Belgian guidelines for laboratory handling and pathology reporting of breast carcinoma after neoadjuvant therapy

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An increasing number of breast cancer patients are being offered neoadjuvant therapy. This has major implications for pathologists, as handling and reporting of breast carcinoma specimens after neoadjuvant therapy requires specific considerations. Several authors have described various methods of evaluation of breast carcinoma specimens after neoadjuvant therapy. Both gross and histological evaluations are important in determining the pathologic response, which has proven to be a powerful prognostic factor. In concordance with the European Community Working Group on Breast Screening Pathology, we recommend to standardise the handling and reporting of breast carcinoma after neoadjuvant therapy. A standard set of reporting elements is proposed. Additionally, some useful scoring systems for the evaluation of response are summarised. The choice of a scoring system should be determined in agreement with the treating oncologist. In neoadjuvant trials using chemotherapy, the RCB system (MD Anderson, USA) is one of the scoring systems that will increasingly be used.

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Introduction

Neoadjuvant treatment (NAT) is increasingly being offered to patients with primary invasive breast carcinoma. The primary aim of NAT is to enhance the likelihood of breast conservation and to eradicate micro-metastatic disease. Another important benefit of NAT is the opportunity to measure the efficacy of systemic therapy in vivo. This is assessed by means of pathologic response, illustrating the importance of accurate pathological examination. The degree of pathological response after NAT is an important prognostic factor, with patients obtaining a pathological complete response (pCR) having the best prognosis. We give an overview of the pathological changes due to NAT and the different systems to evaluate pathologic response, and we propose guidelines for handling and reporting specimens after NAT.

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Pathological changes due to neoadjuvant treatment

Changes in neoplastic breast tissue

Microscopic evaluation of the tumour bed reveals a fibrotic area of scarring. In general, no normal breast ducts and lobules are present, but they can be entrapped at the periphery of the tumour bed. The stroma may become fibrous to fibromyxoid or fibroelastotic and shows vascular hyalinisation (*Figure 1*). The fibroblasts may have enlarged, bizarre nuclei. Oedema and areas of necrosis may be present. A patchy chronic inflammatory infiltrate mainly contains foamy histiocytes, lymphocytes, eosinophilic granulocytes and foreign-body-type giant cells. Hemosiderin deposition and cholesterol clefts can be found throughout the tumour bed.

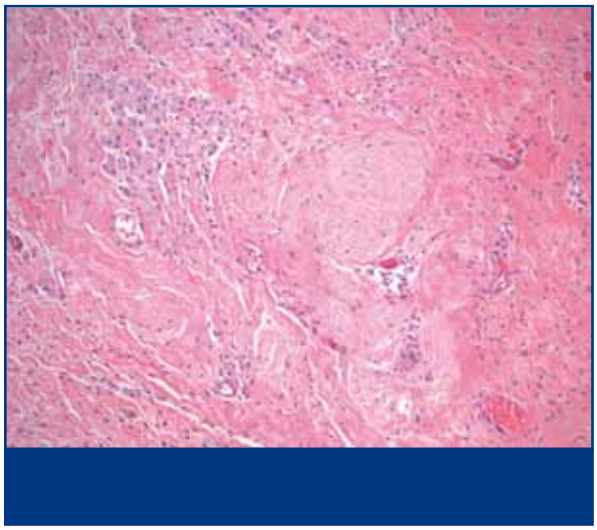

Figure 1. Characteristic fibroelastotic stroma of the tumour bed with foamy macrophages, not to be mistaken for residual carcinoma cells. Haematoxylin-eosin staining, original magnification x100.

The most fundamental manifestation of treatment effect is a total absence of tumour cells or a significant decrease in tumour cellularity (*Figure 2*). Residual carcinoma can be seen as widely dispersed single cells or small clusters of tumour cells. Larger masses of residual tumour show more pronounced distortion of glandular architecture, but with decreased cellularity. The altered cancer cells may resemble histiocytes, however, a frankly high grade of nuclear atypia with hyperchromasia is indicative of malignancy. Immunostaining for pancytokeratins and CD68 may be helpful to make the differential diagnosis between modified cancer cells and histiocytes. The tumour cells often exhibit conspicuous epithelial atypia, with increased cytoplasm, eosinophilic change, cytoplasmic and/or nuclear vacuolisation, pleiomorphic nuclei, multinucleation, and vesicular chromatin. Cell borders are typically well defined, as the cells tend to shrink (artefactual clefts). Accurately subtyping into ductal or lobular carcinoma can become difficult: after NAT an invasive carcinoma can mimic a lobular architecture with single cell files only. Intralymphatic tumour emboli may be unusually prominent, independent of the degree of response in the primary tumour.[1]

As a result of the aforementioned changes, histologic grading may be affected by NAT. An increased nuclear grade and a decreased mitotic activity are frequently encountered. However, other publications indicate either increased, decreased or unchanged nuclear pleiomorphism, proliferative rate and tubule formation.[2,3] Grading of the pretreatment biopsy is necessary, as this histological grade has been proven to be an independent prognostic factor for disease-free survival and overall survival. Recently, the histological grade of the residual invasive tumour after NAT was also shown to have prognostic significance.[4]

In general, tumour markers remain unaltered after NAT. Progesterone receptor (PR) positivity is frequently lost after treatment with aromatase inhibitors, but not with tamoxifen.[5] Both diminished and enhanced expression of HER2/neu have been documented.[6,7] It is unknown whether this is due to downregulation of the receptor or due to selection of tumour cells not expressing HER2/neu.

Carcinoma in situ has repeatedly been noticed to have a relative resistance to chemotherapy.[1] Cytonuclear changes of ductal carcinoma in situ (DCIS) are similar to those described above in the invasive tumour. Lobular carcinoma in situ with chemotherapy changes can mimic DCIS.[8]

Changes in lymph nodes

A reduced number of lymph nodes compared to classical axillary dissection has been reported. Atrophy of lymphoid tissue with associated enhanced sinus histiocytosis may be prominent after treatment. Areas of sclerosis within the lymph node, sometimes with myxoid change and aggregates of foamy macrophages, are indicative of regression of metastatic carcinoma. Cytokeratin immunostaining

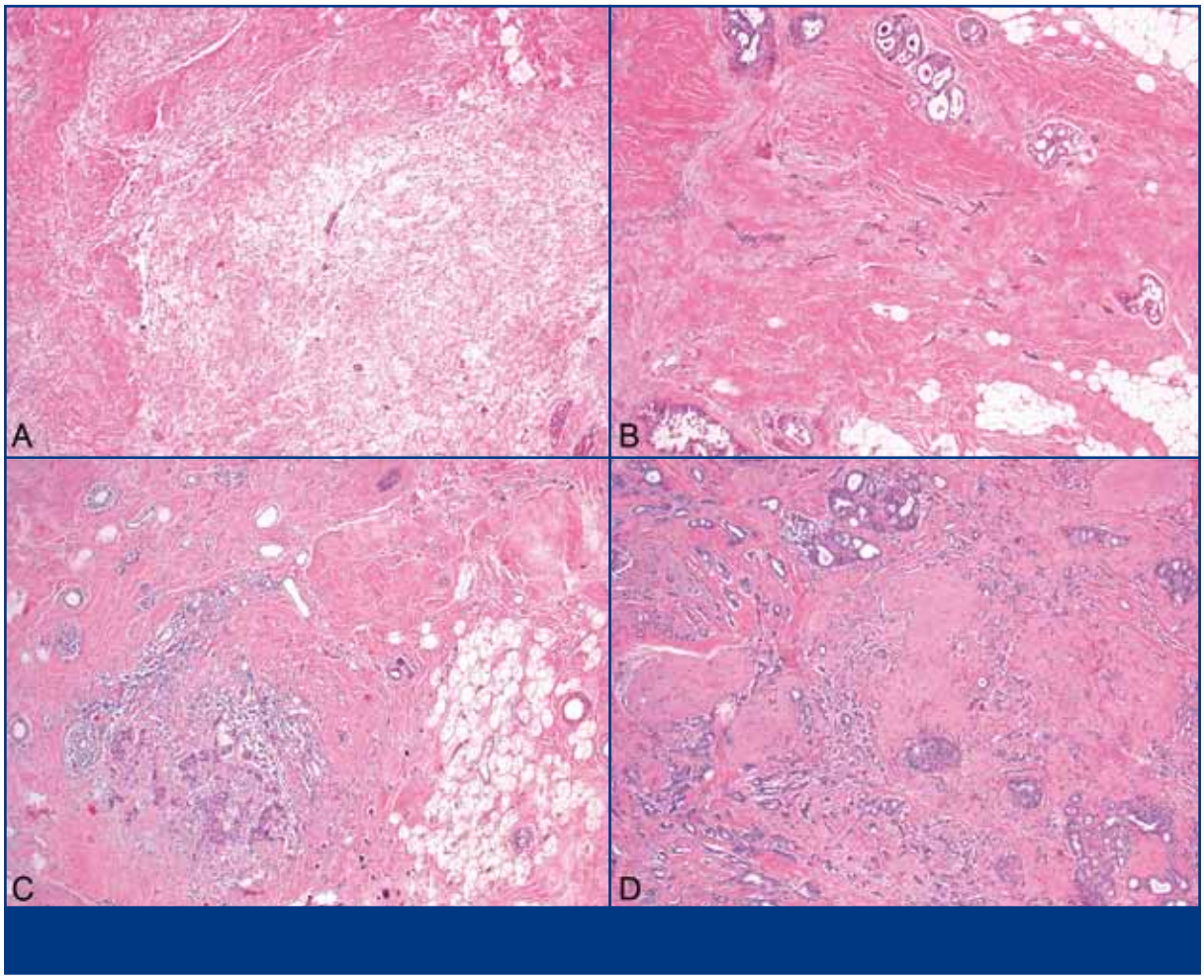

Figure 2. Tumour response. A. Complete pathological response, no residual carcinoma. B. Partial response (i), minimal residual disease with <10% of tumour remaining; note the resistance to chemotherapy of the in situ carcinoma. C. Partial response (ii) with 10-50% of tumour remaining. D. Partial response (iii) with more than 50% of tumour cellularity remaining when compared with the pretreatment biopsy. Haematoxylin-eosin staining, original magnification x50.

is essential to allow the detection of minimal numbers of residual carcinoma cells.

Changes in non-neoplastic breast parenchyma

A frequently encountered feature in the adjacent normal breast tissue is atrophy of the glandular elements, with a considerable reduction in the volume of lobular tissue. Moderate to marked sclerosis and thickening of the basement membranes is often seen. The non-neoplastic breast tissue can show apparent cytonuclear atypia, which should not be mistaken for residual carcinoma in situ. The preserved cellular polarity, the diffuse homogenous increase in chromatin, the heterogeneous appearance of the atypical cells intermixed with normal cells and the lack of stratification, mitotic figures and necrosis, may help to distinguish chemotherapy-related changes in normal ducts from residual DCIS.

Handling and reporting specimen after neoadjuvant treatment

Pretreatment biopsies and preoperative multidisciplinary oncology conference

The pathologist should be present at the multidisciplinary meeting in case of NAT indication. The needle core biopsies should be evaluated to be representative for the tumour. As 10-50% of patients will have no residual tumour after NAT, it is important to have an adequate pretreatment biopsy. The elements to be reported in this biopsy are summarised in *Table 1*.

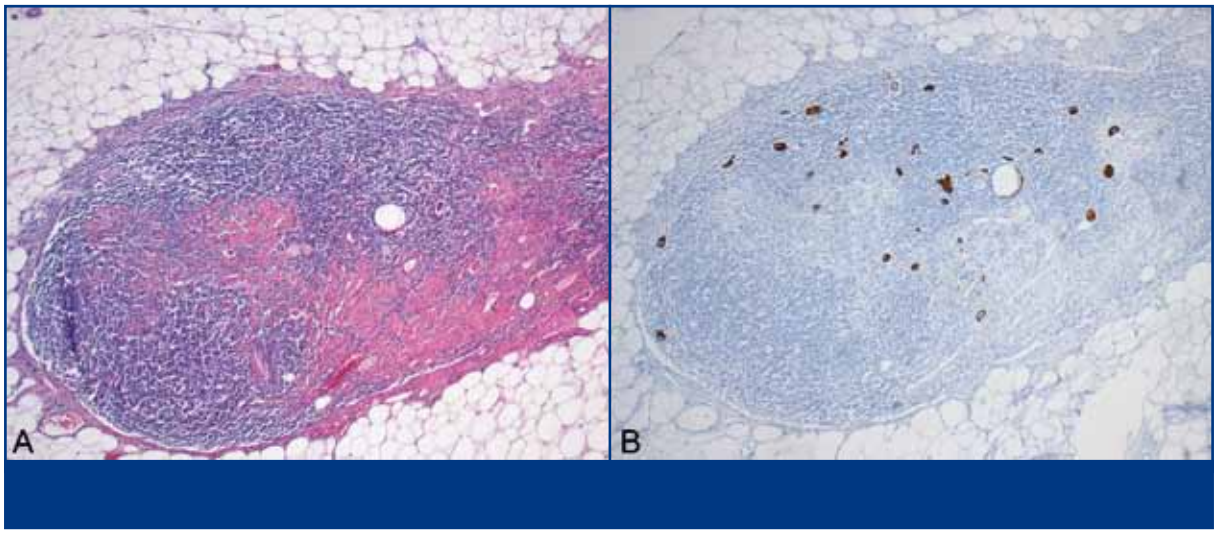

Figure 3. Nodal response. **A.** On the haematoxylin-eosin staining, the area of nodal fibrosis indicates evidence of response, but **B.** metastatic carcinoma cells are still present and can be highlighted with cytokeratin immunohistochemistry. Original magnification x25.

High histological grade, non-lobular histological type, oestrogen receptor (ER) negativity, HER2/neu positivity and extensive tumour necrosis are predictors of pCR.[10] It is therefore helpful to report these features on the pretreatment biopsy, as well as the presence of in situ carcinoma and lymphovascular invasion, to enable comparison to the posttreatment specimen.

ER-positive tumours are known to be less sensitive to chemotherapy than ER-negative tumours. Moreover, pCR is infrequently obtained after endocrine NAT. However, since many patients with residual disease have a favourable outcome, pCR cannot be considered the optimal endpoint for evaluation of the efficacy of NAT in ER-positive disease. Alternative biomarkers are needed for this subgroup of patients. The proliferation marker Ki-67 seems to be the most promising. Therefore, immunohistochemistry for Ki-67 on the pretreatment biopsy is recommended in order to allow comparison to the percentage of Ki-67-positive invasive tumour cells in the posttreatment biopsy.[11]

Placement of markers such as clips or tattoos at the time of initial biopsy or during therapy is useful to ensure accurate localisation of the tumour bed at surgery. Bracketing of the tumour edges, e.g. 3 bracketing tattoos on the skin and in the depth, is preferred to a single central marker. This will give guidance to the surgeon even if the tumour totally disappeared during NAT.

It is obvious that tumour response in lymph nodes can only be evaluated when the lymph nodes have not been removed before NAT. Therefore, pretreatment pathological evaluation of the lymph nodes can only be performed by fine needle aspiration cytology or core needle biopsy.

Requisition form

Accurate gross evaluation requires adequate clinical information, even more so in preoperatively treated specimen. The requisition form should mention the following information: clinical presentation, localisation and size of the lesion(s) before treatment, prior diagnostic procedure, presence of calcifications, presence of a clip or other marking methods (e.g. wire coil, tattoo), prior lymph node evaluation, type of neoadjuvant therapy, and clinical/radiological response.

Gross evaluation

After inking the margins and thinly slicing the specimen, the tumour bed should be localised. This can be readily identified as an area of rubbery fibrous tissue, but can often be very challenging in cases of good response or in fibrous breasts. Information on tumour localisation, comparison between the radiological aspect and size before and after NAT and the presence of marking methods or calcifications is mandatory in such cases. Any residual tumour should be recognised as fleshy nodules within the tumour bed. The tumour bed and residual tumour focus/foci should be measured. The distance of the

Table 1. Reporting elements in the pretreatment biopsy.
tumour type
tumour grade (according to the Nottingham grading system)[9]
presence of in situ carcinoma
lymphovascular invasion (LVI)
tumour viability: percentage of necrosis
tumour markers: ER, PR, HER2/neu, Ki-67

Table 2. Reporting elements in the posttreatment resection specimen.
Invasive Carcinoma
size of the tumour bed (on gross and microscopy): 2 dimensions
size of residual invasive carcinoma (TNM 7th edition 2009)[14]
estimated percentage of tumour bed that contains invasive carcinoma
tumour type
tumour grade (according to the Nottingham grading system), including mitotic activity index[9]
percentage of necrosis in invasive carcinoma
fibrotic reaction: presence/absence
lymphovascular invasion (LVI)
margins size with respect to tumour bed and invasive carcinoma
tumour markers: ER, PR, HER2/Neu, Ki67
In situ carcinoma
size of residual in situ carcinoma
estimated percentage of tumour bed that contains in situ carcinoma
histological type and nuclear grade
margins size with respect to in situ carcinoma
Lymph nodes
number of lymph nodes
number of lymph nodes with metastasis
size of largest metastasis
presence of extranodal extension
number of lymph node metastases with evidence of treatment response
number of lymph nodes with evidence of treatment response, without metastasis
Final pathological TN stage (ypTN)
Optionally
evaluation of response in tumour and lymph nodes e.g. according to Pinder[15]
estimation of the residual disease after NAT e.g. RCB[16]

surgical margins to the tumour bed and to any macroscopically visible residual tumour should be measured as well. The pathologist should also describe the presence and number of bracketing tattoos in relation to the surgical margins, the tumour bed and potential residual tumour focus/foci. The axillary lymph nodes should be carefully searched for.

Sampling

At least 1 block per cm tumour bed should be submitted. If no residual tumour is identified microscopically, additional sampling is recommended, preferably of the entire tumour bed.[10,12,13] If the tumour bed cannot be reliably recognised, breast conserving therapy specimen after NAT are totally embedded; in mastectomy specimen the area of the tumour pretreatment is totally embedded. The pathologist must look meticulously for residual tumour before a pCR can be confirmed. Sampling of the margins of breast conserving therapy specimen and mastectomy specimen should include at least one block of any margin closer than 2 cm of the

Table 3. Evaluation of response in tumour and lymph nodes according to Pinder.[15]

Tumour response

1. Complete pathological response, either (i) no residual carcinoma or (ii) no residual invasive carcinoma but DCIS present.
2. Partial response to therapy, either (i) minimal residual disease/near total effect (e.g. <10% of tumour remaining) or (ii) evidence of response to therapy but with 10-50% of tumour remaining or (iii) >50% of tumour cellularity remains evident, when compared to the previous core biopsy sample, although some features of response to therapy are present.

Points (ii) and (iii) are somewhat subjective especially when the pretreatment biopsy cannot be reviewed.

3. No evidence of response to therapy.

Nodal response

1. No evidence of metastatic disease and no evidence of changes in the lymph nodes.
2. Metastatic tumour not detected but evidence of response/down-staging, e.g. fibrosis.
3. Metastatic disease present but also evidence of response, e.g. nodal fibrosis.
4. Metastatic disease present without evidence of response to therapy.

When there is a mixture of categories, e.g. 1 node with a metastasis showing no response and 1 node showing fibrosis, the worst category should be used (e.g. category 4)

tumour bed or any residual tumour. Axillary lymph nodes should be thinly sectioned in 2 mm sections and completely submitted. One hematoxylin-eosin staining slide of each lymph node should be evaluated. If no tumour is detected, but reactive changes are present, immunohistochemistry for pancytokeratins is recommended (*Figure 3*).

Reporting the posttreatment resection specimen

The elements to be reported in the posttreatment resection specimen are summarised in *Table 2*. The definition of posttreatment final pathology status (ypT) remains controversial and an area in transition. Posttreatment ypT will be defined as the largest contiguous focus of invasive cancer as defined histopathologically with a subscript to indicate the presence of multiple tumour foci.[14,17] Difficulties arise when several small foci of residual carcinoma are seen within 1 tumour bed. Pinder et al. suggest that, if the foci are clearly initially part of a single tumour pre-NAT, one should report the overall dimension of the foci.[15] Posttreatment (ypT) size should therefore be estimated based on the best combination of imaging, gross and microscopic histological findings. The size of some invasive cancers may be unapparent to any imaging modalities or gross pathologic examination. In these cases, invasive cancer size can be estimated by carefully measuring and recording the relative positions of tissue samples submitted for microscopic evaluation and determining which contain invasive cancer. The same method should be used for estimating the size of the DCIS component.

Typing and grading a treated tumour may be difficult, considering the aforementioned histological changes. Tumour grade may be altered compared to the pretreatment biopsy. Both pretreatment and posttreatment histological grade have been proven to be independent prognostic factors.[18]

In some cases, lymphovascular tumour emboli are the sole tumour remnants. As this has recently been proven to have strong independent prognostic significance, it should be clearly stated in the posttreatment pathology report.[19]

Importantly, the prognostic significance of the ypN stage is not the same as that of pN stage: micrometastases or isolated tumour cells in lymph nodes after NAT may represent macrometastases that have partially responded to chemotherapy. Posttreatment nodal metastases not exceeding 0.2 mm in size are classified as ypN0(i+). However, patients with this finding are not considered to have achieved a pCR.

At the end of the report, based on the recommended reporting elements, an evaluation of the response to NAT and/or an estimation of the residual disease after NAT may be given using 1 of the systems described below. The choice of the scoring system used in a particular hospital setting should be determined upon formal agreement between oncologists and pathologists.

Systems for evaluating pathological response to neoadjuvant treatment

Several methods for the histopathological evalua-

Key messages for clinical practice

1. Neoadjuvant treatment (NAT) is increasingly being offered to patients with primary breast cancer.
2. Pathological response is a powerful prognostic factor. Its assessment requires meticulous macroscopic and microscopic evaluation of the resection specimen by the pathologist.
3. The Belgian Working Group for Breast Pathology recommends standardising handling and reporting breast carcinoma after NAT and proposes a list of reporting elements for the pretreatment biopsy and the posttreatment resection specimen.
4. Several systems exist for response evaluation and estimation of residual disease. The choice of a particular scoring system should be determined upon agreement between oncologists and pathologists.

tion of response after NAT have been suggested. In general, all systems recognise a category of pCR and a category of little or no response. The number of categories of partial response and the criteria used to evaluate response as a categorical or continuous variable vary in the different systems. In most studies the degree of response to treatment has been shown to correlate with disease-free survival and/or overall survival, best outcomes always being obtained in the pCR category. However, the different methods for grading the response to treatment have not been compared to each other in large clinical trials, and there is no current consensus favouring any particular method. Systems evaluating both the response of the primary tumour and the response in the lymph nodes are conceptually most likely to be more accurate since the prognostic significance of a pCR in the breast is marginal or absent in models that control for pathological response in lymph nodes.[10,20] In some patients, there is total or almost total tumour response in the breast but little effect on nodal disease. Therefore, pCR is best defined as the absence of residual invasive cancer in both breast and lymph nodes. Below we briefly describe the advantages and disadvantages of the different systems.

The Chevallier system, the NSABP B-18 system and the Miller-Payne system only evaluate response in the primary tumour.[21-23] They do not include the response in the lymph nodes, although in a clinical study published the year before, Miller and Payne assessed the axillary lymph nodes for the degree of response to chemotherapy using a four-point scale and the Chevallier Class 2 requires negative lymph nodes.[21,24]

In the AJCC system, carcinomas are assigned a posttreatment T and N category indicated by the prefix 'y'.[14,17] The system gives an indication of the response to treatment when the ypTN is compared to the pretreatment cTN. However, tumours with scattered residual nests of carcinoma cells in a tumour bed are assigned the same ypT category as large contiguous carcinomas. This system does not take changes in cellularity into account.

The Sataloff system classifies treatment response for both the primary tumour and the lymph nodes.[25] Patients with total or near total therapeutic effect had a better survival at 5 years than all other categories of patients.

The neoadjuvant response index (NRI) system calculates the NRI by adding a breast response score (a five-point scale) to an axillary response score (a three-point scale) and dividing this by the score that would have been obtained in case of a pCR in both breast and axilla.[26] Assessment of the NRI requires clinical information, e.g. on palpability of the

axillary lymph nodes, which is not always available to the pathologist. The follow-up of the patients was too short to correlate the different NRI values with recurrence-free or overall survival.

The European Community Working Group on Breast Screening Pathology recommends using the system described by Pinder et al.[15,27] Although this system has not been corroborated by outcome studies, it is a logical classification of response along the lines of that proposed by Sataloff. It is a descriptive system already used by many pathologists. It includes both nodal and tumour response (*Table 3*).

The RCB system combines the size of the primary tumour bed (measured in the resection specimen after neoadjuvant therapy), the cellularity of the residual invasive carcinoma, the number of lymph nodes with metastases and the size of the largest metastasis mathematically into a continuous index to define 4 categories of residual cancer burden (RCB).[16] It is the only system considering size and cellularity separately. All other systems conceptually summarise the reduction in extent and cellularity of the tumour into remaining disease or treatment effect. Assessment of the RCB requires careful pathology review. Estimates of cellularity are somewhat subjective and more difficult when a marked response has occurred, because islands of highly cellular carcinoma may be interspersed within a large, difficult-to-delineate tumour bed. However, the website *http://www.mdanderson.org/breastcancer_RCB* contains computer generated diagrams of the percentage cellularity per area to assist pathologists.

Although the RCB system has been shown to be an independent predictor of distant relapse-free survival, it is not a good indicator of response to NAT particularly for smaller tumours: small tumours with little response and large tumours with good response may have similar RCB values. Surgical excision of a positive sentinel lymph node before the neoadjuvant treatment would invalidate the accuracy of measuring RCB after the treatment to assess response. If all sentinel lymph nodes were negative before treatment, this would not affect the assessment of RCB after treatment.

The residual disease in breast and lymph nodes system (RDBN) is another system to evaluate residual disease after NAT.[4] It combines residual tumour size, the number of involved lymph nodes, and the histological grade of the tumour into an index that was shown to be a strong predictor of overall and disease-free survival. It cannot be used for patients who did not undergo axillary dissection.

New systems to evaluate response or residual disease after NAT are constantly being developed. Recently, a new pathological response index (PRI) including size of residual tumour, N stage, lymphovascular invasion and any evidence of fibrotic reaction following NAT was shown to accurately predict the disease progression rate.[19]

Very few studies have compared the practical application and predictive use of the different methods for the evaluation of response to treatment. Recently, several classification schemes were compared in a randomised phase II clinical trial. Classification schemes strongly weighing lymph node involvement and posttreatment tumour grade appeared to show better correlation with long-term outcome than those based only on tumour size or cellularity.[28]

Conclusion

NAT has become widespread practice in the treatment of breast carcinoma. The role of the pathologist is crucial, as pathological response is an important prognostic indicator. Meticulous macroscopic and microscopic evaluation of the resection specimen is therefore mandatory, which is cost- and effort-consuming. Adequate clinical information is of the utmost importance. We propose a standardised method of handling the specimen and a list of reporting elements for the pretreatment biopsy and the posttreatment resection specimen.

Several systems exist for response evaluation to treatment and estimation of residual disease after NAT and new systems are being developed. The aim of these systems is to improve the sensitivity of pathological response to predict tumour response/resistance to NAT in other patient groups as those reaching pCR after NAT. The system used should be determined in agreement between oncologists and pathologists.

In neoadjuvant trials using chemotherapy, the RCB system (MD Anderson, USA) is one of the scoring systems that will be used increasingly. The best method to classify response has not yet been determined. The minimal requirements of a grading system for response should include the following: several categories of partial response evaluating

both primary tumour and lymph nodes, cellularity and size of residual invasive tumour, lymphovascular invasion, grading of residual carcinoma according to Nottingham and prognostic markers (ER, PR, HER2, Ki-67).

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