Reviewer's report

Title: Using additional immunohistochemical markers can refine prognosis in triple negative breast cancer

Version: 1 Date: 7 April 2007

Reviewer: William Anderson

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General comments:
The article entitled Using additional immunohistochemical markers can refine prognosis in triple negative breast cancer by Tischkowitz et al used IHC to classify 456 breast cancer cases from JGH and VGH as TNP (ER-, PR-, and HER2-) or CBP (ER-, HER2-, CK5/6+, and EGFR+). Results showed greater impact of so-called "triple negative" (TNP) breast cancer upon breast cancer-specific survival at 3 than 10 years following initial breast cancer diagnosis. That is, the relative risk for breast cancer death for TNP compared to non-TNP was greater at 3 years (RR=4.06) than at 10 years (RR=1.71). A similar pattern was observed for CBP versus non-CBP tumors. In contrast, when CK5/6- and EGFR- were compared to CK5/6+ and EGFR+ tumors, survival differences increased over time.

The manuscript is interesting, confirming that breast cancer is a heterogeneous disease with non-proportional cancer-specific survival or hazard rates for different breast cancer expression patterns or phenotypes. The paper, however, could be strengthened in several specific areas, as described below.

Major Compulsory Revisions by section (that the author must respond to before a decision on publication can be reached)
1) Introduction: Technically, gene expression patterns have identified three (not two) ER negative tumors, i.e., basal-like (TNP), HER2+, and the so-called normal-like or unclassified tumors. This distinction is relevant given that “normal-like” tumors can also have the TNP expression pattern (ER-, PR-, and HER2-). So, without CK5/6 and EGFR, the normal-like tumors might be misclassified as basal-like TNP. With that said, it would be useful to know how many basal-like TNP tumors also were CBP. The difference between TNP and CBP might reflect the number of normal-like or unclassified breast cancers. Additionally, although most basal-like tumors do not express ER, PR, and HER2, some do; and so, the overlap between basal-like and TNP breast cancer also is not complete (Cleator et al The Lancet Oncology 8: 235, 2007). These issues could be discussed in either the introduction or discussion sections of the manuscript.

2) Materials and Methods: Sample size information is difficult to follow. For example, the authors state that treatment data were available for 380 cases in the combined series (VGH and JGH), yet ChemoRx data was available for 448 cases (table 1). Case contribution also is more clearly defined for JGH than for VGH.

3) Materials and Methods, tables 3a and 3b: I find the tables confusing. Some of the columns should be widen to accommodate data in a single row. For example, the word “yes” is split over two lines. I also am not convinced that the parsimonious model adds much beyond their multivariate model. Additionally, why is parsimonious model 1 only adjusted for tumor size and LN, whereas model 2 is adjusted for tumor size, LN, and grade while model 3 is adjusted for tumor size, LN, and center? What is meant by the statement that all of the models accounted for missingness? To “account” for missing data, the authors would need to know the cause of missingness, e.g., did missingness occur at random or was it non-random based upon race, tumor characteristics, etc. In sum, their method of accounting for missing data should be clearly described.

4) Materials and Methods, figure 1: The description of the Poisson model is confusing. I think that the authors are saying that although TNP cases were less likely to be node positive than non-TNP cases (OR=0.44, table 1), nodal status was positively associated with tumor size for both TNP+ and TNP- cases (figure 1). If this is correct it should be clearly described.

5) Results/discussion, page 6: Given that the non-proportional survival effects at 3 and 10 years for TNP tumors is their main finding, the authors should probably provide some statistical test for trend. This will be especially important since the confidence intervals overlap for TNP versus non-TNP, i.e., RR=4.06 (2.11-7.82) at 3 years and 1.71 (1.05-2.78) at 10 years.

6) Results/discussion, page 7: The authors state that models 1 and 2 share ER- negative status, but fail to acknowledge that these two models also share HER- expression.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
Discretionary Revisions (which the author can choose to ignore)
The abstract would be easier to read if it were structured (Background, Methods, Results, Discussion).
Similarly, the results and discussion sections probably should be separated.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
I have no competing interests.