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Distribution and prognosis of molecular breast cancer subtypes defined by immunohistochemical biomarkers in a Spanish population-based study

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HIGHLIGHTS

- This study showed differences in clinicopathological features and survival rates among breast cancer molecular subtypes classified by immunohistochemical biomarkers.
- We confirm that molecular subtypes defined by immunohistochemical biomarkers provide useful prognosis information for guiding and evaluating clinical treatment.
- The prognosis value of molecular subtype persists when adjusting by age, stage and histological grade.

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ABSTRACT

Background. The objective of this study is to analyze the distribution, clinicopathological features, relative survival rate and excess risk of death among females diagnosed with invasive breast cancer and classified by molecular subtype from ten Spanish cancer registries.

Method. Three thousand four hundred and eighty incident cases of women – mostly diagnosed in 2005 – were classified into five molecular subtypes according to immunohistochemical status of hormonal receptors and HER2 (human epidermal growth factor receptor 2): estrogen receptor (ER) and/or progesterone receptor (PR)+ and HER2−, ER+ and/or PR+ and HER2+, HER2-overexpressed (ER−, PR− and HER2+), triple negative (ER, PR and HER2−) and unclassified (hormonal receptor or/and HER2 unknown). Relative survival rates at 1, 3 and 5 years and relative excess risks (RER) of death adjusting for molecular subtype, age, stage and histological grade were estimated.

Results. Marked differences in clinicopathological characteristics and relative survival rate were observed between molecular subtypes. Compared with women with ER+ and/or PR+ and HER2−, ER+ and/or PR+ and HER2+ cases had an RER of 1.00 (95% CI: 0.66 to 1.52) after adjusting for age, stage and histological grade, whereas HER2-overexpressed, triple negative and women with unclassified subtypes presented an RER of 1.72 (95% CI: 1.15 to 2.57), 3.16 (95% CI: 2.26 to 4.41) and 2.55 (95% CI: 1.96 to 3.32), respectively.

Conclusion. The prognostic value of molecular subtype persists when adjusting for age, stage and histological grade. Hormone receptor-positive tumors were associated with a better prognosis when compared with HER2-overexpressed and triple negative subtypes. Further research is required to improve triple negative prognosis.

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Introduction

Breast cancer mortality has decreased in recent years in Spain due to the use of population prevention strategies and improved treatments. Although breast cancer incidence has also been declining since 2003, it is still the most common neoplasm among Spanish women and the most frequent cause of cancer mortality [1,2]. Breast cancer is a highly heterogeneous disease with regard to its clinical features, biological behavior and treatment response [3,4]. Analyses based on breast cancer gene expression patterns using microarray technology have identified the following molecular subtypes: luminal A, luminal B, basal-like and HER2-overexpressed (human epidermal growth factor receptor 2) [5,6]. Several differences have been described between these groups in reference to incidence, survival and risk factors [5–14]. Current knowledge of molecular breast cancer subtypes has provided a better approach to treatment.

Hormone receptor-positive tumors have been associated with a better prognosis and non-aggressive behavior compared to basal-like and HER2-overexpressed subtypes, whereas basal-like tumors have been associated with the worst prognosis. In clinical terms, this subtype is known as triple negative due to the lack of ER and PR protein expression and the absence of HER2 overexpression. However, approximately 25% of basal-like tumors are not triple negative [4]. Remarkably, there is a lack of target treatment for women diagnosed with a basal-like tumor.

Luminal subtypes are characterized by high expression of hormonal receptors. Luminal A is defined by low expression of HER2 and low proliferation and luminal B by high proliferation. Proliferation is assessable using the Ki-67 index and/or histological grade [8,15]. Among these two subtypes, luminal B tumors have a worse prognosis than luminal A [9,13,16].

Genotyping assays used to classify breast cancers into molecular subtypes according to gene expression are costly and not affordable for most hospitals. A relatively high concordance (75–90%) is considered to exist between molecular subtypes defined by genomic methods and immunohistochemical (IHC) phenotype [17]. Currently, IHC biomarkers of the estrogen receptor (ER), progesterone receptor (PR) and HER2 are routinely tested and have been used as a surrogate to stratify breast cancer into ER+ and/or PR+ and HER2−, ER+ and/or PR+ and HER2+, triple negative and HER2-overexpressed tumors.

There is a lack of information regarding population-based distribution and survival by molecular subtype in Spain. The present work analyzes the distribution, clinicopathological features, survival and excess risk of death among females diagnosed with breast cancer classified by molecular subtypes from ten Spanish cancer registries, mostly diagnosed in 2005.

Materials and methods

Data

Data were extracted from the ten Spanish population-based cancer registries participating in the "Spanish High Resolution Breast Cancer Study" (Albacete, Castellón, Cuenca, Gipuzkoa, Girona, Granada, La Rioja, Murcia, Navarra and Zaragoza), covering 20% of the Spanish female population, mainly located in the east of Spain. These cancer registries collect incident of invasive breast cancer (IBC) according to guidelines from the International Agency for Research on Cancer (IARC) and European Network of Cancer Registry (ENC) recommendations [18,19].

All cancer registries provided data of incident cases from 2005 ($n = 2{,}869$). The four cancer registries covering the smallest population (Albacete, Castellon, Cuenca and La Rioja) also collected incident cases from 2004 ($n = 611$). Cases included in the study were all those coded as C50.0–C50.9 according to the International Classification of Diseases, 10th revision [20]. First tumor data were recorded for women with non-synchronous bilateral IBC. A total of 3,480 women were identified. Of these, 3,378 (97.1%) were histologically confirmed cases of IBC, 12 (0.3%) were only diagnosed by death certificate (DCO), and 1 was diagnosed by autopsy. These 13 cases were excluded from the survival analysis. Women were followed up to 31 December 2010. Vital status was determined by means of the National Death Index, and clinical reports were used as information sources to this end. The use of the National Death Index allows survival rate comparisons between registries [21]. Cancer-specific mortality was not available.

Five molecular subtypes were defined according to ER, PR and HER2 status: ER+ and/or PR+ and HER2−, ER+ and/or PR+ and HER2+, HER2-overexpressed (ER−, PR− and HER2+), triple negative (ER−, PR− and HER2−) and unclassified (hormonal receptor or/and HER2 unknown). Hormonal receptor status and HER2 overexpression were recorded from pathology and clinical reports. ER, PR and HER2 status were assessed by means of IHC. The cutoff for RE and PR positivity used by most hospitals participating in the study was >10%. Tumors were considered as HER2+ when cells presented strong membrane staining (3+). Tumors exhibiting 0 or 1+ staining for HER2 protein overexpression were considered to be HER2−. In cases of equivocal membrane staining (score 2+) for HER2, fluorescence in situ hybridization (FISH) was used to evaluate gene amplification.

Age, tumor size, stage of tumor at diagnosis [22], histological grade, multifocality and/or multicentricity of tumor, neoadjuvant treatment (yes, no) and surgery (conservative, mastectomy, unknown) were also recorded. For women who had received neoadjuvant therapy, tumor stage was categorized as unclassified/unknown. The Scarff, Bloom and Richardson (SBR) classification, which was modified by Elston and Ellis, was used by most hospitals to define histological grade. This study did not require ethical approval and no consent was needed from patients involved in the study.

Statistical analysis

Differences in the clinicopathological features of ER+ and/or PR+ and HER2−, ER+ and/or PR+ and HER2+, HER2-overexpressed and triple negative subtypes were assessed using one-way analysis of variance (ANOVA) and the Bonferroni method for mean age (years) and χ^2 test for age groups, menopausal status, tumor size, stage, histological grade, multifocality and/or multicentricity of the tumor, neoadjuvant treatment and surgery. Statistical significance was determined at $p < 0.05$. Version 18 of the SPSS was used for the statistical analyses.

Relative survival (RS) rates were estimated using the Pohar–Perme method as the ratio of observed survival in the study population to expected survival in the general population of the same age, sex, year and province (Albacete, Castellón, Cuenca, Gipuzkoa, Girona, Granada, La Rioja, Murcia, Navarra and Zaragoza) [23]. RS rates were estimated for all cases and according to molecular subtype 1, 3 and 5 years after diagnosis, as well as at their 95% confidence intervals (CI). Expected survival rates were taken from life tables for the population covered by the ten registries participating in the study. Relative excess risks of death (RERs) were estimated using a multivariable generalized linear model with a Poisson distribution. The adjusted variables used were molecular subtype (ER+ and/or PR+ and HER2−, ER+ and/or PR+ and HER2+, HER2-overexpressed, triple negative and unclassified), age at diagnosis (<40, 40–49, 50–59, 60–69, 70–79, ≥80), stage at diagnosis (I, II, III, IV and unknown) and histological grade (I, II, III, and Unknown/Unclassified). Survival analyses were calculated using R, version 2.14.0.

Results

Distribution

A total of 3,480 incident cases of female IBC were identified in the ten Spanish cancer registries participating in the study. The women's ages ranged from 25 to 97 years old. There were 79.6% ($n = 2{,}771$) of