Insulin-Like Growth Factor-I (IGF-1), IGF-Binding Protein-3 (IGFBP-3) and mammographic features

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SUMMARY: Insulin-Like Growth Factor-I (IGF-1), IGF-Binding Protein-3 (IGFBP-3) and mammographic features.

P. DI CELLO, V. CIPOLLA, A.R. FORCIONE, A. PALIOTTA, L. DOMENICI, A. BOLOGNESE


Introduction. The IGF system has recently been shown to play an important role in the regulation of breast tumor cell proliferation. However, also breast density is currently considered as the strongest breast cancer risk factor. It is not yet clear whether these factors are interrelated and if and how they are influenced by menopausal status.

The purpose of this study was to examine the possible effects of IGF-1 and IGFBP-3 and IGF-1/IGFBP-3 molar ratio on mammographic density stratified by menopausal status.

Patients and methods. A group of 341 Italian women were interviewed to collect the following data: family history of breast cancer, reproductive and menstrual factors, breast biopsies, previous administration of hormonal contraceptive therapy, hormone replacement therapy (HRT) in menopause and lifestyle information. A blood sample was taken in each case for determination of IGF-1, IGFBP-3 levels. IGF-1/IGFBP-3 molar ratio was then calculated. On the basis of recent mammograms the women were divided into two groups: dense breast (DB) and non-dense breast (NDB). Student’s t-test was employed to assess the association between breast density and plasma level of IGF-1, IGFBP-3 and IGF-1/IGFBP-3 and their concentration molare in the two groups.

Results. The analysis of the relationship between mammographic density and plasma level of IGF-1, IGFBP-3 and IGF-1/IGFBP-3 molar ratio showed that IGF-1 levels and molar ratio varied in the two groups, results showing higher mean values in the DB group (IGF-1: 109.6 ng/ml versus 96.6 ng/ml; p=0.001, molar ratio 29.4 versus 25.5 ng/ml; p=0.001) whereas IGFBP-3 showed similar values in both groups (DB and NDB). Analysis of plasma level of IGF-1, IGFBP-3 and IGF-1/IGFBP-3 molar ratio compared to breast density after stratification of the study population by menopausal status (premenopausal versus 96.6 ng/ml; p=0.001 and molar ratio 29.4 versus 25.5 ng/ml; p=0.001) whereas IGFBP-3 showed similar values in both groups.

Conclusion. The present study suggests a possible correlation between breast density and plasma level of IGF-1 and IGFBP-3 and their concentration molare in the two groups.

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Introduction

Insulin-like growth factor-1 (IGF-1) is an essential growth factor for the regulation of proliferation and apoptosis in normal mammary cells (1-4). There is also increasing evidence of the role of IGF-1 in the growth of tumors in a number of different cancers (prostate, colon and lung cancer) including breast cancer (2,5,6). The IGF-1 mechanism of action in the carcinogenesis and development of breast cancer is complex and multifactorial and has led to numerous studies reported in the literature. IGF-1 is a growth factor that circulates in the blood and it is known to have a very short lifetime in free form, approximately 12 minutes (4). Its action is strongly influenced by the association with one of six existing different insulin-like growth factor binding proteins (IGFBP) which increases its average lifetime to about 12 hours (2). Over 90% of IGF-1 in circulation is bound to IGFBP-3 (7). This complex remains stable in the blood due to the presence of a binding protein, specific protease inhibitor.

In the extravascular space, the lack of this inhibitor allows specific metalloproteases to break the link between IGF and IGFBP-3 thus favoring the association between IGF and its specific cellular receptor expression in the breast tissue where IGF-1 fulfills its regulatory role (8).

In addition to the regulatory effect on the action of IGF-1 through modulation of the association with IGF receptors (IGFR), the IGFBP-3 also seems to directly promote cell apoptosis independently of IGF-1 (8-10). Several studies have demonstrated an association between the IGF system and breast cancer risk, but only in premenopausal women (6, 11-14), suggesting that IGF-1 might interact with the estrogen signal to increase cell proliferation (15-17). Other more recent studies have reported an association between the IGF system and carcinogenesis also in postmenopausal women (18).

Schernhammer et al. demonstrated a weak association between IGF-1 and breast cancer in premenopausal women but did not find a significant association between IGFBP-3, IGFBP-1, free IGF and breast cancer risk in pre- and postmenopausal women (19). Also in a later study of premenopausal women, the same authors found no significant association between IGF-1, IGFBP-1, IGFBP-3, growth hormone levels and breast cancer risk (20). The debate concerning the association between menopausal status, IGFBP, IGF-1 and breast cancer risk is therefore still open. On the other hand, breast density is currently considered as the strongest breast cancer risk factor (21). Women with mammographic density \(\geq 75\%\) have a five-fold increased risk of developing breast cancer compared to women with fatty breast tissue and density \(<5\%\) (22-25).

Given the regulatory function of IGF-1 on the proliferation of normal breast tissue, the question has been raised whether there is a possible association between IGF and breast density. Some authors have shown a significant association between IGF and breast density in premenopausal women (8, 26-29). However, the results reported in the literature related to postmenopausal women are still discordant, as most authors found no correlation between IGF and breast density (8, 26, 27, 30), whereas some authors reported a weak relationship also in postmenopausal women (31) whether they were receiving hormonal therapy (32) or not (31).

The present study had three objectives. The main objective was to analyze whether there was a relationship between plasma levels of IGF-1, IGFBP-3, IGF-1/IGFBP-3 molar ratio and mammographic density in a study population of Italian Caucasian women. Secondary objective was to assess whether this relationship...
was similar in subgroups of pre- and postmenopausal women. Tertiary objective was to assess whether there were confounding factors that might influence this relationship after dividing the groups according to reproductive factors and lifestyle, today considered among the factors that influence IGF-1 and IGFBP levels along with breast density.

Patients and methods

Ethical approval for this single-center, prospective, observational study was granted by the Medical Research Ethics Committee of our institution and written informed consent was obtained from all patients.

The sample was built up continuously in the order of presentation and 7,000 women were selected among those who spontaneously turned to the Breast Care section of the Department of Gynecology, Perinatology and Childcare of the University of Rome “Sapienza” for a breast examination between March 2005 and March 2007.

According to the protocol we selected only Italian women of childbearing age (regular menstrual cycles during the past year) or naturally postmenopausal women (absence of menstrual cycles for at least 12 months) who had performed mammographic examination, negative for breast cancer pathology, at the section of radiology of our department maximum 3 months prior to recruitment. Premenopausal women were enrolled in the study only if they had undergone mammographic examination within the first ten days of the menstrual cycle.

After recruitment the women were interviewed by a medical doctor (trained in medical research). Collected information included: age at mammography, family history of breast cancer (those with at least two first degree relatives with breast cancer were considered positive), reproductive and menstrual factors such as age at menarche, menopause, parity (nulliparous or with at least one full-term pregnancy), age at first pregnancy, lactation and infertility, previous breast biopsies (yes/no), previous administration of hormonal contraceptive therapy (yes/no), HRT in menopause (yes/no), smoking status (never, past or current; only current smokers were considered positive), alcohol consumption (yes/no; intake of ≥15 g per day during the past year was considered positive), chest X-ray examinations before the age of 20, physical activity before the age of 20 and current physical activity (yes/no; ≥3 hours of physical activity per week for at least one year was considered positive) and previous slimming diets. Height without shoes (cm) and weight in light clothes (kg) were registered by a trained nurse for the calculation of body mass index (BMI).

Women with a clinical history positive for breast cancer and/or for colon and lung cancer, administration of hormone therapy for up to 12 months before recruitment such as menopause hormone replacement therapy (HRT) and hormonal contraceptives, pre-menopausal status (irregular menstrual cycles with or without menopausal symptoms), surgical menopause, participation in assisted fertilization programs and previous breast reduction or augmentation surgery were excluded from the study.

The objective of the study was explained to all the selected subjects. The first phase of the study included signing of an informed consent form, collection of recent mammograms as well as drawing of blood samples for the evaluation of serum IGF-1 and IGFBP-3 and calculation of IGF-1/IGFBP-3 molar ratio. Patients were divided into two groups: dense breast (DB) or non-dense breast (NDB) according to the mammographic parenchymal category assigned at the evaluation of the presented mammograms. Subsequently patients were stratified by menopausal status.

Mammographic classification

To determine the mammographic parenchymal category all mammograms were examined by three physicians (two radiologists and a gynecologist and breast specialist) all blinded to the clinical data and to the classification already assigned. Particular attention was paid to the craniocaudal projections of both breasts, and the distribution of glandular parenchyma was qualitatively evaluated in percentage of the total area of the breast. The patients were then assigned to one of the four categories of breast parenchyma density distribution established by the Breast Imaging Reporting and Data System (BI-RADS): type 1, the breast is almost entirely fat (glandular parenchyma <25% of the total area of both breasts); type 2, scattered fibroglandular densities (25%-50%); type 3, heterogeneously dense breast tissue (51%-75%); type 4 extremely dense (>75% glandular).

It is well-known that the sensitivity of mammography is decreased in type 3 and 4 (33-34), and the patients participating in our study were therefore divided into two groups: DB which included BI-RADS type 3 and 4, and NDB which included BI-RADS type 1 and 2. In case of contradictory judgments, the classification assigned by at least two readers out of three was considered correct.

Peptide assays

At recruitment, a peripheral venous blood sample was drawn for determination of IGF-1 and IGFBP-3 levels. One sample was considered enough as previous studies have shown that a single assessment accurately reflects long-term concentration of IGF-1 (35).

All blood samples were drawn between 8 am and 11 am after an overnight fast; in women of childbearing age samples were drawn between the 6th and 10th day of the menstrual cycle. Serum obtained by centrifuging the blood samples was immediately frozen at -25°C until analysis which was performed in a single block. Blood samples were analyzed by a laboratory technician who was blinded to the parenchymal group (DB or NDB) assigned to the patients. A serum sample of each patient was stored for possible later tests. Determination of IGF-1 and IGFBP-3 levels was performed using Immulite 2000 (Siemens Medical Solutions Diagnostics) based on automated sandwich chemiluminescence immunoassay. Values were determined and calibration was performed on a laboratory instrument according to the producer’s instructions.

Statistical analysis

In order to assess whether classification of DB and NDB was consistent, agreement between the three readers was evaluated using Cohen’s kappa before further statistical analysis.

Univariate analysis, involving examination of each of the considered variables was carried out; particularly percentages, mean values and standard deviations of quantitative and qualitative variables in the two subgroups were calculated. All data were also graphically represented in a scatter diagram to provide an overview of the series and also to identify any outliers or incorrect data. To assess the association or dependence relation between categorical variables, Pearson’s chi-square test was employed.

Group mean values were compared using the Student’s t-test. Significance level was set at 0.05. Multivariate analysis was performed by building logistic regression models using the Enter method and subsequently the Stepwise procedure on the variables selected using univariate analysis. When a dichotomous dependent variable had been identified, logistic regression selected, from a pool of independent variables, those variables that had relevantly and significantly influenced the outcome. The odds ratio (OR) which expresses how much greater the probability of an outcome is among those that are exposed to a factor compared to those that are not exposed, as well as interval estimate 95%. The goodness of fit of the logistic regression model was assessed using the Hosmer-Lemeshow test.
Results

A total of 7000 women were assessed for eligibility; 3099 were excluded because they did not meet the inclusion criteria and 3560 were excluded according to exclusion criteria. This selection produced a final sample of 341 women.

Evaluation of mammographic features showed the presence in the sample of 196 (57.5%) patients with DB (BI-RADS 3 and 4) and 145 (42.5%) patients with NDB (BI-RADS 1 and 2). Assessment of inter-operator variability did not show statistically significant differences; Cohen’s kappa values ranged from 0.85 to 0.89 (p = 0.001) thus indicating a high level of agreement.

Table 1 lists the data collected at the anamnestic interview: demographic information, reproductive history, family medical history, anthropometric measurements and life style related to the two groups DB and NDB. Women with DB were generally younger than those with NDB (mean age about 49 and 59 years, respectively; p = 0.001) and the patients in the DB group were less frequently postmenopausal (36.7% versus 81.4%; p = 0.001).

Women with DB were more frequently nulliparous (32.7% versus 11.3%; p = 0.001), among the women who had had at least one full-term pregnancy, pregnancy had occurred at a later age (mean age 27.1 versus 24.8; p = 0.001) and they had lactated less (49.5% versus 68.3%; p = 0.001); women with DB had a lower BMI (22.5 versus 26.6; p = 0.001).

As regards lifestyle factors a positive association was found between breast density and current smoking status (19.9% versus 11.7%; p = 0.041) and sports activities both at a young age (37.8% versus 24.8%; p = 0.01) and at the time of recruitment (27.0% versus 15.2%; p = 0.008). An inverse association was furthermore found between DB and previous slimming diets (18.9% versus 35.2%; p = 0.001).

Analysis of the relationship between mammographic density and plasma levels of IGF-1, IGFBP-3 and IGF-1/IGFBP-3 molar ratio showed that IGF-1 levels and molar ratio varied in the two groups resulting in higher density and plasma levels of IGF-1, IGFBP-3 and IGF-1/IGFBP-3 molar ratio in the DB group (Fig. 1, Table 2).

Comparing the association between plasma level of IGF-1, IGFBP-3 and IGF-1/IGFBP-3 molar ratio and breast density after stratification of the study population by menopausal status (premenopausal and postmenopausal), it was observed that there was no association neither in premenopausal nor in postmenopausal patients (Table 3).

After division into tertiles of plasma level of IGF-1, IGFBP-3 and IGF-1/IGFBP-3 molar ratio it was observed that the premenopausal women with DB showed the highest percentage distribution in tertile III of molar ratio (50.8%) compared to tertile II (29.8%) and tertile I (19.4%) whereas the premenopausal women with NDB showed the highest percentage distribution in tertile I (42.3%) and in tertile III (46.2%) compared to tertile II (11.5%) (p = 0.025). Among postmenopausal women, the NDB group showed a higher percentage distribution than the DB group in tertile I (38.7% versus 33.3%) and in tertile III (39.5% versus 27.8%) whereas the DB group showed a higher percentage distribution than the NDB group in tertile II (38.9% versus 21.8%) (p = 0.035) (Table 3).

Table 4 shows the results of logistic regression analysis related to the main variables. The binary logistic regression model built up using the enter method and subsequently the stepwise procedure, selected three variables among those found significantly associated with mammographic density when submitted to univariate analysis: BMI being inversely related and menopausal status and parity being directly related to mammographic density.

Each of these factors, BMI, menopausal status and parity affected breast density with an odd ratio of 0.76 (95% CI: 0.69 - 0.82), 3.88 (95% CI: 2.20 - 6.86) and 2.38 (95% CI: 1.16 - 4.89), respectively. Particularly me-
nopausal status and parity ≥1 showed a direct associa-

tion with mammographic density; thus menopausal wo-

men or women with parity ≥1 should have less dense

breasts and women with low BMI should have denser

breasts. Logistic regression analysis did not reveal IGF-

1 and molar ratio as determinants of breast density. The

goodness of fit of the logistic regression model was as-

sessed using the Hosmer-Lemeshow test (p 0.38).

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>Non-dense breast</th>
<th>Dense breast</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at mammography (years)</td>
<td>58.8 ± 10.2</td>
<td>49.5 ± 10.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Reproductive data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity (at least one full-term pregnancy; %)</td>
<td>130 (89.7%)</td>
<td>132 (67.3%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Nulliparity (%)</td>
<td>15 (11.3%)</td>
<td>64 (32.7%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Age at menarche (years)</td>
<td>12.3 ± 1.6</td>
<td>12.6 ± 1.5</td>
<td>NS</td>
</tr>
<tr>
<td>Age at first pregnancy (years)</td>
<td>24.8 ± 4.1</td>
<td>27.1 ± 5.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Breast feeding (yes)</td>
<td>100 (68.5%)</td>
<td>97 (49.5%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Menopausal status (menopause)</td>
<td>119 (81.5%)</td>
<td>72 (36.7%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Family risk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of breast cancer (yes)</td>
<td>28 (19.3%)</td>
<td>36 (18.4%)</td>
<td>NS</td>
</tr>
<tr>
<td>Anthropometric data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.6 ± 4.2</td>
<td>22.5 ± 2.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Lifestyle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol (yes)</td>
<td>46 (31.7%)</td>
<td>49 (25%)</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking status (current)</td>
<td>17 (11.6%)</td>
<td>39 (19.9%)</td>
<td>0.041</td>
</tr>
<tr>
<td>Past physical activity (yes)</td>
<td>36 (24.8%)</td>
<td>74 (37.8%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Current physical activity (yes)</td>
<td>22 (15.2%)</td>
<td>53 (27.0%)</td>
<td>0.008</td>
</tr>
<tr>
<td>Previous slimming diet (yes)</td>
<td>51 (35.2%)</td>
<td>37 (18.9%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Ever on oral contraception (yes)</td>
<td>19 (13.1%)</td>
<td>16 (8.2%)</td>
<td>NS</td>
</tr>
<tr>
<td>Ever on HRT (yes)</td>
<td>16 (11.0%)</td>
<td>10 (5.1%)</td>
<td>NS</td>
</tr>
<tr>
<td>X-ray exposure in childhood (&lt;18 yrs)</td>
<td>38 (26.2%)</td>
<td>69 (35.2%)</td>
<td>NS</td>
</tr>
<tr>
<td>Past biopsies (yes)</td>
<td>18 (12.4%)</td>
<td>20 (10.2%)</td>
<td>NS</td>
</tr>
<tr>
<td>Serum peptide assays</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-1 (ng/ml; mean)</td>
<td>96.6 ± 35.0</td>
<td>109.6 ± 36.1</td>
<td>0.001</td>
</tr>
<tr>
<td>IGFBP-3 (ng/ml; mean)</td>
<td>3.8 ± 1.0</td>
<td>3.8 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>Molar ratio (mean)</td>
<td>25.5 ± 7.6</td>
<td>29.4 ± 8.6</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Discussion

There is an increasing interest in early detection of risk factors for developing breast cancer. Mammo-
graphic density is one factor (21, 22, 25), but the IGF
system has recently been shown to have a role in the de-
velopment of breast cancer (2, 5, 6). However, it is not
yet clear whether these factors are interrelated and if and
how they are influenced by menopausal status (8, 26-30).

The purpose of this cross-sectional study was to exa-
mine the possible effects of IGF-1, IGFBP-3 and IGF-
1/IGFBP-3 molar ratio on mammographic density and
assess whether this relationship was similar in subgrou-
ps of pre- and postmenopausal women.

The study sample was fairly homogeneous as only Ita-
lian Caucasian women were enrolled, while women of
different ethnic origins were excluded due to the possi-
bility that plasma levels of IGF-1 and IGFBG-3 and pa-
renchymal density might vary among different ethnici-
ties. This choice was dictated by the need to build a ho-
mogeneous study sample, as previous studies of IGF-1
and IGFBG-3 reported in the literature seem not to have
paid attention to ethnic differences but only to geographic
location thereby suggesting environmental rather than
genetic influence, whereas parenchymal density is thou-
ted to differ according to ethnicity rather than geo-
 graphical location (28,36-37). Also women who had re-
ceived HRT for up to 12 months before recruitment were
excluded from this study because the use of postmeno-
pausal hormones has been reported to lower circulating
IGF-1 levels and increase breast density (38-40).

Particular attention was paid to the uniformity of
blood sampling for determining IGF-1 and IGFBP-3 le-
vels. Analysis of a single sample was considered sufficient,
as most authors claim that one evaluation can predict
long-term levels of these peptides (41,42) although not
all authors are of the same opinion (43). In premenopausal
women, the blood sample was drawn between 8am
and 11am after an overnight fast between the 6th and
10th day of the menstrual cycle as these values may vary
according to the menstrual cycle; however also in this case
not all authors are of the same opinion (44-46). Blood
analysis was carried out in a single block by one single
laboratory technician who was blinded to the paren-
chymal classification. Using this strategy, peptide levels
measured in our group of Italian women were generally
lower than those reported by other authors (14).

This study has some limitations. One concerns the
reliability of mammographic classification which was
performed qualitatively and not by a computer-assisted
method. However, BI-RADS mammographic classifi-
cation is the most common technique used in the USA
for the assessment of mammographic density (33). In or-
der to further reduce the risk of measurement error, both
premenopausal and postmenopausal women were en-
rolled in the study only if they had undergone mam-
mographic examination at our center not more than th-
ree months before recruitment and blood sampling. Ri-
gid criteria were furthermore used for assessing breast den-
sity (47). Using this method, inter-operator variances were
not statistically significant as there was a high level of con-
cordance in the evaluation carried out by the three blin-
ded readers. Furthermore, BI-RADS was developed to
alert the referring clinician that the ability to detect small
cancers in the dense breast is reduced and it not related
to the risk per se (34).

A second limitation is that blood sampling and mam-
mographic examination were not carried out at the same
time. A third limitation is that the temporality of the re-
lation between growth factors and breast density cannot
be determined due to the cross-sectional design. A fourth
limitation is that the study population was inhomoge-
neous when stratified by menopausal status. Among the
premenopausal women 82.7% had DB and only 17.3%
had NDB, and among the postmenopausal women
63.4% had NDB and only 36.6% had DB.

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>Non-dense breast</th>
<th>Dense breast</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-1 Tertile I (≤85)</td>
<td>62 (42.8%)</td>
<td>44 (22.5%)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>41 (28.3%)</td>
<td>70 (35.7%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>42 (29%)</td>
<td>82 (41.8%)</td>
<td></td>
</tr>
<tr>
<td>IGFBP-3 Tertile I (≤3.1)</td>
<td>54 (37.2%)</td>
<td>62 (31.6%)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>37 (25.5%)</td>
<td>81 (41.3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>54 (37.2%)</td>
<td>53 (27%)</td>
<td></td>
</tr>
<tr>
<td>Molar ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertile I (≤24)</td>
<td>63 (43.2%)</td>
<td>52 (26.5%)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>46 (31.5%)</td>
<td>60 (30.6%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>37 (25.3%)</td>
<td>84 (42.9%)</td>
<td></td>
</tr>
</tbody>
</table>

1. Insulin-Like_Izzo:- 14-05-2012 9:57 Pagina 158
Finally the analysis of the potential confounders of the relationship between mammographic density and plasma level of IGF-1, IGFBP-3 and molar ratio was carried out using logistic regression instead of linear regression because mammographic density was not expressed as a continuous variable.

Univariate analysis showed that IGF-1 values and molar ratio were higher in the DB group compared to the NDB group. The same results were obtained after division into tertiles. IGFBP-3 values were similar in the two groups.

The distribution of IGFBP-3 tertiles in the two groups (DB and NDB) is not easy to interpret as the NDB showed a higher percentage distribution in tertile I than in tertile III, whereas the DB women showed the highest percentage distribution in tertile II. Furthermore, the association between IGFBP-3 expressed in tertiles and breast density is less strong than the association between IGF-1 and molar ratio (also expressed in tertiles) as demonstrated by the p-values (0.01 versus 0.001).

When the levels of IGF-1, IGFBP and molar ratio were compared to breast density stratifying by menopausal status, no association was found. However after division in tertiles we found that among the premenopausal women, the highest percentage of DB was in tertiles III and II whereas the highest percentage of NDB was in tertile I (p = 0.025).

However, also in this case the results are not unambigously interpreted.

### TABLE 3 - ASSOCIATION BETWEEN PLASMA LEVEL OF IGF-1, IGFBP-3 AND IGF-1/IGFBP-3 MOLAR RATIO WITH BREAST DENSITY IN PREMENOPAUSAL AND POSTMENOPAUSAL WOMEN.

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>PREMENOPAUSAL</th>
<th>POSTMENOPAUSAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=150; 43.9%)</td>
<td>(n=191; 56.1%)</td>
</tr>
<tr>
<td></td>
<td>NDB (n=26; 73.7%)</td>
<td>DB (n=124; 27.7%)</td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>107.9±39.3</td>
<td>115.7±36.2</td>
</tr>
<tr>
<td>IGFBP-3 (ng/ml)</td>
<td>3.8±0.9</td>
<td>3.7±0.7</td>
</tr>
<tr>
<td>IGF-1/IGFBP-3 molar ratio</td>
<td>28.7±8.7</td>
<td>31.0±9</td>
</tr>
<tr>
<td>Tertile I (&lt;85)</td>
<td>7 (26.9%)</td>
<td>16 (12.9%)</td>
</tr>
<tr>
<td>Tertile II (85.1-110)</td>
<td>10 (38.5%)</td>
<td>46 (37.1%)</td>
</tr>
<tr>
<td>Tertile III (&gt;110)</td>
<td>9 (34.6%)</td>
<td>62 (50%)</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>87 (26.9%)</td>
<td>38 (30.6%)</td>
</tr>
<tr>
<td>Tertile I (&lt;3.1)</td>
<td>11 (42.3%)</td>
<td>53 (42.7%)</td>
</tr>
<tr>
<td>Tertile II (3.2-3.7)</td>
<td>8 (30.8%)</td>
<td>33 (26.6%)</td>
</tr>
<tr>
<td>Tertile III (&gt;3.7)</td>
<td>24 (9.4%)</td>
<td>19 (9.5%)</td>
</tr>
<tr>
<td>Molar ratio</td>
<td>11 (42.3%)</td>
<td>24 (9.4%)</td>
</tr>
<tr>
<td>Tertile I (&lt; 24)</td>
<td>3 (11.5%)</td>
<td>37 (29.8%)</td>
</tr>
<tr>
<td>Tertile II (24-29)</td>
<td>7 (26.3%)</td>
<td>63 (50.8%)</td>
</tr>
</tbody>
</table>

### TABLE 4 - STEPSWISE LOGISTIC REGRESSION: VARIABLES IN THE MODEL.

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>B</th>
<th>Wald test</th>
<th>Odd ratio</th>
<th>95% CI odd ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>-0.283</td>
<td>p=0.001</td>
<td>0.76</td>
<td>0.69 - 0.82</td>
</tr>
<tr>
<td>Menopause</td>
<td>1.358</td>
<td>p=0.001</td>
<td>3.88</td>
<td>2.20 - 6.86</td>
</tr>
<tr>
<td>Parity</td>
<td>0.866</td>
<td>p=0.02</td>
<td>2.38</td>
<td>1.16 - 4.89</td>
</tr>
</tbody>
</table>

Hosmer-Lemeshow test: p = 0.38.
biguous, since 48.1% of the women with NDB were located in tertile III. Although this percentage is lower than the percentage found in the DB group, this result is not sufficient for asserting that among premenopausal women molar ratio levels were related to breast density, also in view of the fact that an association was not found when the molar ratio was expressed as a continuous variable. This also counts for IGFBP-3 levels.

These results might be due to the lack of homogeneity in the sample related to breast density after stratification by menopausal status. Among the premenopausal women 82.7% had DB and only 17.3% had NDB, and among the postmenopausal women 63.4% had NDB and only 36.6% had DB.

Multivariate logistic regression showed that nulliparity and premenopausal status are positively associated with mammographic density, whereas BMI is inversely associated. It is particularly interesting to note that the analysis did not reveal IGF-1, IGFBP-3 and molar ratio plasma level as determinant of breast density.

This might explain the lack of association between mammographic density and growth factors when the analysis was stratified by menopausal status.

Previous studies showed that breast cancer risk rose steadily with increased percentage of the breast area with a dense appearance on a prediagnostic mammogram, and this association was not explained by other breast cancer risk factors such as age, weight, age at first child birth, family history of breast cancer, alcohol intake, prior benign breast disease, age at menarche, and age at menopause (22,25). It is still not known through what mechanism breast density is related to cancer risk.

On the other hand current breast density reflecting the proportion of stromal and epithelial proliferation, may simply indicate the area of susceptible tissue (number of epithelial cells) or may represent the interaction between stromal and epithelial proliferation influenced by local growth factors, including epidermal growth factor, transforming growth factors, IGF-1, and IGF-2 (53). Growing evidence indicates that breast development and involution are influenced by IGFs (which increase proliferation) and IGFBPs (which reduce proliferation) (54). Thus, greater breast density may be a consequence of higher IGF and molar ratio levels and an associated increase in proliferation and/or of decreased IGFBP levels with a resulting reduction in the involution process.

Our study provides a strong evidence of a crude association between breast density and plasma levels of IGF-1 and molar ratio, but unlike previous studies by other authors, they do not confirm that IGF-1 can be considered determinant in breast density neither in premenopausal (8, 26-29) nor in postmenopausal women (30).

Conclusions

On the basis of our results it is reasonable to assume that the role of IGF-1 and molar ratio in the pathogenesis of breast cancer is mediated through mammographic density. Thus IGF-1 and molar ratio might increase the risk of cancer by increasing the mammographic density.

Further studies are required to clarify these issues, particularly the mechanisms regulating the IGF bioavailability in the biological systems which may explain the development of not only breast cancer, but also prostate, colon and lung cancer in which growth factors have been implicated.

References

Insulin-Like Growth Factor-I (IGF-I), IGF-Binding Protein-3 (IGFBP-3) and mammographic features


