Prediction of histologic grade in breast cancer using an artificial neural network.

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Abstract — **Histological grade is a historically used and well-documented prognostic indicator in breast cancer. There are three categories of grade (G1, G2 and G3) based on the degree of tubule formation, nuclear pleomorphism and mitotic count. A number of studies have reported that histological assessment is not uniformly reported. As a result of low inter-pathologist correlation associated with pathological diagnosis and non-standardised grading systems, patients are not always allocated into the correct grouping, and G2 has often been considered a "safe" group if one is unsure. A previously published study used real-time polymerase chain reaction (RT-PCR) for 5 genes to molecularly classify the G2 tumours into either G1 or G3. Due to the workflow constraints within pathology laboratories it was not considered feasible to molecularly profile every G2 tumour. In light of this we obtained the antibodies that corresponded to the 5 genes (BUB1B, CENPA, RACGAP1, RRM2 and NEK2) and performed immunohistochemistry (IHC) on formalin fixed paraffin embedded (FFPE) sections of 43 tumours (11 G1 and 32 G3). Results for all tumours were randomly divided into training and testing sets and an artificial neural network (NeuralSight and NeuralWare Predict) was used to classify the grade of tumours. Thirty-three additional G2 tumours were used for validation of the ANN. The ANN classified these tumours into 5 G1 and 28 G3 tumours. This predicted grade showed significant correlation with patient survival. Neural networks can be used to reclassify breast cancer G2 tumours into G1 and G3 using a panel of 5 IHC markers. This has the potential to impact on patient care, treatment decisions and outcome.**

Keywords- Breast cancer, grade, artificial neural network.

I. INTRODUCTION

Breast cancer is the leading cause of cancer-related deaths in women world-wide [1] and is the second most common cancer in Australian women. More than 13,000 new cases are diagnosed each year in Australia [2], increasing from 11,000 in 2000 [3], of which 2,200 are in Queensland. Approximately 2,700 deaths from breast cancer occur annually in Australia of which 465 were in Queensland in 2003. The number of hospital separations in Australia for women increased from 15,831 in 1995-96 to 23,598 in 2003-4 [2].

In current clinical practice for breast cancer, the standard prognostic factors that guide adjuvant systemic treatment decisions include tumour size, histologic subtype, histologic grade, oestrogen and progesterone receptor status, HER2 status and axillary lymph node status. A measure of the limitation of these factors in predicting outcome is exemplified by the observation that recurrence rates are approximately 25% in lymph node negative breast cancer patients who will often not receive adjuvant therapy [4].

Despite the published reports of new biomarkers to predict prognosis or response to therapy in breast cancer patients, only a limited number have entered clinical practice. A predictive factor is defined as a clinical or pathologic feature that determines the likelihood of a response to a particular treatment. A prognostic factor is defined as a clinical or pathologic biomarker that determines patient outcome or survival. A biomarker may be both predictive and prognostic. For a factor to be useful, it must be technically validated, clinically validated and influence clinical decision making. A large proportion of studies have used immunohistochemistry (IHC) to assess the expression of different antigens in breast cancer cells compared to normal tissue. Limitations of these studies include variation in patient selection, different primary antibody clones, different cut-off criteria, inter-laboratory variation and reproducibility of the methodology.

In addition, molecular techniques including DNA microarray analysis have indicated a large number of genes are involved in the progression of breast carcinoma. More than 3,000 genes have been implicated in distinguishing oestrogen receptor (ERp) positive tumours from ERp-negative tumours [5].

Breast cancer is graded according to the Elston-Ellis modified Scarff, Bloom and Richardson grading system also known as the Nottingham modified Bloom and Richardson system [6, 7]. There are three components assessed for assigning a grade to a tumour: tubule formation, nuclear pleomorphism and mitotic rate. Each component is assigned a number from 1 to 3 and the components are tallied to obtain a value out of 9. Tumours with a score of 5 or less are graded as Grade 1 (G1), scores of 6-7 are Grade 2 (G2) and scores of 8-9 as Grade 3 (G3) tumours. The grade of tumour

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shows significant correlation with breast cancer specific survival [8-11] for patients with lymph node metastases and those without nodal metastases at 5, 10 and 15 years (Fig. 1). The evaluation of tubule formation and mitotic rate also provide independent prognostic information [12].

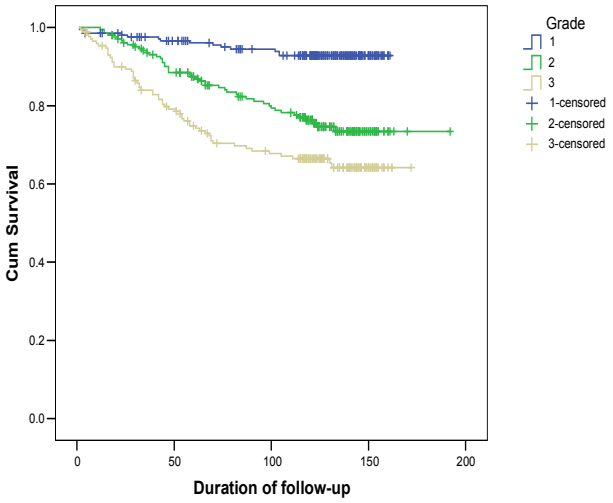

Figure 1: Kaplan-Meier survival curves for 591 breast cancer patients according to tumour grade over 200 months.

Evaluation of large patient cohorts with multivariate analysis has shown that grade is a significant predictor of disease recurrence and patient death [10, 11, 13, 14]. Untreated G1 patients have a 5 year survival rate of approximately 95%, whereas, those patients with G2 or G3 tumours have 5 year survival rates of approximately 75% and 50% respectively.

There is, however, significant inter-observer variability in the evaluation of histological grade by pathologists [15-19] and the usefulness in patient prognosis has been questioned [20, 21]. Grade 2 tumours comprise approximately 50% of all breast cancers and it has been proposed that refinement of this group may improve the prognostic value of tumour grading [22, 23].

Gene expression profiling has been used to more accurately grade tumours to overcome the limitations of the current system [22, 23]. Sotiriou et al [22] developed a 97-gene expression profile associated with histological grade and found that the gene expression profile correlated more strongly with relapse-free survival compared to histological grade [22]. Similarly, Ma et al, [24] and Ivshina et al, [23] have also developed histologic grade signatures and the latter enabled the refinement of the G2 tumours into different sub-grades with clinical implications [23]. The Genomic Grade Index [25-27] has been further developed for both frozen and formalin fixed paraffin embedded tissue (FFPE). Ma et al, [28] developed a polymerase chain reaction (PCR) based five gene molecular grade index (MGI) for applications with FFPE material. The rationale for this approach was that it may be more easily implemented into a diagnostic laboratory. The MGI has been validated in a separate patient cohort [29].

In this study, the five gene panel of the MGI was used to develop IHC staining protocols for patient samples and an artificial neural network was used to develop an algorithm for determination of tumour grade.

II. Materials and Methods

Tissue microarrays (TMAs) were constructed using an automated Beecher ATA-27 and semi-automated Beecher Galileo (Beecher Instruments, Sun Prairie, USA). Areas of tumour were identified on haematoxylin and eosin (H & E) stained slides and tissue cores were punched from these areas and inserted into recipient blocks. 4 µm sections were cut onto SuperFrost Plus slides and IHC was performed using primary antibodies against BUB1B, NEK2, CENPA, RacGAP1 and RRM2. Slides were digitally scanned on a Hamamatsu Nanozoomer (Hamamatsu City, Japan) at 40x magnification and attached to image maps in a database, SlidePath Distiller (Leica Microsystems, Wetzlar, Germany). The images were scored on-line using SlidePath Distiller. Each TMA core was scored for percentage of tumour staining (0-100%), intensity of tumour staining (0 – 3) and the localization in the cellular compartments; membrane, cytoplasmic or nuclear. Data, including morphologic grade, patient follow-up, survival and cause of death, was extracted from the database into Microsoft Excel (Microsoft Corporation, USA).

43 patients with complete data sets were randomly divided into training and teaching sets. Within this patient population there were 11 Grade 1 tumours and 32 Grade 3 tumours.

NeuralSight (NeuralWare, Carnegie, PA) was used to evaluate 20 models through NeuralWare Predict (NeuralWare, Carnegie, PA). In NeuralSight, the Model Type selected in the program was Classification and build options included cascaded variable selection with noisy data and comprehensive and exhaustive variable selection options. In NeuralWare Predict, a multilayer perceptron neural network, the selected options included adaptive gradient learning rule, cascaded variable selection, an output layer function of SoftMax and an evaluation function of average classification rate. Cascaded variable selection was performed before the main variable step and was used to estimate the probability that a particular variable was present in a good solution, eliminating those variables that have a very low probability of inclusion in an optimum solution. Input variable selection used a genetic algorithm with multiple regression to select the model's input variables. The adaptive gradient learning rule used back-propagated gradient information to guide an iterative line search algorithm with weight decay used to avoid overfitting. The neural network architecture selected used 22 input nodes, 3 hidden nodes and 1 output node. NeuralWare Predict was used to run predicted grade and validate the algorithm on a separate data set of 33 Grade 2 tumours through a plugin in Microsoft Excel 2007. SPSS 19 (IBM Australia) was used

for Kaplan-Meier survival curves for Breast Cancer Specific Survival (BCSS).

III. Results

Photomicrographs of morphologic G1 and G3 tumours are illustrated in Figure 2.

IHC staining showed variable intensity and percentage of cytoplasmic staining for BUB1B, RRM2 and NEK2 and variable intensity and percentage of nuclear staining for RacGAP1 and CENPA (Figure 3). Kaplan-Meier survival curves for BCSS for the tumours used in this study show differences in survival for the morphologic G1, G2 and G3 tumours with the poorest outcome for G3 tumours (Figure 4).

a)

b)

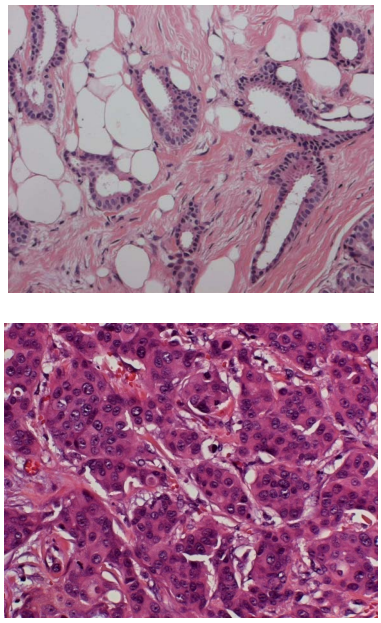

Figure 2: Haematoxylin and eosin stained sections illustrating (a) Grade 1 and (b) Grade 3 infiltrating ductal carcinoma (x20 magnification).

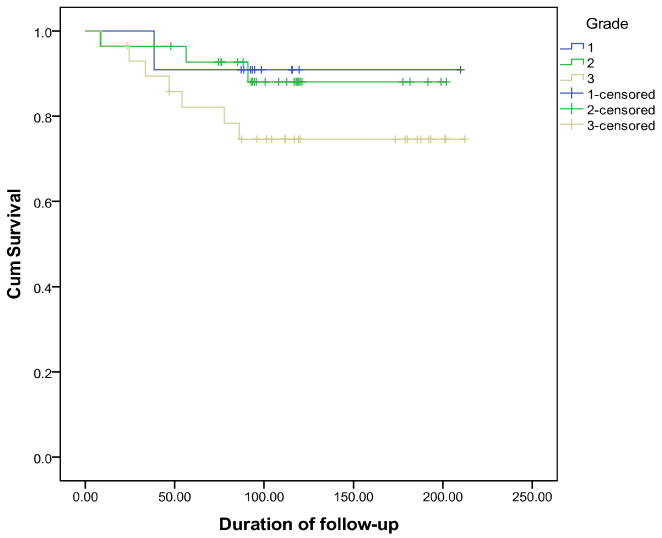

Figure 3: Kaplan-Meier BCSS survival curves for morphologic G1, G2 and G3 tumours over 250 months.

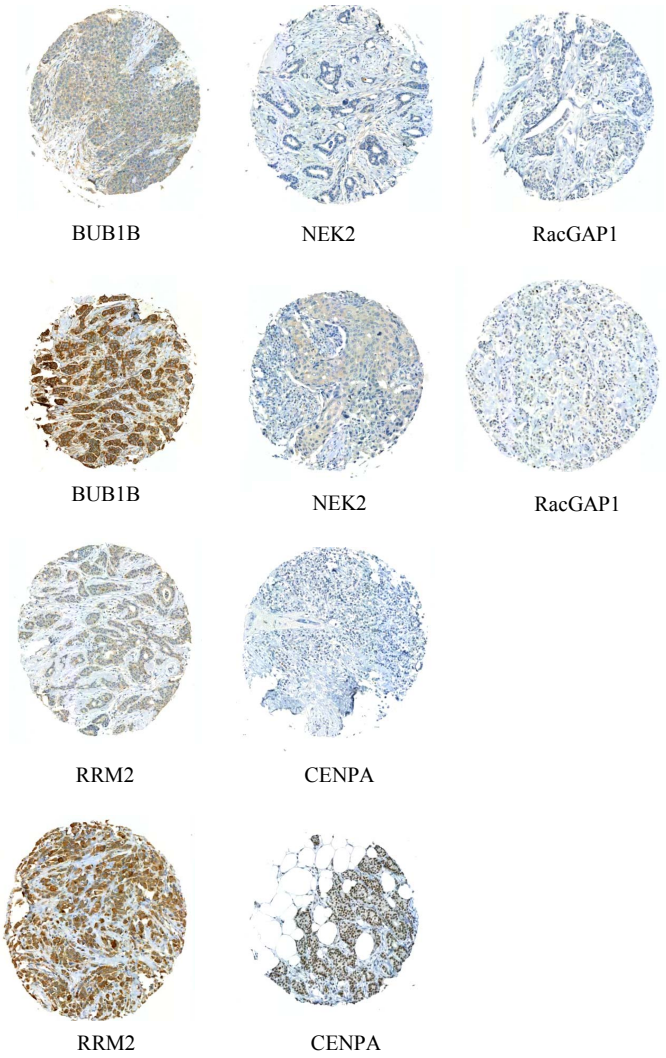

Figure 4: Immunohistochemical detection of BUB1B, NEK2, RacGAP1, RRM2 and CENPA in breast cancer TMAs (x20 magnification).

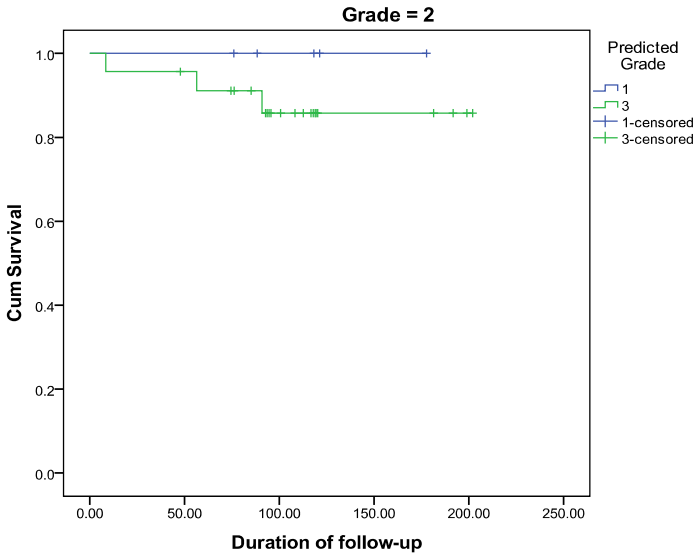

Figure 5: Kaplan-Meier BCSS survival curves for morphologic G2 tumours stratified into predicted G1 and G3 tumour over 250 months.

NeuralSight generated 20 models and these were evaluated using ROC modeling. Model 10 showed an ROC of 1 and this model was used to evaluate the G2 tumours. Of the 33 G2 tumours, 5 were predicted to be G1 and 28 were predicted to be G3 tumours. Kaplan-Meier survival curves showed that the predicted G1 tumours had a 100% long term BCSS, whereas, the predicted G3 tumours had a BCSS similar to morphologic G3 tumours (Figure 5).

IV. Discussion

Breast cancer grade is a significant prognostic factor in breast cancer patients; however, morphologic assessment of grade is subjective and there is variability in interpretation between reporting pathologists. To overcome these limitations, molecular profiling has been used to develop algorithms to stratify G2 tumours into those tumours that are similar to morphologic G1 tumours and those similar to morphologic G3 tumours. The rational for this is that it will enable more accurate prognostic information to improve patient care and outcome.

Gene expression profiling and even real-time polymerase chain reaction (RT-PCR) are not easily integrated into a diagnostic anatomical pathology laboratory and for this reason, based on a small gene list, IHC was used to ascertain if it was feasible to use this technology to achieve the same goals.

This study shows that it is possible to use a panel of five IHC biomarkers, in conjunction with an artificial neural network, to stratify G2 breast cancers into two separate groups with differing survival outcomes. The neural network model in this application uses biomarkers that had previously been reported to be useful in predicting grade stratification. The parameters included a mixture of percentage of tumour cells staining and the intensity of staining which correlates with the protein expression of that particular biomarker. This format forms part of routine patient care in breast cancer for other biomarkers including oestrogen receptor, progesterone receptor and HER2. In addition, the design of model parameters enables variations in the interpretation of the IHC results to be accommodated by the network model. Further validation studies are in progress. If successful, this methodology has the potential to be readily integrated into the diagnostic laboratory to improve patient care.

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