

## HISTOPATHOLOGICAL STANDARDS FOR BREAST CANCER

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### Summary

Diagnosis and treatment of breast cancer has progressed rapidly in recent 20 years. The diagnosis was first based on clinical appearance that changes after the introduction of mammography in the diagnosis. Development of radiological techniques has led to the detection of a small and non-palpable lesions, and surgeons are increasingly applied conserving procedures for breast cancer. Therefore, today is a very important multidisciplinary team in the treatment of patients with breast cancer. A pathologist is a key member of the multidisciplinary team who determines the prognostic and predictive factors for patients with breast cancer which require some standardized protocols and processing of tissue samples.

KEY WORDS: *breast cancer, pathology, prognostic and predictive factors*

### PATOHISTOLOŠKI STANDARDI ZA KARCINOM DOJKE

#### Sažetak

Dijagnostika i liječenje karcinoma dojke je posljednjih 20 godina vrlo brzo napredovalo. U prvo vrijeme dijagnoza se postavljala na temelju kliničkih nalaza što se promijenilo uvođenjem mamografije. Razvoj radioloških metoda doveo je do otkrivanja malih i nepalpabilnih lezija te su kirurzi počeli primjenjivati poštudne kirurške zahvate nakon kojih slijedi adjuvantno liječenje. Zbog toga je danas vrlo važan multidisciplinarni tim u liječenju bolesnica s karcinomom dojke. Patolog je ključni član multidisciplinarnog tima jer mora odrediti brojne prognostičke i prediktivne čimbenike za bolesnice s karcinomom dojke što sve zahtjeva odgovarajuće standardizirane protokole i postupke za uzorke tkiva.

KLJUČNE RIJEČI: *karcinom dojke, patologija, prognostički i prediktivni čimbenici*

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### INTRODUCTION

The diagnosis and treatment of breast cancer has rapidly evolved over the past 20 years. In the first part of the 20<sup>th</sup> century, treatment of breast cancer consisted of radical mastectomy, but adjuvant systemic treatment and adjuvant radiotherapy did not play a major role. Diagnosis of breast cancer was mostly made based on clinical presentation, later aided by mammography and often combined with frozen section pathology confirmation. Starting in the 1980s, there have been important alterations in the diagnosis and treatment

of breast cancer, having an important impact on the diagnostic procedure employed by pathologists.

Radiological techniques have greatly improved, and in addition, population-based mammography screening is increasingly offered, especially to women over 50 years of age. These developments have led to the detection of many small non-palpable lesions, including ductal carcinoma in situ.

Histopathological features play an important role in guiding the treatment decisions. In addition, genetic research is starting to have an in-

creasing impact on therapy by providing prognostic and predictive factors (1).

### PRE-TREATMENT DIAGNOSIS

A palpable mass is the most common clinical sign of invasive breast carcinoma, although skin retraction, nipple inversion, nipple discharge and less commonly, a change in the size or shape of the breast may still be found. Rarely, we can see a change in the colour or texture of the skin. All the symptoms of breast cancer may also be caused by benign breast disease. Therefore, diagnosis of breast lesions is based on clinical examination, radiology and pathology. When abnormalities are found, the diagnostic findings should be discussed among specialists from three disciplines involved in the diagnostic work-up: the surgeon, the radiologist and the pathologist. The evaluation with imaging and histological sampling with core biopsy (CB) or fine-needle aspiration cytology (FNAC) are indicated to establish a definitive diagnosis.

FNAC and CB have been extensively used for years in the diagnosis of breast lesions and have good sensitivity for the diagnosis of malignancy in palpable lesions (2). There is evidence that CB is more sensitive for the detection of impalpable lesions and is recommended for the evaluation of microcalcifications and FNAC findings that are suspicious (3). Experience to date has indicated that there is excellent correlation between the findings of CB with those of open biopsy. The use of strict diagnostic criteria, coupled with immunohistochemistry, is useful to avoid a misdiagnosis. However, a definitive diagnosis is not always possible and a classification that includes borderline categories such as 'suspicious', 'equivocal' or 'uncertain malignant potential' is useful in patient management (4). When a clearly distinguishable mass is present, two or three biopsies are usually sufficient to obtain a definite diagnosis. When the radiological/clinical finding is an architectural distortion or microcalcifications, more biopsies are usually required to obtain a certain diagnosis. Histological grade can be assessed on CB with about 70% of agreement with the grade determined in the surgical specimen (5). Both FNAC and CB can give an indication of histological type, but neither is definitive because of the

existence of tumours with mixed types and heterogeneity of tumour. Estrogen receptor status and HER-2 status can be reliably assessed on CB, with agreements of about 98-99%. The analysis of prognostic and predictive factors using FNAC should be limited to cases of distant metastasis and cases with no available tissue material. Ultrasound-guided CB or FNAC of axillary lymph nodes can be helpful in the management of invasive carcinoma, for the preoperative diagnosis of metastases (6). The B ( B1-B5) category system is applied to tissue samples of the primary breast cancer obtained by CB. Pathologists need to be highly qualified and experienced in breast diseases as they are key players in interdisciplinary communication and decision-making (7).

### EXAMINATION OF SURGICAL SPECIMEN CONTAINING NONINVASIVE BREAST CANCER

Knowledge of the lobular architecture of the mammary gland is a prerequisite for understanding noninvasive ductal cancer (DCIS). DCIS takes its origin from the terminal duct lobular unit (TDLU), starting with distension of the ductular structure and unfolding of lobules by the proliferating tumor cells. Further expansion then leads to the involvement of the extralobular ductal system. The ductal-lobular system forms segments from the nipple to the periphery, which appear to be pyramid-shaped with peak pointing towards the nipple (8). Therefore, these segments do not follow the geometry of the artificial system of quadrants. DCIS most frequently occurs within one segment, very rarely foci are found in clearly different segments. Radiological microcalcifications are the only clue for detection of DCIS, but can also be detected as incidental finding showing no microcalcifications (8). The pathologist must rely on radiologically detected microcalcifications to identify areas suspicious for DCIS. The pathologist must: find a lesion, to determine the size of the lesion and the most important condition of the resection margins. Therefore, immediately after removing the surgical specimen the surgeon should mark the margins (lateral – long stitch, medial- medium stitch, superior- 2 short stitches) and then places the excision specimen on a foil with schematic drawing of breast outlines fixed the specimen

with cannulas. The radiologist then performs specimen radiography and marks the margins of the microcalcification area with several pins. This marking for the pathologist is done independently of the preoperative marking for surgeon. Marking the surface of excision tissue is essential for microscopic identification of the original resection margins. Therefore, pathologist should paint the specimen surface with marker that adhere to the tissue during fixation, dehydration, embedding, cutting and staining and must be visible at microscopy. The specimen should be serially sectioned from mamillary pole to the periphery of the segment (9). Standardized sampling enables the pathologist to identify all lesions and determine their size. Size of breast carcinoma is the most important factor for management of patients eligible for breast-conserving therapy. Data on the margins give highly reliable information about residual tumors in the event of re-excisions. Immunohistochemically, the pathologist must determine the status of estrogen and progesterone receptors.

Intraoperative frozen section examinations can only be justified in two situations. Firstly, in the case when an invasive tumor was detected neither by mammography nor by ultrasound. Secondly, when DCIS is centrally located and when is operation with preservation of the nipple.

The pathologist responsibilities include assessment of suspicious findings, participation in preoperative therapy planning for carcinomas, intra and postoperative diagnostics as well as quality assurance.

#### **EXAMINATION OF SURGICAL SPECIMEN CONTAINING INVASIVE BREAST CANCER**

Nowadays, 60-70% of patients undergo breast-conserving therapy. Standardized work up of surgical specimens, obtained as part of breast-conserving therapy, is extremely important. The surgeon should mark the excision specimen so that the pathologist can reconstruct the orientation of the resection margins ( lateral – long stitch, medial - medium stitch, superior- 2 short stitches). The resection margins should be marked with ink by a pathologist. After this procedure, the pathologist must measure the size of the tissue sample in three dimension, determine the dimensions of the skin if it is present, to determine the size of the

tumor in three dimensions and must record the localization wire if present in cases of small tumours (10).

To obtain optimal morphology in the histology sections, and to obtain optimal immunohistochemical staining results, the resection specimen should be cut into thin slices immediately after surgery. For microscopic examination the pathologist should be obtained and processed for paraffin sections full diameter of the tumour and its surroundings, small part of the tumour to perform immunohistochemistry, if there are macroscopical or radiological abnormalities in the tissue surrounding the invasive tumour, these areas should be sampled. If the surrounding tissue is without abnormalities, it is necessary to take at least two sections from macroscopically normal breast tissue.

On slides stained with hematoxylin eosin (HE), pathologist must determine the prognostic and predictive factors for breast cancer. This includes the histological type of cancer (11), the degree of tumour differentiation (12), mitotic counts, lymphovascular invasion, estrogen and progesterone receptors (13), protein HER-2 (14) and proliferative index Ki-67 (15).

Receptors are determined by immunohistochemistry and the results are expressed as the percentage of positive cells and intensity of staining. Staining for estrogen and progesterone receptor is always nuclear in localization and in most institutes all patients with a tumour in which more than 10% or more 1% of the tumour cells show positive staining regardless of the intensity of staining are candidates for adjuvant hormonal therapy. According to the consensus of the St Gallen 2014. cut-off of the progesterone receptors is 20%. This value best separating luminal type A from luminal type B breast cancer. Values below 20% indicate that the progesterone receptors are negative or low<sup>16</sup>. When negative staining for estrogen and/or progesterone receptor is seen, it is important to confirm that staining of the hormone receptor-negative case has been successful. This can usually be tested, since the majority of normal breast tissues contain some nuclei ducts and lobules that are positive for estrogen and progesterone receptor. If no normal breast epithelial cells are found to show positive staining, the hormone receptor assays should be repeated on another tumour block.

HER-2 gene amplification is observed in 15-30% of invasive breast cancers and leads to HER-2 receptor overexpression. HER-2 positive invasive breast cancers respond favourable to therapies that specifically target the HER-2 protein, therefore it is very important today to identify candidates for this type of targeted therapy. Several technologies are available for determining HER-2 status, but the two most commonly used are immunohistochemistry (IHC), which measures HER-2 protein expression, and CISH (chromogen in situ hybridisation) which detects HER-2 gene amplification a method that is often used today in the pathology than FISH (fluorescence in situ hybridisation). The interpretation of the results is based on the intensity and percentage of stained cells. The most commonly used score system is 0, 1+ (negative results) , 2+ and 3+ (positive results). A 2+ is considered equivocal and should be followed by retesting by CISH. Women with IHC 3+ tumours are candidates for therapy with trastuzumab, but women with 2+ tumour should be retested and if the results show amplification of gene of those are candidates for trastuzumab. To ensure the highest possible accuracy, pathology centers must standardise methodologies and testing procedures.

Proliferative index is also very important and is determined by immunohistochemistry by monoclonal antibody Ki-67. Positive reaction is nuclear reaction and are counted positive nuclei in 1000 tumour cells on the high magnification and the results obtained is expressed as a percentage of positive nuclei. According to St Gallen consensus cut of value is 20% of positive cells, which means that below this value is low and value above 20% is high proliferative index (16).

Based on the receptors, HER-2 status and proliferative index breast cancers are classified immunophenotypically into five subgroups: luminal type A, luminal type B HER-2 negative, luminal type B Her-2 positive, HER-2 positive ( non-luminal type) and triple negative tumours. Based on the immunophenotype of the cancer patients receive appropriate therapy. The multi-gene testing remains inaccessible for the majority of women with early breast cancer, therefore is adopted clinico-pathological testing, now expressed in surrogate IHC-based classification.

In the widest sense, post-therapy effects include morphological and biological alterations in

cancer and normal tissue after any treatment. The patients have no or little response, and the majority has a partial response to therapy. The extent of this response is associated with outcome. Identifying stage after treatment is important and provides additional prognostic information. Histopathologically, then staging a label y. The response in lymph nodes has more prognostic importance than does response in the breast. Small metastases after treatment, including isolated tumour cells, are representative of an incomplete pathological response (17).

## EXAMINATION OF AXILLARY LYMPH NODES

### Sentinel node biopsy

One to three sentinel node biopsies are obtained from axilla. In some institutes, frozen section evaluation of the sentinel node biopsy is performed, but many institutes prefer evaluation on paraffin section. There is agreement that one hematoxylin and eosin (HE) stained section of the sentinel node should always be evaluated. At various levels of the sentinel node is recommended to do immunohistochemistry with antibodies directed against keratin (pancytokeratin, cytokeratins of low molecular weight and cytokeratins of high molecular weight). The size of lymph node metastasis should be categorized as follows:

>2 mm = a macrometastasis

0.2-2 mm = a micrometastasis

< 0.2 mm = isolated tumour cells (the tumour cells can often only be detected by immunohistochemistry)

### Axillary dissection

When an axillary dissection is performed, fatty tissue is removed which contains the lymph nodes. The pathologist should carefully identify all the lymph node that are present in the resection specimen, and each of these lymph nodes should be embedded for histological examination. Lymph nodes up to 1cm can be totally embedded, larger lymph nodes should be bisected or lamelated and fully embedded. The number of lymph nodes containing metastases should be recorded. In addition, the diameter of the largest lymph node metastasis should be recorded and the invasion of the capsule.

## CONCLUSION

Numerous studies in recent years have identified many prognostic and predictive factors for breast cancer. Most of them determined pathohistologically, which resulted in a large responsibility for pathologists. In addition, the pathologist has become a key person in a multidisciplinary team of breast cancer and the key person in implementation of specific individual therapy.

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