
Preanalytic Variables, Tissue Quality and Clinical Samples from Breast Cancer Patients: Implications for Treatment Planning, Drug Discovery and Translational Research

2

David G. Hicks

Abstract

There are an increasing number of cancer therapies that target specific molecular pathways that drive disease progression in a number of solid tumors requiring companion diagnostic assays. The testing for these tumors usually involves analyzing the tumor on a molecular level for the presence or absence of certain cancer gene signatures or biomarkers. In order for the correct treatment regimen to be determined, the test results need to be an accurate picture of what the patient's tumor is in vivo. With the increase of this type of molecular testing on solid tumors, there has developed an urgent need to preserve the integrity of these molecular markers in the tissues being tested. A long delay between removal of the tissue from the patient and preservation of the tissue can result in degradation or alterations in the molecular integrity of the tissue, confounding analysis. This chapter will review the data about these important preanalytic variables; discuss the need for standardized tissue handling procedures, and offer solutions.

Keywords

Pre-analytical variables • Tissue fixation • Processing

2.1 Introduction

The introduction of targeted cancer therapies into routine clinical practice, in which new treatment regimens are selected based on companion diagnostic testing of tumor tissues, is rapidly ushering in a new era of individualized 'precision' cancer care (Hicks 2012). The analysis of human tissue with high throughput molecular technologies has been used to help define new prognostic and predictive biomarkers and gene

D.G. Hicks (✉)

Surgical Pathology Unit, Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, NY, USA
e-mail: David_Hicks@URMC.Rochester.edu

signatures that have been shown to outperform the standard clinical/pathologic variables used in current clinical practice (Van de Vijver et al. 2002). These discoveries are rapidly being translated into new treatment paradigms, and going forward we will see cancer diagnoses and treatments which include companion diagnostic molecular testing performed to guide the selection of the most appropriate therapies for individual patients, on a case by case basis (Hicks et al. 2008). For hematologic malignancies and certain solid tumors such as breast, lung, gastric and colon cancer, this new era has already arrived (Romond et al. 2005; Hicks and Whitney-Miller 2013). These advancements in diagnosis and management are being driven by rapid technologic advances in our ability to profile routine clinical samples on a molecular level, as well as by ever present and increasing economic pressures. Rising health care costs, along with the increasing number of new and costly targeted cancer drugs, necessitates the development and adoption of new diagnostic strategies and the implementation of new standards that will enable more effective patient selection, therapeutic decisions and treatment options that improve patient outcomes (Parkinson et al. 2014; Barron et al. 2009). As the molecular analysis of diagnostic human tissue samples enters into clinical practice, the accuracy, reliability and relevance of this approach need to be critically evaluated. These important issues apply equally to the discovery of new targeted cancer drugs and to the development of companion diagnostic molecular tests that will help identify the most suitable patients and guide the use of new targeted agents.

The procedures for collecting and preserving diagnostic clinical samples in current medical practice are for the most part decades old and involve the use of 10 % neutral buffered formalin as a fixative to stabilize and preserve tissue for morphologic evaluation. This preservation of human tissue for the morphologic analysis of routinely prepared hematoxylin and eosin stained sections has historically been the gold standard

for diagnosis in anatomic pathology. The methods of tissue handling, fixation, processing and sectioning were developed to ensure adequate tissue preservation and acceptable morphology, and it is important to emphasize that accurate morphologic assessment will continue to be relevant as an important part of the diagnostic evaluation in this new era of individualized or 'precision' cancer care (Hicks and McMahon 2010).

These standard practices, however, have paid little attention to the suitability of these tissues for further molecular analysis (Hicks and Boyce 2012). Increasingly the evaluation of diagnostic tissue samples demands further information beyond pure morphology, including an assessment of tumor biology and the level of expression of important target molecules within the diseased tissues. Significant variability in tissue handling and fixation in clinical laboratories has the potential to adversely affect the quality of these clinical samples for both diagnostic evaluation and translational research. This is particularly true in breast cancer, where the evaluation of molecular pathways involving the estrogen receptor (ER), progesterone receptor (PR) and the tyrosine kinase human epidermal growth factor receptor-2 (HER2) is now a part of the standard initial work up for all newly diagnosed breast cancer patients and is important for clinical decisions concerning the selection of the most appropriate adjuvant treatment regimen. These critically important biomarkers help to identify subsets of patients who are appropriate candidates for treatments that target these specific molecular drivers of disease progression (Yaziji et al. 2008; Hicks and Kulkarni 2008a, b). The tissue sample now needs to be considered an analyte, and specification of tissue quality becomes an important aspect of any validated molecular assay performed on these samples (Hewitt et al. 2008). However, the accurate, reliable and reproducible assessment of these biomarkers in clinical specimens represents a significant challenge for surgical pathology laboratories.

2.2 Pre-analytical Variables

The emerging field of biospecimen science has recognized the significant impact of tissue handling and other preanalytical variables on the expression of biomarkers and the suitability of biospecimens for molecular analysis (Moore et al. 2011; Betsou et al. 2009; De Cecco et al. 2009). Preanalytical procedures affecting tissue quality are not generally standardized and have historically been poorly controlled. In addition to prolonged ischemia, many other preanalytic variables have been identified; including the type of fixative used, the size of the tissue, time of fixation, temperature during fixation and processing as well as the type of tissue processing. The ligation of the blood supply to a living tissue being resected during a surgical procedure will induce hypoxia and metabolic stress, resulting in progressive changes in the levels of gene expression along with the degradation of macromolecules that are of potential clinical interest (Liu et al. 2013). The time interval between arterial ligation and tissue removal from the patient has been termed the ‘warm ischemic time’ and can vary considerably depending on the experience of the surgeon and the complexity of the surgical procedure (Liu et al. 2013). The ‘cold ischemic time’ is the interval from removal of the sample from the surgical field until incision of the tissue in the laboratory and placement into a suitable fixative. While the ‘warm ischemic time’ is difficult to control because it is dependent on the surgeon and the surgical procedure, the ‘cold ischemic time’ is dependent only on the proximity of the operating rooms to the laboratory and having procedures in place to quickly transport the tissue to the laboratory so that it can be prepared for tissue fixation (Hicks et al. 2011). Differing intervals of cold ischemic time due to variable tissue handling remains an important technical hurdle for the study of molecular targets in clinical samples. The current reality is that specimen handling can be quite diverse across different institutions and most routine clinical

practices and lacks strict standardization or well-defined standard operating procedures (Hicks and Boyce 2012). Furthermore, in many places both the time interval and the degree of variability are virtually unknown. With this in mind, recent national guidelines have recommended both prompt gross examination and the establishment of minimal and maximal fixation times for breast samples in an attempt to reduce the reported variability that exists in breast tumor predictive assays such as HER2 (Wolff et al. 2007). New ER, PR and HER2 testing guidelines from the ASCO/CAP task force have taken this one step further and now require that breast biopsies and excised breast tissue samples be assessed grossly as rapidly as possible, sectioned and placed in formalin, ideally within 1 h from excision and removal from the patient, and that these times be recorded for each specimen (Hammond et al. 2010; Wolff et al. 2014). While the authors of the guidelines attempted to have each of the recommendations supported by scientific evidence, quantitative data are sparse for many preanalytic variables, including the effects of cold ischemic times. Subsequently, the new guidelines have prompted a re-evaluation of protocols and procedures involving tissue handling for breast specimen and more globally for all surgically removed tissues in a number of institutions. With the rising importance of being able to obtain molecular and genetic information from clinical samples, both surgeons and pathologists will need to be more cognizant of these changes and reevaluate the traditional ways in which tissue samples coming from the operating rooms have been handled as they are transported to the pathology laboratory (Balch 2011). In light of these national guidelines, we have assessed the degree of variability in tissue handling from the operating rooms in our institution and, on the basis of our findings, took steps to try to standardize this potentially important preanalytic variable for all tissue samples that are handled and processed in the surgical pathology unit at our medical center.

2.2.1 The Rapid Tissue Acquisition Program

To address the problem of variable tissue handling in our medical center, we have implemented a new standard operating procedure that we call the “rapid tissue acquisition program” (RTAP), in which pathology has assumed the responsibility for the collection and transport of tissue samples coming from the operating room to the pathology laboratory (Hicks and Boyce 2012; Hicks and Kulkarni 2008a; Hicks 2014). Technical personnel from pathology are stationed in the operating room area during regular hours, equipped with cell phones. These ‘pathology-runners’ are notified when a specimen has been removed from a patient and are responsible for pickup and rapid transport of the resected tissue to the laboratory. The ‘runner’ verifies that the specimen is properly labeled, that clinical history is provided and that the collection time, laboratory receipt time and fixation start time are all recorded and tracked for each specimen. Since implementation of this new system, we have seen significant improvement in the median time to fixation and the total number of specimens incised and placed into formalin within one hour from surgical removal from the patient.

2.2.2 How Important Is Standardizing Tissue Handling?

With the implementation of a rapid tissue acquisition program, we have shown that the proactive, rapid retrieval and delivery of tissue specimens from the operating room to the surgical pathology laboratory is possible and can significantly reduce the time interval from collection to the start of fixation for tissue samples removed during surgery (Hicks and Boyce 2012; Hicks and Kulkarni 2008a; Hicks 2014). But the question remains, how important is this for patient care, translational research and potential molecular analysis?

The increasing utilization of molecular analysis and biomarkers in clinical practice as well as

in translational research has begun to raise awareness of issues surrounding tissue quality and has led to an increasing emphasis on optimal sample preparation for molecular interrogation (Sherman et al. 2010). The ligation of the blood supply to living tissues being excised during surgery will lead to hypoxia, ischemia and the progressive degradation of macromolecules that are of potential clinical interest. Studies have shown that the level of expression of gene transcripts and proteins can change significantly during this ischemic interval (Hewitt et al. 2008; Liu et al. 2013; Dash et al. 2002; Miyatake et al. 2004). These changes include degradation of RNA, increased levels of expression of hypoxia-induced factor, as well as markers of post-translational modification as a consequence of ischemia and delayed time to formalin fixation (Neumeister et al. 2012). The degradation and/or changes in expression of different target molecules with increasing ischemia will almost certainly confound research studies that are performed on clinical samples with variable or unknown tissue handling (van Maldegem et al. 2008). Additionally, for solid tumors such as breast cancer in which current targeted therapies represent a potential treatment option, the potential impact of the quality of the tissue for diagnostic evaluation remains unclear, and there are few studies available dealing with the impact of tissue handling on the accuracy of breast predictive factor assays (Khouri et al. 2009). Pinhel et al. (2010) have shown that while ER, PR and Ki67 immunohistochemical expression levels were similar between needle core biopsies (which are typically placed into fixative immediately after removal) and breast cancer excisions. However, the immunohistochemical reactivity for phospho-Akt and phospho-Erk1/2 was markedly reduced in the latter specimen type from the same patient. These differences are most likely attributable to variations in tissue handling of excisional samples. Vassilakopoulou et al. (2015) have evaluated the change in antigenicity of a series of phosphoproteins in paraffin-embedded samples for breast tumors as a function of time to formalin fixation. The analysis was performed using the AQUA technology for quantitative

immunofluorescence, and showed that the majority of epitopes tested revealed changes in expression with increasing cold ischemic times. Some phosphorylated proteins, such as phosphor-HSP27 and phosphor-S6 RP, which are involved in posttranslational modification and stress response pathways, showed an increase in expression or phosphorylation levels. Other phosphor-epitopes, like phosphor-AKT, phosphor-ERK1/2, phosphor-Tyrosine, and phosphor-MET, were found to be quite labile with loss of antigenicity within 1–2 h of cold ischemic time. This data strongly suggests that there is an important dephosphorylation of proteins in surgical specimens as a result of endogenous tumor phosphatase activity related to delayed fixation, and that the subsequent results of these assay might not be reflective of the *in vivo* status of the tumor (Vassilakopoulou et al. 2015; Espina et al. 2008). Given that protein kinases are targets for a significant number of new drugs under development for oncology, potentially important pharmacodynamic end points may be deleteriously affected by tissue handling practices that are routine but suboptimal. These differences will have major implications for future translational research, drug development, companion diagnostics test development, as well as clinical management, and must be taken into consideration in the design of ongoing clinical trials of new therapeutic agents, which have a linked companion diagnostic and a correlative science component.

Neumeister et al. (2012) have studied changes in antigenicity as a function of cold ischemic time in a series of 93 breast cancers with known time to fixation using the AQUA method of quantitative immunofluorescence. They found no evidence of loss of antigenicity with time-to-fixation in a 4 h time window. However, with a bootstrapping analysis, they observed a trend toward loss for ER and PR, a statistically significant loss of antigenicity for phosphorylated tyrosine ($P = 0.0048$), and trends toward loss for other proteins. They reported evidence of significantly increased antigenicity in acetylated lysine, AKAP13, and HIF1A, which are proteins known to be expressed in conditions of hypoxia.

The loss of antigenicity for phosphorylated tyrosine, and increase in expression of AKAP13 and HIF1A, were confirmed in another cohort of biopsies compared with resection specimens from the same patients. These authors concluded that there is a need for further studies that extend the time range and normalize for intratumoral heterogeneity that can provide more comprehensive information on preanalytic variation due to cold ischemic time and its potential impact on protein biomarker analysis. Neumeister et al. (2014) have also used the AQUA technology to attempt the construction of a tissue quality index (TQI) that could serve as an intrinsic control that would allow a global assessment of protein status based on quantitative measurement of a small number of selected, informative epitopes based on observed changes as a function of delayed time to formalin fixation. Using the quantitative expression levels of three epitopes on separate cohorts of training and validation specimens, these investigators were able to show an association of negative TQI values (an indicator for loss of tissue quality) with increasing cold ischemic times. The authors conclude that although this work is preliminary and requires further optimization and validation, it represents a proof of concept for the potential to provide a surrogate for monitoring tissue quality that could help to inform companion diagnostic testing for clinical trial enrollment or clinical decision making.

2.3 Pre-analytical Variable and Breast Cancer Diagnosis

Accurate and reliable assessment of estrogen and progesterone receptors is important in adjuvant treatment planning for breast cancer patients (Yaziji et al. 2008; Hammond et al. 2010; Goldstein 2010). ER and PR are thermolabile proteins whose levels of expression have been shown to be altered by prolonged cold ischemic times (Nkoy et al. 2010; Yildiz-Aktas et al. 2012). Recent studies have suggested that delays from tissue collection to the initiation of formalin fixation may adversely affect ER and PR

assessment (Khoury et al. 2009; Nkoy et al. 2010) as well as HER2 analysis (Khoury et al. 2009). In a study reported by Yildiz-Aktas et al. (2012), breast resection specimens were subjected to variable cold ischemic times within the refrigerator and at room temperature. These samples were processed and stained for ER, PR and HER2 and the results compared with the prior needle core biopsies from the same patient, which would have had a negligible cold ischemic time period before fixation. Significant reduction in IHC staining for hormone receptors and HER2 were not detected until 4 h for refrigerated samples and after 2 h for non-refrigerated samples. The authors concluded that the ASCO/CAP guideline of a cold ischemic time period of <1 h is a prudent guideline to follow and that refrigeration of specimens that may encounter delays until the start of fixation may be warranted. In a similar study, Khoury et al. (2009), showed that the staining for ER and PR were negatively impacted between 1 and 2 h and that fluorescence in situ hybridization for HER2 started to be significantly compromised after 2 h. The negative effect of delay to fixation for ER and PR were also seen when different antibody clones for these proteins were investigated (Qiu et al. 2010).

The results of these studies suggest that variable tissue handling leading to excessive cold ischemic times for excised breast tumor specimens could potentially result in patients being falsely classified as receptor negative. The consequence of invalid breast cancer testing for these important therapeutic targets has the potential to change the type of adjuvant therapeutic regimen offered, which in turn could adversely affect patient outcome. New ER, PR and HER2 testing guidelines from the ASCO/CAP task force now require that breast biopsies and excised breast tissue samples be incised and placed into formalin within 1 h from excision (Wolff et al. 2007, 2014; Hammond et al. 2010). Accomplishing this goal will require that collection times and the fixation start times are recorded and tracked so that the cold ischemic time for each clinical sample can be calculated and monitored. Our experience after implementing the ‘rapid

tissue acquisition program’ at our institution suggests that the one-hour window proposed by the ASCO/CAP task force is challenging and will require an emphasis on standardization of tissue handling as well as a commitment of resources and personnel, along with close collaboration with surgeons and operating room staff to accomplish this goal (Kulkarni and Hicks 2008).

2.3.1 Conclusions

There is a growing need for high quality human biospecimens for translational research as well as in clinical care. Advancements in our ability to profile human tissues on a molecular level has led to a better understanding of tumor biology, which in turn has led to new therapeutic targets and novel treatment approaches. This movement toward “precision medicine” and individualizing treatment to address disease biology will be heavily dependent on high quality tissue samples removed for diagnosis and molecular analysis. Efforts to minimize and document the cold ischemic time will be important as we move forward with clinical/translational research and will allow investigators to determine which genes and proteins are potentially valid and reliable as biomarkers for clinical decision-making (Hewitt et al. 2008). A greater emphasis needs to be placed on developing standardized methods of tissue procurement for diagnosis and molecular testing that are evidence-based, and this issue must be addressed by future biospecimen research. Defining the preanalytical requirements for the molecular analysis of clinical samples will be critical for moving next generation molecular testing from the research laboratories into clinical practice, and will help achieve the goals and potential of ‘precision’ cancer care. Such studies will in all likelihood lead to evidence-based guidelines for best practices in surgical pathology with regards to tissue handling and molecular testing. Taking ownership of specimen acquisition and transport on the part of our laboratory has led to improved standardization in tissue handling in our institution. Ensuring proper tissue handling of clinical samples is the joint

responsibility of pathologists, surgeons, radiologists, and their respective staff (Kulkarni and Hicks 2008) and will require good communication, collaboration and partnership to help ensure the best possible clinical care. The standardization of tissue handling will, in all likelihood, lead to improvements in the overall quality of patient specimens, which will benefit both clinical and research efforts.

Acknowledgments The author would like to thank Ms. Mary Jackson for her assistance in manuscript preparation. The author would also like to thank and acknowledge the work of Emma Tillett, Kristin McCarthy, Robyn Sage, Laurie Baxter, and Michelle Fuller from the department of Pathology as well as the operating room staff for their help and support for improving the quality of human tissue samples collected in our institution.

References

- Balch CM (2011) Reexamining our routines of handling surgical tissue in the operating room. *J Natl Cancer Inst Monogr* 2011(42):39–40
- Barron JJ, Cziraky MJ, Weisman T, Hicks DG (2009) HER2 testing and subsequent trastuzumab treatment for breast cancer in a managed care environment. *Oncologist* 14:760–768
- Betsou F, Barnes R, Burke T et al (2009) International Society for Biological and Environmental Repositories (ISBER) Working Group on Biospecimen Science. Human biospecimen research: experimental protocol and quality control tools. *Cancer Epidemiol Biomarkers Prev* 18:1017–1025
- Dash A, Maine IP, Varambally S et al (2002) Changes in differential gene expression because of warm ischemia time of radical prostatectomy specimens. *Am J Pathol* 161:1743–1748
- De Cecco L, Musella V, Veneroni S et al (2009) Impact of biospecimens handling on biomarker research in breast cancer. *BMC Cancer* 9:409
- Espina V, Edmiston KH, Heiby M et al (2008) A portrait of tissue phosphoprotein stability in the clinical tissue procurement process. *Mol Cell Proteomics* 7:1998–2018
- Goldstein NS (2010) Standardized predictive receptor immunohistochemical assays: the view forward from our past. *Am J Clin Pathol* 133:681–683
- Hammond ME, Hayes DF, Dowsett M et al (2010) American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J Clin Oncol* 28:2784–2795
- Hewitt SM, Lewis FA, Cao Y et al (2008) Tissue handling and specimen preparation in surgical pathology: issues concerning the recovery of nucleic acids from formalin-fixed, paraffin-embedded tissue. *Arch Pathol Lab Med* 132:1929–1935
- Hicks DG (2012) Targeted therapies in breast cancer: tailoring treatment to the molecular drivers of disease progression. *MLO Med Lab Obs* 44:38–39
- Hicks DG (2014) Standardization of tissue handling from the OR to the laboratory. *AORN J* 99:810–813
- Hicks DG, Boyce BF (2012) The challenge and importance of standardizing preanalytical variables in surgical pathology specimens for clinical care and translational research. *Biotech Histochem* 87:14–17
- Hicks DG, Kulkarni S (2008a) Trastuzumab as adjuvant therapy for early breast cancer: the importance of accurate human epidermal growth factor receptor 2 testing. *Arch Pathol Lab Med* 132:1008–1015
- Hicks DG, Kulkarni S (2008b) HER2 + breast cancer: review of biologic relevance and optimal use of diagnostic tools. *Am J Clin Pathol* 129:263–273
- Hicks DG, McMahon L (2010) The pros and cons of automation for immunohistochemistry from the perspective of the pathology laboratory. In: Shi S, Taylor C (eds) *Immunohistochemistry & Antigen Retrieval Technique Based Research & Diagnostics*. John Wiley & Sons, Inc., Hoboken, NJ, pp 151–163
- Hicks D, Whitney-Miller C (2013) The evolving role of HER2 evaluation for diagnosis and clinical decision making for breast and gastric adenocarcinoma. *Biotech Histochem* 88:121–131
- Hicks DG, Kulkarni S, Hammond ME (2008) The role of the indispensable surgical pathologist in treatment planning for breast cancer. *Arch Pathol Lab Med* 132:1226–1227
- Hicks DG, Kushner L, McCarthy K (2011) Breast cancer predictive factor testing: the challenges and importance of standardizing tissue handling. *J Natl Cancer Inst Monogr* 2011:43–45
- Khoury T, Sait S, Hwang H et al (2009) Delay to formalin fixation effect on breast biomarkers. *Mod Pathol* 22:1457–1467
- Kulkarni S, Hicks DG (2008) HER2-positive early breast cancer and trastuzumab: a surgeon's perspective. *Ann Surg Oncol* 15:1677–1688
- Liu NW, Sanford T, Srinivasan R et al (2013) Impact of ischemia and procurement conditions on gene expression in renal cell carcinoma. *Clin Cancer Res* 19:42–49
- Miyatake Y, Ikeda H, Michimata R et al (2004) Differential modulation of gene expression among rat tissues with warm ischemia. *Exp Mol Pathol* 77:222–230
- Moore HM, Compton CC, Alper J, Vaught JB (2011) International approaches to advancing biospecimen science. *Cancer Epidemiol Biomarkers Prev* 20:729–732
- Neumeister VM, Anagnostou V, Siddiqui S et al (2012) Quantitative assessment of effect of preanalytic cold ischemic time on protein expression in breast cancer tissues. *J Natl Cancer Inst* 104:1815–1824

- Neumeister VM, Parisi F, England AM et al (2014) A tissue quality index: an intrinsic control for measurement of effects of preanalytical variables on FFPE tissue. *Lab Invest* 94:467–474
- Nkoy FL, Hammond ME, Rees W et al (2010) Variable specimen handling affects hormone receptor test results in women with breast cancer: a large multi-hospital retrospective study. *Arch Pathol Lab Med* 134:606–612
- Parkinson B, Pearson SA, Viney R (2014) Economic evaluations of trastuzumab in HER2-positive metastatic breast cancer: a systematic review and critique. *Eur J Health Econ* 15:93–112
- Pinhel IF, MacNeill FA, Hills MJ (2010) Extreme loss of immunoreactive p-Akt and p-Erk1/2 during routine fixation of primary breast cancer. *Breast Cancer Res* 12:R76
- Qiu J, Kulkarni S, Chandrasekhar R et al (2010) Effect of delayed formalin fixation on estrogen and progesterone receptors in breast cancer: a study of three different clones. *Am J Clin Pathol* 134:813–819
- Romond EH, Perez EA, Bryant J et al (2005) Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med* 353:1673–1684
- Sherman ME, Howatt W, Blows FM et al (2010) Molecular pathology in epidemiologic studies: a primer on key considerations. *Cancer Epidemiol Biomarkers Prev* 19:966–972
- Van de Vijver MJ, He YD, van't Veer LJ et al (2002) A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med* 347:1999–2009
- van Maldegem F, de Wit M, Morsink F et al (2008) Effects of processing delay, formalin fixation, and immunohistochemistry on RNA Recovery From Formalin-fixed Paraffin-embedded Tissue Sections. *Diagn Mol Pathol* 17:51–58
- Vassilakopoulou M, Parisi F, Siddiqui S et al (2015) Preanalytical variables and phosphoepitope expression in FFPE tissue: quantitative epitope assessment after variable cold ischemic time. *Lab Invest* 95(3):334–341
- Wolff AC, Hammond ME, Hicks DG, et al, American Society of Clinical Oncology; College of American Pathologists (2014) Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *Arch Pathol Lab Med* 138(2):241–256
- Wolff AC, Hammond ME, Schwartz JN et al, American Society of Clinical Oncology/College of American Pathologists (2007) American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Arch Pathol Lab Med* 131:18–43
- Yaziji H, Taylor CR, Goldstein NS et al (2008) Consensus recommendations on estrogen receptor testing in breast cancer by immunohistochemistry. *Appl Immunohistochem Mol Morphol* 16:513–520
- Yildiz-Aktas IZ, Dabbs DJ, Bhargava R (2012) The effect of cold ischemic time on the immunohistochemical evaluation of estrogen receptor, progesterone receptor, and HER2 expression in invasive breast carcinoma. *Mod Pathol* 25:1098–1105

Molecular Pathology of Breast Cancer

Badve, S.; Gökmen-Polar, Y. (Eds.)

2016, VIII, 428 p. 54 illus. in color., Hardcover

ISBN: 978-3-319-41759-2