Received: 1 May 2024 | Accepted: 4 May 2024

DOI: 10.1002/jso.27717

REVIEW ARTICLE

Journal of SURGICAL ONCOLOGY WILEY

Guidelines of the Brazilian Society of Surgical Oncology for anatomopathological, immunohistochemical, and molecular testing in female tumors

Reitan Ribeiro[1] | Filomena Marino Carvalho[2] | Glauco Baiocchi MD, PhD[3] | Rodrigo Santa Cruz Guindalini[4] | Juliano Rodrigues da Cunha[5] | Carlos Henrique dos Anjos[6] | Caroline de Nadai Costa[7] | Ana Carolina Leite Vieira Costa Gifoni[8] | Renato Cagnacci Neto MD, MSc[9] | Allyne Queiroz Carneiro Cagnacci MD[10,11] | Vandré Cabral Gomes Carneiro[12,13,14] | Aknar Calabrich[15] | Renato Moretti-Marques[16] | Rodrigo Nascimento Pinheiro[17] | Heber Salvador de Castro Ribeiro[18]

[1]Department of Gynecology Oncology, Erasto Gaertner Hospital, Curitiba, Paraná, Brazil

[2]Department of Pathology, Faculdade de Medicina (FMUSP), Universidade de São Paulo, São Paulo, São Paulo, Brazil

[3]Department of Gynecologic Oncology, AC Camargo Cancer Center , São Paulo, São Paulo, Brazil

[4]Department of Genetics, Instituto D'or de Pesquisa e Ensino (IDOR), Rio de Janeiro, Brazil

[5]Department of Surgical Oncology, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil

[6]Department of Oncology, Oncology Center, Hospital Sírio Libanês, São Paulo, São Paulo, Brazil

[7]Department of Oncology, Paraná Institute of Oncology, Curitiba, Paraná, Brazil

[8]Department of Medical Oncology, Oncologia D'Or Fortaleza, Fortaleza, Ceará, Brazil

[9]Department of Mastology, Breast Cancer Reference Center, AC Camargo Cancer, CenterSão Paulo, São Paulo, Brazil

[10]Department of Oncology, Oncology Center, Hospital Alemão Oswaldo Cruz, São Paulo, São Paulo, Brazil

[11]Hereditary Cancer Department, Instituto do Câncer do Estado de São Paulo (ICESPSP), São Paulo, São Paulo, Brazil

[12]Department of Gynecology Oncology, Instituto de Medicina Integral Professor Fernando Figueira (IMIP), Recife, Pernambuco, Brazil

[13]Research Department, Hospital de Câncer de Pernambuco, Recife, Brazil

[14]Department of Oncogenetic, Oncologia D'OR, Recife, Pernambuco, Brazil

[15]Department of Oncology, Clínica AMO/DASA, Salvador, Bahia, Brazil

[16]Department of Oncology, Albert Einstein Israelite Hospital, São Paulo, São Paulo, Brazil

[17]Department of Surgical Oncology, Base Institute Hospital, Brasília, Federal District, Brazil

[18]Abdominal Surgery Department, AC Camargo Cancer Center, São Paulo, São Paulo, Brazil

Correspondence
Reitan Ribeiro, Department of Gynecology Oncology, Erasto Gaertner Hospital, Rua Dr Ovande do Amaral 201, Curitiba, PR 81200-100, Brazil.
Email: reitanribeiro@hotmail.com

Funding information
AstraZeneca; GlaxoSmithKline

Abstract

Introduction: Precision medicine has revolutionized oncology, providing more personalized diagnosis, treatment, and monitoring for patients with cancer. In the context of female-specific tumors, such as breast, ovarian, endometrial, and cervical cancer, proper tissue collection and handling are essential for obtaining tissue, immunohistochemical (IHC), and molecular data to guide therapeutic decisions.

Objectives: To establish guidelines for the collection and handling of tumor tissue, to enhance the quality of samples for histopathological, IHC, genomic, and molecular analyses. These guidelines are fundamental in informing therapeutic decisions in cancer treatment.

Method: The guidelines were developed by a multidisciplinary panel of renowned specialists between June 12, 2013 and February 12, 2024. Initially, the panel deliberated on critical and controversial topics related to conducting precision medicine studies focusing on female tumors. Subsequently, 22 pivotal topics were identified within the framework and assigned to groups. These groups reviewed relevant literature and drafted preliminary recommendations. Following this, the recommendations were reviewed by the coordinators and received unanimous approval. Finally, the groups made the final adjustments, classified the level of evidence, and ranked the recommendations.

Conclusion: The collection of surgical samples requires minimum quality standards to enable histopathological, IHC, genomic, and molecular analyses. These analyses provide crucial data for informing therapeutic decisions, significantly impacting potential survival gains for patients with female tumors.

KEYWORDS
anatomopathological, cancer, immunohistochemical, molecular testing

1 | INTRODUCTION

Advancements in molecular biology have explained the onset and progression mechanisms of various types of cancer. Consequently, novel therapeutic agents have been developed to target specific anomalies. Some medications now transcend traditional indications, which were solely based on neoplasm origin and staging. Instead, they are prescribed based on marker testing results, regardless of tumor origin. An example of this progress is the use of immunotherapy, particularly in cases where microsatellite instability (MSI) and/or deficient DNA mismatch repair (dMMR) are present.[1]

In the context of female tumors, the acquisition of tissue, in which immunohistochemistry (IHC) and genetic testing can be performed, are essential for guiding therapeutic decisions that lead to real survival gains for patients with breast,[2,3] ovarian,[4,5] endometrial,[6] and cervical cancer.[7,8] Thus, it is imperative that samples used in these tests are collected properly, ensuring an adequate quantity of high-quality tissue for diagnostic evaluation. Inadequately collected biopsies may yield false-negative results, potentially leading to delays in treatment.[9]

These guidelines establish standardized protocols for the collection of biopsy samples, aiming to ensure both the quality and quantity of tumor material necessary for histopathological, IHC, and molecular analysis. They offer guidance to healthcare professionals on optimal practices in the precision medicine approach to treating female tumors.

2 | METHODS

The guidelines for biopsy sample collection for precision medicine tests, endorsed by the Brazilian Society of Oncological Surgery were developed by a multidisciplinary panel of 13 renowned specialists. Spanning from June 12, 2013 and February 12, 2024, the process commenced with comprehensive discussions among the experts, addressing pivotal topics such as collection methods, sample storage, and collection time. Subsequently, 22 key topics were identified and allocated to specialized groups, each consisting of two specialists. These groups reviewed relevant literature and drafted preliminary recommendations related to the assigned topic. The initial recommendations underwent thorough scrutiny by the coordinators, who provided suggestions to standardize the text and align the recommendations with the work purposes. Following this phase, each working group presented their recommendations and listened to suggestions from all participants in a video conference. The suggestions were incorporated by the groups, and the final version was created by the coordinators.

The adapted version of the Infectious Diseases Society of America-United States Public Health Service Grading System[10] was used to define the level of evidence and rank each recommendation proposed by the group (Table 1). Subsequently, all recommendations underwent a comprehensive evaluation of their grade of recommendation and level of evidence. During the grading process, panel members were allowed to abstain if they felt they lacked sufficient knowledge to agree or disagree with the recommendations, or if they

TABLE 1 Level of evidence and recommendation grading.

Quality of evidence	
I	Evidence from at least one large randomized, controlled trial by sound methodological rigor (indicating low potential for bias) or from meta-analyses of well-executed randomized trials without heterogeneity
II	Small randomized trials or large randomized trials with suspicion of bias (indicating lower methodological quality) or from meta-analyses of such trials or of trials with demonstrated heterogeneity
III	Prospective cohort studies
IV	Retrospective cohort studies or case-control studies
V	Studies without control group, case reports, or expert opinions
Grade of recommendation	
A	Strong evidence for efficacy with a substantial clinical benefit, strongly recommended
B	Strong or moderate evidence indicates efficacy, although with a limited clinical benefit, generally recommended
C	Insufficient evidence for efficacy or benefit does not outweighing the risk or the disadvantages (such as adverse events or costs), optional
D	Moderate evidence against efficacy or for adverse outcome, generally not recommended
E	Strong evidence against efficacy or for adverse outcome, never recommended

harbored any conflicts of interest that could potentially bias their decision-making. The voting results were then used to define agreement among participants, with a consensus threshold set at more than 80% agreement for recommendation approval. Recommendations receiving less than 80% approval would be excluded from the guideline.

3 | RESULTS

3.1 | General guidelines for tissue collection and processing for precision medicine tests

The attending physician responsible for requesting tissue collection should conduct a complete clinical assessment of the patient to define the best biopsy collection approach, including physical examination, medical history, and imaging tests. When tissue collection is warranted, image-guided biopsy techniques, such as ultrasound, mammography, computed tomography should be employed to help precisely locate the tumor. This approach minimizes the risk of sampling necrotic tissue areas or collection of tissue that is not representative of the tumor.

Adequate local anesthesia should be administered to minimize discomfort and ensure patient cooperation throughout the procedure. Sedation may be necessary to ensure patient comfort and safety, based on the discretion of the attending physician.

Samples must be sent for analysis and identification, and the request should include relevant clinical information of the patient. This should include details such as confirmed or suspected genetic syndromes, primary tumor site, stage, previous treatments, and previous diagnostic tests. Furnishing these data is imperative for a precision medicine laboratory to conduct a complete and integrated analysis.

In addition to these basic guidelines, the panel highlighted four central questions applicable to all tissue samples:

1. What is the minimum quantity of tumors required for diagnostic and somatic tumor testing (STT) to define the treatment of female tumors?

Given that tumor samples may undergo various tests and manipulations at different times, they are susceptible to unpredictable factors that can affect their long-term quality. Therefore, a robust and accurate pathology report requires a sufficient volume of viable tumor cells for testing purposes.

The first sections of tissue samples, processed and embedded in paraffin in the laboratory, are used to assess tumor morphology via routine hematoxylin and eosin (H&E) staining. In many cases, additional sections are required for ancillary testing with IHC, because special staining may be required to confirm or refine the diagnosis. Subsequently, any remaining tissue is designated for molecular testing, if deemed necessary.[11] A German study[12] reported that, of 190 women eligible for homologous recombination deficiency (HRD) testing, 27 (14%) lacked sufficient tumor tissue necessary for evaluation. The authors showed that surgeons have improved sample collection over time, reducing cases of insufficient samples. The same reasoning can be applied to cytological samples, which can be fixed and paraffin-embedded for molecular testing; however, they are less accurate [13]; thus advocating their use only in the absence of an appropriate tissue sample.

Individual samples can be embedded in separate blocks to preserve the tissue for molecular testing, which is conducted after H&E staining for morphology assessment.[11] Each subsequent test will require a part of this material, such as one block for IHC and another for molecular testing.

Given the exponential advancements in tests and treatments, coupled with the likelihood of new tests to be developed, tissue samples collected must anticipate future needs.

Recommendation: Multiple female tumor samples must be collected in a significant volume for both diagnostic and molecular testing. Cytology collection alone is not recommended.

Level of evidence: II

Grade of recommendation: A

2. What is the ideal preservation method for female tumor tissue analysis in precision medicine?

Tissue preservation is a crucial factor for high-quality anatomo-pathological and molecular testing accuracy, influenced by several factors such as cold ischemia time, fixation, decalcification, paraffin embedding, and paraffin block storage.[14] Proper fixation varies according to the type of fixative used, the ratio of specimen to fixative volume, and the duration of fixation. For histological samples and IHC testing, the preferred fixative is 10% buffered formalin with pH 7. Surgical specimens are the most difficult to fix properly because they present a speed of tissue penetration of 1 mm/h at room temperature; hence, meticulous preparation and slicing are necessary.[14,15] The specimen must be completely immersed in the fixative, with a specimen-to-fixative volume ratio of at least 1:4, preferably 1:10.[14] The ideal fixation time ranges between 6 and 48 h. Optimal fixation times for DNA, RNA, and IHC testing are 6–72, 8–48, and 6–24 h, respectively. However, depending on the type of tissue, the interval for IHC testing can reach 72 h.[16] Decalcification of samples using ethylenediaminetetraacetic acid (EDTA) is crucial to enable molecular testing and improve antigen preservation for IHC testing.[16,17] Paraffin blocks can be stored at room temperature (18–25°C), preferably in a cool, dry environment protected from light exposure.[18]

Recommendation: Biopsy and surgical specimens must be fixed in buffered formalin for 6–72 h, initiated within 1 h of collection.

Level of evidence: III

Grade of recommendation: A

3. Are there any specific recommendations for specimen manipulation at the time of collection?

Biopsy samples are generally quickly fixed in buffered formalin, whereas surgical specimens require special care. The critical factor to consider is the cold ischemia time, defined as the duration between specimen collection and immersion in the fixative. This time window must occur immediately or within 1 h to optimize results, mainly for IHC and RNA testing.[15] Two distinct scenarios must be considered to ensure ideal fixation conditions: specimen preparation at the laboratory or specimen manipulation in the operating room. In the first scenario, the surgical specimen is promptly dispatched to the pathology laboratory postcollection, where it undergoes proper fixation. This approach necessitates either the presence of a pathologist in the operating room or the proximity of the laboratory to the operating room.

In instances where the pathology laboratory is distant, the recommendation is to immediately send the sample sealed in a vacuum at 4°C.[15] However, in Brazil, it is more common to send the specimen to laboratories, which may not always be close or available for proper preparation. In this situation, it is the responsibility of the surgical team to prepare the specimen properly. The preparation process involves proper identification of the sample, followed by immediate placement in a large container with an appropriate volume of buffered formaldehyde, as described before.[15] For example, in the case of hysterectomy specimens, it is essential to open the specimen to expose the entire endometrial surface, ensuring full immersion in the fixative, with no risk of deformation. The specimen should preferably be opened along the lateral walls (3 and 9 h) to expose bilateral tubal insertions and the entire surface. If the tumor deeply involves the myometrium, additional cuts can be made perpendicularly to the surface in thicker areas, without penetrating the serosa.[19]

Recommendation: Surgical specimens should be ideally sent to the pathology laboratory intact and right after collection, with the fixation process commencing within 1 h.

Level of evidence: IV

Grade of recommendation: B

Recommendation: If a pathology laboratory is not close to the operating room, the specimen can be sent sealed in vacuum at 4°C or prepared in buffered formaldehyde by the surgical team, following the instructions provided by the reference pathology service.

Level of evidence: IV

Grade of recommendation: C

4. Is there a time limit to perform tumor testing for precision medicine in paraffin-embedded tissue samples?

The acceptable paraffin block storage time is ≤5 years for DNA extraction, ≤1 year for RNA extraction, ≤25 years for IHC testing, and <10 years for protein extraction.[16] However, the literature presents different results. For instance, Fujii et al.[20] reported a significant decline in DNA quality extracted from blocks stored for ≥3 years. Conversely, other studies have reported that sample quality for DNA, RNA, and protein extraction can be maintained for 12 years with ideal paraffin block preparation and storage.[21] Additionally, Carrick et al.[22] demonstrated a 90% success rate in next-generation sequencing (NGS) using high-grade serous carcinoma samples stored for 3–32 years.

Recommendation: DNA samples for NGS should be preferably extracted from paraffin blocks stored for up to 3 years.

Level of evidence: IV

Grade of recommendation: C

3.1.1 | Breast cancer

5. Which basic biomarker tests are required to properly treat early breast cancer?

The eighth edition of the American Joint Committee on Cancer (AJCC) Cancer Staging Manual (2017) introduced the Nottingham

modification of the Scarff-Bloom-Richardson grading system and established the Tumor Node Metastasis (TNM) classification as the gold-standard staging system for breast cancer. Additionally, the manual emphasizes the importance of IHC testing for estrogen receptor (ER) and progesterone receptor (PR) biomarkers, as well as HER2 status assessment. In cases where high HER2 amplification level confirmation is necessary, in situ hybridization techniques are employed.[23] The inclusion of IHC testing has provided important information to define the best adjuvant systemic treatment and predict response to neoadjuvant treatment. The AJCC now requires the inclusion of these biomarkers in the prognostic and predictive staging of breast cancer.[24] Randomized clinical trials such as the CREATE-X[2] and KATHERINE[3] have demonstrated increased survival with neoadjuvant treatment and differentiated complementary treatment in cases of incomplete pathological response in early triple-negative and HER2-positive disease, respectively.

In a scenario where gold-standard systemic therapy is readily available, the results of IHC staging—mainly HER2-positive and triple-negative, indicate the potential benefit of neoadjuvant treatment, even in cases of initially operable disease. This approach has been shown to increase patient survival. Approximately 15% of primary invasive breast cancers exhibit HER2 amplification or overexpression, initially assessed via IHC and then confirmed, if necessary, using fluorescence in situ hybridization (FISH).[3]

Given the prognostic and predictive value of these tests, all newly diagnosed primary breast cancers or those presenting with recent metastases undergo testing to identify ER, RP and HER2 status, to identify, for example, patients who can benefit from treatment with HER2-targeted agents.[25]

Although widely used, Ki-67 protein testing is not routinely recommended by the AJCC,[23] the American Society of Clinical Oncology (ASCO),[26] or the College of American Pathologists (CAP).[25] This stance is justified by several factors, including the absence of a consensus value, interobserver variability, analytical validation problems, and a lack of data on the effect of preanalytical variables.[27] European entities support limited use of Ki-67 testing due to the frequent unavailability of genomic signatures in countries with public health systems. The European Group on Tumor Markers (EGTM)[28] suggests the routine use of Ki-67 as a prognostic marker and proposes that values <10% represent low risk, and values >25% represent high risk (level of evidence IB, grade of recommendation B).

In 2021, the International Ki67 in Breast Cancer Working Group[27] proposed the use of Ki-67 for prognostic assessment, but with values <5% and >30% representing low and high risk, respectively. They suggested using Ki-67 levels to guide the decision on adjuvant chemotherapy in HER2-negative luminal tumors (not uses when <5%, uses when >30%). However, when asked about the use of Ki-67 in the indication of adjuvant chemotherapy in breast cancer, the panelists at the 2021 St. Gallen International Breast Cancer Consensus Conference[29] indicated that 35.6% declared not knowing the Ki-67 cutoff point to define adjuvant chemotherapy, 42.4% suggested a cutoff point of 30% to indicate adjuvant chemotherapy, 6.8% suggested 25%, 13.6% suggested 20%, and 1.7% suggested 15%, which reinforced the difficult use of this biomarker in breast cancer treatment.

Recommendation: The diagnosis of breast cancer should include IHC testing to assess at least ER, PR, and HER2 status.

Level of evidence: I

Grade of recommendation: A

Recommendation: IHC testing can include Ki-67 at the first diagnosis of breast cancer, especially in Brazil, where genomic signatures are scarcely available.

Level of evidence: III

Grade of recommendation: C

6. Which genomic signatures are recommended in breast cancer management?

Except for IHC testing, genomic signatures have not yet established a defined role in cases of very small invasive tumors (<0.5 cm), in situ carcinomas, or in fragile patients with no clinical conditions to undergo chemotherapy.[24,29]

At the time of this publication, axillary-negative invasive carcinomas <1.0 cm were considered eligible for upfront surgery, regardless of the molecular subtype. However, defining the molecular subtype remains essential for determining appropriate adjuvant treatment strategies.[2,28]

In cases characterized by hormone-positive, HER2-negative status, or absence of nodal involvement (N0), the identification of a low-risk genomic signature can identify tumors with better prognosis, thereby facilitating the delineation of appropriate adjuvant management strategies.[24] More recently, the National Comprehensive Cancer Network (NCCN)[30] has suggested extending the recommendation for genomic signatures utilization to include axillary-positive patients (1–3 positive lymph nodes).

However, unlike IHC, there is no evidence to support the development of genomic signatures for indicating neoadjuvant treatment. Existing literature contains only small retrospective studies on this subject. Although recent studies, such as the one conducted by Freeman et al.,[31] have shown a moderate correlation between complete pathological response and high-risk OncotypeDx© and Mammaprint© tests, the reported results are not sufficiently robust to base global consensuses. In Brazil, genomic signatures are not covered by the Unified Health System or listed in the National Regulatory Agency for Private Health Insurance and Plans (ANS). However, national literature already demonstrated favorable cost-effectiveness for genomic signatures in our population, both in private[32] and public[33] healthcare settings.

Recommendation: Genomic signatures are recommended for defining adjuvant therapy in hormone receptor (HR)-positive and HER2-negative patients.

Level of evidence: I

Grade of recommendation: A

Recommendation: Genomic signatures should not be used to define neoadjuvant treatment in breast cancer.

Level of evidence: III

Grade of recommendation: D

7. Should IHC testing be repeated in breast surgery specimens?

According to the College of American Pathologists (CAP),[34] IHC testing should not be routinely repeated on surgical specimens, as the treatment plan is typically defined based on the IHC results from the first core needle biopsy. While the literature reports different results, several publications have indicated good agreement between IHC results from core needle biopsies and those from surgical specimens.

Meattini et al.[35] published a review of 15 studies on this topic, of which 10 studies demonstrated >90% agreement on ER status, and 13 studies showed high agreement on HER2 status, with five showing >90% agreement and six showing approximately 85% agreement. The CAP[34] suggests repeating IHC testing in individualized cases, where the pathologist has doubts about the test result, as summarized below:

- IHC testing should be repeated on a subsequent specimen, mainly surgical, in all cases of ER-, PR-, and HER2-negative tumors, regardless of whether or not the patient is undergoing neoadjuvant therapy.
- IHC testing should be repeated on a subsequent specimen, mainly surgical, in cases where there is a discrepancy between IHC results and histopathological characteristics. This includes scenarios such as tumors that are low-grade but ER-negative, or that are grade 2 or 3, PR-negative, and exhibit high Ki-67 but are HER2-negative.
- IHC testing should be repeated on a subsequent specimen, mainly surgical, in cases where sampling was insufficient, especially if the initial IHC results are negative.
- IHC testing should be repeated on a surgical specimen in multifocal and multicentric tumors, especially when additional foci present histopathological characteristics different from the index tumor.
- IHC testing should be repeated on a subsequent specimen in case of suspected false-negative results due to analytical or preanalytical problems, such as when a negative HR tumor with negative internal control generates an indeterminate result.
- IHC testing should be repeated after neoadjuvant therapy in case of large residual disease in the breast or lymph node.

There is ongoing debate regarding the necessity of repeating IHC testing, reevaluating slides, and repeating biopsies in cases of HER2-low tumors. This debate stems from the growing understanding that HER2-low tumors are spatially and temporally heterogeneous, and that sample quality issues require new evaluation. Therefore, these cases must be individually analyzed.

Recommendation: IHC should not be routinely repeated on surgical specimens, regardless of neoadjuvant treatment. The decision to retest for IHC should be made on a case-by-case basis, especially in cases where the first test has inconclusive results.

Level of evidence: III

Grade of recommendation: C

8. Which therapeutically relevant biomarkers should be assessed in advanced breast cancer?

Germline pathogenic variants in breast cancer susceptibility genes 1 and 2 (BRCA1 and BRCA2) were detected in 5% of patients with metastatic breast cancer.[36,37] Patients with pathogenic variants may benefit from therapy with poly (ADP-Ribose) polymerase (PARP) inhibitors such as olaparib[38] or talazoparib[39] particularly if they have been previously treated with chemotherapy and received at least one line of hormone therapy in case of luminal disease.

IHC testing for PD-L1 expression can aid in selecting the initial therapy for patients with advanced metastatic triple-negative tumors. The combination of immunotherapy and chemotherapy has been beneficial for first-line patients with metastatic triple-negative breast cancer exhibiting a programmed cell death ligand 1 (PD-L1) combined positive score (CPS) of ≥10[40] or a Ventana® PD-L1 SP142 assay of ≥1.[41]

Patients with HR-positive disease should be investigated for PIK3CA somatic mutation using validated NGS tests or qualitative real-time single-gene polymerase chain reaction (PCR). This mutation is present in 40% of patients with HR-positive and HER2-negative breast cancer, indicating a worse prognosis and predicting response to combined alpelisib-fulvestrant therapy after initial endocrine-based therapy.[42] Testing can be performed at the initial diagnosis of metastatic disease or at the time of tumor progression with first-line therapy. Based on the approval study, it is advisable to test the tumor sample; however, plasma samples (ctDNA) can be tested if tumor tissue is not available. If the plasma is negative for PIK3CA mutations, more tissue should be collected to repeat the test in tumor tissue, when possible.[42]

Recommendation: In addition to ER, PR, and HER2 status, we recommend routinely investigating germline BRCA1/2 pathogenic variants (gBRCAm) in HER2-negative advanced tumors, as well as PD-L1 status by IHC in triple-negative subtypes, and PIK3CA mutations in HER2-negative luminal tumors.

Level of evidence: I

Grade of recommendation: A

9. Is it necessary to repeat any tests for metastatic or recurrent breast cancer?

A biopsy of the metastatic site is recommended as part of the investigation in case of suspected metastatic disease or first recurrence. This biopsy can be used to accurately determine the presence of disease, identify tumor histology, and search for biomarkers with prognostic and predictive value that are important in treatment selection.[43] It is important to repeat hormone receptor (HR-ER and PR) and HER2 testing in all cases where diagnostic tissue is available.[30,44]

However, patients who are not clinically fit to undergo biopsy and who show strong evidence of recurrence can be treated based on the primary tumor's ER/PR/HER2 status.

Receptor status disagreement between primary and recurrent disease has been reported in several studies with disagreement rates between primary and metastatic lesions typically ranging between 5% and 30%.[45,46] This disagreement can be related to disease biology changes, differential effects of prior treatment on clonal subsets, tumor heterogeneity, or imperfections in assay accuracy and reproducibility.[45]

Currently, there are no established guidelines regarding which result should be used in treatment decision-making when tumor

biology results differ between the metastatic lesion and the primary tumor. Until a clinical trial analyzes this issue, the use of targeted therapy (endocrine or anti-HER2 therapy) is recommended when receptors are positive in at least one biopsy, regardless of the time of testing.[47]

HER2 status should be reassessed in a repeated biopsy specimen in patients with recurrent or metastatic breast cancer[25] since the HER2 status disagreement between the primary and metastatic breast tumor ranges between 2% and 25%.[48–51] Notably, a significant number of women with HER2-negative primary tumors exhibit protein overexpression at tumor recurrence.[51–54]

In metastatic tumors, differentiation between HER2 IHC 0 and 1 is clinically relevant since patients with HER2 1+ or 2+ unamplified FISH results in primary or metastatic specimens which may be eligible for targeted treatment with trastuzumab deruxtecan.[55]

Recommendation: Whenever possible, the metastatic lesion should be biopsied at the first diagnosis of advanced or recurrent breast cancer to confirm histology and reevaluate tumor biology, including ER, PR, and HER2 status.

Level of evidence: I

Grade of recommendation: A

10. Which tumor-agnostic biomarkers may have therapeutic application in later-line treatment of unresectable or metastatic breast cancer?

Patients with unresectable or metastatic breast cancer who exhibit deficient dMMR on IHC testing, high microsatellite instability (MSI-H), or high tumor mutational burden (TMB, ≥10 mutations/megabase), may also benefit from pembrolizumab after tumor progression with other treatments.[56] The evidence supporting this therapeutic application has mostly been provided by basket studies on pembrolizumab use with different primary tumors, which typically include a small number of patients with breast cancer.[56–58]

Patients with solid tumors lacking satisfactory alternative treatments can be treated with entrectinib [59] or larotrectinib,[60] when NTRK gene fusions are identified. A search for NTRK gene fusions using FISH, NGS, or PCR should be conducted as part of the diagnostic workup.[61] Structural rearrangements involving one of the NTRK genes result in oncogenic transformation and cause tumor growth. However, the incidence of NTRK gene fusions in breast cancer is low, being estimated at <1%.[62]

Furthermore, patients with RET gene fusion and no alternative treatment options may benefit from selpercatinib,[63] although the data is still preliminary. This drug was approved by the FDA in 2022 based on its overall response rate and duration in 41 patients with multiple primary tumors treated in the LIBRETTO-001 study.[63] Notably, a large-scale genomic profiling study identified RET gene fusions in only 16 of 9693 (0.17%) patients with breast cancer.[64]

Recommendation: Agnostic-tumor markers such as MSI, TMB, NTRK, and RET fusion should be assessed in later-line treatments when these targeted therapies are recommended.

Level of evidence: II

Grade of recommendation: B (confirm)

3.1.2 | Ovarian cancer

11. What is the minimum information required in ovarian cancer anatomopathological analysis and IHC testing?

The College of American Pathologists (CAP) [65] recommends the report of the following data for surgical specimens: tumor site; specimen integrity, size, histologic type, and histologic grade (if applicable); ovarian and uterine tube surface involvement, presence of implants (low-grade serous borderline carcinoma); size of the largest pelvic focus; size of the largest extrapelvic focus; peritoneal or ascitic fluid; chemotherapy response score (CRS) (for postneoadjuvant high-grade serous carcinoma; CRS1, no or minimal response; CRS2, moderate response; CRS3, significant response, minimal disease or complete response); regional lymph node status, anatomical site (pelvic, para-aortic), and number of lymph nodes with metastasis (define size ≥10 or <10 mm); pathologic TNM (pTNM) classification; p53 IHC. These data, when combined with the surgical report, play a crucial role in defining the prognosis and provide relevant information for clinical decisions, such as the use of maintenance therapy.[66] In the case of biopsy, the data are restricted to specimen site, type, and histological grade.

The use of IHC to determine p53 status can differentiate low or high grade ovarian serous carcinoma. An aberrant IHC pattern serves as a surrogate marker for TP53 gene mutations. The most common aberrant patterns are overexpression (diffuse, strong nuclear positivity) and null type (complete absence of nuclear reactivity), which usually arises from the insertion or deletion of the TP53 gene. To avoid confusion, p53 expression should be reported as either normal (wild type) or abnormal, with the aberrant expression pattern in parentheses.[67]

For all endometrioid and clear cell ovarian carcinomas, IHC testing for the MMR proteins MLH1, MS2, MSH6, and PMS2 should be performed to screen Lynch syndrome.[68–70] In cases where there is no expression of one or more of these proteins, further investigation to confirm hereditary cancer syndrome are needed.[71]

Recommendation: The anatomopathological report should include details such as tumor site; specimen integrity, size, histological type, and histological grade; ovarian and uterine tube surface involvement, presence of implants; size of the largest pelvic focus; size of the largest extrapelvic focus; peritoneal or ascitic fluid; metastatic lymph node status and number, and pTNM classification.

Level of evidence: II

Grade of recommendation: A

Recommendation: p53 and MMR IHC should be performed for serous and endometrioid/clear cell histology, respectively.

Level of evidence: II

Grade of recommendation: A

12. Which STT are required to define ovarian cancer treatment at the time of diagnosis?

The advent of PARP inhibitors and their substantial benefits have made assessment of HRD status, which includes BRCA1/2 testing, mandatory in serous and endometrioid ovarian carcinomas. For those patients with advanced disease stages III and IV who demonstrate

responsiveness to platinum-based chemotherapy and exhibit HRD-positive, combination maintenance therapy with olaparib and bevacizumab has proven beneficial.[4,72] Additionally, the use of niraparib as a standalone maintenance therapy has shown significantly prolonged progression-free survival compared with placebo, regardless of HRD[5] status. Therefore, determining HRD status is essential in defining prognosis and guiding therapeutic decision-making. Moreover, tumor HRD status is a screening tool for gBRCAm, as one in four patients with HRD-positive tumor has a gBRCAm.[73]

Despite the comparable incidence of homologous recombination mutations in both serous and nonserous carcinomas,[74] there is currently no evidence demonstrating clinical benefits in testing these patients.

Recommendation: HRD should be tested at the time of diagnosis in high-grade advanced serous and endometrioid carcinomas.

Level of evidence: II

Grade of recommendation: A

13. Is there an ideal time for STT in ovarian cancer?

HRD testing should be conducted immediately upon confirmation of the diagnosis of high-grade EC III or IV serous and endometrioid carcinomas. This is crucial as information regarding genetic mutations such as BRCA1, BRCA2, and HRD score can significantly impact decision-making regarding systemic maintenance treatment, such as the use of PARP inhibitors.[4,5,72] It is essential not to delay HRD testing, as molecular tests may lose sensitivity according to CRS.[75] In addition, carboplatin and paclitaxel (initial treatment of choice) have a response rate greater than 50%[76]; therefore, there may be no residual tumor for testing after systemic therapy is initiated.

Patients with gBRCAm should undergo genetic counseling, when breast and ovary risk-reducing surgeries and intensive surveillance will be discussed for them and their relatives.[71,77] However, the tumor molecular profile does not influence surgical planning.

Recommendation: HRD should be tested at the time of diagnosis for high-grade advanced ovarian serous and endometrioid carcinomas.

Level of evidence: I

Grade of recommendation: A

Recommendation: Patients in follow-up, with treated high-grade serous and endometrioid carcinoma, and without signs of recurrence should be referred for genetic evaluation if not previously tested.

Level of evidence: III

Grade of recommendation: B

14. Is it necessary to repeat any STT in case of ovarian cancer recurrence?

Genetic testing, including germline and somatic testing, should not be repeated in cases of cancer recurrence, as it currently lacks therapeutic implications.[78] Although secondary BRCA1/2 mutations have been identified in patients developing resistance to platinum or PARP inhibitor therapy, these mutations do not influence subsequent treatment decisions and should not be used to exclude PARP inhibitor therapy in eligible patients who have never been treated with PARP.[79]

Patients with high-grade epithelial ovarian cancer who were not tested for HRD at the time of diagnosis should undergo germline testing for BRCA1/2 and others associated with hereditary ovarian cancer risk, as these genetic factors have a significant impact on management strategies in hereditary ovarian cancer syndrome carriers.[80] Therefore, germline panels should be offered for all patients not assessed at the time of diagnosis, regardless of recurrence.[78]

Recommendation: STT should not be repeated in ovarian cancer recurrence.

Level of evidence: I

Grade of recommendation: A

Recommendation: Patients with previous negative gBRCAm testing should undergo BRCA1/2 STT in case of relapse.

Level of evidence: II

Grade of recommendation: B

Recommendation: Genetic testing for resistance mutations has not been incorporated into routine clinical practice for patients who progressed to PARP inhibitor therapy. Therefore, such testing should be conducted in the context of research protocols.

Level of evidence: III

Grade of recommendation: B

3.1.3 | Endometrial cancer

15. What is the minimum information required in endometrial cancer anatomopathological analysis and IHC testing?

Pathological reports of surgical specimens in all cases of endometrial carcinoma should include the following information[81]: histological type, according to the updated World Health Organization (WHO) classification[82]; histological grade; myometrial infiltration; vascular embolization; uterine serosal involvement; extension to the uterine cervix and extrauterine extension; and lymph node status.

Despite wide variations between populations, almost a quarter of patients with endometrial cancer have dMMR tumors.[83] Therefore, all patients with endometrial cancer must undergo IHC testing to identify repair enzyme deficiency, including MLH1, MSH2, MSH6, and PMS2, which may indicate Lynch syndrome.

In addition, the recent association of standard-of-care chemotherapy with checkpoint inhibitors dostarlimab[84] or pembrolizumab[6] significantly increased progression-free survival in patients with advanced (EC III or IV) or recurrent primary endometrial cancer who exhibit dMMR, reinforcing the importance of testing in these scenarios.

ER and PR IHC testing is recommended to predict the response of endocrine therapy in stages III and IV, as well as in cases of relapsed disease.[85,86] Moreover, HR expression is a valuable prognostic factor, particularly when associated with histological grade, especially in tumors exhibiting a nonspecific molecular profile. A study with 904 patients reported a risk of death of 1.6% in low-grade tumors with positive HR (>1%) across all stages, compared with the 1.4% in stage I tumors. Conversely, high-grade tumors or those

with negative HR showed a significantly higher mortality risk of 22.9%.[87]

The assessment of p53 using IHC is important in both early- and advanced-stage endometrial tumors. It is advisable to request this assessment during diagnostic endometrial biopsy procedures. A positive result requires a peritoneal evaluation similar to the one required in ovarian cancer, including omentectomy.[88] However, this can be disregarded in cases of low-grade endometrial tumors. Patients with abnormal p53 status are classified as high risk, even in instances of minimal myometrial invasion, with an indication for adjuvant therapy.[86]

HER2 overexpression and/or amplification testing is indicated for serous carcinoma and other high-grade p53 mutations.[89–91] A phase 2 randomized multi-institutional clinical trial is currently evaluating the efficacy of carboplatin/paclitaxel versus carboplatin/paclitaxel/trastuzumab in advanced and recurrent HER2-positive endometrial serous carcinoma.[92] Updated survival results published in 2020 showed that trastuzumab increased overall survival in this group of patients, mostly in stages III and IV.[93]

Additionally, other IHC markers may hold significance in pathological diagnosis (PTEN, p16, ER, Napsin A, Racemase, Pax8, and E-Cadherin) or prognosis assessment (L1CAM).[86] However, their clinical utility is under investigation and is not essential for treatment definition.

Recommendation: IHC testing to identify repair enzymes (MLH1, MSH2, MSH6, and PMS2) and p53 should be conducted for all patients with endometrial cancer.

Level of evidence: III

Grade of recommendation: B

Recommendation: ER, PR, and HER2 IHC testing is recommended in stages III and IV and relapsed disease.

Level of evidence: II

Grade of recommendation: A

16. Which STT are required to define initial endometrial cancer treatment?

Several medical societies have recommended molecular profiling for subtype definition in endometrial cancer, including analysis of POLE mutation, dMMR, aberrant p53, and nonspecific molecular profiling.[19,81,82,86,94–96] However, molecular classification requires POLE mutation sequencing analysis (NGS, Sanger, etc.), which may not be universally accessible in Brazil. Considering that management changes can only be individualized according to the molecular profile in some cases, this test may be indicated in endometrioid tumors restricted to the uterine body, MMR proficient, and meeting at least one of the following criteria: infiltration in ≥ 50% of the myometrium, multifocal vascular embolization, high grade, or aberrant p53. In cases where these criteria are not met, the test may not be necessary, particularly in advanced stages (II or IV) or low-risk disease.[91]

Although MSI analysis by PCR can identify the majority of patients with dMMR, this test is reserved for cases with inconclusive MMR IHC,[97] as it is more widely available.

Recommendation: Consideration may be given to POLE mutation analysis in endometrioid tumors restricted to the uterine body, MMR proficient, and meeting at least one of the following criteria: infiltration in ≥50% of the myometrium, multifocal vascular embolization, high grade, or aberrant p53.

Level of evidence: III

Grade of recommendation: C

17. How can sentinel lymph node ultrastaging be performed in endometrial cancer?

Ultrastaging is indicated for analyzing sentinel lymph nodes that test negative for metastasis in the routine histopathological analysis of endometrial cancer. This method significantly improves the detection of lymph node metastasis during surgical staging, especially in identifying low-volume metastases such as micrometastases. Studies have demonstrated that conventional techniques may overlook 37%–50% of positive sentinel lymph nodes.[98,99] Considering that patients with micrometastasis have a worse prognosis without adjuvant treatment,[100] identifying these micrometastases is essential for informing treatment decisions.

Several ultrastaging protocols have been established, yet there is no standardized technique [81] preferred universally due to reports of insignificant differences between protocols.[98,101–103] These protocols vary in terms of lymph node cutting intervals (40–250 µm), but all commonly involve a combination of H&E analysis and cytokeratin AE1/AE3 IHC.[104–108] Among the recommended methods, two have been compared and showed to have no significant differences[101]: five H&E levels with 250 µm tissue block cuts, with two unstained slides at each level; pankeratin IHC at level 1 in cases with negative H&E levels; one H&E level with two unstained slides with 250 µm tissue block cuts and pankeratin IHC in cases with negative H&E levels. Another protocol uses H&E and pankeratin IHC with 50 µm tissue block cuts, resulting in a total of five sections per block.

Recommendation: Micrometastasis should be systematically investigated by sentinel lymph node ultrastaging in patients with endometrial cancer.

Level of evidence: II

Grade of recommendation: A

Recommendation: Ultrastaging should be performed on 40–200 µm lymph node cuts using combined H&E analysis and cytokeratin AE1/AE3 IHC.

Level of evidence: III

Grade of recommendation: B

18. Which IHC or STT are required for assessing relapsed endometrial tumors?

Patients who have not undergone the previously suggested **IHC or STT** testing at the time of diagnosis should undergo all recommended IHC tests for newly diagnosed tumors in case of recurrence. The status of MMR is essential because it influences the decision on whether to use immunotherapy or not, as it has been shown to significantly increase disease-free survival in relapses.[6,84] HER2 overexpression and amplification should be investigated in patients with serous carcinoma or other high-grade aberrant p53 since it may indicate the inclusion of trastuzumab in standard chemotherapy for increasing overall survival in this group of patients.[93] Likewise, ER and PR IHC testing is recommended in relapsed disease to predict the response to endocrine therapy.[85,86]

Some of these tests may need to be repeated in patients who have been previously tested since their status may have changed, requiring treatment changes. Loss of HER2 expression is common in metastatic endometrial tumors; thus, HER2 levels should be assessed in metastatic lesions to define the potential benefit of anti-HER2 treatment in patients with endometrial cancer.[109] Notably, in 11% of cases, primary tumors with low HER2 levels may present corresponding metastases with high HER2 expression.[109]

ER and PR IHC tests should be repeated in patients with previous positive results, as their expression is significantly lower (approximately 30%) in metastases compared with the corresponding primary tumors.[110]

MMR, p53, and p16 results often agree between primary tumors and recurrences or metastases.[111] However, approximately 7% of endometrial tumors may present somatic loss of MMR protein expression at recurrent or metastatic sites compared with a paired primary tumor.[112] In selected cases, MMR IHC testing may be considered in tumor recurrence or metastasis to guide immunotherapy.

Recommendation: Patients with relapsed endometrial cancer should undergo MMR (MLH1, MSH2, MSH6, and PMS2), HER2, ER and PR IHC testing.

Level of evidence: I

Grade of recommendation: A

Recommendation: Patients with relapsed endometrial cancer and HR-positive primary tumors eligible for hormonal therapy should repeat ER and PR IHC at the site of recurrence or metastasis.

Level of evidence: III

Grade of recommendation: A

3.1.4 | Cervical cancer

19. What is the minimum information required in cervical cancer anatomopathological analysis and IHC testing?

The CAP[113] recommends including the following data for surgical specimens for cervical cancer: tumor size, histological type, degree of differentiation, stromal invasion, involvement of adjacent structures (parametrium/organs), margins, lymphovascular invasion, regional lymph nodes, TNM staging, and additional findings.

While IHC testing may not impact therapeutic decisions for most histological types of cervical cancer, it can be crucial for differential diagnosis and prognostic assessment.[114] One exception is p16 analysis, which helps determine if the tumor is human papillomavirus (HPV)-dependent or independent, serving as an important prognostic factor.

Additionally, cytokeratin AE1/AE3 IHC in sentinel lymph node ultrastaging is recommended, as it significantly increases the detection of low-volume disease from 5.3% to 9.1%.

Recommendation: Ultrastaging with cytokeratin IHC should be used to analyze sentinel lymph node micrometastasis in patients with cervical cancer.

Level of evidence: III

Grade of recommendation: B

20. Which STT are required to define initial cervical cancer treatment?

Although STT is not routinely indicated for patients with cervical cancer, it may be considered in certain situations.

From a somatic perspective, the Precision Medicine Working Group of the European Society of Medical Oncology (ESMO)[115] has recommended TMB analysis in all cervical tumors since 2020. TMB ≥ 175 mutations/exome is associated with clinically significant improvements in the efficacy of pembrolizumab monotherapy. Additionally, higher TMB levels have shown better results for pembrolizumab compared with chemotherapy in a wide range of previously treated advanced solid tumor types.[116] However, this indication is only valid for selected cases.

Recommendation: NGS panel analysis is acceptable for the somatic assessment of TMB in previously treated advanced cervical tumors to select patients eligible for immunotherapy.

Level of evidence: IV

Grade of recommendation: C

21. Is HPV testing necessary in confirmed cervical cancer?

Almost all cervical cancers are linked to HPV infection. However, the clinical applicability of HPV detection is limited to cervical cancer screening by in situ hybridization or HPV genotyping using PCR.[117]

HPV detection is not required for the diagnosis or staging of cervical carcinoma; however, may be useful in the differential diagnosis of metastatic lesions suspected of primary cervical cancer.[118] Although the gold standard for HPV testing is in situ hybridization or HPV DNA PCR, p16 IHC has been used.[119] Notably, unlike in oropharyngeal tumors, HPV detection in cervical tumors does not change the prognosis or influence therapeutic strategies.[120]

Recommendation: HPV detection is not routinely recommended and has no therapeutic implications in cervical tumors.

Level of evidence: II

Grade of recommendation: B

22. Which IHC testing are required to define first-line treatment in recurrent or advanced cervical cancer?

PD-L1 expression has not been demonstrated in normal cervical tissue, however, it is detected in 95% of cervical intraepithelial neoplasia and in various cell types within cervical cancer, including T cells, antigen-presenting cells (APCs), and tumor cells.[121] In cervical squamous cell carcinoma (SCC), PD-L1 expression rates vary widely, ranging from 19% to 88%, whereas it is less prevalent in cervical adenocarcinoma (14%).[122] Assessing PD-L1 expression using the CPS can help with the decision to add immunotherapy to systemic treatment when analyzed by a validated test and preferably in a freshly obtained biopsy or stored tumor tissue sample collected from a nonirradiated lesion.[7,8]

A phase III study reported that adding pembrolizumab to first-line chemotherapy, with or without bevacizumab, improved disease-free survival and overall survival in patients with persistent, recurrent, or metastatic disease, without adverse effects on their quality of life.[7,8] Specifically, the risk of death decreased by 40% in patients with PD-L1 CPS ≥ 1, 37% in the general population, and 42% in those with CPS ≥ 10.[123]

Recommendation: PD-L1 IHC analysis should be used to identify eligible patients with recurrent, progressive, or metastatic disease who may benefit from the addition of pembrolizumab to first-line systemic therapy. The sample should be preferably from the metastatic site or the primary tumor.

Level of evidence: I

Grade of recommendation: A

4 | CONCLUSION

Ensuring proper tissue collection, manipulation, and analysis is fundamental for the successful implementation of precision medicine in female tumors. These updated and standardized guidelines improve the quality and utility of samples for histopathological, IHC, and STT. By establishing clear protocols, we can significantly advance the personalization of cancer care, ultimately improving clinical outcomes for patients with female tumors.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, Reitan Ribeiro, upon reasonable request. The data are not publicly available due to their containing information that could compromise the privacy of research authors.

ORCID

Reitan Ribeiro http://orcid.org/0000-0002-4323-3854
Filomena Marino Carvalho http://orcid.org/0000-0002-5838-3636
Glauco Baiocchi http://orcid.org/0000-0002-8193-5582
Carlos Henrique dos Anjos http://orcid.org/0000-0002-2815-0882
Renato Cagnacci Neto http://orcid.org/0000-0002-5812-3084
Allyne Queiroz Carneiro Cagnacci https://orcid.org/0009-0004-8926-5626
Vandré Cabral Gomes Carneiro https://orcid.org/0000-0001-8776-8837
Aknar Calabrich http://orcid.org/0000-0002-0641-5683
Rodrigo Nascimento Pinheiro http://orcid.org/0000-0002-2715-7628

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How to cite this article: Ribeiro R, Carvalho FM, Baiocchi G, et al. Guidelines of the Brazilian Society of Surgical Oncology for anatomopathological, immunohistochemical, and molecular testing in female tumors. *J Surg Oncol*. 2024;1-14. doi:10.1002/jso.27717