

Topical Update – The Hong Kong College of Pathologists

The Hong Kong College of Pathologists, Incorporated in Hong Kong with Limited Liability

Volume 17, Issue 2

July 2022

Editorial note:

New molecular techniques have contributed to the ever-expanding armamentarium for breast cancer diagnosis, treatment and prognostication. Since the molecular classification of breast cancer was established, pathologists have been using immunohistochemistry and DNA sequencing techniques to routinely grade and subtype breast cancer. RNA expression profiling using various platforms such as microarrays, quantitative PCR and Nanostring has also been used to guide patient treatment in early diseases. This topical update provides a concise review on the current diagnostic and prognostic modalities in breast cancer management. We welcome any feedback or suggestions. Please direct them to Dr. Alvin Cheung (e-mail: acheung@cuhk.edu.hk) of Education Committee, the Hong Kong College of Pathologists. Opinions expressed are those of the authors or named individuals, and are not necessarily those of the Hong Kong College of Pathologists.

Molecular diagnostics for breast cancer

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Introduction

Since the seminal report on breast cancer classification in 2000[1], increased understanding in the molecular biology of breast cancer has led to numerous immunohistochemical markers and molecular panels used as adjunct biomarkers. These biomarkers mainly serve the following purposes: As prognostic markers, to gauge the likelihood of a clinical event, disease recurrence or progression; as predictive markers, to assess the

likelihood of favourable or unfavourable effect from exposure to a medical product or a therapeutic agent[2]. In this review, the classical biomarkers of breast cancer will be briefly discussed, followed by a more detailed elaboration of molecular panels which are based on DNA alterations and gene expression levels.

Hormonal receptor and proliferative index markers

Unlike other cancers, the molecular classification of breast cancer (luminal A/B, HER2 positive, and basal-like cancers) have been translated well to the clinic[3], and immunohistochemical markers have been established to facilitate such classification without resorting to molecular methods[4, 5]. Some authorities believe that normal-like breast cancer are an artifact of contamination by normal cells[6, 7]. The Estrogen Receptor (ER) and Progesterone Receptor (PR) are predictive biomarkers for endocrine therapy[8]. ER or PR-expressing tumours tend to have a better outcome than those lacking the receptors.

The expression of Human Epidermal growth factor Receptor 2 (HER2) defines the molecular basis of the “HER2-positive” group of cancer. They account for slightly less than 20% of breast cancers, and have a worse prognosis compared to ER+/PR+/HER2- cancers[9]. It serves as a therapeutic target for trastuzumab and pertuzumab. While ER and PR are routinely detected by immunohistochemistry (IHC), HER2 expression can be detected by IHC, Dual in situ hybridization (DISH) or Fluorescence in situ hybridization (FISH)[10].

The proliferation marker Ki-67 serves as a useful adjunct investigation in the grading of breast cancer[11]. Calculated as the percentage of nuclear staining in cancer cells, the prognosis is said to be better when Ki-67 is <5% and significantly worse when it is >30% for early disease[12].

Molecular tests at the DNA level

Some genetic aberrations in breast cancer are worth mentioning because they may be susceptible to targeted therapy and can predict treatment response. *PIK3CA* mutation occurs in about 36% of breast cancer[13, 14]. In advanced or metastatic hormonal receptor-positive cancer, or in patients with disease progression on endocrine-based regimen, combination therapy with the PI3K inhibitor, alpelisib, together with fulvestrant may be a treatment option if there is *PIK3CA* mutation[15]. The mutation can be detected by the companion diagnostic kit Therascreen, with Sanger sequencing, or with next generation sequencing. In secretory

carcinoma, *NTRK* fusion is targetable by larotrectinib or entrectinib[16]. The presence of translocation can be detected with immunohistochemistry, next generation sequencing (NGS), Reverse-transcriptase (RT)-PCR or FISH. In non-secretory type breast cancers, *NTRK* fusion is very rare[17], such that the routine testing of this gene is unnecessary. For triple-negative breast cancer, *BRCA* aberrations can be present in about 6.5-34% of the cases[18]. The *BRCA* proteins constitute a part of the homologous recombination repair pathway. They are encoded by relatively large genes, with *BRCA1* being present on chromosome 17q21, having 23 exons; and *BRCA2* on chromosome 13q13.1, having 27 exons. The incidence of aberrations is markedly higher among Ashkenzi Jews (2.5%) than the general population (0.1%)[19]. *BRCA*-mutated tumour highly depends on PARP, another DNA repair protein, to maintain the tumour genome integrity. Therefore, PARP inhibitor therapy are useful in *BRCA*-mutated tumours, and this treatment approach is termed a “synthetic lethality”[20]. Due to the size of these genes, NGS would be the preferred detection platform, while multiplex ligation-dependent probe amplification (MLPA) is also suitable[21].

For other advanced cancer or triple negative breast cancer, immune checkpoint inhibitor may be indicated in some patients. Besides testing for PD-L1 expression by the companion diagnostic kits for atezolizumab and pembrolizumab, some data support the testing for microsatellite instability (MSI) and tumour mutation burden (TMB) as well[22]. MSI-high breast cancer may be treated with pembrolizumab, as are tumours with high TMB as assessed by the FoundationOne companion diagnostic or other NGS platforms[23].

Molecular tests at the RNA expression level

Oncotype Dx

Oncotype Dx was launched in year 2004. It involves mRNA extraction from formalin-fixed paraffin-embedded (FFPE) tissues[24]. The detection panel includes 21 genes (16 cancer-related genes and 5 reference genes), and the

detection platform is by quantitative-PCR (qPCR). The test had been studied in several trials, including the NASBP trial (National Surgical Adjuvant Breast and Bowel Project)[25], TAILORx trial (including 10273 women), and TxPONDER trial (5018 women)[26]. The test generates a recurrence score (RS) in the range of 0-100. In the TAILORx trial, patients of age >50 years had a substantial benefit from chemotherapy when RS \geq 26, whereas younger patients may be benefited when RS \geq 16.

MammaPrint

MammaPrint was launched in 2007. It consists of a 70-gene microarray, which accepts both fresh frozen or FFPE tissue for testing. It categorizes patients into “High risk” or “Low risk”. The MINDACT trial included 6693 patients and the RASTER trial included 427 patients for this test[27, 28]. There are some preliminary data to suggest systemic treatment can be recommended for the patients in the “High risk” group.

Blueprint

The Blueprint assay was developed by the same company as MammaPrint, and the test can be used together with MammaPrint. It involves a 80-gene panel, and serves to categorize tumour into luminal-A, luminal-B, HER2, or basal subtypes. Although this may overlap with the objective of IHC study described above, one important difference is that the luminal A and B groups can be associated with a different chemosensitivity and prognosis according to the Blueprint schema. Particularly, in the luminal B, Her2, and basal subtypes, chemotherapy can be beneficial to some patients with an improved survival. In contrast, for the luminal A group, the benefit for chemotherapy is not pronounced[29].

Prosigna (PAM50)

The Prosigna assay was launched in 2013. Following RNA extraction from FFPE tissue, the expression of a panel of 50 genes are detected by the NanoString “nCounter” platform[30]. This test is indicated for post-menopausal patients. Two large trials were conducted, including The ABCSG-8 study (Austrian Breast and Colorectal Cancer Study Group 8) and TransATAC study (translational arm of the anastrozole or tamoxifen

alone or combined)[31]. While a scoring scheme of 0-100 is used, the risk stratification is different depending on the lymph node status. For node-negative cancers, they are classified as low (0-40), intermediate (41-60), or high (61-100) risk; as for node-positive cancers, they are classified as low (0-40) or high (41-100) risk. The suggested treatment for low risk disease is hormonal therapy alone, while for high risk disease, chemotherapy in addition to hormonal therapy may be beneficial.

The Breast Cancer Index

The Breast Cancer Index was launched in 2008[32]. As an RT-PCR assay on FFPE tissue, it features a 11-gene panel with two major testing endpoints: Whether there is a benefit of extended endocrine therapy (for 5 years), and the risk of recurrence 5 to 10 years after diagnosis. The ratio of expression between estrogen signaling pathway genes HOXB13 and IL17BR (H/I ratio) is an important parameter, as in the MA.17 trial, high H/I indicated a higher risk of late recurrence and a benefit from extended letrozole therapy. Another trial, the aTTom study, included H/I high patients for an extended therapy and found up to 15% reduction in recurrence risk[33, 34]. The test results for the Breast Cancer Index are simple enough to be interpreted even by patients, with “Yes” and “No” to the question of whether extended endocrine therapy is beneficial, and recurrence risk in percent to report the chance of late distant recurrence.

Comparisons between test modalities

When the included genes are compared, it is noted that the Oncotype Dx and PAM50 panels have the most overlap. 11 genes are in common for Oncotype Dx and PAM50, for example *BCL2*, *CCNB1*, *MMP11*, which are markers for apoptosis, cell cycle, and tumour invasiveness[35]. Interestingly, for the 70 genes included in MammaPrint, only one gene, *SCUBE2*, overlaps with Oncotype DX, and two genes, *MELK* and *ORC6L*, overlap with Prosigna PAM50[35]. It remains to be studied whether the results in one test can be correlated with another test, but some key differences are still worth to be noted. For hormonal receptor positive stage I-II invasive breast cancer, all the tests have some use for prognostication. However, concerning whether chemotherapy is recommended, only Oncotype DX

has an established predictive value, while there is insufficient evidence for Mammaprint, Blueprint and Prosigna [36]. The Breast Cancer Index has predictive value for extended endocrine therapy. Some efforts have also been taken to translate some of these tests to hormonal receptor positive DCIS. Oncotype DX DCIS and DCISionRT have some use in patient prognostication, however, both tests have insufficient evidence to guide chemotherapy [37, 38].

For the regulatory status, Oncotype DX has been included in the NCCN/ASCO guidelines for the management of breast cancer patients. As for Mammaprint and Prosigna, these kits have been FDA-cleared for specific clinical settings. Logistically, both Oncotype DX and Mammaprint require end users to deliver specimens to a central laboratory for testing. Prosigna is available in a kit format for local laboratories to perform the test.

Conclusions and future perspectives

Unlike most other cancer types, RNA expression profiling has found remarkable translational use in breast cancer treatment. This can be attributed to an increased understanding of molecular classifications, hormonal receptor functions and breast cancer biology. While panels including other RNA expression signatures can be expected to emerge, it is important to understand the indications and differences for each testing system, as an increased number of testing options can be confusing to patients, while contradicting results among platforms can complicate the interpretation. Because RNA expression level has an inherent variability among patients, the subgrouping of patients into risk groups may not be ideal, and some patients may be placed in the wrong group using only one particular panel. Hopefully, with further elucidation of the breast cancer genome, novel molecular targets based on DNA alterations can be uncovered, as the presence of a particular mutation or translocation is a more consistent marker of susceptibility to targeted therapy. As we enter the era of personalized medicine, histologic assessments, immunohistochemical studies such as hormonal receptors and PD-L1 status, and molecular diagnostics can be expected to go hand

in hand in the formulation of management plans and prognostication in breast cancer patients.

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