MOLECULAR ONCOLOGY XXX (2014) 1–12

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/molonc

Comparison of frequencies and prognostic effect of molecular subtypes between young and elderly breast cancer patients

Esther M. de Kruijf[a], *Esther Bastiaannet*[a,b], *Francesca Rubertá*[a], *Anton J.M. de Craen*[b], *Peter J.K. Kuppen*[a], *Vincent T.H.B.M. Smit*[c], *Cornelis J.H. van de Velde*[a], *Gerrit Jan Liefers*[a,*]

[a]*Department of Surgery, Leiden University Medical Center, Albinusdreef 2, 2300 RC Leiden, the Netherlands*
[b]*Department of Gerontology & Geriatrics, Leiden University Medical Center, Leiden, the Netherlands*
[c]*Department of Pathology, Leiden University Medical Center, Leiden, the Netherlands*

ARTICLE INFO

Article history:
Received 24 December 2013
Received in revised form
10 March 2014
Accepted 28 March 2014
Available online ■

Keywords:
Breast cancer
Prognostication
Molecular subtypes
Elderly

ABSTRACT

Purpose: To compare the distribution and prognostic effect of the breast cancer molecular subtypes in young and elderly breast cancer patients.
Patients and methods: Our study population ($n = 822$) consisted of all early breast cancer patients primarily treated with surgery in our center between 1985 and 1996. A total of 142/822 fresh frozen tissues were available with good quality RNA and analyzed by gene expression microarray. Gene expression molecular subtypes were determined by correlation to the expression centroids of 534 "intrinsic" genes. Sections of a tissue micro array containing formalin-fixed paraffin-embedded tumor tissue of 714/822 patients were immunohistochemically (IHC) stained for Ki67, EGFR, CK5/6. Tumor expression of ER, PR, HER2 was previously determined. IHC molecular subtypes were defined based on expression of these markers: Luminal A: ER+ and/or PR+, HER2− and Ki67−; Luminal B: ER+ and/or PR+ and ki67+; ERBB2: ER−, PR− and HER2+; Basal-like: ER−, PR−, HER2− and EGFR+ and/or CK5/6+; Unclassified: ER−, PR−, HER2−, EGFR− and CK5/6−. IHC molecular subtypes were validated against gene expression defined molecular subtypes. Assessment of distribution and prognostic effect of molecular subtypes was stratified to age (<65 versus ≥65 years).
Results: Validation of molecular subtypes determined by IHC against gene expression revealed a substantial agreement in classification (Cohen's kappa coefficient 0.75). A statistically significant association ($p = 0.02$) was found between molecular subtypes and age, where Luminal tumors were more often found in elderly patients, while ERBB2, basal-like and unclassified subtypes were more often found in young patients. Molecular subtypes showed a prognostic association with outcome in young patients concerning relapse-free period (RFP) ($p = 0.01$) and relative survival (RS) ($p < 0.001$). No statistically significant prognostic effect was found for molecular subtypes in elderly patients (RFP $p = 0.5$; RS $p = 0.1$). Additional analyses showed that no molecular subtypes showed a statistically significant difference in outcome for elderly compare to young patients.

Abbreviations: 95%CI, 95 percent confidence interval; BCS, breast conservative surgery; CT, chemotherapy; EGFR, epidermal growth factor receptor; ER, estrogen receptor; ET, endocrine therapy; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; IHC, immunohistochemistry; MAST, mastectomy; N, number of patients; PR, progesterone receptor; RER, relative excess risk; RFP, relapse-free period; RS, relative survival; RSN, robust spline normalization; RT, radiotherapy; VST, variance stabilizing transformation.

* *Corresponding author*. Tel.: +31 71 526 2309; fax: +31 71 526 6750.
E-mail address: g.j.liefers@lumc.nl (G.J. Liefers).

1574-7891/$ – see front matter
http://dx.doi.org/10.1016/j.molonc.2014.03.022

Conclusion: We have shown that molecular subtypes have a different distribution and prognostic effect in elderly compared to young breast cancer patients, emphasizing the fact that biomarkers may have different distributions and prognostic effects and therefore different implications in elderly compared to their younger counterparts. Our results support the premise that breast cancer clinical behavior is significantly affected by patient age. We suggest that competing risks of death in elderly patients, ER-driven differences and micro-environmental changes in biology are underlying these age-dependent variations in patient prognosis.

1. Introduction

Breast cancer is increasingly becoming a disease affecting older women. However, evidence based treatment guidelines specific for this aged breast cancer population are lacking (Petrakis and Paraskakis, 2010). Decisions regarding breast cancer treatment are based on prognostic and predictive patient and tumor characteristics discovered and analyzed in relatively young patient populations (Anderson et al., 2009; Beadle et al., 2011; Early breast cancer Trialists' Collaborative Group, 2005; Goldhirsch et al., 2007). These characteristics have been found to differ considerably between elderly and young breast cancer, i.e. elderly breast cancer patients present more often with tumors positive for hormone receptor expression, no overexpression of human epidermal growth factor receptor 2 (HER2), lower proliferation rates, diploidy, normal p53 expression and bcl-2 overexpression (Daidone et al., 2003; Diab et al., 2000; Remvikos et al., 1995). This may be indicative for differences in underlying tumor biology and it has indeed often been suggested that elderly breast cancer is a biologically different tumor type of a more indolent character compared to young breast cancer (Diab et al., 2000; Remvikos et al., 1995; Thomas and Leonard, 2009). Moreover, it suggests that biomarkers may show different prognostic and predictive effects in the elderly compared to young breast cancer patients. In addition, due to competing causes of death, life expectancy is significantly shorter in elderly breast cancer patient (Bastiaannet et al., 2010, 2011; Wildiers et al., 2007). Therefore, since breast cancer relapses can occur after long periods of time, this further suggests that the impact and significance of prognostic and predictive biomarkers may vary significantly in this patient population. Nevertheless, as this patient population is often underrepresented in translational studies and randomized trials, little is known about the implications on outcome of prognostic and predictive biomarkers in elderly (Anderson et al., 2009; Beadle et al., 2011).

Gene expression studies have identified several distinct breast cancer subtypes based on gene expression patterns, that showed marked differences in patient prognosis (Perou et al., 2000; Sorlie et al., 2001, 2003). This "intrinsic" classification proposes four different classes of breast tumors: Luminal A and B, which are mostly hormone receptor positive and show high expression of genes characteristic of the luminal epithelial cell layer, including expression of estrogen receptor (ER), GATA3 and genes regulated by these (Sorlie et al., 2001, 2003). Compared with Luminal A tumors, Luminal B tumor often express genes associated with high tumor proliferation (Sorlie et al., 2001, 2003). The "intrinsic" subtypes further include 2 main subtypes of hormone receptor negative tumors: Basal-like tumors, which typically are triple negative tumor (ER, progesterone receptor (PR), and HER2 negative) and exhibit high expression of genes characteristic of the basal epithelial cell layer such as cytokeratin (CK) 5, 6 and 17 (Perou et al., 2000) and the ERBB2 tumor subtype, which clusters near the basal-like tumor, are mostly hormone receptor negative and show high overexpression of HER2 and high HER2 gene amplification (Sorlie et al., 2001, 2003). Concerning outcome, hormone receptor positive tumors result in the best patient outcome where, compared to Luminal B tumors, Luminal A tumors seem to be the most indolent tumors (Sorlie et al., 2001). Hormone receptor negative "intrinsic" subtypes, ERBB2 and Basal-like tumors have an aggressive natural history, resulting in an unfavorable patient outcome (Sorlie et al., 2001). In a large study on almost 500 breast cancer patients Perou et al. (2000) found the molecular subtypes, determined with immunohistochemistry (IHC), to be significantly associated with tumor histological grade, lymph node status and patient age, where ERBB2 and Basal-like subtypes showed to correlate with unfavorable tumor characteristics and younger patient age (Carey et al., 2006). The distribution and prognostic effect of molecular breast cancer subtypes specific in the elderly breast cancer population compared to younger breast cancer patients is still unknown.

We used immunohistochemical (IHC) surrogates, which we validated against gene expression determined molecular subtypes, to identify breast tumor molecular subtypes in a large cohort of breast cancer patients. The aim was to investigate the distribution and prognostic effect of molecular subtypes of breast cancer in elderly patients compared to their younger counterparts.

2. Patients and methods

2.1. Patients and tumors

The patient population comprised all non-metastasized breast cancer patients primarily treated with surgery in the Leiden University Medical Center between 1985 and 1996 ($n = 822$). Patients with bilateral tumors or a prior history of cancer (other than basal cell carcinoma or cervical carcinoma *in situ*) were excluded. The following data were known: age,

Please cite this article in press as: de Kruijf, E.M., et al., Comparison of frequencies and prognostic effect of molecular subtypes between young and elderly breast cancer patients, Molecular Oncology (2014), http://dx.doi.org/10.1016/j.molonc.2014.03.022