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SHORT REPORT

Invasive breast cancer: stratification of histological grade by gene-based assays: a still relevant example from an older data set

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Invasive breast cancer: stratification of histological grade by gene-based assays: a still relevant example from an older data set

Aims: A Netherlands Kanker Institute data set provided the results of gene-based assays (GBAs) and histological grades of 295 patients with invasive breast cancer. Grade is the first prognostic assay available after a cancer diagnosis. Given this time-line of actual practise, the aim was to study how gene-based assays further stratify histologic grade.

Methods and results: Emphasis was placed on evaluation of a simple decision tree and on study of the recurrence score (RS). The decision tree determined three risk stratifications. Tumours that were both intermediate grade (IG) and low-RS were grouped with low grade, and tumours that were IG and high-RS were coupled with high grade. IG and intermediate-RS tumours comprised the third category. Survival analysis was performed with respect to the three stratifications. Cramer's V statistic was used for concordance analysis. The mixed grade-RS classifier showed significant survival stratification ($P < 0.00001$). The mixed classifier was concordant with the 70-gene assay (Cramer's V = 0.57). Recurrence score alone had a 0.59 Cramer's V with the gene assay. Because two-thirds of tumours were of either low or high grade, concordance was maintained despite the majority of classifications having been determined by grade alone.

Conclusion: There is no compelling reason to test low- and high-grade tumours further by GBAs.

Keywords: breast cancer, gene, grade recurrence score, molecular prognosis

Introduction

The availability of open data sets is viewed as a positive trend.[1] Open data sets allow for others to expand upon findings not reported by original authors. It has not been established how new information obtained from old (but open) data sets should be reported if the data set corresponds to a previous paper that had already been subjected to peer review. As per a suggestion,[2] this effort will be brief, in keeping with data that has largely been reported elsewhere.[3] Supporting information is provided that should be of particular interest to pathologists who diagnose breast cancer.

An open data set is available from a study of the concordance of gene-based assays.[3] The data corresponded to the analysis of 295 tumours from a series of patients who were followed by the Netherlands Kanker Institute (NKI). The tumours had been used in the validation of the 70-gene expression profile[4] and in evaluation of the wound response signature.[5]

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Particulars of the NKI data set are available in the relevant papers,[3–5] but of interest is that all 295 patients were <53 years of age. It was also noted[4] that histological grading was reported to have been assessed by the Elston and Ellis method,[6] but no other details of how grading was performed were described.

Not recognized in the concordance study (nor would it have been an expectation) was the significance of histological grade being the first prognostic assay reported after a diagnosis of cancer is given. Often, grading has a turnaround time of less than an hour, as grade is usually assessed at the same microscopic session as the original cancer diagnosis. The results of a gene-based assay would arrive much later. The grade-first perspective is important in the reality of the time-line of everyday practise. Additional testing by assays having the same general goal of grade may yield difficult-to-interpret discrepancies.

The NKI data were studied from the grade-first perspective.

Methods

The data set was copied from a listing provided in the online supplementary findings of the *New England Journal of Medicine (NEJM)* concordance paper.[3] Among several comparisons, it was verified that survival curves built from the transcribed data matched curves shown in the concordance paper.

The approach used in examination of the data was based largely on findings from the Nottingham group. They had evaluated breast cancer staining using the immunohistochemical Ki-67 biomarker.[7] Specifically, they found that Ki-67 staining can divide intermediate grade into two distinct subgroups. They did not conclude that the results of Ki-67 staining should be used to further subdivide low or high grades.

For the purpose of the current study, the Nottingham work was considered to have outlined a simple decision tree of low and high grades serving as terminal nodes. However, intermediate grade would be divided further by a single biomarker into two terminal nodes or, if the biomarker had an intermediate-risk stratification, three terminal nodes would be built. Recurrence score has an intermediate tier.

The decision tree approach was not proposed by the Nottingham group in their paper. The approach was conceived by the author as a means to study the NKI data using an analysis that was derived extrinsic to the NKI data, and would not have been trained from the NKI data. Integral in following the decision tree path is the common theme of Ki-67 and gene-based assays serving as measures of tumour proliferation,[8,9] tumour proliferation being considered the most important prognostic feature in the prediction of survival.

The patient data were first divided into low-, intermediate- and high-grade subsets. Then, low-grade tumours were grouped with intermediate-grade tumours that were low recurrence score. An intermediate stratification was maintained, as intermediate-grade tumours were grouped with tumours having an intermediate recurrence score. High-grade tumours were placed together with intermediate-grade tumours that had a high recurrence score. A final classifier was built that relied upon grade alone for the majority of risk assignments. Two-thirds of the patients had either low- or high-grade tumours.

The R statistical package was used for analysis.[10] Statistical significance in the prediction of survival was assessed by the log-rank method available in R library survival.[11] Concordance was evaluated by the Cramer's V statistic. The Cramer's V statistic was the chief measure of concordance in the *NEJM* concordance study.[3] A Cramer's V of 0.5 or greater was considered as two attributes showing strong concordance. Calculations for Cramer's V can be obtained from the R library vcd.[12]

Results

Figure 1 shows the survival curves of histological grade, recurrence score and the relevant curves after recurrence score has divided intermediate-grade tumours into low-, intermediate- and high-risk stratifications. As seen from examination of Figure 1, the decision tree classifier provides statistically significant survival stratification. There is a considerable reduction in the number of patients placed into an intermediate-risk stratification. There is an increase in the number of patients placed into a low-risk category, and this is with respect to both low grade and low recurrence score.

Recurrence score was able to provide statistically significant stratification of intermediate-grade tumours ($P = 0.007$). As detailed further in the Supporting information (Figure S1), recurrence score was unable to stratify low- and high-grade tumours significantly.

The Cramer's V of the RS-grade decision tree classifier with the 70-gene profile was 0.57, while high recurrence score as a stand-alone had a 0.59 Cramer's V with the 70-gene assay. Thus, the majority of cases could be stratified based on grade alone without loss of strong concordance.

Given strong concordance, the simple decision tree approach paralleled 70-gene and recurrence score in

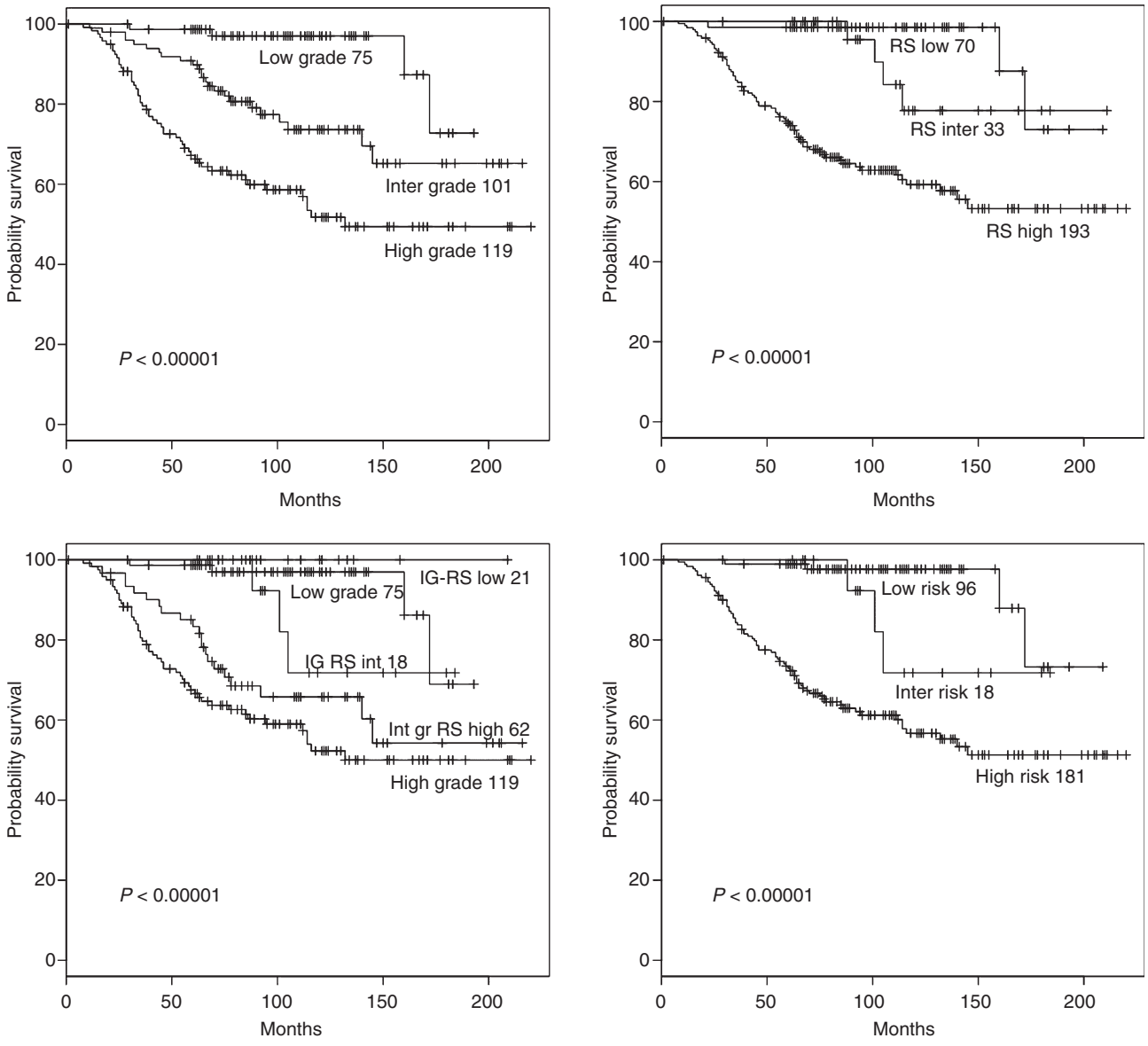

Figure 1. The left upper panel is the survival curve for grade and in the right upper is the survival curve for recurrence score. The left lower panel shows the relevant curves after intermediate grade (IG) has been subdivided by recurrence score (RS). The right lower panel shows the final classification. Low grade has been grouped with IG-RS low, and high grade has been grouped with IG-RS high. An intermediate-risk stratification remains of IG being placed together with RS-intermediate. The final classifier has 21 more patients in low-risk stratification (28% increase) compared to low-grade, and 26 more compared to low recurrence score (38% increase). R library survival was used in constructing the Kaplan–Meier survival curves, and in evaluating statistical significance of survival by the log-rank method.

predicting tumour recurrence, and not just overall survival. The decision tree approach showed equivalent prognostic ability in various patient subsets, such as those patients who were oestrogen receptor-positive and lymph node-negative. Also, it did not matter if intermediate grade was stratified by the 70-gene profile rather than by recurrence score.

In that the data set is freely available,[3] the above summarizations can be verified. Additional permutations can also be easily studied. Some additional analysis is provided in the Supporting information: additional survival curves to include those of wound response signature and 70-gene assay (Figures S2–S5), a cluster dendrogram (Figure S6), likelihood ratio test and receiver operator curve analysis (Figure S7), integrated discrimination improvement analysis (Table S1), a three-way table comparing grade with recurrence score (Table S2) and relevant R script (Table S3).

Conclusion

That gene-based assays can offer added value is not disputed. What has been shown is an important clarification of where the value is most significant.

Further testing of low- and high-grade tumours by gene-based assays is dubious, and such testing may simply confuse matters. It might be assumed that a modern molecular test is superior to old-fashioned morphology, but it has been shown that low-grade tumours may have high recurrence scores[13] due to the proliferation of background stromal and/or inflammatory cells. Discrepancies due to sampling and/or tumour heterogeneity[14] are additional concerns. Also, pathologists studying morphology, and the molecular biologists reviewing gene-based results, may be viewing much the same. A capable gene-expression profile can be built with low and high grades as target variables.[15]

Results from Nottingham,[7] as well as from others,[16] show that immunohistochemical markers may also serve the same role of subdividing intermediate grade. Shown here has been an approach derived from the use of an immunohistochemical biomarker in the Nottingham data set that guided a successful approach in the NKI data set.

It may be that more recent studies have now rendered the information reported here as being nothing new. If so, this provides a historical perspective, if not a lesson. The NKI data have been available for many years, and predate studies that offer a similar opinion. The *NEJM* concordance paper[3] is often referenced, and in the author's experience reprints of the paper have been included with vendor marketing materials. The NKI data set is high-profile, and thus the findings here are not necessarily limited to a conclusion, but are of particular interest given the source.

That the NKI data set is available[3] speaks to a wish for all to have the opportunity to study the data in ways not originally anticipated. This is to be applauded; it has allowed for a perspective that should be of special interest to practising pathologists, and should foster discussion with their clinical colleagues.

Competing interests

There are no conflicts of interest. This work was self-funded, and all work was performed by the author. The author has no ownership or derivative interest in a molecular and/or histopathology laboratory.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Figure S1. The left panel shows survival of the low-grade subset stratified by recurrence score.

Figure S2. The left panel shows survival stratification offered by the seventy gene profile (70G).

Figure S3. If intermediate grade is grouped with high grade (left panel) a binary classifier is the result.

Figure S4. The left panel corresponds to low-grade stratification by the wound response signature, and the middle corresponds to intermediate-grade stratification.

Figure S5. The left panel shows further low-grade stratification by luminal-A subtype.

Figure S6. Cluster analysis provides a way to quickly visualize association among attributes.

Figure S7. There were 249 patients who had died within 5 years or had survived 5 years.

Table S1. Integrated discrimination improvement [IDI] and net reclassification index [NRI] for various attributes.

Table S2. The reclassification of grade by the simple decision tree.

Table S3. R script used for simple survival analysis, calculation of the Integrated discrimination improvement/net reclassification index, cluster analysis, and Cramer's V calculation.

