Utility of a Simplified Molecular Classification of Tumors for Predicting Survival of Patients with Invasive Ductal Breast Carcinoma

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Abstract. Background: In a recent report it was shown that molecular subgroups of early (pT1) breast tumors belonging exclusively to the most common histological variant, ductal-infiltrating carcinoma, showed significantly different clinical and biological features. Objective: To test in a series of patients with ductal-infiltrating carcinoma encompassing all stages of the disease if the above-mentioned biological differences already detected at the earliest stage are ultimately reflected in survival differences. Patients and Methods: All patients with ductal infiltrating carcinoma operated upon at Hospital de Móstoles, Madrid, Spain, between 1997 and 2002 were included into the study, to allow for at least five years of follow-up for survivors. Of 242 studied patients, according to the International Federation of Gynecology and Obstetrics (FIGO) classification, 37.6% were in stage I, 47.2% in stage II, 11.0% in stage III, and 4.2% in stage IV. According to the simplified molecular classification of Carey et al., 116 tumors (47.9%) expressed estrogen receptors, and did not express c-erb-B2 (Luminal A), 67 (27.7%) coexpressed hormone receptors (either estrogen receptors, progesterone receptors or both) and c-erb-B2 (Luminal B), 33 (13.3%) expressed c-erb-B2 in the absence of hormone receptors (HER-2), and 26 (10.7%) neither expressed hormone receptors, nor c-erb-B2 (triple-negative, basal). Results: In a univariate model, both disease-free survival and overall survival of the patients were significantly associated with stage (p=0.0003; p<0.0001), histological grade (p<0.0001; p<0.0001), lymphatic vascular space invasion (p=0.0005; p=0.0044), menopausal status (p=0.04; p=0.034) and molecular subgrouping (p=0.037; p=0.01). In a multivariate model, only stage (p=0.013), grade (p<0.0001), and menopausal status (p=0.007) retained their prognostic power for predicting disease-free survival, and just stage (p<0.0001) and grade (p<0.0001) for predicting overall survival. The molecular classification of the tumors almost reached statistical significance for predicting overall survival (p=0.06).

In a recent report (1), it was shown that molecular subgroups of early (pT1) breast cancer belonging exclusively to the most common histological variant, ductal-infiltrating carcinoma, showed significantly different clinical and biological features. For the molecular classification of the tumors during that investigation, the costly and complicated microarray technology originally employed for the definition of the molecular subgroups was not used (2, 3), but instead we used the much simpler classification subsequently proposed by Carey et al. (4). This is based on the immunohistochemical assessment of hormone receptors and c-erb-B2 expression, which are nowadays routine techniques in every pathological laboratory involved in breast cancer diagnosis. To further verify whether the different biological and clinical features of ductal infiltrating carcinoma such defined do also translate into a significantly different prognosis of the patients harboring the tumors, the present study was conducted. The simplified classification of Carey et al. was retrospectively applied to a series of patients with ductal infiltrating carcinoma, whose fate was well known through a sufficiently long follow-up, and the results were compared with those related to the classical prognostic factors commonly employed in the clinic, using a Cox proportional hazards regression model.

Patients and Methods

The charts and pathological reports of all patients with ductal-infiltrating carcinoma operated upon at the Hospital de Móstoles, Madrid, Spain, between 1997 and 2002 were revised. This time span was chosen to allow for a minimum follow-up period of five years for surviving patients. The total number of patients included in the study was 242. Of them, according to the International Federation of
Gynecology and Obstetrics (FIGO) classification, 37.6% were in stage I, 47.2% in stage II, 11.0% in stage III, and 4.2% in stage IV. According to the classification proposed by Carey et al. (4), 116 tumors (47.9%) expressed estrogen receptors and did not express c-erb-B2, and were thus termed Luminal A tumors, 67 (27.7%) coexpressed hormone receptors (either estrogen receptors, progesterone receptors or both) and c-erb-B2, and were termed Luminal B tumors, 33 (13.3%) expressed c-erb-B2 in the absence of hormone receptors, and were termed HER-2 tumors, and 26 (10.7%) did neither express hormone receptors, nor c-erb-B2, and were termed triple-negative tumors.

For the statistical work-up of the data, as a first step, both disease-free survival and overall survival were studied in a univariate model, using Kaplan-Meier curves and the log-rank test, according to every available clinical and pathological prognostic factor. The pathological variables included were stage, histological grade, lymphatic vascular space invasion, p53 expression and the molecular classification described above. The clinical variables included in the univariate model were age and menopausal status. The results were considered significant, when the corresponding \( p \)-value was <0.05. Subsequently, all significant prognostic factors identified in the univariate model were included in a multivariate Cox proportional hazards model in order to identify those with independent prognostic power. All calculations were performed using the SPSS statistical package (SPSS Inc., Chicago, USA).

**Results**

In the univariate model, both disease-free survival and overall survival of the patients were significantly associated with stage, histological grade, lymphatic vascular space invasion, menopausal status and molecular subgrouping (Table I). The greatest differences in survival between patients were registered between those harboring luminal A and HER-2 tumors, the former presenting the best and the latter presenting the worst survival. Luminal B and triple-negative tumors occupied an intermediate position and showed very similar survival. After inclusion of these variables into a multivariate Cox proportional hazards model, only stage, grade, and menopausal status retained their prognostic power for predicting disease-free survival (Table II), and only stage and grade for predicting overall survival (Table III). The molecular classification of the tumors almost reached statistical significance for predicting overall survival (\( p = 0.06 \)).

**Discussion**

The introduction of microarray technology has revolutionized the study of cancer, both through the identification of distinct biological subtypes for a given tumor, and a refinement of risk assessment besides that provided by conventional prognostic markers. This is especially true for breast cancer. The pioneering work of Perou et al. (5) identifying molecular subgroups of breast cancer, followed shortly thereafter by reports from the same group (6) and by van’t Veer et al. (7),
showing that the molecular classification of the tumors had profound clinical implications is bound to change our diagnostic and therapeutic protocols in the near future. A large international trial (MINDACT) using microarray technology in a prospective randomised fashion to direct treatment in cases where conventional prognostic factors are not resolutive is underway (8), and will answer fundamental questions. Until the results of this and similar trials are published, however, all efforts to apply this new knowledge are purely investigational. Among them, from the very beginning, one of the main aims has been to find simpler alternatives to the costly and time-consuming use of microarrays. Carey et al. (4), from the Perou group which introduced the molecular classification based on microarrays, offer a surrogate molecular classification based on the immunohistochemical determination of hormone receptor and c-erb-B2 expression, which, as has been mentioned earlier, are standard techniques in every pathology laboratory dealing with breast cancer. Their classification approximates the microarray-based one, but they do not completely overlap. The main discrepancy between them is at the expense of the luminal B subvariant. Using the Carey et al. classification, approximately 30-50% of luminal B tumors are misclassified, since this is the proportion of luminal B tumors not expressing c-erb-B2 when identified by means of gene profiling. Another drawback of this classification and similar ones is that they do not account for gene expression patterns peculiar to different histological variants. Here, the greatest source of error again lies in the difference of intrinsic expression of c-erb-B2 between the ductal, lobular and medullary variety within tumors with otherwise similar prognosis. This latter issue prompted our first study, restricting the use of the Carey et al. classification to just one, the most frequent, histological variety of breast cancer, ductal-infiltrating carcinoma (1). In it, by comparatively simple means, we were able to identify biologically different subgroups. However, since the study was carried out on early (pT1) breast carcinomas, with an excellent initial prognosis, we lacked a sufficiently long follow-up and could not draw conclusions as to the prognostic power of the molecular classification. In the present study, we have obviated this drawback by again restricting it to ductal infiltrating carcinomas, but including all stages and closing the recruitment in 2002 and the follow-up in 2007.

As can be seen from our results, the molecular subgrouping of the tumors resulted in significant differences in both disease-free and overall survival in the univariate analysis. Furthermore, it almost retained its independent prognostic power in the multivariate analysis, albeit only for predicting overall survival. The most powerful independent prognosticators emerging from the multivariate analysis, as so often, were stage and grade. Both encompass so many individual biological and clinical features of the tumors, that it is extremely difficult to find a new single factor that is able to retain its independent prognostic value in the face of them. According to the results reported by the Dutch group of van’t Veer et al. (9, 10), this has been the case using the sophisticated technology necessary for gene profiling, but according to our present results, the same goal is not altogether affordable with much simpler means. Nevertheless, our results are encouraging, since the molecular classification used by us almost reached statistical significance in the multivariate model. The addition of perhaps one or two immunohistochemical determinations would not greatly complicate the process from the technical and economical point of view, and could possibly yield clearer results. It has already been shown that immunohistochemical cytokeratin 5/6 and epithelial growth factor receptor (EGFr) measurements help to better define the subgroup of basal-type tumors among the cohort of patients with triple-negative tumors (4, 11). The greatest problem probably lies in identifying a panel of immunohistochemical markers that helps to precisely discern between luminal B type tumors and the rest, because as has been mentioned earlier, the coexpression of hormone receptors and c-erb-B2 may misclassify up to 50% of the cases belonging to this subgroup. Luminal B tumors unrecognised by this marker combination are thus wrongly attributed to the luminal A subgroup, which is the one with the best intrinsic prognosis, and may diffuse significant differences in survival between this group and the remaining three.

In conclusion, the molecular classification of ductal infiltrating breast carcinoma according to the simplified classification of Carey et al. resulted in significantly different survival of the patients belonging to each subgroup in the univariate analysis. However, in the multivariate analysis, the molecular classification is not able to retain its independent prognostic power, although it misses this target by a very narrow margin. This opens the possibility of defining, with a few additions, an immunohistochemical marker panel which may yield similar results to those obtained with microarray technology.

References


