Review Articles

The role of tumor markers in non-palpable breast cancers

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Summary

Screening mammography and clinical breast examination are the best tools available for the diagnosis of breast carcinomas in asymptomatic women. Many studies have attempted to determine the pathological and biological characteristic findings in screening-detected cancers. Tumor size, histologic type, cytological grading and lymph node status have an important role in estimating the biological profile of non-palpable breast cancers. Tissue tumor markers, such as proliferation markers, hormone receptors, c-erbB-2 and p53 oncoproteins, bcl-2 gene and angiogenesis-related markers do not seem to distinguish mammographically detected tumors from clinically presented cancers. Further studies are needed to assess the prognostic role of certain biological factors in well-designed clinical studies along with long follow-up of screened patients.

Key words: Non-palpable breast lesions; Breast cancer; Tumor markers.

Breast cancer is a primary cause of death for women in the West with a 12% lifetime risk of developing the disease and a 3.5% lifetime risk of death [1, 2]. A variety of factors are associated with an increase in breast cancer risk such as genetic, familial, hormonal and environmental factors as well as the existence of benign breast diseases that are associated with atypical hyperplasia [2]. However, most women who develop breast cancer have none of these risk factors, except the risk of aging.

Advances in early diagnosis or appropriate therapy can arrest the disease in most women. In the last decade, screening programs including screening by breast self-examination or clinical examination and mammography can detect breast carcinomas in asymptomatic women in an earlier stage of evolution [3].

Non-palpable breast cancers consist a group of tumors that are detectable by mammography or ultrasound. These tumors are frequently non-invasive or of better differentiated type than clinically presented tumors. In addition, they are smaller in size and tend to have less nodal and distant metastases [4-7]. However, the frequency of nodal metastasis has also been found to be similar to the palpable cancers when adjusted for size and grade [8].

Various biological markers have been studied for their possible prognostic significance in non-palpable breast cancers. The clinically available markers such as size, histological type, cytological grading and lymph node status are important indicators of tumor behavior, but they can not always provide useful information for the management of the patients. Although tumor markers have not proven sufficient as screening tests, several of them have an association with prognosis itself or with established prognostic factors, such as grade. Tissue tumor markers comprise markers of proliferation, differentiation (hormone receptors), transformation (oncogenes and oncosuppressor genes), metastatic potential (proteases, metalloproteinases) as well as apoptotic and angiogenesis-related markers.

Cell kinetic data are important indicators of clinical behavior in many types of cancer and significant correlations between the proliferation rates and metastatic potential, recurrence as well as overall survival have been detected in most studies. Several approaches are used to measure the percentage of proliferating cells (thymidine labelling, bromodexyuridine labelling, proliferation associated antigens, S-phase fraction, DNA ploidy, etc.). In breast cancer, high tumor proliferation has been suggested as an adverse prognostic factor, particularly in node-negative patients in whom it can be used to discriminate those with the highest risk of metastasis [9]. In the study of Moezzi *et al.* [10] in which 117 breast cancer cases were included (39 non-palpable and 78 palpable cases) the investigators found lower expression of Ki-67 in non-palpable tumors. In addition, they observed that S-phase of diploid tumors was similar in the two groups, but S-phase of aneuploid tumors was lower in the mammographically detected tumors. Later, in a larger study by Molino *et al.* [11] in which a total of 1,863 breast cancer patients were investigated (1,247 with palpable and 616 with

non-palpable lesions), it was found that the first group of cancers had higher Ki-67 levels than the second one. Interestingly, in non-palpable tumors the higher Ki-67 rates were associated with tumors of higher grade and mainly with comedo-type carcinomas. Finally, concerning DNA ploidy, a high prevalence of aneuploid tumors was observed among non-palpable cancers with malignant-appearing microcalcifications [12].

Determination of hormone receptors is an important parameter in the management of breast cancer patients. Estrogen and progesterone receptors (ER, PR) have been now employed as prognostic markers to distinguish among breast cancer patients in both nodal groups, those of low and those of high risk of recurrence. In the first available study of Von Rosen *et al.* [12], 66 non-palpable invasive breast carcinomas from the Stockholm Mammography Screening Project were investigated. The majority of the tumors were predominantly of low or intermediate histological grade of malignancy and ER-rich, when compared with a non-selected tumor group. These tumors represented a prognostically favorable subset since only two cases recurred during a follow-up of 51 months. Comparisons of hormone receptor expression in palpable versus non-palpable cancers showed a higher percentage of ER and PR negativity in symptomatic tumors [11]. However, in other studies ER and PR expression was similar in both groups [10].

Taking together the mammographic findings of the tumors, a higher prevalence of hormone receptor negative lesions and aneuploidy was observed among tumors with malignant-appearing microcalcifications [13, 14]. In contrast, in other studies the positivity of the receptors was correlated with the presence of microcalcifications [15].

Overexpression of the c-erbB-2 gene has been suggested as an independent poor prognostic factor in non invasive and invasive breast carcinomas [16]. The c-erbB-2 gene is amplified in 30% of clinically presented tumors and has been associated with lymph node involvement, shorter relapse time, poor survival and decreased response to endocrine therapy and chemotherapy [17]. A higher expression of the gene has been identified in palpable than in non-palpable tumors [18]. Statistically significant correlations were found between malignant-appearing microcalcifications and c-erbB-2 positivity [15]. Moreover, in *in situ* cases c-erbB-2 overexpression, together with Ki-67 and DNA ploidy, was correlated with high grade tumors, mainly with comedo-type [19, 20].

The p53 oncoprotein is the commonest molecular abnormality in human cancers, including breast cancer. Approximately 50% of breast cancers have mutations of the gene, which have been associated with high histologic grade, disease aggressiveness and worse patient outcome [21]. Expression of this oncoprotein has been demonstrated less frequently in mammographically detected cases than in clinically presented cancers [10]. In the first group of tumors the expression was higher in high grade lesions [10]. However, in some studies no statistically significant differences were found [7, 18].

Dysregulation of apoptosis mechanism plays an important role in the pathogenesis and progress of some cancers as well as in response of cancers to therapeutic intervention. Many apoptosis-triggering molecules and apoptosis blockers, such as bcl-2 family proteins have been identified. The bcl-2 gene product is frequently expressed in breast tumors and this expression is associated with favorable prognosis [22]. It has been reported that the gradual decrease of bcl-2 expression in premalignant and malignant lesions results in increased tumor aggressiveness [23]. In this setting, the findings in some studies of c-erbB-2 overexpression and loss of bcl-2 expression combined with low PR positivity as well as the non significant association between microcalcifications and bcl-2 expression could represent an aggressive tumor phenotype that requires intensive treatment [15]. It is also important to report the significant association between the expression of the proapoptotic protein Bax and mammographically detected malignant microcalcifications [15].

Tumor growth and metastasis are critically dependent on tumor angiogenesis. Neoangiogenesis and its mediators, such as vascular endothelial growth factor (VEGF) represent useful indicators of poor prognosis [24]. The most commonly used measurement of angiogenesis is light microscopy counting of intratumoral blood vessels stained with anti-CD31, anti-CD34, anti-Factor VIII or the immunohistochemical VEGF detection. In the study by Moezzi *et al.* [10], lower levels of microvessel density were observed in non-palpable than in clinically presented cancers.

Proteinases and adhesion molecules (matrix metalloproteinases, urokinase-type plasminogen activator and its inhibitor, Cathepsin D, E-cadherin) are all molecules that promote the dissolution of peritumoral stroma and therefore they promote invasion and metastasis [25]. Although these markers or at least some of them

have been reported as useful prognostic indicators in clinical breast cancers, no definite results exist up to now about the prognostic significance in patients with non-palpable cancers [6].

In conclusion, it is difficult enough to evaluate the up-to-date information concerning the prognostic significance of a variety of tumor markers in non-palpable breast cancers. From the existing data two main questions arise. Do non-palpable breast cancers represent a subset of tumors with more favorable characteristics or do they represent the same breast lesions at an earlier stage of evolution? The relationships found in the literature do not appear strong enough to guide patient management. At present, the available markers such as tumor size, histologic type, cytological grade and lymph node status have an important role in estimating the biological profile of non-palpable breast cancers. Further studies are needed to assess the prognostic role of certain tumor markers in well-designed clinical studies along with long follow-up of screened patients.

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