UNIVERSITY OF MEDICINE AND PHARMACY CRAIOVA
DOCTORAL SCHOOL

DOCTORAL THESIS
SUMMARY

THE HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF PRECURSOR LESIONS OF INVASIVE BREAST CARCINOMA OF NO SPECIAL TYPE

SCIENTIFIC COORDINATOR:

PROF. UNIV. DR. SIMIONESCU CRISTIANA EUGENIA

CANDIDATE:

FOTA GEORGIANA LUMINIŢA

CRAIOVA, 2013

CONTENTS

KEYWORDS: PRECURSOR LESIONS, USUAL DUCTAL HYPERPLASIA, ATYPICAL DUCTAL HYPERPLASIA, FLAT EPITHELIAL ATYPIA, DUCTAL CARCINOMA IN SITU, INVASIVE BREAST CARCINOMA OF NO SPECIAL TYPE

INTRODUCTION

Even though intraductal proliferative lesions have been analyzed in depth over time epidemiologically, clinically, morphologically, molecularly, and cytogenetically, some of them still pose problems at the present time in regard to the diagnosis and the approach of a therapeutic protocol, clinical behavior, and evolution, as well as establishing the prognostic.

Several clinical studies have indicated that intraductal proliferative lesions are associated with various risks in the further development of the mammary invasive lesion. Observing female patients with usual ductal hyperplasia (UDH) over the long term evidentiated a slightly increased risk (1.5 to 2 times) of occurrence of invasive mammary carcinoma. At present, there are no prognostic factors available to establish with certitude which of the patients with simple ductal hyperplasia will go on to develop mammary carcinoma. Retrospective studies have evidentiated a low risk of progression toward cancer of the flat epithelial atypia (AEP), lower than that of the atypical ductal hyperplasia. The risk increases 3-5 times in the population with atypical ductal hyperplasia (ADH) and 8-10 times in patients with ductal carcinoma in situ [3].

Due to implementation of mammary screening programs, the identification of ductal carcinoma in situ (DCIS) approaches 30% of the total number of cases of diagnosed mammary carcinomas worldwide. Identifying the mammary lesion in the in situ stage and establishing the proper treatment lead to curing, the recurrence rate of the ductal carcinoma in situ being very low; only 1-2.6% of the patients who were diagnosed with ductal lesion in situ had died of invasive breast cancer 10 years after the initial diagnosis [2].

SCOPE AND OBJECTIVES

The current study aims to provide a complete and detailed evaluation of the factors involved in the early stages of mammary carcinogenesis, with the purpose of identifying the possible prognostic and therapeutic targets. The study has analyzed the poliferative intraductal lesions that are considered precursors to invasive breast carcinoma of no special type (BIC NST)**,** with the help of classical histopathological methods of investigation, as well as with that of more recent immunohistochemical techniques. The identification of complex mechanisms manifesting at the molecular level and of the interactions between them provide useful and valuable information with regard to the tumoral initiation and progression and the prognostic of patients with such lesions. The specific objectives of this project included: enlarging knowledge related to the clinical, histopathological, and immunohistochemical factors which contribute to the

occurrence of precursor lesions of NST invasive carcinoma, for greater understanding of the carcinogenic mechanisms; identifying and defining the morphological parameters characterizing the intraductal proliferative lesions, for a correct diagnosis and categorization, in order to select the most reliable markers of the prognostic; identification of the immunoprofile and orientation of the prognostic of ductal carcinoma in situ; identification of the significantly statistical correlations between the clinical, histopathological, and immunohistochemical parameters for the lesions precursor to BIC NST.

MATERIALS AND METHODS

The first phase of this study began with the scientific documentation with regard to precursor lesions of the invasive breast carcinoma of no special type. I searched for the most recent information concerning precursor lesions of invasive breast NST carcinoma, with a focus on the epidemiological studies and risk factors of the occurrence of the lesions and their progression toward the invasive disease, cytogenetic and molecular characteristics, and histopathological classification, as well as their immunoprofile.

I made a selection of cases with intraductal proliferative lesions stretching over seven years (2005 – 2011) and registered it into a database using Microsoft Excel 2002 software. Next, I established and evaluated some clinical parameters for the selected cases, e.g., gender and age of the patients, by making retrospective studies on the registered cases of the Department of Pathology of the Emergency Clinical County Hospital of Craiova, as well as prospective studies in collaboration with the Oncology and Surgery Departments of the same hospital.

The retrospective studies aimed toward the selection of cases with precursor lesions of the BIC NST, diagnosed in four years (2005-2008), by gathering clinical and morphological data and inputting them into the database. Also, paraffin blocks corresponding to the selected cases have been sorted in order to enter the histopathological and immunohistochemical procedures.

The prospective study aimed the selection of new cases of precursor lesions of the BIC NST, over three years from the period of the study (2009-2011). The mastectomy fragments have undergone histopathological and immunohistochemical examinations.

The first tissular sections have been hematoxilin-eosin stained and on the basis of those stains, the pathological diagnostic has been established.

The histopathological analysis was performed on 478 cases of intraductal proliferative lesions, of which 222 were cases of simple ductal hyperplasia, 44 cases were atypical ductal hyperplasia, 3 cases were flat epithelial atypia, and 209 cases were ductal carcinoma in situ. The histopathological parameters have been stored in a database using Microsoft Excel software; then the histological study groups were determined.

The fragments of mammary tissue were fixed in 10% concentration of formaldehyde, then embedded in paraffin through the usual technique, in the Laboratory of Pathology of the Emergency Clinical County Hospital of Craiova. Successive sections have been stained with hematoxilin-eosin (BioOptica kit) and some of them have undergone the special PAS histochemical staining. The histopathological blocks selected through the retrospective study have been similarly processed.

The histopathological analysis followed the cases of intraductal proliferative lesions on the basis of the histological type (cytologic and architectural characteristics), the growth pattern, the grade of the lesions in situ, the mutual association of the lesions, their association with the invasive breast NST carcinoma, as well as their association with other malignant lesions.

The cases with intraductal proliferative lesions were defined, evaluated, and categorized, according to the most recent data of the World Health Organization [2].

The successive slides from the sections that were histochemically processed (hematoxilin-eozine, PAS) have been immunohistochemically processed within the Laboratory of Pathology of the Emergency Clinical County Hospital of Craiova and the Department of Pathology of the University of Medicine and Pharmacy of Craiova.

The two-step indirect working method has been utilized, by using the Dako En Vision™ Dual Link System-HRP technique of polymeric amplification. This method is based on the use of a secondary marked antibody having specificity against the primary unmarked antibody. In the first step, the primary antibody links to the tissular antigen, the excess being removed through washing, and after that the second enzymatically marked antibody is applied; the second antibody recognizes the antigenic determiners on the first antibody (which became antigen). The formed complex is then viewed with the help of a

chromogen (diaminobenzidine, DAB). The immunohistochemical analysis has been made on 70 DCIS cases that showed the NST invasive component by studying the markers involved in the evaluation of the immunohistochemical profile and the orientation of the prognostic of the lesion in situ. I selected the DCIS cases that were representative for the three histological grades and for the histological types identified in the histopathological analysis.

I used the following antibody panel: c-ErB2 oncoprotein (Her2/neu), hormonal receptors (estrogen and progesteron), p53, Ki67, D1 cyclin (bcl 1), and beta-catenin.

In processing the numerical data obtained within the histopathological analysis, the following statistical indicators have been used: arithmetical mean, standard deviation, variance, median, and quartiles. The statistical analysis of the intraductal proliferative lesions has also used tests that analyze the incidence tables generated through the cross-tabulation of some pairs of factors, in order to identify the relations between the various intraductal proliferative lesions and the relations between these lesions and the malignant lesions.

The statistical analysis of the obtained immunohistochemical results was made through the use of Somers's D and Kendall's *tau* tests. These tests show the strength of the relation between the order of the categories of any two analyzed factors and the intensity of the immunoreactivity of the markers used on the two studied factors [1].

CONCLUSIONS

The study made upon 478 cases of intraductal proliferative lesions of the mammary gland, precursor of BIC NST, selected in the time interval 2005-2011, has led to the follwing conclusions:

• Most of the cases with usual ductal hyperplasia have been diagnosed in 2008 and 2009, of those with atypical ductal hyperplasia in 2008, and of those with ductal carcinoma in situ in 2011. Most of the intraductal proliferative lesions have been diagnosed in female patients, while ductal carcinoma in situ and flat epithelial atypia have been diagnosed exclusively in female patients.

• The intraductal proliferative lesions had their maximal incidence in the age interval of 50-59 years for female patients with UDH, AEP and ADH, and in the age

interval of 70-79 years for those with DCIS. For male patients mammarily investigated, their age was significantly higher than that of female patients.

• UDH was present in 13% of the cases of investigated intraductal proliferative lesions. AEP was the intraductal proliferative lesion present in 0,18% of the cases, being present only in female patients. ADH was observed in almost 3% of the studied cases, representing about 9% from the total of the intraductal proliferative lesions. DCIS has been diagnosed in about 13% of the analyzed cases.

• The cases diagnosed with ADH were associated with BIC NST in 57% of the cases, of which 41% were also associated with DCIS. An association with benign mammary lesions was found in 14% of the cases and in 11% of the DCIS cases.

• About 80% of the cases diagnosed with DCIS were associated with BIC NST and 11% of the cases were associated with other malignant lesions. DCIS was associated with other intraductal proliferative lesions in almost 30% of the cases, most of the cases being also associated with BIC NST (24%). DCIS was found in pure form, not associated with malignant mammary lesions in almost 9% of the cases.

• A strong relation between the occurrence BIC NST and the presence of DCIS has been observed (OR:15,046). There was a statistically significant difference between the association with BIC NST among DCIS with a single histological type and the association with BIC NST among mixed DCIS (p Chi square - 0,039), thus strengthening the hypothesis that the mixed forms of DCIS have a greater tendency of transformation into BIC NST. In addition, there was statistical indication that the existence of a single histological type of DCIS indicates a lower probability of transformation into BIC NST than in the case of mixed DCIS.

• The immunohistochemically analyzed cases showed a statistically highly significant ($p<0,001$) relation between the DCIS grade and the BIC NST grade. The analysis of the relation between the expression of the immunohistochemical markers in DCIS and their expression in BIC NST has evidentiated in most of the cases very strong direct relations from statistical point of view.

• The expression of the hormonal receptors ER and PR was present in over 50% of the DCIS and BIC NST cases that were analyzed immunohistochemically. The immunoreactivity of the hormonal receptors correlated indirectly with the grade of the

lesions, their positivity being maximal in those with low grade, namely in the well differentiated forms.

• Most of the low- and moderate-grade DCIS histological types have shown negative Her2/neu immunoreaction. The overexpression of Her2/neu oncoprotein was present exclusively in the high-grade DCIS cases and in the poorly differentiated invasive ones.

• A high rate of celullar proliferation (Ki67) has been identified mostly in the high-grade DCIS cases, being present in over 70% of the high-grade DCIS cases and in almost 90% of the associated invasive ones.

• The positive immunoexpression of the p53 protein and the negative immunoexpression of the hormonal receptors have been observed in about 31% of the cases, most of the lesions being of high grade (84%).

• The immunoexpression of beta-catenin was normal in most of the analyzed cases, in both existent components (DCIS and BIC NST - 97%). The absence of the immunoexpression of beta-catenin was observed in the comedo-type DCIS G3 cases.

• The immunoreaction of D1 cyclin correlated directly with the grade of the investigated lesions, the fewest cases being of low grade, followed by the moderate and high grade, respectively well, moderately, and poorly differentiated.

• The analysis of the relation between the expression of the immunohistochemical markers in DCIS and their expression in BIC NST has used Somers's D and Kendall's *tau* tests. In most of the cases, I found very strong direct relations (Somer's D >0,80, Kendall's *tau* p value < 0,001), except for the expression Ki67, where the relation was just strong (Somer's D=0,638).

REFERENCES

1. Hill T, Lewicki P. Statistics: Methods and applications, StatSoft Inc 2006;1:3-65;

2. Lakhani SR, Ellis IO, Schnitt SJ, et al. WHO Classification of Tumours of the Breast. IARC Press 2012, Lyon;

3. Schnitt SJ, Ellis IO, van de Vijer MJ, et al. Intraductal proliferative lesions: Introduction and overview. WHO Classification of Tumors of the Breast. IARC Press 2012;5:81-90.