Short Communication

Ki 67 is a major, but not the sole determinant of Oncotype Dx recurrence score

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BACKGROUND: Immunohistological assessment of Ki 67 expression is less expensive than Oncotype Dx, which is currently used to identify patients with lymph node-negative breast cancer, who will benefit from adjuvant chemotherapy.

METHODS: The relationship of immunohistologically measured Ki 67 to Oncotype DX recurrence score (RS) was examined in 53 cases of T1–2 N0 M0 (oestrogen receptor-positive, HER2/neu negative) breast cancer.

RESULTS: There was a strong linear correlation between Ki 67 value and the Oncotype Dx RS. All patients in the low Ki 67 group (Ki 67 of ≤ 10%) had Oncotype Dx RSs of low or intermediate risk. The vast majority of patients (93.8%) in the high-Ki 67 group (Ki 67 > 25%) had oncotype RSs of high or intermediate risk.

CONCLUSION: Ki 67 proliferation value is a major, but not the sole determinant of Oncotype Dx score.

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Ki 67 protein is found in proliferating cells. It is present in the nuclei of cells in G1, S, G2, and M phases of the cell cycle. Ki 67 protein levels are low during G1- and early S-phase, and gradually increase to reach a maximum during mitosis. Therefore, Ki 67 protein expression can be a useful marker of cell proliferation (Urruticoechea et al, 2005).

Multiple studies have shown an association of Ki 67 expression with prognosis and response to systemic treatments in both the neoadjuvant and adjuvant settings. High-Ki 67 expression has been associated with an increased risk of breast cancer recurrence and cancer death (Lee et al, 1997; Colleoni et al, 2004; Kronqvist et al, 2004; Penault-Llorca et al, 2009). Tumours with high Ki 67 before neoadjuvant chemotherapy have a higher rate of pathologic response, and lower Ki 67 after neoadjuvant chemotherapy is associated with favourable disease-free survival (DFS; Nishimura et al, 2010; Yerushalmi et al, 2010). At least two studies have suggested that patients with high-Ki 67 tumours may benefit more from adjuvant chemotherapy and endocrine therapy (Viale et al, 2008a, b; Penault-Llorca et al, 2009).

The Oncotype Dx gene test (Genomic Health, Redwood City, CA, USA) is a commercially available reverse transcriptase PCR assay (RT–PCR) of 21 genes, which uses a specific algorithm to calculate the recurrence score (RS) for oestrogen receptor (ER)-positive breast cancers. On the basis of RS, patients are considered low risk (RS < 18), intermediate risk (18 ≤ RS < 31), or high risk (RS ≥ 31). Although different cancer-related genes including ER and HER-2 expression are included in calculating the RS, five proliferation genes (Ki 67, STK 15, Survivin, CCNB 1, and MYBL 2) are heavily weighted and especially important in this calculation (Paik et al, 2004). This test is currently used for predicting the risk of recurrence in lymph node-negative ER-positive breast cancers, and is considered by the National Comprehensive Cancer Network guidelines as an option to help decision-making in this group of patients. Unfortunately, the high cost of this multigene assay limits its’ use in daily practice in many countries.

The aim of this study was to compare the correlation of immunohistologically measured Ki 67 protein expression with the Oncotype Dx RS in patients with lymph node-negative ER/progesterone receptor (PR)-positive, HER-2-negative breast cancers.

MATERIALS AND METHODS

With approval from the Institutional Research Ethics Committee, we analysed 53 cases of T1–2 N0 M0 (ER/PR-positive, HER-2-negative) breast cancer treated in the Jewish General Hospital, Montreal, Canada. These cases were chosen randomly from a pool of patients with early-stage breast cancer, who had Oncotype Dx analysis of their tumour. Pathology reports were reviewed and histological type, tumour size, Nottingham grade, perineural invasion status, lymphovascular invasion status, invasive tumour necrosis, ER levels, and PR levels were recorded. ER and PR levels were recorded as per the Allred Score (Harvey et al, 1999). All tumours were HER-2 negative, using immunohistochemistry (IHC) or fluorescent in situ hybridisation. Sentinel lymph node sampling data were available in all patients and no lymph node
involvement was reported. The Oncotype Dx RS results were provided by Genomic Health test reports. Patients were stratified to three risk groups as per Oncotype RS: low risk (RS < 18), intermediate risk (18 ≤ RS < 31), and high risk (RS ≥ 31) (Paik et al., 2004).

Ki 67 expression was examined by IHC of formalin-fixed deparaffinised tissue, using prediluted rabbit monoclonal antibody against human Ki 67 (Clone 30-9, Ventana, Tucson, AZ, USA) at a concentration of 2 μg ml⁻¹. Slides were stained on automated immunostainer Benchmark XT from Ventana, using the iView DAB Detection Kit. The same tissue blocks were used for both Oncotype Dx testing and Ki 67 immunostaining. Results were assessed without the use of an image analysis system. The fraction of positive cells (in percentage) with definite nuclear immunostaining, including mild, moderate, and strong was counted. The representative fields were chosen at low magnification and included at least two areas at the most cellular edges of tumour, and one area in the centre. The number of cells counted at high-power magnification varied depending on distribution of Ki 67 immunopositive staining. For cases with even distribution, the Ki 67 staining was determined with 400–600 tumoural cells, but in cases with uneven distribution, up to 2500 tumoural cells were counted. The stained slides were evaluated by two of the authors where the distribution of Ki-67-positive tumoural cells was uneven, or when the percentage of immunoreactivity was near the cut-off points. Patients were divided into low-risk (Ki 67 < 10%), intermediate-risk (10% ≤ Ki 67 < 25%), and high-risk group (Ki 67 ≥ 25%) on the basis of the expression of Ki 67.

Ki 67 < 10% was considered low, based on a cut-off point used by Kronqvist et al. (2004) and Breast International Group Trial 1-98 (Viale et al., 2008a,b). However, as there were other studies that used Ki 67 > 20% as their cut-off point, we decided to consider Ki 67 > 25% as high (Colleoni et al., 2004; Viale et al., 2008a,b). By doing so, we could study the group of patients with Ki 67 values falling between these two numbers (Ki 67 intermediate group).

The SPSS version 19 (Chicago, IL, USA) was used for statistical analysis. Linear regression, univariate analysis, multivariate analysis, and partial correlation analysis were performed.

RESULTS

The pathologic characteristics of patients are presented in Table 1.

The median Ki 67 value was 17.3% (range 2–90%). The median Oncotype RS was 18 (range 7–60). There was a strong linear correlation between Ki 67 expression and Oncotype RS (correlation coefficient = 0.73, P-value < 0.001; Figure 1).

There was also a significant correlation between Nottingham grade and Oncotype RS on univariate analysis (correlation coefficient = 0.52, P-value < 0.001). The correlation of Ki 67 and Oncotype RS remained robust (correlation coefficient = 0.6, P-value < 0.001), even after controlling for the effect of Nottingham grade by using partial correlation analysis and multivariate analysis. On the other hand, there was no significant correlation between Nottingham grade and Oncotype RS when the effect of Ki 67 was controlled (correlation coefficient = 0.064, P-value = 0.65). This suggests that the correlation found between Nottingham grade and Oncotype RS on univariate analysis was most likely due to the effect of Ki 67.

We also analysed the correlation between other histopathological characteristics of tumour with Oncotype RS. Previously, Flanagan et al. (2008) reported significant correlation between nuclear grade, mitotic count, ER score, and PR score. In our study, there was a weak but significant correlation between nuclear grade and Oncotype RS (correlation coefficient = 0.39, P-value = 0.005). This correlation was weaker but still significant, when the effect of Ki 67 was controlled by using partial correlation analysis (correlation coefficient = 0.32, P-value = 0.047). There was a significant correlation between mitosis score (measured as part of Nottingham scoring) and Oncotype RS in univariate analysis (correlation coefficient = 0.39, P-value = 0.005). This correlation

Table 1 Pathological characteristics of tumours

| Size     | Number (%) |
|----------|------------|
| ≤ 1 cm   | 6 (11.3)   |
| > 1 cm   | 47 (88.7)  |

| Histology | Number (%) |
|-----------|------------|
| Ductal    | 48 (90.6)  |
| Lobular   | 5 (9.4)    |

| Nottingham grade | Number (%) |
|------------------|------------|
| 1                | 15 (28.3)  |
| 2                | 29 (54.7)  |
| 3                | 9 (17)     |

| Ki 67     | Number (%) |
|-----------|------------|
| Low (<10) | 16 (30.2)  |
| Intermediate (10 ≤ Ki 67 < 25) | 21 (39.6)  |
| High (Ki 67 ≥ 25) | 16 (30.2)  |

| ER level (Allred score) | Number (%) |
|-------------------------|------------|
| Negative (<3)           | 0 (0)      |
| Weak (3–4)              | 1 (1.9)    |
| Strong (≥5)             | 52         |

| PR level (Allred score) | Number (%) |
|-------------------------|------------|
| Negative (<3)           | 6 (11.3)   |
| Weak (3–4)              | 9 (17)     |
| Strong (≥5)             | 38 (71.7)  |

| Oncotype Dx RS category | Number (%) |
|-------------------------|------------|
| Low (RS ≤ 17)           | 25 (47.2)  |
| Intermediate (18 ≤ RS ≤ 30) | 20 (37.7)  |
| High (RS ≥ 31)          | 8 (15.1)   |

Abbreviations: ER = oestrogen receptor; PR = progesterone receptor; RS = recurrence score.

Figure 1 Correlation of Oncotype DX RS with Ki 67 value. Correlation coefficient = 0.73, P-value < 0.001.
was not significant when the effect of Ki 67 was controlled in multivariate analysis and partial analysis (correlation coefficient = 0.054, P-value = 0.714).

In previous studies, lower expression of PR has been associated with higher Oncotype RS (Flanagan et al, 2008; Tang et al, 2010). Tang et al (2010) demonstrated that among ER-positive tumours, PR-poor tumours had significantly higher Oncotype RS. Our results were consistent with these findings. ER/PR expression had a significant inverse correlation with Oncotype RS, but did not affect the correlation between Ki 67 and Oncotype RS. Lower expression of PR was associated with higher Oncotype RS (correlation coefficient = −0.56, P-value <0.001), which was independent from the effect of ER expression. The combination of ER/PR expression and Ki 67 had a very strong correlation with Oncotype RS (correlation coefficient = 0.84, (0.75–0.93) P-value <0.001).

There was no significant correlation between perineural invasion, lymphovascular invasion, invasive tumour necrosis, and Oncotype RS on multivariate analysis.

Most patients (93.85%) in the high-Ki 67 group (Ki 67 ≥25%) had Oncotype RS of high or intermediate risk. All patients in low-Ki 67 group (Ki 67 of <10%) had RS of low or intermediate risk (Table 2).

### DISCUSSION

Oncotype Dx testing provides valuable prognostic and predictive information in patients with early-stage breast cancers. Unfortunately, the high price limits the accessibility of this test to all patients. Hence, there has been increasing interest to find simple pathology tests, which can help predict the recurrence of disease. As Ki 67 is one of the proliferation genes assessed routinely by IHC in different malignancies, several investigators have studied its prognostic and predictive value in different stages of breast cancer treatment. High-Ki 67 expression detected by IHC has been associated with higher Oncotype RS and was used in combination with the PR level to divide cases into different subgroups.

In summary, our data suggests that Ki 67 is the major but not the sole determinant of Oncotype RS. It will be of great interest to study an immunopanel consisting of Ki 67 with other proliferation markers measured by Oncotype Dx, that is, STK 15, Survivin, CCNB1, and MYBL2.

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