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Pathomorphological assessment of tissue material after pre-operative systemic therapy (neoadjuvant therapy) in patients with breast cancer

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ABSTRACT

Oncological management of patients with breast cancer, with the use of pre-operative systemic therapy in the last decade presented significant increase of effectiveness. The greater the number of cases with complete or major pathomorphological response means that getting the right material for postoperative histopathological assessment is becoming more and more difficult. In addition, the demonstrated correlation between complete pathomorphological response (pCR) and long-term treatment effects (in HER2-positive and triple-negative subtypes of breast carcinoma) makes the standardisation of postoperative pathomorphological examination, both at the gross and microscopic level, a necessity. This article presents the recommended rules for the preparation of such material and the method of its reporting, corresponding to the needs of contemporary oncology.

Key words: pathology, gross pathology, neoadjuvant therapy, pathology report

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Introduction

Due to the increasingly common introduction of neoadjuvant therapy, it has become necessary to modify the method of postoperative material evaluation. Recent publications have shown that the response rate to hormone treatment and/or chemotherapy before surgery is an important predictor of disease-free survival (DFS) [of note, currently the US Food and Drug Administration (FDA) prefers the term “event-free survival” (EFS)] and overall survival (OS). This was considered in the new, currently applicable and generally available materials, such as material assessment paradigms according to the College of American Pathologists (colloquially known as CAP protocols [1]). In the latest materials published in February this year, it was indicated that, due to the systemic treatment used prior to breast resection, the pT0 and pTis categories (ductal carcinoma *in situ*, DCIS) are reserved exclusively for the aforementioned treat-

ment (i.e. neoadjuvant therapy). It means that to describe the status in patients diagnosed with breast cancer, who were then subjected to systemic treatment, and in whom the finally delivered postoperative material could not be found (pCR, pathologic complete response), the category ypT0N0 or ypTisN0 should be used. This wording is currently dedicated to cancers in complete remission after neoadjuvant therapy, and in whom no invasive cancer can be revealed either in breast or in lymph nodes. According to the current interpretation of this classification, the description of ypTX IS NOT ALLOWED to be used in the discussed case.

The need to develop a unified protocol for the assessment of postoperative material from breast cancer patients undergoing neoadjuvant treatment resulted from many previous publications (meta-analysis [2]). In addition, new technologies are now being exploited (using information technology and statistical tools in the form of “machine learning”) to combine many variables

evaluated on baseline magnetic resonance images with features of postoperative material — especially in patients with pCR [3]. Nevertheless, the most precise and consistent recommendations regarding the pathomorphological description in the case of pCR are presented in the publication by Bossuyt et al. [4].

Principles of proceeding

The source of material after systemic treatment (after chemotherapy or hormonotherapy) is currently a tumourectomy (in form of so-called small or large lumpectomy) or mastectomy with a sentinel node procedure or lymphadenectomy. In the case of post-treatment reduction of tumour size in imaging tests, and thus a decrease of clinical stage (primary tumour and lymph nodes shrinkage), surgery may be performed in accordance with the “new” clinical stage. Therefore, more and more often organ-sparing surgery (tumourectomy and sentinel node procedure) can be used following the systemic treatment.

Patients undergoing pre-operative treatment may harbour several groups of breast cancers:

- infiltrating locally advanced breast cancers:
 - breast cancers with lymph node metastases,
 - inflammatory cancers,
 - cancers with breast skin infiltration;
- cancers belonging to subtypes of known aggressive clinical course:
 - triple-negative cancers,
 - HER2-positive cancers (nonluminal),
 - luminal B cancers (HER2-positive).

In these situations, chemotherapy and, if justified, targeted therapy is used. In recent years, hormone therapy has also been used in selected cases of luminal carcinomas.

There are three reasons to use preoperative treatment. The first is the proven benefit found in clinical trials in term of DFS and OS prolongation in locally advanced breast cancer and selected subtypes of breast cancer regardless of clinical stage. The second reason is the possibility of using surgical treatment in initially inoperable cases. The third is the chance to use conservative treatment in patients with the initial indication for mastectomy.

The effect of systemic treatment (including chemotherapy, targeted therapy, and hormone therapy) may be:

- complete pathologic response (cPR);
- partial pathologic response (pPR);
- no pathologic response (nPR) or disease progression.

The macroscopic picture after treatment can therefore be presented in the form:

- total tumour macroscopic regression, where it is impossible even to find the tumour site;
- absence of a tumour (cancer histology) with more or less visible residual lesions (e.g. fibrosis, necrosis foci);

- tumour (cancer histology) with accompanying residual lesions;
- tumour (cancer histology) without any changes after treatment.

This situation means that it is necessary for the pathologist collecting the material to have information about pre-treatment tumour characteristics (tumour size and location [e.g. quadrant]). The original tumour area is referred to as the tumour bed. Such information is available only in the case of close cooperation between teams of radiologists, surgeons, pathomorphologists, and clinical/medical oncologists. Without proper documentation (marking) of tumour size before treatment, it is not possible to determine the degree of tumour response to treatment after completion of neoadjuvant therapy and possible prognosis regarding further course of the disease. Presence of the tumour has to be confirmed before treatment in tissue material assessment (e.g. core needle biopsy) with a full panel of required factors (ER, PgR, HER2, Ki67), and in the presence of metastases in the lymph nodes it should be confirmed with fine needle biopsy. Therefore, it is important to understand the assessment principles of all the above-mentioned specialists.

In the last decade, the results of clinical trials have been published showing that the pathomorphological intensification of pre-operative systemic treatment effects correlate with the long-term clinical outcomes. Triple-negative and HER2-positive breast cancer patients with cPR have longer asymptomatic and overall survival. The consequence of this situation is the recognition of cPR as a surrogate endpoint in clinical trials. This means that when comparing a selected new drug with an old one, according to the currently applied principles of the evaluation of the effectiveness of the therapy, it is not necessary to observe patients for many years in a clinical trial in order to determine the superiority of the use of a new drug. Comparison of cPR rates can replace such long-term observation. The effects may include faster drug registration and lower costs of clinical trials.

A reliable assessment of response to systemic treatment is currently one of the main criteria for its effectiveness in patients, including clinical trials.

Pathomorphological management of tissue material after systemic treatment in patients with invasive breast cancer

Preparation and collection of tissue material

Biopsy material

Preparation and collection of tissue material is not different from that used in other cases of core needle

or surgery biopsy. The microscopic evaluation should be carried out as described later in this paper. The collection of material from breast cancer during systemic treatment is the method of assessing therapeutic response, providing the possibility to modify or change the treatment schedule in the absence of histopathological features of response. This assessment allows the comparison of treatment responses in individual patients. Indications for the use of a specific treatment do not always translate into a reduction of tumour size and cellularity as well as a reduction in the mitotic index or proliferation index Ki67 in tumour cells. A comparison of these clinical and pathomorphological indices enables the identification of patients in whom a change in treatment plan would be justified.

Postoperative material

General recommendations for postoperative material

1. In invasive breast cancer it should be clearly indicated on referral that the material after systemic treatment belongs to this group.
2. Fixation of the material and its initial preparation (cross-sectioning) follow a routine procedure.
3. After material fixation (24–72 h after the surgery) it is recommended to collect and describe the specimens:
 - from the entire largest cross-section of the tumour bed (the tumour bed is the primary area occupied by breast cancer prior to systemic treatment);
 - from the tumour after treatment (if it is clearly visible macroscopically), typically it is recommended to take at least one specimen for every centimetre of the largest tumour dimension — at least two (up to five). The area of the tumour bed may or may not overlap with the tumour area after treatment. The cancer tissue may reduce its diameter or dilute the cellularity with preserved diameter, or an uneven tumour disappearance can occur manifesting itself in pseudo-multifocality in postoperative image.

If lymph nodes are collected after systemic therapy, the principle of taking all lymph nodes according to general rules is applied. This means that all lymph nodes found during pathology processing are taken. They are placed in separate baskets, after cutting them into 2–3-mm-thick slices (for lymph nodes). The exception are lymph nodes over 1 cm with macroscopically visible macrometastases, which can be described macroscopically and taken only partially. This collection scheme applies to both sentinel lymph nodes (taken before and after systemic treatment) as well as lymph nodes after lymphadenectomy. In addition, attention should be paid to fibrosis focuses and the presence of resorption features in adipose tissue of the axillary cavity accompanying the lymph nodes and to collect specimens from such sites.

The varying degree of response to the treatment makes the precise determination of the tumour bed difficult in some cases. In such situations:

- one should reach the description or radiological image (location of the lesion, its size before treatment, number of tumours, their shape);
- one should find a metal marker if it was implemented during a diagnostic biopsy — it allows localisation of the primary tumour site.

Detailed recommendations for postoperative material

1. Evaluation of the tumour bed size and collection of specimens from the tumour bed

After neoadjuvant treatment, the tumour bed is a macroscopically indistinctly bounded, fibrous, often elastic area, instead of clear, solid tumour. A tumour bed can be more easily identified by the combined use of visual assessment and palpation of tissue slices than by visual inspection alone. Therefore, it is important that the pathologist conducts a careful macroscopic examination of the tissues and correlates these results with radiological images (or their descriptions) and the history of the disease to find the primary tumour location.

Macroscopically, the tumour bed should be measured and described in three dimensions. Any additional lesions in the breast should also be measured and described in three dimensions, with an estimated location and measurement of their distance from the main tumour bed. The distance of each tumour bed from the surgical margins should also be reported.

It should be noted that the residual tumour bed may have poorly visible borders, and rather recognise neighbouring “satellite tumours” as part of the main tumour, unless they are distant from it by more than 0.5 cm or form a distinctly separate tumour. Even then, however, the histopathological specimens should be taken from the tissues that separate the tumours, to exclude their connection to the main tumour.

Imaging examinations (e.g. mammography images) or digital photographs of the material constitute an important documentation of macroscopic results and increase the accuracy of specimen collection for histopathological evaluation. They can be very helpful for a pathologist if they are used as a “map” for a macroscopic description and indicate the place of origin of each specimen prepared for histopathological examination. Moreover, the detailed mapping allows more accurate examination of residual disease presence, measurement of tumour size, and residual tumour assessment, including the evaluation of tumour cellularity.

The postoperative material should be dissected as thinly as possible into 3–5-mm-thick slices (material from the breast).

Note: In some international recommendations it is indicated that dissected material should then be

subjected to a radiological examination (mammography) with a radiographic evaluation of the images to determine the presence/extent of the residual disease. The pathologist should examine the sample visually and palpatively to identify suspicious areas and proximity of the margins, and correlate these results with radiological findings. The result of pathological and radiographic examination should be discussed with the surgeon regarding the radicality of tissue removal and the possible need for additional surgical resection. It is best to do this intraoperatively to facilitate a single operation. In the local practice the organisation of such a scheme requires good cooperation between the surgeon and pathologist and radiologist and the full availability of radiological equipment for postoperative material assessment.

In the absence of a tumour, macroscopically demonstrable residual cancer may still be present in the microscopic picture. To confirm that there is no residual infiltrating disease, specimens from the whole area of the tumour bed should be taken.

The residual tumour bed with fibrotic features should be measured macroscopically in the three largest dimensions. It is recommended to prepare a map for the pathologist and provide it with appropriate measurements and take into account the location of each tissue sample collected for histopathological assessment. Specimens for histopathological examination should be taken from suspicious areas and margins. It is best to collect the samples taking into account the entire area of the largest cross-section of the tumour bed and other suspect areas. The macroscopic description should include these specimens together with their orientation.

The number of samples taken results from exact material assessment, radiological characteristics, and overall size of the surgical material. Some pathologists use cytological assessment of a freshly dissected tumour bed (touch imprint or gentle tissue scraping and smear performing) to confirm the presence of cancer cells during material collection, although this is optional.

The largest cross-section of an alleged tumour bed should be subjected to histological evaluation. It is expected that in the case of a macroscopically complete response, at least 10–15 blocks will be needed to rule out the microscopically residual disease. If the primary tumour and/or resected material was large, it is recommended to take at least one block per 1 cm of tumour size before treatment and additional specimens representing the margins of the material. If these blocks do not contain a tumour, another series of material collection should be made. If the residual tumour bed is small (< 3 cm) and there are no clear features of the persistent cancer, it should be subjected to histopathological examination as a whole. If tumour bed is larger than 3 cm, then at least 15 blocks should be taken.

To avoid the absence of residual invasive cancer, two issues need to be addressed. First of all, tags (clips)

are indicators, not real changes. Sometimes metal clips migrate within the breast. In addition, the clips are placed as focal indicators of a more extensive tumour. It is therefore important to carefully examine macroscopically and radiographically surrounding tissues, rather than focusing only on the metal clips. Secondly, microcalcifications remain stable in the treated breast, but can only represent the component of *in situ* tumour. Therefore, microcalcification is a helpful indicator of tumour location, but not necessarily the best indicator of its invasive component. Because HER2-positive breast cancers may have extensive and distant *in situ* components, microcalcification should be taken, but it is not assumed that they represent the site and extent of all residual invasive diseases.

2. Histopathological evaluation

Neoadjuvant systemic therapy can result in many types of response — from non-identifiable to complete absence of cancer. The tumour bed must be identified in order to reliably determine the pCR, which is defined by exclusion, and therefore depends on the appropriate sampling of the correct area in the breast.

Characteristic changes include oedematous fibrous tissue with residual vascularity and dispersed mast cells as well as lymphocytic infiltration, histiocytic cells with degenerative vacuolisation, hyalinised vascular stroma, fatty necrosis, macrophages with haemosiderin, and lack of glandular tissue — all of which may indicate a tumour bed. There is no doubt, however, that the exact clinical-pathological correlation during macroscopic examination and specimen collection remains the most accurate method of identifying the tumour bed.

Residual tumour cells may have an unusual, sometimes bizarre appearance or may contain subtle changes in the form of signet ring cells, plasmacytoid cells, or have a histiocytoid appearance. Sometimes immunohistochemical staining may be required to detect residual tumour cells in the tumour bed, surgical margins, and/or lymph vessels in order to distinguish histiocytes (CD68 +) and epithelial cells (cytokeratin AE1/AE3 + or cytokeratin 7+).

In the case of residual disease, routine histopathological parameters such as type, size, vascular invasion, and margin status should be recorded. A change of histological tumour grade occurs as a possible reaction to treatment, but it has not been confirmed as an independent prognostic factor in residual disease. The assessment of tumour grade in the material after neoadjuvant treatment is currently recommended (College of American Pathologists). Obviously, it cannot be done in the case of complete or near-complete response to treatment (e.g. when only tumour embolism or cancer *in situ* or cancer cells in the lymph nodes are preserved after treatment).

In order to correctly determine the pathological response, it is important to distinguish between

intra-lymphatic or endovascular cancer (without invasive features) from *in situ* cancer, and it should be considered as a residual invasive disease.

Changes in cellularity may lead to a false impression of multifocality. Immunohistochemistry can often show altered cancer cells in fibrous tissue. If multifocality is suspected, it is recommended that samples be taken from the tissue between the focuses to find macroscopically hidden “branches” of the main tumour. In addition, representative fragments of tissues adjacent to the tumour bed are helpful in the search for residual invasive cancer and to ensure accurate measurement of tumour size.

The assessment of residual tumour size should be based entirely on histopathological examination of the tumour after macroscopic and microscopic correlation, but not on the size of the largest single lesion described macroscopically. Schematic mapping of cross-sections based on macroscopic description is the most accurate method of measuring and assessing residual disease. Therefore, the macroscopic dimensions (three) of the residual tumour bed can be changed up or down after histopathological evaluation of the appropriate tissue sections from the tumour bed and representative surrounding tissues.

3. Evaluation of cellularity

Cancer cellularity is the percentage of examined tissue area (usually of tumour or tumour bed) that is occupied by malignant cells, i.e. breast cancer.

It should be emphasised that, according to such a definition, cellularity before treatment does not have to account for 100%, and in most cases is smaller. This definition does not require knowledge about the cellularity before treatment. Naturally, the reduction in cellularity observed in the material after treatment is the result of therapy effectiveness. This parameter is required to correctly calculate the Residual Cancer Burden (RCB). In practice, the calculation of cellularity requires its evaluation in all specimens taken from the tumour bed and/or tumour and the calculation of the mathematical average from the obtained values.

4. Evaluation of percentage of cancer *in situ*

The assessment of *in situ* carcinoma in the material after systemic treatment of breast cancer consists of determining the percentage of *in situ* texture in relation to the entire cancer histological structure (*in situ* and infiltrating).

The percentage of *in situ* cancer is one of the parameters required to calculate RCB. Theoretically, infiltrating cancer responds better to systemic treatment than cancer *in situ*. A better blood supply of infiltrating cancer and a higher mitotic index mean that the percentage of *in situ* cancer increases with effective elimination of infiltrating cancer. In practice, however, various combinations in the proportions of cancer *in situ* and infiltrating tumour tis-

sue are encountered after neoadjuvant treatment. Most commonly, with effective treatment, the area occupied by both components (*in situ* and infiltrating cancer) decreases. However, even with low post-treatment cancer cellularity (e.g. 5%), based on recommended definition, the percentage of cancer *in situ* is 90%.

Microscopic evaluation of the material after systemic treatment

The microscopic evaluation of the material after systemic treatment is more complex and time-consuming than the assessment in the absence of such treatment.

In addition to the routine elements of the pathomorphological report, the response rate should be determined according to the recommended scoring system:

- Pinder classification;
- Residual Cancer Burden (RCB) score.

From a practical point of view, the RCB is the preferred scoring system. The pathomorphologist can use a convenient online calculator containing detailed instructions for evaluation of each individual parameter. The results in the form of estimated RCB and RCB class also allow a more objective comparison of treatment effects in patients. It is also significant that this system is more often used and analysed in the literature, especially American, which in turn increases interest among Polish oncologists.

Both systems include the response within the primary tumour and metastases in lymph nodes. They also take into account the presence of cancer *in situ* after systemic treatment.

The evaluation of responses to systemic treatment in breast cancer according to Pinder classification is presented in Table 1.

The diagnosis description should include all information regarding relevant points, as shown in the microscope image.

The RCB scoring is carried out using an online calculator (<http://www3.mdanderson.org>), which allows conversion of the required data using a complicated mathematical formula into the corresponding RCB result and the RCB class assigned to the appropriate value ranges.

The values required for calculating RCB are listed in Table 2.

It should be emphasised that the presence of *in situ* texture in RCB scoring system does not exclude a complete pathomorphological response:

- additional parameters of histopathological evaluation of the material after systemic treatment include assessment of Ki67 proliferative index after or during treatment (material from core needle biopsy) and comparison with pre-operative proliferative index; the use of this parameter requires an evaluation of at least 500 cells by immunohistochemical staining

Table 1. Pinder classification

Evaluation of the response to systemic treatment in breast cancer according to Pinder classification	
Breast	
1. pCR: (1) with no residual cancer or (2) with no residual infiltrating cancer, but with the presence of cancer <i>in situ</i>	
2. Partial response: <ul style="list-style-type: none"> (1) minimal residual disease (< 10% of residual cancer) or (2) response from 10–50% of persistent cancer, or (3) > 50% of persistent cancer texture with the present features of injury after treatment 	
3. With no features of response to treatment	
Lymph nodes	
1. With no metastases and no response to treatment	
2. Metastases absent but visible features of response to treatment	
3. Metastasis present, but with features of response to treatment	
4. Metastases present, with no response to treatment	

Table 2. Values required for calculating the Residual Cancer Burden (RCB)

1. Primary tumour bed	Values and their units
The area of the primary tumour bed	[mm] × [mm]
Total cancer cellularity (as a percentage of the area)	(%)
Percentage of cancer <i>in situ</i>	(%)
2. Lymph nodes	
The number of positive lymph nodes	
Diameter of the largest metastasis	[mm]
The above values entered into appropriate fields of the calculator allow calculation of the following: <ul style="list-style-type: none"> — Residual Cancer Burden (RCB) — Residual Cancer Burden Class (Table 3) 	

for Ki67 index and determination of the index with an approximation of at least 1%;
 — assessment of necrosis percentage — this parameter is less important than those mentioned above.

The two additional parameters of histopathological evaluation of the material after systemic treatment are now optional in routine diagnostics. They constitute an element of research, including clinical trials. However, their prognostic value is scientifically proven.

The elements of the pathomorphological report described in the points above allow the oncologist to obtain information about the response to treatment. This data

Table 3. Categories of Residual Cancer Burden (RCB) assessment system

Residual Cancer Burden class	Residual Cancer Burden (RCB)
RCB 0	With no cancer in the breast or lymph nodes
RCB 1	Partial response, minimal residual cancer
RCB 2	Partial response, moderate residual cancer
RCB 3	Chemoresistance, extensive residual cancer

can be used to calculate RCB, determine the Pinder classification, and give an indication of the degree of pathomorphological response according to AJCC/UICC (American Joint Committee on Cancer) (TNM, Tumour, Nodules, Metastases) and FDA, German Group.

The inclusion of the above data also allows the assessment of complete pathomorphological response (pCR), regardless of its definition:

- pCR in the form of absent infiltrating cancer (in the breast and lymph nodes) — AJCC/UICC (TNM);
- pCR in the form of absent infiltrating cancer (in the breast and lymph nodes) and absent cancer *in situ* — FDA, German Group.

Summary

The introduction of neoadjuvant therapy imposes a change in the management of the surgical material. The proper principles at the pre-analytic stage, as well as modifications in the integrated pathomorphological diagnosis, are the main elements to properly establish patient’s further prognosis. The principles presented in this report should be used in all cases of breast cancer patients subjected to systemic treatment before surgery.

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