The role of Ki-67 in breast cancer

A Mannell

Department of Surgery, University of the Witwatersrand, Johannesburg, South Africa

Corresponding author: *Aylwyn Mannell (mannell@mweb.co.za)*

The proliferative marker, Ki-67, is a human nuclear antigen, and forms an integral part of cell division in both normal and malignant tissue. Since the hallmark of cancer is uncontrolled and relentless cell proliferation, the Ki-67 proliferative index is increasingly used to assess and manage breast cancer. The value of Ki-67 as a prognostic indicator, a guide to the selection of therapy, and a method of measuring response to ongoing treatment, is examined in this review.

S Afr J Surg 2016;54(2)

The Ki-67 antigen, a non-histone protein, was first described by Gerdes et al.[1] when raising mouse monoclonal antibodies to the nuclei of a Hodgkin's disease cell line. This work was performed at Kiel University in Germany, hence the "Ki". The "67" refers to the clone number in a 96 well plate.

The Ki-67 antigen can be identified by immunostaining with a monoclonal antibody in all phases of cell proliferation. Non-existent in the resting (G0) phase, it appears within the nucleus in the S, G1 and G2 phases. The level increases on the surface of the chromosomes, reaching a peak in mitosis in both normal and malignant tissue.[2]

The Ki-67 score or index is the percentage of positively stained cells among the total number of malignant cells scored. The use of the original anti Ki-67 monoclonal antibody was restricted to fresh frozen tissue, but by using another anti-human monoclonal antibody, N1B-1 (clone 42), the Ki-67 can be measured in formalin-fixed, paraffin-embedded sections, archived over decades.[3]

Ki-67 and breast cancer prognosis

The value of Ki-67 as a prognostic index was examined in a retrospective study of 3 658 cases of invasive breast cancer entered in the Regenburg clinical cancer registry, Bavaria, Germany, from 2005–2011.[3] In addition to the receptor status and commonly recorded histopathological features, the Ki-67 percentage was part of the routine workup for these patients. In a univariate analysis, a Ki-67 >25%, together with unfavourable clinical and histopathological parameters, conferred a worse prognosis on the studied population. A low Ki-67 (≤15%) was associated with five- year disease-free survival and overall survival of 87% and 89%, respectively, whereas patients with a high Ki-67 (>45%) had disease-free survival and overall survival of 76% and 83%. These findings confirm those of an earlier meta-analysis by De Azambuja,[4] in which it was shown in a univariate model that a high Ki-67 percentage correlated with decreased survival in both node-negative, node-positive and untreated breast cancer patients.

While aggressive clinical and histopathological features (receptor negativity, high grade cancer, a positive nodal status, a young age and lymphovascular invasion) are significantly associated with worse outcomes, a multivariate analysis of the Regenburg data showed that a high Ki-67 percentage (> 25%) remained an independent prognostic parameter for disease-free and overall survival, irrespective of the clinical and histopathological features of the cancer.[3]

Ki-67 and racial differences in breast cancer

In the USA, black women are 40% more likely to die than white women with breast cancer, and in Africa, Nigerian women with breast cancer have higher mortality than British women.[5] Many local factors may contribute to this difference, for example, patients presenting at an advanced stage owing to the unaffordability of available transport, the distance from the healthcare facility, patient delays because of low levels of health education and an initial reliance on traditional therapies. These factors result in delays in the diagnosis and treatment of breast cancer,[6] which, in turn, negatively impacts on survival. While socio-economic disparities in the delivery of health care are well recognised, there is an increasing awareness of differences in tumour biology which exist between ethnic nationalities.

A study was made of young African-American and white women in Atlanta, USA, with newly diagnosed unilateral invasive breast cancer seen from 1996–2000.[7] In addition to documenting the USA National Cancer Institute (NCI) Surveillance, Epidemiology and End Results (SEER) Program

grade and stage of the cancer, archival tumour tissue was re-examined for the degree of tumour necrosis, the mitotic rate, receptor status, proliferative markers and cell cycle regulatory proteins. Racial differences in tumour characteristics were identified and correlated with outcome data from the Georgia Center for Cancer Statistics (part of the NCI-funded SEER programme).

After adjusting for age, grade and stage, breast cancer in African-American women was more likely to have a higher proliferative rate and abnormal expression of cell cycle regulatory proteins, cyclin E and D, than breast cancer in white women. A more aggressive breast cancer phenotype in African-American women underlies the poor survival in this population group.

These findings that identify racial differences in tumour biology are supported by a later study from Nigeria and Nottingham, UK.[5] The clinic pathological features and biomarkers, including Ki-67, were examined in the breast cancer of 302 Nigerian women. This series was then stage and grade matched to a cohort of British women of Caucasian descent. All patients were assessed and managed in a standard fashion by primary surgery, followed by adjuvant therapy determined by receptor status. Survival data were maintained on a prospective basis and the outcome recorded for each case.

The levels of Ki-67 were significantly higher in the breast cancer of Nigerian women than those in British women, independent of stage, grade and receptor status. Mortality was greater in the Nigerian patients. It is likely that the greater proliferative fraction, identified by high levels of Ki-67, contributed to the racial differences in survival.

Ki-67 and the molecular subtyping of breast cancer

Ki-67 has been used as a marker to define the molecular subtypes of breast cancer. Cheang et al.[8] combined the Ki-67 with a panel of receptors [oestrogen receptor (ERs), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2)], and found that a Ki-67 level of 13% could separate luminal A cancer with a good prognosis from luminal B when the prognosis was worse. Nine hundred and forty-three patients with node-negative breast cancer, who did not receive systemic therapy, were subtyped using these four immunohistochemical markers (IHC4), i.e. ER, PR, HER2 and Ki-67, and followed to document relapse and 10-year cancer-specific survival. Those with luminal B cancer with a Ki-67 of >14% had a significantly worse prognosis for recurrence and death than those with luminal A tumours, where the Ki-67 was <14%.

The cut-point in the previous study, separating high risk from low risk was a Ki-67 of 14%. However, in the literature, cut-points used to make this distinction have ranged from a Ki-67 of 5–30%.[4] This wide variation of cut-points in the Ki-67 assays has made a comparison of the measurements of proliferative activity from different breast cancer centres very difficult. Added to this difficulty has been continuing debate regarding methods of staining and counting neoplastic cells in paraffin sections. Some pathologists have elected to count stained nuclei in "hot spots" and at the invasive edge of the malignancy, while others score cell numbers in a field considered to be representative of the entire section.[9] Such has been the controversy that the Ki-67 assay was omitted from the list of recommended biomarkers for clinical practice in the 2007 guidelines of the American Society of Clinical Oncology.[10]

Subsequently, an international Ki-67 in breast cancer working group was set up in 2010 to examine the value of Ki-67 as a reproducible prognostic marker and to address the problems of methodology.[9] The group published guidelines for the measurement of Ki-67, specifying the type of biopsy, fixative to be used, times of storage, as well as methods advised for antigen retrieval. The best monoclonal antibody to use as reagent for immunohistochemistry and staining techniques was outlined. Guidelines to standardise scoring, data analysis and interpretation of the results have also been published by the working group.

However, as yet, consensus on a single cut-point or a range of cut-point values has not been reached. This is partly owing to the fact that the Ki-67 displays a continuous distribution, and persisting variations in preanalytic and analytical methodology.[11] These difficulties underlie the continued debate regarding the value and reproducibility of the Ki-67 assay.

However, the majority of experts who expressed an opinion on the treatment-orientated classification of subgroups of breast cancer reported that Ki-67 scores should be interpreted in light of local laboratory values.[12] The panel gave the example of a laboratory with a median Ki-67 for receptor-positive cancer of 20%, so that cancers with a Ki-67 (measured by that laboratory) of ≤10% clearly have a low proliferative index, and those with a Ki-67 of ≥30%, a high proliferative rate.

Nevertheless, the 2015 St Gallen International Breast Cancer Conference consensus statement[12] recognised the importance of measuring and comparing hormone receptor levels and proliferative activity to determine prognosis, and as a guide to adjuvant chemotherapy.

It was also agreed in the consensus statement that "international collaboration has led to improvements in concordance of the Ki-67 scoring", encouraging continued use and standardisation of this marker.

Ki-67 and the distant recurrence of breast cancer

Improved surgical techniques and extended radiation fields, together with advances in cytotoxic drugs and targeted therapy, have increased disease-free survival and reduced overall mortality from breast cancer. But it is still not possible to characterise a subset of patients as "cured" of cancer. Node-negative ER positive cancers relapse at the rate of 2% per annum for at least 15 years after prolonged anti-oestrogen therapy.[13] This led to the search for a scoring system to separate patients at very low risk of relapse who would not benefit from adjuvant chemotherapy from those with a sufficiently high

risk of recurrence to justify cytotoxic therapies. The Genomic Health 21-gene recurrence score (RS) (6H1-RS), available commercially as Oncotype DX®, is one such scoring system, developed from an assay of tumour-related genes.[14] The 6H1-RS score was calculated for node-negative, ER-positive and HER2-negative patients on the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial who did not receive adjuvant chemotherapy.[15] These investigators found the Oncotype DX® was a more accurate predictor of distant recurrence than age, stage, grade and ER expression. However, six of the cancer-related genes in the Oncotype DX® are associated with proliferation, and the IHC4 score was found to be as good as this 21-gene score in predicting distant disease in the five years after completion of the treatment.[16]

The most obvious advantage of the IHC4 score is that these parameters are routinely measured in the tertiary referral hospitals in South Africa in which the cost of an immune stain, including Ki-67, is R350 in the public health sector (as per information supplied by the Department of Histopathology, Charlotte Maxeke Johannesburg Academic Hospital, Johannesburg, South Africa). The 21-gene Oncotype DX® score, at a cost of R28 780.00 (as per information supplied by Drs Gritzman and Thatcher Inc Laboratory, Johannesburg, South Africa) is prohibitively expensive for the resource-poor environment of Africa, and is available only in private health laboratories.

Multicentre trials of prolonged hormonal therapy for ER-positive breast cancers [the MA.17 trial[17] and Adjuvant Tamoxifen: Longer Against Shorter (ATLAS) trial] have run concurrently with these analyses of predictive factors.[18] These clinical trials have confirmed that continuing anti-oestrogen for 10 years significantly reduces the mortality of breast cancer in the second decade after diagnosis.

Selection of therapy

Apart from the contribution of Ki-67 to prognosis, the Ki-67 index is used on a daily basis in the selection of therapy. Dividing cells have increased sensitivity to cytotoxic drugs, and a high Ki-67 is associated with a good response to neoadjuvant chemotherapy (NAC).[11,19] Conversely, strongly ER-positive cancers with a low Ki-67 are better managed with 4–8 months of neoadjuvant hormonal therapy.[12] However, the power of baseline Ki-67 values to predict response to a specific adjuvant chemotherapy regimen has not been established.[11,20]

While baseline Ki-67 staining may guide the initial selection of therapy, assessment of response to ongoing treatment has become an important issue in patient management. The Ki-67 in a core biopsy was measured two and 12 weeks after commencing treatment in the Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen (IMPACT) trial.[21] The suppression of Ki-67 by anastrozole at both time intervals was greater than that recorded for tamoxifen or the combination. The greater suppression of Ki-67 by anastrozole correlated with the significantly improved recurrence-free survival of patients on anastrozole at the 31-month review of the ATAC trial.[22]

The aim of the Perioperative Endocrine Therapy for Individualizing Care (POETIC) trial is to test the hypothesis that a change in Ki-67 after two weeks of anti-oestrogen treatment can predict the final outcome of endocrine therapy.[23] This study is ongoing as the recruitment of 4 000 patients with non-metastatic ER-positive breast cancer is planned.

A reliable marker to assess response to ongoing chemotherapy is needed. A 20–25% decrease in baseline Ki-67 after a single cycle of cyclophosphamide, epirubicin and 5-fluorouracil correlated significantly with a decrease in the risk of recurrence in a small study from Karolinska University Hospital, Stockholm, Sweden.[24] The failure of a chemotherapy regime to decrease the proliferative fraction of a cancer signals the need for a change in therapy.

Assessment of residual risk

Chemotherapy achieves a clinical response in the majority of patients with invasive breast cancer, but a complete pathological response occurs in a minority only. It is important to profile the residual cancer in the surgical excision specimens as a guide to adjuvant therapy. A study of Ki-67 before and after NAC was conducted in 283 patients with ER-negative, invasive non-metastatic breast cancer who did not have a pathological response. Patients with a high baseline Ki-67 had a better response to NAC, but those with a high Ki-67 in the surgical excision specimen experienced significantly worse recurrence-free survival.[25] A high Ki-67 in residual cancer is an indication for further, non-cross-resistant treatment.

Conclusion

The Ki-67 is a human nuclear antigen closely associated with the cell cycle and mitosis, so that the Ki-67 percentage represents the proliferative fraction of a cancer. The Ki-67 is a durable antigen which can be easily and economically retrieved from paraffin-embedded sections of tumour tissue. Numerous studies have endorsed its value as a prognostic marker of breast cancer.

The Ki-67 is used in daily practice to select therapy, and offers the potential to measure response to ongoing treatment. Most patients with breast cancer do not have a complete pathological response after NAC so reassessment of the receptors and Ki-67 in the surgical excision specimen helps in the choice of a second-line, non-cross-resistant regimen. Equally important is the measurement of Ki-67 and receptor expression in loco-regional and distant breast cancer recurrences to facilitate the selection of appropriate systemic therapy.

With a wide variation in methodology, scoring and cut-off points, standardisation and laboratory accreditation is essential for the Ki-67 to reach full clinical potential, as has been achieved with hormonal and HER2 receptors.[26]

Declaration

Part of this review was presented at the Durban breast cancer forum on 18 August 2015.

Conflict of interest

The author confirms that there was no conflict of interest which may have inappropriately influenced her when writing this article.

REFERENCES

1. Gerdes J, Schwab U, Lemke H, Stein H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer. 1983;31(1):13–20.
2. Jonat W, Arnold N. Is the Ki-67 labelling index ready for clinical use. Ann Oncol. 2011;22(3):500–502.
3. Inwald EC, Klinkhammer-Schalke M, Hofstädler F, et al. Ki67 is a prognostic parameter in breast cancer patients: results of a large population-based cohort of a cancer registry. Breast Cancer Res Treat. 2013;139(2):539–552 .
4. De Azambuja E, Cardoso F, de Castro G, et al. Ki67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12155 patients. Br J Cancer. 2007;96(10):1504–1513.
5. Agboola AO, Banjo AA, Annunobi CC, et al. Cell proliferation (Ki67) expression is associated with poorer prognosis in Nigerian compared to British breast cancer women. ISRN Oncol. 2013;675051.
6. Iqbal J, Ginsburg O, Rochon PA, et al. Differences in breast cancer stage at diagnosis and cancer specific survival by race and ethnicity in the United States. JAMA. 2015;313(2):165–173.
7. Porter PL, Lund MJ, Ming GL, et al. Racial differences in the expression of cell cycle-regulatory proteins in breast carcinoma. Cancer. 2004;100(12):2533–2542.
8. Cheang MCU, Chia SK, Voduc D, et al. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst. 2009;101(10):736–750.
9. Harris L, Fritsche H, Mennel R, et al. The American Society of Clinical Oncology 2007 update of recommendations for the use of tumour markers in breast cancer. J Clin Oncol. 2007;25(33):5287–5312.
10. Dowsett M, Torsten NO, A'Hern R, et al. Assessment of Ki67 in breast cancer: recommendations from the international Ki67 in breast cancer working group. J Natl Cancer Inst. 2011;103(22):1656-1664.
11. Yerushalmi R, Woods R, Ravdin PM, et al. Ki67 in breast cancer: prognostic and predictive potential. Lancet Oncol. 2010;11(2):174–183.
12. Coates AS, Winer EP, Goldhirsch A, et al. Tailoring therapies – improving management of early breast cancer: St Gallen International Expert Consensus on the primary therapy of early breast cancer 2015. Ann Oncol. 2015;26(8):1533–1546.
13. Sestak I, Dowsett M, Zabaglo L, et al. Factors predicting late recurrence for estrogen receptor-positive breast cancer. J Natl Cancer Inst. 2013;105(19):1504–1511.
14. Paik S, Shak S, Tang G, et al. A multi gene assay to predict recurrence of tamoxifen-treated node-negative breast cancer. N Engl J Med. 2004;351(27):2817–2826.
15. Dowsett M, Cuzick J, Wale C, et al. Prediction of risk of distant recurrence using the 21-gene recurrence score in node-negative and node-positive postmenopausal patients treated with anastrozole or tamoxifen: a trans ATAC Study. J Clin Oncol. 2010;28(11):1829–1834.
16. Cuzack J, Dowsett M, Pineda S, et al. Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67 and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the Genomic Health recurrence score in early breast cancer. J Clin Oncol. 2011;29(32):4273–4278.
17. Goss PE, Ingle JN, Martino S, et al. Randomized trial of letrozole following tamoxifen as extended adjuvant therapy in receptor-positive breast cancer: updated findings from NCIC CTG MA.17. J Natl Cancer Inst. 2005;97(17):1262–1271.
18. Davies C, Pan H, Godwin J, et al. Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomized trial. Lancet. 2013;281(9869):805–816.
19. Fasching PA, Keusinger K, Haeberle L, et al. Ki67, chemotherapy response and prognosis in breast cancer patients receiving neoadjuvant therapy. BMC Cancer. 2011;11:486.
20. Sheri A, Dowsett M. Developments in Ki67 and other biomarkers for treatment decision making in breast cancer. Ann Oncol. 2012;23 Suppl 10:x219–x227.
21. Dowsett M, Smith IE, Ebbs SR, et al. Prognostic value of Ki67 expression after short-term presurgical endocrine therapy for primary breast cancer. J Natl Cancer Inst. 2007;99(2):167–1170 .
22. Cuzick J, Sestak I, Baum M, et al. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer. 10 year analysis of the ANAC trial. Lancet Oncol. 2010;11(12):1135–1141.
23. Dowsett M, Smith I, Robertson J, et al. Endocrine therapy, new biologicals, and new study designs for presurgical studies in breast cancer. J Natl Cancer Inst Monogr. 2011;2011(43):120–123.
24. Billgren A-M, Rutqvist LE, Tani E, et al. Proliferating fraction during meoadjuvant chemotherapy of breast cancer in relation to objective local response and relapse-free survival. Acta Oncol. 1999;38(5):597–601.
25. Jones RL, Salter J, A'Hern R, et al. The prognostic significance of Ki67 before and after neoadjuvant chemotherapy in breast cancer. Breast Cancer Res Treat. 2009;116(1):53–68.
26. Hammond ME, Hayes DF, Wolff AC, et al. American Society of Clinical Oncology/College of American Pathologists guideline. Recommendations for immune histochemical testing of estrogen and progesterone receptors in breast cancer. J Oncol Pract. 2010;6(4):195–197.