEL LYMPH NODE

According to SIAPEC-GIPaM Protocol, "sentinel" lymph node (SNL) is examined on paraffin sections conducted at intervals of 200 microns until depletion of the tissue, with microscopic examination conducted on 10 H&E-stained sections and by immunostaining (OPTIONAL) [n. #] with anti-pancytokeratin antibodies (clone #).

If the macroscopic reduction is conducted with 2 mm deep sections, for each section 10 cut layers will be obtained. At this point, two sections in parallel can be collected, one for H&E stain, the other as reserve for any further immunohistochemical staining (especially in case of lobular carcinoma, in doubtful cases or in case of unexpected lymphomas). On the whole, 10 H&E and 10 unstained sections will be obtained, with exhausted material. It is also possible to increase the number of unstained sections for each level.

The microscopic report should include:

- The total number of SNL received and examined.
- Macroscopic metastases, if present.
- The number of lymph nodes with metastatic disease.
- The amount of the metastatic lesion expressed in mm, particularly in case of micrometastases, and adopting the pN categories of AJCC 2010, VII edition.
- If several metastatic foci are found within a lymph node, the larger one should be taken into account. Using the categories of AJCC 2010, VII edition, the suffix (sn) should be used in case the nodal status is determined on the basis of the biopsy of the SNL only (that is, without axillary dissection).
- Specify the adopted protocol and whether the positivity was assessed only on the basis of the H&E, or using immunohistochemistry with antibodies anti-pancytokeratin.
- If present, isolated tumour cells (ITC) should be reported, but their systematic search is not recommended.

AXYLLARY LYMPH NODES

- Total number of examined lymph nodes: #
- Number of metastatic lymph nodes: #
- Extracapsular extension: #
- Lymph node Staging (pN according to AJCC 2010, VII edition): #

Reporting of microscopical diagnosis after neoadjuvant chemotherapy or primary systemic therapy (PST)

<u>On the needle biopsy, before neoadjuvant chemotherapy or PST, the report should specify</u>:

1. Number of diagnostic frustules
2. Histological typing
3. Histological grade (or, if it is not possible, the nuclear grade)
4. Presence or absence of vascular invasion
5. Presence of *in situ* carcinoma
6. ER, PgR, HER2 and Ki67
7. If a skin sample is present in the frustules, specify the

possible presence of infiltration, ulceration, dermal vascular invasion.

_._._._._._._._._._._._.

For surgical specimens after neoadjuvant chemotherapy or primary systemic therapy (PST) the diagnostic microscopic checklist is comparable to that for invasive carcinoma, with the following additions and indications:

Residual tumour size

In case of absence of pathological response to therapy, the size of the tumour and the histology result almost unchanged.
In case of partial pathologic response, histological changes may be of different degrees; most carcinomas show a reduced cellularity, with nests of neoplastic cells (more or less cellular) scattered in the tumoral bed. It often happens that the only residual tumour can be found in the lymph-vascular spaces, and this is an important finding, since it is associated with an increased risk of recurrence.
In case of a pathologic complete response the morphological picture is characterized by an oedematous connective tissue and high vascularization, with chronic inflammation and macrophage infiltration. In difficult cases, the use of immunohistochemical staining with anti-pancytokeratin antibodies is useful in differentiating histiocytes from any residual malignant epithelial cells.
Usually, carcinoma *in situ* appears more resistant to therapy, and foci can be encountered even in the absence of an invasive component.
If there are multiple invasive residual foci separated by loose fibro-elastotic or myxoid stroma with any presence of foci of necrosis, the size of the entire area affected by the residual neoplastic foci should be reported. If there are no residual foci of invasive carcinoma, the area of fibrous regression must be measured taking into account also the possible preoperative placement of markers. The use of anti-pancytocheratin antibodies can be useful to confirm the absence of residual tumour and a pathologic complete response (pCR).

Cellularity of the residual tumour

It is expressed as the percentage of neoplastic cells compared with fibrous tissue and it is evaluated by comparing the surgical post-PST sample with pre-PST pre-surgical biopsies.

If pre-PST biopsies are not available, cellularity can be expressed as a percentage of neoplastic cells compared to fibro-myxoid tissue evaluated in the surgical post-PST specimen.

For a detailed description and for the calculation of the residual cellularity, refer to the website:
http://www.mdanderson.org/breastcancer_RCB

Nodal status

Lymph nodes usually show large areas of fibrosis, which may be associated with foci of necrosis and abundant macrophage infiltrate. These histological changes are interpreted as the response of the metastatic disease, induced by therapy. However, it may happen that complete response in a metastatic lymph node doesn't leave any histological evidence of neoplasm.
Both the metastatic lymph nodes and those with areas of fibrosis or foci of necrosis, along with lymph nodes in which the two findings coexist, should be described and quantified. In cases where there is no evidence of residual neoplastic cells on the H&E slides, the use of anti-cytokeratins antibodies (AE1/AE3 or CAM 5.2) can disclose micrometastases or residual isolated tumour cells.

ER, PgR and Ki67 expression and HER2 status

Since no univocal scientific data are available on the modification in the expression of ER, PgR and Ki67 and on HER2 status after PST, these parameters must be determined again on residual tumour in the surgical sample after PST in case of partial response (pPR) or no response (pNR).

STAGING

The pathological report should be completed with the pathological staging according to the AJCC 2010, VII edition, adding the prefix "y" to pT and pN.

REPORT ON TUMOUR RESPONSE

Various grading systems to assess tumour response to neoadjuvant therapy or PST have been published, and there is currently no consensus on which has the best prognostic impact.

It is here reported the system proposed by **Pinder et al.** (*Pinder et al., Histopathology 2007; 50:409-417*), which has been adopted by the 2012 European Guidelines.

Tumour response

1. Complete pathological response to therapy, further divided into:
   i) absence of residual carcinoma;
   ii) absence of residual infiltrating carcinoma, but presence of *in situ* carcinoma.

2. Partial response to therapy, further divided into:
   i) minimal residual disease / near total effect (e.g. <10% of tumour remaining);
   ii) evidence of response to therapy, but residual disease equal to 10-50% of tumour remaining;
   iii) > 50% of tumour cellularity remains evident with areas of fibrosis, inflammation and macrophages with hemosiderin.

3. No evidence of response to therapy.

Response in the lymph nodes

1. No evidence of metastatic disease and no evidence of changes in the lymph node parenchyma.

2. No evidence of metastasis but evidence of response (fibrosis, inflammation, etc.) that indicates a down-staging linked to neo-adjuvant chemotherapy.

3. Presence of metastatic disease associated with alterations indicative of partial response to therapy.

4. Presence of metastatic disease not associated with alterations indicative of partial response to therapy.

References

[1] Lakhani SR, Ellis IO, Schnitt SJ, et al, eds. *WHO Classification of tumors of the breast.* Lyon: IARC Press 2012.

[2] *European guidelines for quality assurance in breast cancer screening diagnosis.* 4th ed. European Communities 2006.

[3] Edge S, Byrd DR, Compton CC, et al. *AJCC Cancer staging manual.* 7th ed. New York: Springer 2010.

[4] *Raccomandazioni AIFA/AIOM-SIAPEC 2010.*

[5] Elston CW, Ellis IO. *Pathological prognostic factors in breast cancer. The value of histological grade in breast cancer: experience from a large study with long-term follow-up.* Histopathology 1991;19:403-10.

[6] Pinder SE, Provenzano E, Earl H, et al. *Laboratory handling and histology reporting of breast specimens from patients who have received neoadjuvant chemotherapy.* Histopathology 2007;50:409-17.

IN SITU HYBRIDIZATION

BASED ON ASCO/CAP 2013 RECOMMENDATIONS

Currently licensed ISH techniques are: FISH, CISH and SISH with double probe (*HER2* gene and CEP17) or single probe (*HER2* gene only).
Pathologist has to guarantee the correspondence of all morphological, immunohistochemical and *in situ* hybridization data.
It is mandatory to participate in external quality control programs.

ISH: interpretation of results

Careful examination of the whole ISH sample to evaluate if different populations of cells, with different number of *HER2*/nucleus signals, are present; otherwise, a selection of potentially positive areas can be performed referring to immunohistochemical samples.
If ISH samples are homogenous, at least 20 cells for field need to be evaluated, on at least 2 fields of the invasive component of the carcinoma previously identified on H&E stained slides.

If two different populations of cells are identified, one of which showing an increased number of *HER2*/nucleus signals and being representative of more than 10% of the entire sample, a separate evaluation has to be performed, counting at least 20 cells of the population showing an increased number of signals. In case of heterogeneous neoplastic population, ISH testing has to be considered positive if the percentage of amplified cells is >10% of the neoplastic cells present on the examined slide. Data of both populations of cells, with percentages, must be reported.
NOTE: if overexpression/amplification is present in a percentage of neoplastic cells equal to or less than 10% of an inclusion, additional inclusions of the primary tumour and/or lymph node metastases should be tested.

POSITIVE *HER2* ISH CASES (DOUBLE PROBE):

- ***HER2*/CEP17 ratio ≥ 2.0**
  with mean *HER2*/nucleus signals ≥ 4.0
  with mean *HER2*/nucleus signals < 4.0

or

- ***HER2*/CEP17 ratio < 2.0**
  with mean *HER2*/nucleus signals ≥ 6.0

or

- **Heterogeneous case in which the amplified population** (nuclei with number of *HER2* signals ≥ 6.0) **is >10%** of the neoplastic cells present on the examined slide

POSITIVE *HER2* ISH CASES (SINGLE PROBE):

- ***mean HER2*/nucleus signals ≥ 6.0**

or

- **Heterogeneous case in which the amplified population** (nuclei with number of *HER2* signals ≥ 6.0) **is >10%** of the neoplastic cells present on the examined slide

NEGATIVE *HER2* ISH CASES (DOUBLE PROBE):

- ***HER2*/CEP17 ratio < 2.0**
  with mean *HER2*/nucleus signals < 4.0

NEGATIVE *HER2* ISH CASES (SINGLE PROBE):

- **mean *HER2*/nucleus signals < 4.0**

EQUIVOCAL *HER2* ISH CASES (DOUBLE PROBE)

- ***HER2*/CEP17 ratio < 2.0**
  with mean *HER2*/nucleus signals ≥ 4.0 and < 6.0

EQUIVOCAL *HER2* ISH CASES (SINGLE PROBE)

- **mean *HER2*/nucleus signals ≥ 4.0 and < 6.0**

NOTE:
In case of equivocal *HER2* ISH cases, both using double and single probe, it is necessary to perform further examinations:
- Reflex test 1: on the same sample, to perform ISH evaluation using alternative probes on chromosome 17.
- Reflex test 2: on the same sample, to perform HER2 IHC reactions, if they have not been previously performed.

OR

- New tests: if available, to perform ISH and/or IHC evaluations on a different sample (core biopsy, metastatic lymph node and/or metastasis sample) of the same patient.

If the additional ISH and/or IHC examinations are not useful to clarify the HER2 status, the case must be reported as EQUIVOCAL.
Equivocal cases, both at IHC and ISH examination, are the most critical: in this situation, oncologist is allowed to consider the use of anti-HER2 therapy. The final clinical decision has to be personalized on the basis of the characteristics of each patient and disease, and must be discussed and shared with every single patient.

INDETERMINATE *HER2* ISH CASES:

If it is not possible to evaluate as positive, negative or equivocal one or both of the tests (IHC and or ISH) performed on a tumoral sample, due to technical problems (inadequate fixation and/or sample processation, presence of crush artefacts or artefacts on the borders) or to the failure of analytic testing, such a case should be reported as "indeterminate".

References

[1] Wolff AC, Hammond ME, Hicks DG, et al. *Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update.* Arch Pathol Lab Med 2013;138:241-56.

[2] Consensus AIOM/SIAPEC 2014. *Best possible care in Breast Cancer (BICE).*

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Orru Sandra - *Cagliari*
Orsaria Maria - *Udine*
Palma Fabiola - *Bari*
Paglierani Milena - *Firenze*
Panarelli Marzia - *Taranto*
Paradiso Beatrice - *Ferrara*
Pastena Maria Irene - *Bari*
Pedriali Massimo - *Ferrara*
Perin Tiziana - *Aviano*
Perracchio Letizia - *Roma*
Pertoldi Barbara - *Alto Vicentino*
Petroni Stella - *Bari*
Pietribiasi Francesca - *Moncalieri*
Piubello Quirino - *Verona*
Poletti Alessandro - *Bassano*
Popescu Ondina - *Bari*
Pulcini Federica - *Roma Responsabile Pagina Web*
Querzoli Patrizia - *Ferrara*
Ravarino Alberto - *Cagliari*
Reale Domenico - *Rovigo*
Reghellin Daniela - *Vicenza*
Rizzo Antonio - *Castelfranco Veneto (TV)*
Romeo Francesco - *Cosenza*
Rossi Esther Diana - *Roma*
Roz Elena - *Palermo*
Sabò Claudio - *Lucca*
Salvi Sandra - *Genova*
Santinelli Alfredo - *Ancona*
Santini Donatella - *Bologna*
Santoro Angela - *Foggia*
Sapino Anna - *Torino*
Saragoni Luca - *Forlì*
Scelsi Mario - *Pavia*
Sebastio Anna - *Taranto*
Simone Gianni - *Bari*
Sironi Maria - *Chiavari*
Spina Donatella - *Siena*
Stumpo Michela - *Grosseto*
Tinacci Galliano - *Firenze*
Tozzini Silvia - *Pistoia*
Truglia Maria Caterina - *Prato*
Truini Mauro - *Genova*
Vezzosi Vania - *Firenze*
Viberti Laura - *Torino*
Villani Laura - *Pavia*
Visonà Alfonso - *Alto Vicentino*
Zanconati Fabrizio - *Trieste*
Zirillo Maurizio - *Alto Vicentino*