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Commentary

Ki-67, an elusive marker in the prognosis of breast cancer

Proliferative activity has been traditionally assessed by counting mitotic figures at high magnification and is subsequently substantiated by immunohistochemical detection of Ki-67[1]. It is an antigen, having a short half-life and is accumulated in the S-phase of the proliferating cells. It is considered as a useful marker of cancer cells, owing to its relatively selective cell cycle phase expression pattern[2].

The immunohistochemical (IHC) detection of Ki-67 using the MIB-1 monoclonal antibody gained importance in routine breast cancer diagnosis. Its use was recommended by the St. Gallen consensus conference, when defining an adjuvant treatment plan for cancer patients[3]. IHC assessment of Ki-67 has been proposed as a potential marker to distinguish luminal A and luminal B breast cancer and guide adjuvant chemotherapy in hormone receptor-positive/HER2-negative (HR+/HER2−) early breast cancer[4]. It is helpful in accurate risk stratification to ensure the appropriate management of patients with chemotherapy so as to avoid unnecessary exposure. This calls for a strict standardization and quality control of its estimation[2,5]. Not only a high Ki-67 level correlated with some classical unfavourable prognostic factors, it also independently predicted poor clinical outcome in patients with HR+/HER2− carcinomas. Its role in triple-negative breast cancer is yet to be established[5].

Despite the standardization, this antibody remains a cause of concern. Variability in Ki-67 results is attributed in part to pre-analytical components including antigen retrieval, fixations, storage and staining techniques, with additional concerns of epitope loss[6]. In a study on the quality control of immunohistochemical staining and inter-observer variability of MIB-1 labelling in breast carcinomas, it was found to be not only dependent on obvious confounders *i,e.,* immunostaining technique and the selection of the tumour area but also on additional parameters[7]. It was not even corrected by methods of meticulous counting *vis-à-vis* rapid eyeballing. Efforts to standardize by exactly following the strict guidelines for identifying MIB-1-positive nuclei including steps of where and how to count them also showed no improvement. Interobserver variability of MIB-1 labelling index in breast cancer was found to be more problematic than expected by the authors[7].

The problem is compounded by the lack of clearly defined criteria for scoring/evaluation as well as cut-offs for Ki-67 interpretation. The general method has been average score across the sections, but some studies have focussed on hotspot and compared the predictive value of both techniques[5,8]. Hottest spot predicted clinical outcome better than the average score across the sections in 388 patients with HR+/HER2− cancers, as reported by the authors[5]. They attributed the results to the hottest spots, being the most biologically active part of the tumour which drives the outcome of the disease. It has also been postulated that the hottest spot may reflect the area, where the fixation condition is most suitable for Ki-67 staining. Assessment at the hottest spot is also more convenient and relatively free from issues of area selection as in assessment across the slide[5]. The cut-offs for Ki-67 have also been controversial ranging from 10 to 25 per cent and have been arbitrary[9].

In the 14th St. Gallen International Breast Cancer Conference (2015)[10], a majority of the Panel accepted a cut-off of Ki-67 within the range of 20-29 per cent to distinguish ‘luminal B-like’ disease, but one-fifth of the Panel did not agree with this recommendation. Still the definition of a single useful cutpoint has proved elusive because Ki-67 displays a continuous distribution[10].

The new addition to the prognostic parameters is 21-gene recurrence score (RS) (Oncotype DX)[4,11]; it is based on quantitative reverse transcriptase polymerase chain reaction (RT-PCR) on formalin-fixed, paraffin-

embedded tissues. This assay comprises of 16 cancer-related genes and five reference genes and consists of *Ki-67, STK15, Survivin, CCNB1* and *MYBL2*[11]. It was developed as a multigene array to assess the residual risk after surgery in early breast cancer patients with HR+, HER2− and node-negative disease from three independent cohorts[4,11]. This score stratifies patients into three risk groups: low (RS <18), intermediate (RS 18-30) and high (RS >30). High RS predicts benefit from addition of chemotherapy to hormonal therapy; however, whether intermediate RS predicts benefit from addition of chemotherapy or not is yet unknown[12]. Oncotype DX may also be used to predict response to chemotherapy in endocrine-responsive patients, when uncertainty remains even after consideration of other tests, as recommended by St. Gallen consensus conference[3,4]. RS was correlated with the incidence of recurrence of breast cancer as well as with the likelihood of benefit from adjuvant chemotherapy, significantly[4]. Later on, only one-fourth of the Panel of the 14th St. Gallen International Breast Cancer Conference believed that subtype determination could be replaced by risk scores derived from multiparameter molecular markers[10].

While IHC assesses protein expression, RT-PCR detects mRNA transcription levels, a high degree of concordance between the two assays has been reported on oestrogen receptor, progesterone receptor and HER2[13]. However, in a study by Gao *et al*[14], RT-PCR assessment of Ki-67 showed a weak concordance with IHC assessment. They attributed this to tumour heterogeneity. This could be because the authors followed the hotspot method according to the International Ki-67 in Breast Cancer Working Group's standards[8], whereas RT-PCR assessment was based on a non-specific portion of the tumour. Another possible reason considered was the role of epigenetic factor, leading to the differences between protein and mRNA expressions[14]. They did not see any significant association between Ki-67 status by IHC and disease outcome in the overall group of patients. Only 3.5 per cent absolute difference in three-year disease free survival between the patients with low and high Ki-67 expression was noted. It was argued that this poor performance of Ki-67 status by IHC could be because of insufficient adjustment for clinicopathological factors and adjuvant treatment. On the contrary, a significant prognostic value for Ki-67 status by RT-PCR was reported only in the training cohort. Low Ki-67 expression defined by RT-PCR was associated with a decrease in the relative risk of relapse[14]. The authors concluded that RT-PCR might be the potential solution to problems of interobserver variability and analytical subjectivity in Ki-67 scoring[14].

Mehta and colleagues[15] in this issue have reported a significant correlation between Ki-67 labelling index (LI) determined by IHC and mRNA determination by RT-quantitative PCR (RT-qPCR). The optimal cut off obtained by receiver operating characteristic (ROC) curve analysis for RT-qPCR in this study was 22.23 per cent, unlike 5.68 optimal cut-off point, using X-tile programme cut-off point for Ki-67 by RT-PCR[15]. The comparison was done in the IHC group as <25 per cent with RT-qPCR, whereas Gao *et al*[14] used 14 per cent as cut-off in IHC group. Interestingly, in the study by Mehta *et al*[15], nine patients were found to have high proliferation (>25%) by IHC but low by RT-PCR (<22.23%) while 14 cases were found to have high proliferation (>22.23%) by RT-PCR but low by IHC (<25%). The application of RT-qPCR has been reported with mixed results in the literature[14,16]. Suryavanshi *et al*[17] in their comparative study of RT-PCR HER2 versus IHC and fluorescence in situ hybridization (FISH) reported suboptimal performance of RT-PCR to IHC in terms of discriminative ability and clinical benefit.

Although RT-qPCR technique has been projected as a promising alternative to IHC for Ki-67 estimation in the present study, it yet needs to be confirmed by larger clinical outcome-based studies. The need for specialized equipment, cost factor and unfamiliarity with a new procedure in resource-poor settings are major impediments before this technique is considered as an alternative to the standard IHC.

***Conflicts of Interest*:** None.

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