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A plea for appraisal and appreciation of immunohistochemistry in the assessment of prognostic and predictive markers in invasive breast cancer

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ABSTRACT

This viewpoint is a personal reflection on the values and merits of immunohistochemistry in current breast cancer diagnosis. Immunohistochemistry is a validated mainstay in molecular subtyping of invasive breast cancer. Immunohistochemical assessment of hormone receptor status and HER2 expression is used to determine the clinico-pathological surrogate of breast cancer intrinsic subtypes, which guide neoadjuvant and adjuvant therapy. The advent of genomic prognostic signatures and qualitative mRNA-based assays makes some clinicians and researchers wonder whether immunohistochemistry should be abandoned. However, the perils and pitfalls of these mRNA-based tests cannot be neglected. This viewpoint offers a brief overview of quality issues in immunohistochemistry and qPCR, as well as a concise summary of currently available evidence on the correlation of immunohistochemistry and mRNA-based testing for prognostic and predictive markers in invasive breast cancer. We strongly advocate the use of immunohistochemistry as it integrates valuable spatial information with quantification of protein expression.

1. Introduction

Immunohistochemical assessment of hormone receptor (HR) status and HER2 expression in invasive breast tumors is performed daily world-wide. Determination of the clinico-pathological surrogate of breast cancer intrinsic subtypes is a mainstay as it guides adjuvant therapy [1,2]. At the 15th Sankt Gallen International Breast Cancer Conference (Vienna, March 2017) [3], some eminent researchers alluded that immunohistochemistry (IHC) could easily be replaced by mRNA-based assays.

Their motivation is mainly based on the observation that central review of IHC within large randomized controlled clinical trials resulted in discordant results regarding estrogen receptor (ER), progesterone receptor (PR) and HER2 testing. For instance, central review within a phase III ALLTO trial revealed up to 21,4% false-negative cases for ER and up to 14,5% false-positive cases for HER2 [4]. Orlando et al. reported significant discordance after central pathological review of locally diagnosed early invasive breast cancers, which impacted the subsequent decision-making process regarding therapy [5]. We noticed significant discrepancies between local and central HER2 IHC results, as central re-testing resulted in higher IHC scores [6,7]. Nevertheless, not all reports on central reviewing or re-testing are pessimistic. A report on the OPTIMA prelim trial showed that central re-testing of HR and HER2 status resulted in high levels of reproducibility, even raising questions concerning its cost-effectiveness [8]. Moreover, the central laboratory is not necessarily always right, since a benefit

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of trastuzumab therapy was shown for patients who had a locally tested HER2-positive, centrally tested HER2-negative tumor [9].

The number of reports on high concordance between IHC and RT-qPCR for the assessment of HR status and HER2 increases steadily [10,11]. RT-qPCR-based assays are propagated to be more sensitive than IHC. Has the era of IHC come to an end?

2. Quality issues in immunohistochemistry

IHC requires stringent quality control, but so does RT-qPCR. The ASCO/CAP panels for HR and HER2 IHC testing highlighted that it is essential to keep the cold ischemic time as short as possible, and that fixation duration should take at least 6 h and maximum 72 h [12,13]. Both prolonged cold ischemic time and insufficient time in fixative can cause false-negative results, leading to inadvertently denying these patients adjuvant therapies. Although the ASCO/CAP guidelines state that cold ischemic time should be limited to maximum 1 h [13], most studies assessing the effect of cold ischemic time generally observe no significant reduction in IHC staining until 4 h for refrigerated samples and 2 h for non-refrigerated samples [14,15]. It can be assumed that prolonged cold ischemic time is not only detrimental for proteins, but for RNA quality too. Evidence on the effects of cold ischemic time on RNA yield and RNA integrity in breast cancer is rather limited, but available reports indicate that time to fixation should be kept as short as possible to prevent RNA fragmentation [16]. Central re-testing, either IHC-based or RNA-based, will not mend the consequences of a sloppy pre-analytical phase.

In the past decade, increasing efforts have been made to assure quality in IHC. The use of both intrinsic and external positive controls provides information on possible failures in the pre-analytical and analytical phase [17]. The use of batch-controls is discouraged, as on-slide controls allow for the accurate detection of false-positive and false-negative tests on automated IHC platforms [18]. The participation in external proficiency testing programmes as well as audits by valid accrediting agencies allow critical monitoring of laboratory performances [13]. In the long term, laboratories can use positivity rates as quality indicators. Laboratories that perform HER2 testing should have an average positivity rate of 14%, ranging from 7 to 27% depending on patient characteristics [19]. Both HR status and HER2-positivity rates are stable in populations with stable clinical and histopathological characteristics when standardized assays are used [20]. As the average HER2-positivity rate is influenced by histological grade, HR status, age and nodal status, standardized assessment of these tumor- and patient-related features should be performed to obtain an “adjusted” HER2-positivity rate, which could then be used to identify centers with HER2 testing quality issues [19,21].

3. Discrepancies between IHC-based and RNA-based testing

Can IHC easily be replaced by RNA-based testing? In a subgroup of ductal carcinoma in situ (DCIS) patients included in the ECOG-E5194 study, the OncotypeDX DCIS recurrence score was used to determine HER2-positivity, which amounted only 7,6% [22]. Although this highly selected study population is not entirely representative for the “total” DCIS population, this HER2-positivity rate is incredibly low. This observation might partly be explained by the low nuclear grade and limited tumor size, which influence the HER2-positivity rate in DCIS [23]. However, a similarly low HER2-positivity rate of 17,5% was noted in the Ontario population-based DCIS cohort when the OncotypeDX DCIS recurrence score was used [24]. Contrary, the average IHC-based HER2-positivity rate was shown to amount 34,9% in the large multicenter NSABP B-43 trial [25]. We observed an IHC-based HER2-positivity rate ranging from 52 to 59% in single-center DCIS cohorts [23,26]. Therefore, the ability of the OncotypeDX DCIS recurrence score to adequately assess HER2 status might be questioned. Unfortunately, comparison between RT-qPCR-based HER2 status and conventional HER2 IHC or FISH was beyond the scope of the study of Solin et al. [22]. In agreement with these observations in DCIS, Dabbs et al. reported an unacceptably high false-negative rate for RT-qPCR-based HER2 status in invasive breast cancer, when OncotypeDX was compared with HER2 amplification status [27], and similar false-negative results were observed by others [28]. It must be acknowledged that the OncotypeDX assay has not been developed to assess HER2 status, as its primary goal is to quantify the risk of distant recurrences in patients with HR-positive early breast cancer. Contrary to OncotypeDX, the aim of the Mammatyper assay is to examine ER, PR, HER2 and Ki67 RNA expression in invasive breast cancer. The founders of STRATIFYER Molecular Pathology GmbH stated that RT-qPCR assessment of ER, PR and Ki-67 as provided by the Mammatyper assay is more accurate than quantitative IHC [11]. However, when Mammatyper was compared with two other RNA-based multiparameter assays (MammaPrint/BluePrint and the PAM50-based Prosigna) to determine intrinsic subtypes in the independent OPTIMA Prelim trial, discordant results across these tests were observed in 41% of patients [29]. Alvarado et al. reported poor correlation between OncotypeDX and Prosigna test results, with only 54% agreement between risk classifications [30]. Different tests apply different sets of genes, and different algorithms are used to obtain final results, which probably account for these differences in risk stratification. For this reason, we believe it is too early to abandon IHC in favor of RNA-based assays. After an extensive review of available literature, Liu et al. concluded that transcript levels alone do not suffice to predict protein expression [31]. Before we solely rely on RNA-based assays in daily clinical practice, more thorough investigations on correlation between IHC-based and RNA-based techniques should be performed and the cause of these discrepancies should be explored.

4. Perils and pitfalls in RNA-based testing

Many causes have been suggested to explain discordant results. Intratumor heterogeneity can affect gene expression profiles [32]. One of the major disadvantages of RNA-based testing is the loss of spatial information. Intratumor heterogeneity can be observed in IHC, and this information is lost upon use of RNA-based assays. Moreover, breast tumors do not consist of malignant cancer cells only, but comprise a complex ecosystem, which is populated by carcinoma-associated fibroblasts (CAFs), immune cells, adipocytes and endothelial cells, embedded within an extracellular matrix scaffold [33]. A significant number of invasive breast cancers is admixed with an in situ component [34]. When RNA is extracted, either from frozen tissue samples or paraffin-embedded tissue sections, RNA of invasive tumor cells is inevitably admixed with RNA of other tumor ecosystem components. Breast cancer itself is a heterogenous disease. Many factors can cause RNA sample dilution: some tumors have extensive in situ components, some tumors are rich in tumor-infiltrating lymphocytes or macrophages, and others evoke significant desmoplastic stromal reactions with numerous CAFs.

Sample dilution caused by stromal components might cause both false-negative and false-positive results. An increased stromal cellularity with or without associated inflammatory cells was shown to increase OncotypeDX recurrence scores, which was attributed to ‘contamination’ of the sample by stromal RNA [35]. Mammary fibroblasts have been shown to express ER [36]. Theoretically, a tissue sample of a triple-negative tumor which is admixed with large amounts of peritumoral fibroblasts might