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Summary

Biochemical assessment of tissue prognostic factors in breast cancer. Blankenstein MA. Ned Tijdschr Klin Chem 1995; 20: 305-311.

Information on prognosis and probability of response to treatment of breast cancer patients can be derived in part from an assessment of prognostic factors in the tumour tissue.

Ned Tijdschr Klin Chem 1995; 20: 311-313

Molecular biological tools in the diagnosis and prognosis of mammary carcinoma

J. ten KATE

Breast cancer has received a great deal of attention in the last few decades in which much progress has been made in characterising the alterations at the DNA level in the different types of breast cancer. These developments offer new hope for patients having breast cancer and for members of families showing a predisposition for this disease.

Neoplastic transformation in general has been shown to be caused by multiple mutations in oncogenes (dominant) and suppressor genes (recessive). The accumulation of mutations leads to the development of invasive, metastasising cancers (1). 40% of patients suffering from breast cancer finally die of the disease. Breast cancer research aims at reducing this figure. One important issue is to develop markers which make it possible to recognise the group with bad prognosis at the time of diagnosis, so that special therapeutical regimens can be developed for this group of patients.

Breast cancers can be subdivided into 4 categories: (1) hereditary, (2) familial, (3) sporadic, and (4) as

Oestrogen and progesterin receptors were among the first and still remain important. The generalized use of adjuvant treatments and the diversification of techniques for receptor assays have caused many hospitals to abandon quantitative receptor assay or to refrain from receptor assays completely. New prognostic factors are being proposed more and more frequently, but prior to their generalized acceptance, they should be validated both analytically and clinically to a greater extent than is usually done. Standardization of techniques and establishment of external quality control procedures are part of the technical validation. Among these potentially useful new prognostic factors are the components of the urokinase system, like urokinase type plasminogen activator and its inhibitors, thymidine kinase, oestrogen receptor variants with constitutive, i.e. hormone-independent, transactivation properties, and (even?) prostate specific antigen. Techniques for the assessment of new prognostic factors are at the protein, RNA and DNA level and thus require specialized laboratories with dedicated staff, willing to commit themselves to guidelines emerging from international cooperative efforts to provide the best possible laboratory care to the breast cancer patient. To maintain the momentum in the research of such laboratories, it is imperative that they are provided with tissue specimens whenever tumour size allows for it.

Key-words: breast cancer prognostic factors, oestrogen receptors, progesterin receptors.

part of a predisposing syndrome (2,3). This paper will deal with the cancers of categories 1 and 3.

Hereditary breast cancer

Three publications in *Science* are a milestone in the field of hereditary breast/ovarian cancer (4-6). These papers present the research concerning the discovery of BRCA-genes (Breast Cancer-genes). BRCA1 was located on chromosome 17 and was found to be mutated in 50% of the hereditary breast cancer families (4). Four mutations are described, of which three inactivate the gene and one is an amino acid mutation. The product of this gene was described to be expressed in breast as well as in ovarian epithelial cells. The mutations in this gene did not cause tumors in breast cancer only, but also predispose for the development of ovarian cancer. In sporadic cases, however, these mutations were not observed (5). In contrast to the findings in colon cancer, the mutations in the hereditary form of breast/ovarian cancer do not occur in the sporadic cases.

The third paper in this series describes the BRCA2-gene, which was located on chromosome 13 (6). Mutations in this gene were predisposing for the development of breast cancer, also in males. Predisposition for ovarian cancer seems to be less pronounced in comparison with mutations in the BRCA1-gene.

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About 85% of the hereditary breast cancer families can be investigated with the BRCA1- and BRCA2-genes, but other BRCA-genes have to be found for the remaining 15% of the families. The family investigations are carried out by the Clinical Genetical Centers. A careful investigation of the families, followed by a discussion concerning the risks and possible preventive options are typical in the field of the geneticist. However, in 85% of the cases of breast cancer there is no hereditary pattern, and DNA analyses of these cases have to be developed in the coming years. This may yield new prognostic markers.

Sporadic breast cancer

In the last two decades it has become clear that cancer is a disease caused by changes in the DNA. In breast tumors, the sites of oncogenes are marked by regions of DNA amplification and gene over-expression. Three well-characterized examples are MYC on chromosome 8 (amplified in 15% of breast tumors), CYCLIN D1 on chromosome 11 (amplified in 15% of breast tumors) and ERBB2 on chromosome 17 (amplified in 20% of breast tumors). No inherited alterations in any of these loci have been shown to be responsible for familial breast cancer (1).

Another line of research focuses on suppressor genes. P53, molecule of the year 1994, is mutated in 13%-15% of breast cancers, causing a high S-phase activity and decreased apoptosis. A relationship with prognosis is rather controversial, tending to no correlation at all (7).

I would like to focus on the ERBB2 oncogene, a gene from the epidermal growth factor receptor family. In 1989 Slamon et al reported the relation between ERBB2 amplification or overexpression and the disease free interval (8). In the years following this publication, many investigations were devoted to this oncogene. The outcomes of these studies are rather contradictory, although it seems to become clear now that ERBB2 amplification is an important prognostic marker (see reference 9). One of the main problems with these studies is that all of them apply different techniques, such as Southern, Northern and Western-blotting, PCR analysis and immunohistochemistry. Hence it seems to be important that standardised methods are developed, because the lack of standardisation hampers our insight into the usefulness of this and many other markers.

In several recent studies the usefulness of ERBB2 amplification was shown as a marker to define a group of patients, who benefit from a specific therapeutic intervention, while other patients do not (10,11). It is important that such lines of investigation are developed and are indicators of our role, as clinical chemists, in this field.

The role of the clinical chemist

Development of diagnostic/prognostic tools for mammary cancer will require: (1) standardisation of methodologies and interlaboratory quality control, (2) definition of a limited set of markers which are independently predictive and (3) systems that integrate

this information and provide a kind of prognostic index, on the basis of which decisions concerning the follow-up of cancer patients can be made (12).

There is an important role for the EORTC and the regional cancer centers in defining the above-mentioned item 2 and coordinating efforts in this direction. For the clinical chemistry laboratories it is important to develop DNA facilities, where PCR, Southern blotting and DNA electrophoresis can be performed. Within the NVKC there is a Task Force for this field, where discussions concerning these items are going on.

However, currently there are no clear-cut applications in the field of breast cancer and molecular biology. This will have changed within several years. If we do not want to miss this boat, we will have to develop this field.

We can score rather easily on the above-mentioned items 1 and 3, because they are in line with our traditions. I hope that these techniques will be integrated in our laboratories in the coming decade, since it makes our discipline still more interesting and will strengthen our position in the hospital.

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Summary

Molecular biological tools in the diagnosis and prognosis of mammary carcinoma. Kate J ten. Ned Tijdschr Klin Chem 1995; 20: 311-313.

Alterations at DNA level have been detected in hereditary and

sporadic breast cancer. In hereditary cancer, breast-cancer genes were located on chromosomes 17 and 13 in 85% of the cases. In the sporadic form, no such genetical mutations could be detected, but the sites of the oncogenes show regions of DNA amplification and gene over-expression. Selection of patient groups based on these findings can be beneficial in order to offer the right therapeutic intervention.

Key-words: breast cancer, hereditary, cancer genes, sporadic, oncogenes.