GENERAL REVIEW

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Large-format histology in diagnosing breast carcinoma

Histološka tehnika velikog formata u dijagnostici karcinoma dojke

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Introduction

Breast cancer comprises a remarkably diverse group of diseases in terms of morphology, molecular phenotype, clinical and radiological manifestations, and response to therapy. Management of breast cancer patients is based on prognostic and predictive parameters, which are essential for therapy planning. Key prognostic parameters are tumour size, histological grade, histological type, lymph node status, lymphovascular invasion, and presence or absence of distant metastases [1, 2]. In addition, a few next-generation prognostic parameters have been introduced into routine practice, of which the status of oestrogen and progesterone receptors (ER and PR, respectively), human epidermal growth factor receptor 2 (HER2/neu), and proliferation of cancer cells are the most prominent [3, 4]. Whereas steroid receptors, HER2 status, and proliferative activity are the major parameters for oncological therapy planning, breast cancer subgross morphological parameters such as tumour size, disease extent, and lesion distribution are essential for planning tailor-made surgery and radiation therapy [5, 9].

While breast cancer subgross morphological parameters can be determine with both pathological and radiological methods separately, the most effective is combination of these methods in the form of radiological-pathological correlation [9, 10]. The small block histology, which is the standard pathohistological method, is based on taking 1–2 cm sized representative tissue samples from breast specimens, which are selected under the control of only a pathologist's naked eye and, sometimes, using radiological marks. These samples represent selected pieces of the specimen, and interrelations of different tumour components, which are not present in the same block, are destroyed. Even though the examination of small standard histological samples enables precise determination of the type, grade, and hormone receptor status of the tumour, as well as detection of other molecular markers, this analysis may lack the adequate correlation with the radiological image [9–12].

Opposed to standard small sections, large-format histology is based on embedding and processing continuous tissue slices representing the entire cross-section of a quadrantectomy specimen that includes not only the tumour but also surrounding tissues together in one plane [9]. At the same time, this technique retains the advantages of standard sections and fulfils the requirements of multidisciplinary team needs for therapy planning. Large-section method is considered the most adequate modern diagnostical procedure in breast pathology [9, 13–18]. Some of the opponents of large-format histology state the costs of such technique as the principal argument against it. Recent cost-benefit analyses have shown that the large-format technique is less expensive in daily routine use than the conventional small block technique demonstrating equal tissue surface. The time needed for final pathology report is two weeks at average, which satisfy needs of medical oncologists as it does not prolong the time till adjuvant therapy or change therapeutic options for patients [17, 19].

Methodological and technical aspects

The large-format histology technique has a long history, but only with the introduction of mammography screening, its importance has been realized, since this method allows precise access to all mammographically detected lesions [16, 18, 20–22]. Large-section method is a routine technique in a several

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pathology centres worldwide. This technique has been incorporated in the everyday practice at our institution, and the procedure has been successfully used to study neoplasms affecting various organs since 2011. Technical aspects of obtaining large-sections have been previously described in detail [23–25].

Gross examination of the specimen

In order to obtain an appropriate large-format histology section it is necessary to carefully plan the cut-up of the specimen. The size of the specimen (quadrant resection or mastectomy) and the type of the lesions (microcalcifications, solitary or multiple tumours) determine the way the unfixed specimen is processed.

A pathologist needs to have access to the specimen radiogram, which shows the location of abnormalities in the specimen. The specimen should be delivered to the pathology laboratory properly oriented in a way that enables correct determination of each surgical margin. The specimen radiogram helps a pathologist to map out a way of cutting-up that will include the entire lesion and surrounding tissue in a single cross-section. The specimen is macroscopically described and measured with special indication of present suturing and other markers, if any [22].

In cases where a surgical specimen have only radiologically detected microcalcifications or a non-palpable tumour, the cut-up depends entirely on mammographic findings. It is recommended that specimens should be cut horizontally, in the plane of the specimen radiogram, parallel to the skin and pectoral muscle. This type of cutting is recommended even in cases of solitary tumour masses that are clearly-defined. Multiple tumours are more difficult to obtain in a single large-format section, and in those cases the way of cutting must be selected on the basis of palpation findings of a surgical specimen and findings of the mammogram [22].

The thickness of obtained slices should amount to approximately 3–4 mm. All slices are examined macroscopically and visible tumours are measured in millimetres and described. At the same time, the relation between the tumour and margins is defined [22–25].

Radiologist's markings on images help a pathologist in selecting representative tissue slices for further processing. After the formation of large tissue sections, the position of the selected section is marked on the radiogram, and at the same time, margins of the specimen are inked.

The recommended number of selected tissue slices is 2–4 *per* case. In addition to the formation of large specimens, a small tissue sample is usually taken for immunohistochemical and molecular analyses, but the most representative large sections must remain intact. If a tumour is smaller than 1 cm in the largest diameter, small slices should not be taken.

Slicing of mastectomy specimens for large-section histology is different for two reasons: dimensions of the slices are usually larger than dimensions of available large-format glass slides, and – more importantly – the dorsal resection margin, corresponding most often to the pectoral fascia (not the circumferential as in quadrant-resection) is the only important one. Therefore, the large-format section must show the dorsal surgical margin, so the specimen is sliced perpendicular to the skin. In this case, a pathologist must bear in mind that the radiogram of the entire specimen and the second specimen radiogram are taken in two different planes [22].

Tissue processing

The selected tissue slices are processed in the laboratory, using specific protocols for large-format histology. Process itself requires appropriate equipment and techniques adapted for handling large slides. Fixation is performed in formaldehyde solution for 24 hours as standard. The processing (dehydration) of large tissue sections is performed conventionally in any commercially available automated tissue processor for 22 hours. After the processing is completed, paraffin blocks are made using metal brackets placed on a glass plate. Cutting is performed on a special macrotome. The sections, 3-4 μm thick, are placed on commercially available large microscope slides, dimensions 12x9 cm. Staining is performed in modified slide racks, in the same automated stainer as for the small-block sections. Before archiving, the large sections must be properly dried in a well ventilated room, for 2–3 months [23–25].

Morphological prognostic parameters – interpretation of findings in large section histology

Many of the prognostic parameters used in assessing breast carcinomas are based on precise histopathological examination of specimens. It has also been shown that large-format sections are effective in the everyday breast routine practice for detailed evaluation of the size, extent, and distribution of the tumour, as well as the resection margins [9, 16, 26–29]. Foster et al. [26] demonstrated that large-sections gave more information than conventionally small blocks in 172 out of 656 cases, as they documented with additional findings of clinical significance, as minute multiple foci of carcinoma, involved margins, or change in size and extent of the disease [18, 26].

Correct tumour size measurement is very important and can be precisely determined on large format histology [16]. Foschini et al. [18] compared the tumour size of 102 consecutive quadrantectomies analyzed with both large-sections and standard small blocks. In 8.8% (9/102) of the cases, large-sections helped to definitely determine the size of the tumour better than conventional blocks, especially in invasive lobular carcinoma, where macroscopic borders of the tumour were ill defined and difficult to be measured at macroscopic level only [18]. Jackson et al. [16] demonstrated that the tumour size measured on large-format histology was larger at average than that measured with small blocks which was corresponding to the large-section size in only 63% of the cases in this series. In addition, ductal carcinoma *in situ* (DCIS) was found more frequently in association with invasive carcinomas in cases documented with large-sections than in cases assessed using conventional histological method.

If the surgical specimen is dissected into smaller pieces while grossing, the interrelation of multiple lesions is compromised, and the assessment of the extent of the tumour

disease becomes impossible. The extent of the disease is defined as the area including all invasive and *in situ* tumour foci, and is a robust prognostic parameter on its own. Tumours with limited extent have an extent less than 4 cm in largest dimension. Patients with this type of tumours are candidates for breast conserving surgery, as opposed to extensive breast carcinomas, where distance between tumour foci is greater, and often require mastectomy [30, 31]. Another important prognostic parameter is the distribution of breast carcinoma foci, which can be unifocal, multifocal and diffuse (Figures 1–3). *In situ* breast carcinomas, much like invasive carcinomas, show unifocal, multifocal and diffuse distribution [22, 32, 33].

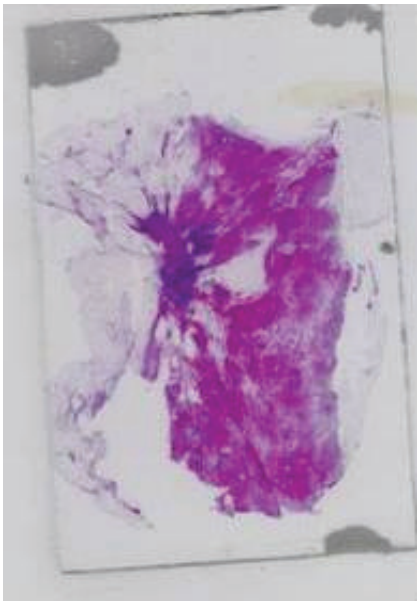

Fig. 1 – Large-format histology section of an unifocal breast carcinoma.

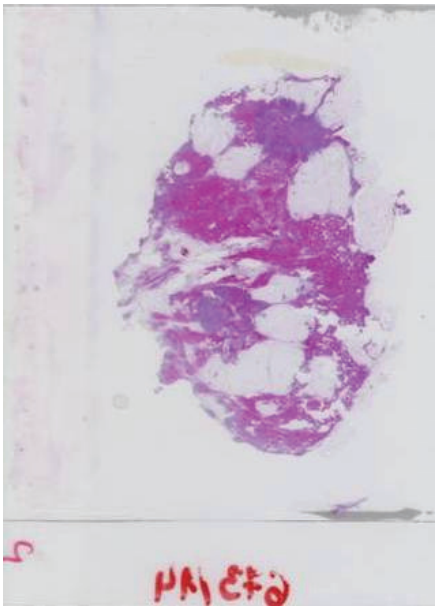

Fig. 2 – Large-format histology section of a multifocal breast carcinoma.

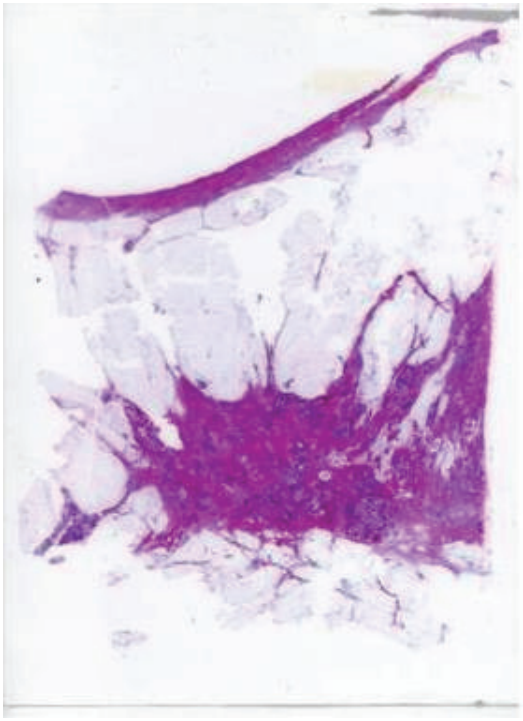

Fig. 3 – Large-format histology section of a diffuse breast carcinoma.

In everyday practice, pathologists should begin the gross examination of a specimen by summarizing radiological findings including the radiological disease extent.

For precise determination of the disease extent, the slice with the greatest disease area should be chosen and processed. Analysis of large-section slides begins on the peripheral part and is then directed towards the central area of the sections [22]. The most peripheral malignant lesions in the sample are marked. In order to properly determine the extent of the disease, a pathologist must summarize all findings related to the specimens and reconstruct the lesions as a whole. After this step the distribution of malignant lesions is assessed in the described area (*in situ* component and invasive tumour foci are identified).

The proportions of multifocal breast cancer cases vary among studies, depending on definition and methodology of assessment. According to large-format histopathology studies, multifocality of the invasive component occurs in ~35% of breast tumours [9, 30, 34]. Modern breast imaging techniques also support these results [35]. Several studies performed on large-sections have shown the prognostic value of the extent and distribution of lesions in breast carcinomas, as the metastatic capacity is higher in patients with multifocal and diffuse tumours when compared to those with unifocal carcinomas [36, 37]. Pekar et al. [34] using large-sections, have demonstrated that multifocality and diffuse distribution of invasive tumours were associated with significantly poorer survival in breast cancer patients compared to those with unifocal tumour disease.

The goal of histopathological examination of specimens resected after neoadjuvant chemotherapy is the detection of either the residual viable tumour or documenting the presence of the tumour bed. Large-format technique is especially

useful in the evaluation of post-neoadjuvant therapy surgical specimens with complete or near-complete response to therapy since these types of lesions are usually not visible on gross examination**.** Thus, residual cancer foci of small size may remain undetected on conventional blocks. The use of large-sections improves the accuracy of the assessment and increases the chance to detect even the smallest residual tumour foci [18, 38].

Precise evaluation of resection margins has become a very important issue especially in quadrantectomy cases. Standard blocking is based on gross inspection of the lesion and on palpation of the tissue. Small tumour foci may remain undetected during this examination. Large-format histology samples are ideal for determining the circumferential resection margins. Superficial and deep resection margins (close to the skin and close to the pectoral muscle, respectively) are not displayed directly due to the recommended way of slicing the specimen obtained with conservative surgery. The absence of any radiological or gross abnormalities in the first and the last horizontal slice is useful evidence of sufficient radicality in these directions. If one or both slices include suspicious macroscopic or radiological abnormalities, it is necessary to take small samples from that zone, based on which the status of margins over and under the tumor will be determined. Clarke et al. [25] reported that the large-sections method is more sensitive than conventional method for identifying positive margins or multifocal tumour disease in breast quadrantectomy specimens. The use of large-sections also helps distinguishing between the real inked margin and migration of ink through tissue clefts and fissures in the specimen surface [30].

Breast carcinoma is not necessarily composed of a identical monoclonal cells, but may represent a population of diverse tumour cell clones. This may result in different morphology in various parts of a tumour focus (intratumoural heterogeneity), or varying morphology of different tumour foci within the same breast (intertumoural heterogeneity). Heterogeneity is of the greatest importance when interpreting biomarkers, especially HER2 status, because many invasive breast cancers contain at the same time cells with and without HER2 amplification. Large-format histology allows a simple insight to inter- and intratumoural heterogeneity.

All cases of breast cancer documented with large-format histology should be regularly analysed at multidisciplinary meetings of pathologists, radiologists and surgeons, using an overhead projector [39].

Oncology Institute of Vojvodina experience with large-format histology

Large-format histology has been introduced in routine use atthe Oncology Institute of Vojvodina in 2011, and since then it has become an integral part of our diagnostics protocols. Our six-year results with diagnostics of breast carcinoma are shown in Table 1.

In some of the cases, the use of large-format histology has led to detection of pathological changes that were undetected prior to surgical intervention and pathological work-up (Figure 4). Also, since the growth pattern of invasive carcinoma is sometimes infiltrative without clearly visible borders, macroscopic tumour size needed a substantial correction after analysis of large-format slides, which was in accordance to the observations of Foster et al. [26]. In our experience, tumour multifocality is easy to detect on large sections; its incidence in our series is comparable with that of other reported series. The status of the surgical margins is also accurately defined in large sections [9, 30, 40].

Table 1
Six year experience with large-format histology at the Institute for Oncology of Vojvodina

Demographics	Values
Total number of patients (n)	289
Average age of patients (years)	55.04
Neoplastic lesions (n)	215
Non-neoplastic lesions (n)	74
Large-format slides (n)	520
Large-format slides *per* patient (n)	1.78
Tumour distribution (n)	
unifocal	92
multifocal	73
diffuse	50
Histological type (n)	
ductal carcinoma in situ	41
invasive carcinoma NST	122
only invasive carcinoma	36
invasive and in situ carcinoma	86
lobular	28
only invasive carcinoma	14
invasive and *in situ* carcinoma	14
mixed	8
papillary	7
mucinous	4
tubular	2
metaplastic	2
medullary	1
Complete response to NAT(n)	4
Histological grade (n)	
1	46
2	114
e 3	55
T stage (TNM) (n)	
T0	3
Tis	39
T1	82
T1mi	1
T1a	20
T1b	22
T1c	39
T2	60
T3	22
T4	8

***NST – no special type; †NAT – neoadjuvant therapy; TNM – tumor, node, metastasis.**

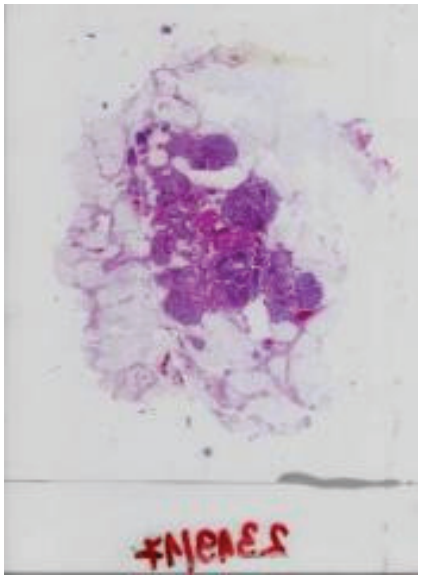

Fig. 4 – Large-format histology section of an accidental finding of submilimeter invasive breast carcinoma surrounded with diffuse *in situ* intracystic papillary carcinoma.

Large-format histology is a histo-technical method that allows pathologists to correctly identify the important morphological prognostic factors such as tumour size, the extent of the disease, the distribution of lesions, inter- and intratumoural heterogeneity, and the status of the circumferential surgical margin, while all of these parameters can be directly compared with radiograms. This technique is especially useful in the diagnosis of i*n situ* and early invasive carcinomas, as well as post-neoadjuvant therapy surgical specimens with complete or near-complete response to therapy, since these types of lesions are usually not visible while sampling the tissues. The too often expressed criticism regarding the presumed high costs and prolonged laboratory turn-around time due to this technique is not justified. Our experience and the growing body of scientific evidence show that it is the only cost-effective histo-technical method that meets the needs of modern multidisciplinary diagnostic approach in breast pathology. We propose including large-format histopathology into work-up of all breast surgical specimens as the standard method.

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