Estrogen Receptor, Progesterone Receptor, and Nuclear Size Features in Female Breast Cancer in Libya: Correlation with Clinical Features and Survival

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Abstract. *Background: The features of Libyan patients with breast cancer have not been fully investigated. The aim of this study was to evaluate the expression patterns of estrogen (ER) and progesterone receptor (PR), as well as nuclear morphometric features, in patients with breast cancer, and to correlate them with clinicopathological features and prognosis. Patients and Methods: Data for a total of 62 female Libyan patients with breast cancer, diagnosed between 2000 and 2006, were retrospectively studied. Their clinical and pathological data were collected and analysed. Immunohistochemical evaluation of ER and PR expression was also performed. Further more nuclear morphometry was carried out. Results: Of the 62 patients, disease in 10 was of the lobular type, 43 had invasive ductal and 9 had other carcinoma types; 47 out of 62 had lymph node involvement. Positive hormonal receptor expression was more common among those with lymph node-negative than lymph node-positive tumours. ER- and PR-positive patients appeared to have a better survival than ER- and PR-negative patients. The most significant difference, with respect to survival, was found between those bearing tumors with completely negative hormonal staining (J score 0) and those with positive staining (J score 1, 2 and 3). Larger nuclear size was associated with lymph node involvement and high-grade tumours ($p<0.01$ and $p<0.0001$, respectively), with shorter survival, larger tumour size and higher stage. Conclusion: The cut-off points for defining the groups with good or worse prognosis might be set, between score 0 and 1 (corresponding to 1% or fewer positive cells). Patients with ER- and PR-positive cancer had better overall survival than patients with hormonal receptor-negative cancer. In our hospital setting, ER and PR expressions and mean nuclear area (MNA) in breast carcinoma may be prognostically useful markers in guiding future treatment in prospective studies.*

Female breast carcinoma is one of the most common malignant diseases in the world, with approximately one million new cases diagnosed annually (1). In Libya and in developing countries in general, breast cancer management constitutes a major medical, social and economic issue. The hallmarks of the detection and treatment level of breast cancer in most developing countries are advanced stage, lack of mammographic screening programs, preponderance of younger pre-menopausal patients, and high morbidity and mortality (2-5).

However, improvements in methods of detection have significant influence on disease outcome (6). Prognostic factors are important in understanding the course of a disease, in predicting the outcome for the individual patient, in selecting appropriate therapy and in planning further additional treatment.

The determination of estrogen (ER) and progesterone receptor (PR) status in breast cancer is important in treatment selection, but also prognostic and predictive factors (7-9) when hormone therapy is administered.

Several different methods of evaluating hormone receptor status (HR+ or HR–), based on the intensity and/or percentage of positively stained cells have been reported, such as the J-score (9) and Allred's score (10). Breast cancer

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in both Africa and Europe has similar histological types and the same risk factors (11, 12). However, there may be differences in ethnic genetic markers, in the role of women in the family and their professional life, the number of pregnancies, age at first pregnancy (13-17), and other factors, such as the disease pattern at presentation, prognosis, and the course of disease. For example female breast cancer in Libyans, when diagnosed, is generally more advanced than those of Finnish and other European patients. These tumours also have larger nuclear size (18).

Molecular markers are available for the better understanding of breast carcinogenesis, cancer progression, and as a guide to treatment. However, few studies have been performed in Libyans regarding breast cancer; the tumour phenotypic alterations in the Libyan population are not well-studied.

Baak *et al*. were the first to introduce morphometry in the prognosis of breast cancer (19). The independent prognostic value of nuclear variables was established in several studies regarding infiltrating breast cancer (20-23). Nuclear area and diameter were shown to be useful prognostic factors. However, nuclear diameter failed to distinguish between the primary tumour and its metastasis (23). Since patients whose tumours have high nuclear area and high standard deviation (SD) of nuclear area values tend to have a poor prognosis (4, 18, 19), the evaluation of nuclear area can potentially be used in morphometric grading of breast cancer (4, 18, 19, 23). Nuclear area was a significant prognostic factor among lymph node-positive (LN+), but completely lacked significance among lymph node-negative (LN–) patients (19). Some studies showed that as the size of tumour cell nuclei increased, the HR expression decreased (24, 25).

The aims of this study were: Firstly to evaluate the clinical and pathological manifestations of Libyan patients with breast cancer; secondly to estimate the prognostic role of ER and PR expression, as well as the morphometric features in female breast cancer patients who received first-line treatment, with or without adjuvant therapy, in clinical centers in north-western Libya [Tripoli Medical center (TMC) and African Oncology Institute (AOI)].

Patients and Methods

Patient selection and clinical pathological data. A total of 171 samples from Libyan female patients with breast cancer, diagnosed between 2000 and 2006 in the AOI and TMC were collected for this retrospective study. Paraffin blocks were available from all patients but 40 cases lacked blocks with malignant tissue. This left 131 samples for the study of nuclear morphometry. Since needle biopsies did not allow for enough tissue acquisition for immunohistochemistry, only 90 samples were available for receptor studies. Only samples with positive internal control tissue were accepted for this study. This left 62 histological samples available for ER and PR analysis. The details of patients are shown in Table I. The follow-up period ranged from 9 to 72 months, with the average being 36.7 months, and the median 33 months. The death rates were 1 out of 62 (1.6%) and 15 out of 62 (24.2%) after one year and five years, respectively. Clinical and pathological data were obtained from the Pathology Departments, the Cancer Registry, and patients' medical files. The latter files were the major source for data on mortality. The permission for tumour sample collection was obtained from the Libyan National Authority for Medical Affairs. This study is a part of breast cancer studies, carried out under permission from the local Ethical Committee of the National Cancer Institute at AOI.

Table I. *The characteristics of 62 cases of Libyan female breast carcinoma*.

Pathological characteristics	Number of patients	Percentage
Histological type		
Infiltrating ductal carcinoma, NOS	43	69.4
Infiltrating lobular carcinoma	7	11.3
Mixed ductal and lobular carcinoma	3	4.8
Invasive papillary carcinoma	2	3.2
Medullary carcinoma	3	4.8
Mucinous carcinoma	3	4.8
Metaplastic carcinoma	1	1.6
Nuclear grades		
1	8	12.9
2	34	54.8
3	20	32.3
Lymph node status		
N–	15	24.2
N+	47	75.8
Clinical stage		
0	0	
1	1	1.6
2	23	37.1
3	30	48.4
4	8	12.9
Clinical characteristics at presentation		
Breast mass	30	48.4
Mass, lymph node involvement	20	32.3
Ulcerated mass	3	4.8
Mass, lymph node involvement and distant metastasis	9	14.5

NOS, Not otherwise specified.

Histopathological examination. Paraffin sections were routinely stained with hematoxylin and eosin (H&E) to diagnose the cases, and were re-graded by FBEA (certified pathologist), according to the modified Bloom and Richardson system (26). The predominant histological type was infiltrating ductal carcinoma, with 43 cases (69.4%); 10 cases (16.1%) were lobular (7 pure lobular, 3 mixed type); and 9 cases (14.5%) were other carcinoma types (three of the medullary type, three mucinous, two papillary, one metaplastic carcinoma).

Immunohistochemistry (IHC). Sections were cut serially at 5 μm for routine hematoxylin and eosin staining and for IHC analysis; IHC analysis was carried out using an automatic system (BenchMark

XT; Ventana Medical Systems, Inc. Tucson, Az, USA). This fully automated processing of bar code labeled slides included baking of the slides, solvent-free deparaffinization, antigen retrieval in a cell conditioning buffer CC2 (mild: 30 min conditioning, and standard: 60 min conditioning, at 95˚C), incubation with both anti-ER rabbit monoclonal antibody (clone: SP1, isotype: IgG; Zymed Laboratories, San Franscisco, CA, USA), and anti-PG rabbit monoclonal antibody (clone 1E2, isotype: IgG; Zymed Laboratories) for 32 min at 37˚C. UltraView™ (Ventana Medical Systems, Inc. Tucson, Az, USA) universal diaminobenzidine (DAB) detection kit, a biotin-free, multimer-based detection system for the specific and sensitive detection of mouse IgG, mouse IgM, and rabbit IgG primary antibodies, was applied. Counterstaining with blueing reagent took four min, as did post-counterstaining. After staining, the sections were dehydrated in ethanol, cleared in xylene, and covered with Mountex and cover-slips. The anti-ER and anti-PR (antibodies) react directly with human ER and PR proteins located in the nuclei.

Scoring system. ER and PR expression were determined according to the J-Score method (9), as follows: 0, no stained cells; 1+, stained cells ≤1%; 2+, stained cells >1% to <10%; 3+, stained cells >10%. Assessment of staining: negative, score of 0; indeterminate; score of 1 or 2; positive; score of 3.

When the ER and PR status differed from those reported in the original pathology report, new staining results were recorded in the database. This occurred only in three out of 62 cases (4.8%). The original IHC was not carried out in hospitals where the patients were admitted at the time of the original reports.

Nuclear morphometry. The most representative sections were analyzed by using an interactive digital image overlay measurement system run by the Prodit morphometry program (Prodit 3.1; Promis Inc, Almere, and Buro medische Automat serving, De Meern, the Netherlands). The system consists of a light microscope, a personal computer (Compaq Deskpro 386/20e; Compaq Computer Corporation, Houston, TX, USA), a video camera attached to the microscope (JVC TK-870U; JVC, Japan) and a PIP-512B video digitizer board (Matrox Electronic Systems, Dorval, Quebec, Canada). Analog images of the nuclear profile were outlined on the monitor screen using a computer mouse, and consequently a digital database was created from the nuclear features in the computer. The system produced the basic statistics of the variables measured (27). The latter included size variables (*e.g.* mean area, perimeter), and several shape factors. The instrument was calibrated in two perpendicular directions with a micrometer scale before each session of measurement. Outlining of nuclei was carried out at ×2,500 magnification on the monitor screen (×40 objective lens magnification, ×10 video ocular and ×1.25 internal magnification).

Treatment protocols and outcomes. Fifty-eight patients underwent surgery (58/62=93%). The types of surgery included modified radical mastectomy (n=46), simple mastectomy (n=11), and excisional biopsy only (n=1). A total of 82% of the patients chose to have surgery as first line of therapy. Adjuvant therapies included chemotherapy in 57 patients (91.9%). A total of 43 cases out of 62 underwent anthracycline regimes and 12 cases out of 62 a taxane-based chemotherapy regime. Only two patients underwent therapy with cyclophosphamide, methtroxate and 5-fluorouracil, along with prednisone and other supportive management. Hormonal therapy was given in 39 (62.9%) cases, when samples were positive for HR; radiotherapy was applied in 52 cases (83.9%). A total of 15 out of 62 patients died and an additional 7 out of 62 patients progressed in stage.

Table II. *Distribution of (ER) and (PR) expression as determined by immunohistochemistry in 62 Libyan cases of breast cancer.*

IHC J-score	ER-positive		PR-positive	
	Number	Percentage	Number	Percentage
0	27	43.5	30	48.4
1	4	6.5	5	8.1
2	14	22.6	14	22.6
3	17	27.4	13	21.0

Statistical analysis. Statistical analyses were performed using the SPSS for Windows, version 16.0. (SPSS, Inc., Chicago, IL, USA) software packages. Frequency tables were analysed using the Chi-square test, with likelihood ratio (LR), or the Fischer's exact test to assess the significance of association between the variables. Comparison of numerical data was performed with the *t*-test if the distribution of samples was normal, or the Mann-Whitney *U*-test, if the sample distribution was asymmetrical. Analysis of variance (ANOVA) was only used for deriving the mean values and their 95% confidence interval (CI) of each individual stratum. Univariate survival analysis was performed with Kaplan Meier curves and significance was determined by the log-rank test (KM-LR). Values with $p<0.05$ were regarded as being statistically significant.

Results

Staining characteristics are shown in Figures 1 and 2. The nuclear staining of benign proliferative lesions and normal breast tissue were used as internal controls for ER and PR staining. The number of completely negative cases was 27 and 30 ER and PR staining, respectively. As shown in Table II, positive cases were usually of moderate intensity.

Hormonal receptors. A total of 58% and 51% of all tumours exhibited positive epithelial nuclear staining for ER and PR, respectively. Three cases (4.8%) were positive for ER only and negative for PR; 32 cases were recognized as being positive for both ER and PR. Therefore, altogether 27 (43.5%) cases were negative for both ER and PR. The results of the immunohistochemical analysis for both ER and PR in the whole material are summarized in Table II.

ER and PR expression and clinicopathological features. Neither ER nor PR expression had any significant relationship with age, menopausal status, or histological type. The time between onset of symptoms and diagnosis by histopathology, exceeded six months in 36 out of 62 (58.1%)

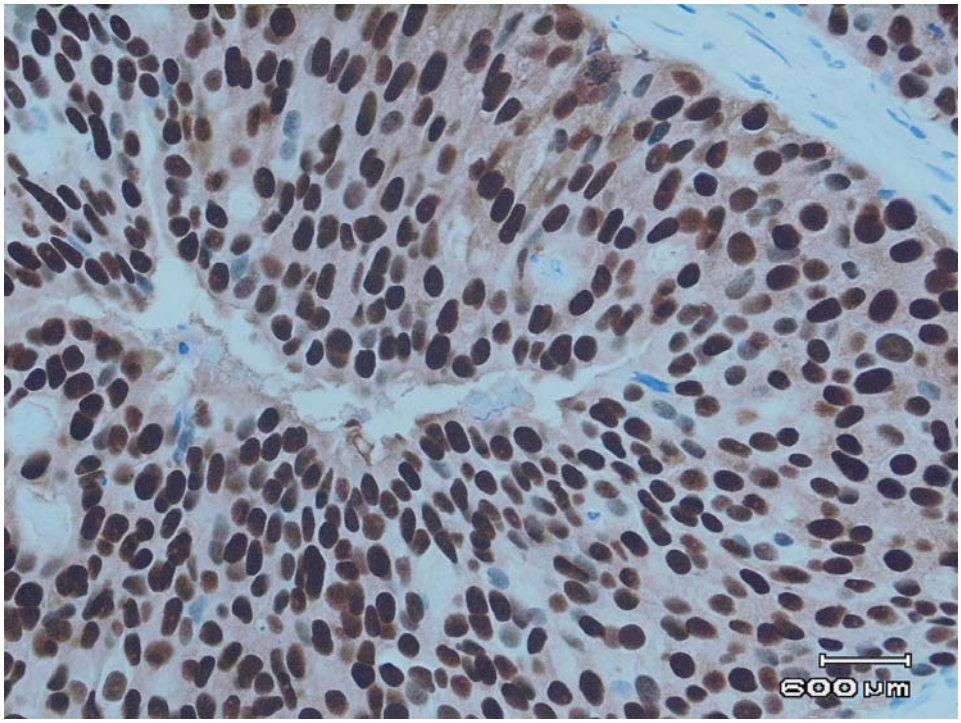

Figure 1. *Invasive ductal carcinoma showing strong estrogen receptor (ER) expression, J-score-3. Magnification, ×400.*

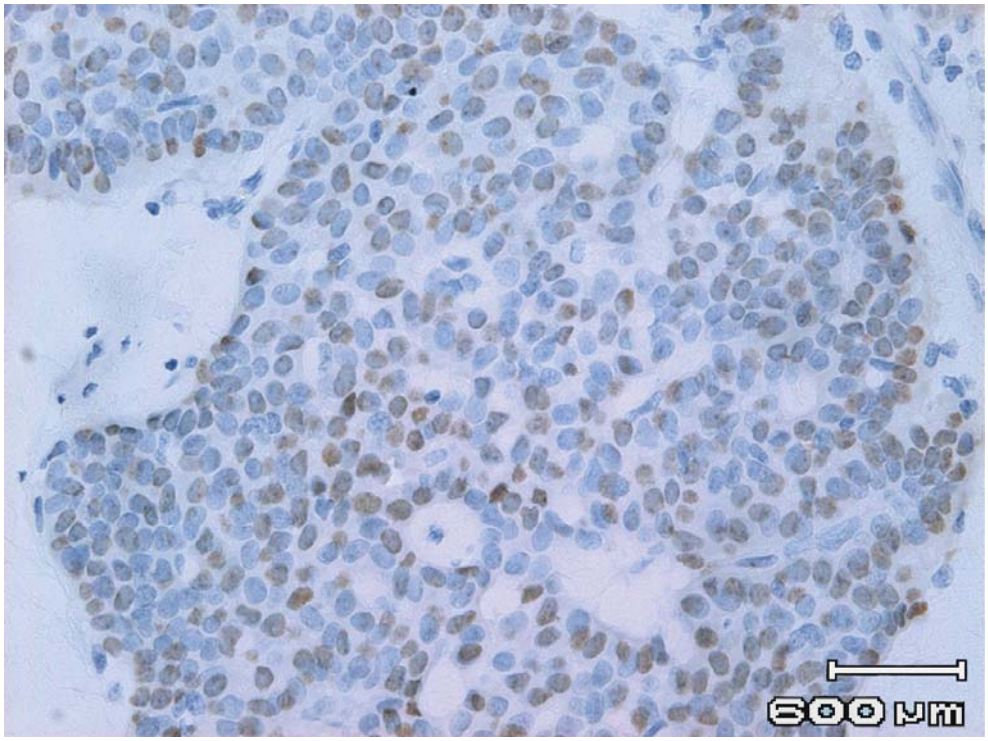

Figure 2. *Invasive ductal carcinoma showing weak estrogen receptor (ER) expression, J-score-1. Magnification, ×400.*

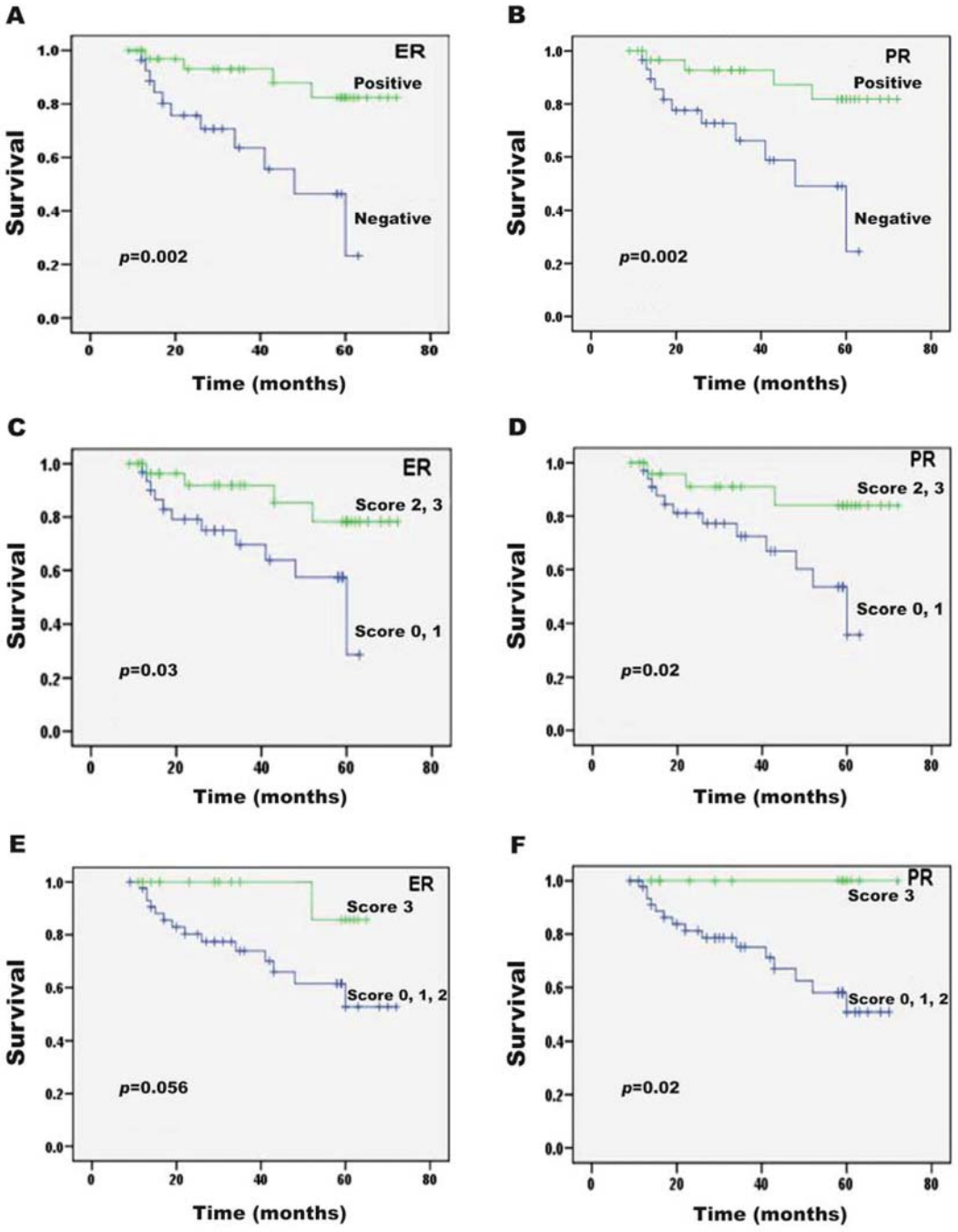

Figure 3. *Overall survival according to the expression of estrogen receptor (ER) and progesterone receptor (PR) in primary breast cancer at three different cut-off points. A, B: Expression status was evaluated by J score, with cut-off between score 0 and 1. Positive ER and PR are associated with better survival. In this study on Libyan samples, the receptors are much better prognostic factors than in the previous studies on European samples. C, D: Cut-off between score 1 and 2. E, F: Cut-off between score 2 and 3. The difference between all present pairs of curves are statistically significant (p=0.005-0.05, but at a higher level of significance in A, B.*

cases, but this did not appear to influence the staining positivity. The size of the tumor mass was variable: 53 (85.5%) were >3 cm and 14.5% <3 cm (p=0.27). High-stage tumors (stages 3-4) on average, were negative for hormonal staining and low-stage tumors (stages 1-2) were positive (ER, p=0.017; PR, p=0.015). Overall, 47 out of 62 cases (75.8%) had lymph node involvement; 32 out of 62 (51.6%) had N1 status; 14 cases (22.6%) were of N2; and one case

Table III. I*mmunohistochemical staining in subgroups divided according to histological grade, lymph node status, clinical stage, menopausal status, tumor size and histological type. p-Values are from t-test and ANOVA.*

Pathological characteristics	PR		*p*-Value	ER		*p*-Value
	–	+		–	+	
Grade			0.041			0.053
1	2	6		2	6	
2	14	20		12	22	
3	14	6		13	7	
Lymph node status			0.05			0.03
Negative	4	11		3	12	
Positive	26	21		24	23	
Stage			0.015			0.017
I-II	7	17		6	18	
III-IV	23	15		21	17	
Histological type			NS			NS
Ductal	23	20		22	21	
Lobular	3	7		3	7	
Other	4	5		2	7	
Menopausal status			NS			NS
Pre-menopausal	15	22		13	24	
Post-menopausal	15	10		14	11	
Tumour Size			NS			NS
≤3 cm	3	5		3	5	
>3 cm	27	27		24	30	

NS; Not statistically significant; PR: progesterone receptor; ER: estrogen receptor.

Table IV. *The mean nuclear area (MNA) in relation to grade, estrogen receptor and progesterone receptor expression, lymph node status and histological type the cut-off point used refers to an earlier study (18).*

	MNA (μm^2)		
Pathological characteristic	<71	≥71	*p*-Value
Grade			0.0001
1	8	0	
2	26	8	
3	2	18	
Estrogen receptor status			0.015
Negative	11	16	
Positive	25	10	
Progesterone receptor status			0.022
Negative	13	17	
Positive	23	9	
Lymph node status			0.006
Negative	13	2	
Positive	23	24	
Histological type			0.03
Ductal	21	22	
Lobular	9	1	
Other	6	3	

was N3. The prevalence of lymph node involvement was significantly lower in patients with tumours which had positive HR status than in those with negative HR status (p=0.03 for ER and p=0.05 for PR). The significant relationships are shown in Table III. Among the 35 ER-positive and 32 PR-positive cases there were 32.1% of ER-positive and 28.6% of PR-positive cases with metastases, after an average follow-up of approximately 41.0 months.

Nuclear morphometry and clinicopathological features. Nuclear morphometric features were analysed in the whole group, and in groups defined by the histological type, ER and PR status, and lymph node status, as shown in Table IV. A statistically significant correlation between the mean nuclear area (MNA) and most clinicopathological features was observed. The strongest association was observed for nuclear grade (p<0.0001). There was also correlation between MNA and node status (p=0.006). The difference in the MNA between invasive ductal carcinoma and lobular carcinoma was statistically significant (p=0.02). The MNA was larger in HR-positive tumours, but was less statistically significant than the relationship between lymph-node status and HR positivity. The MNA was higher in pre-menopausal patients than in post-menopausal patients, but the difference was not statistically significant. The MNA was also higher in larger tumours and in tumours of advanced stage (stages 3 and 4); however, the difference was not statistically significant. A corresponding relationship was found with the other nuclear size-related features but not with the shape-related features.

Survival analysis. Among Libyan patients, the menopausal status, histological type of tumour, and age of the patient did not seem to influence survival. However, advanced tumour stage, lymph-node involvement and high grade were strongly associated with shortened survival (log rank (p<0.0001, p=0.002 and p=0.05 respectively). The survival analysis showed that a short survival time was associated with high nuclear morphometric values (p=0.04).

Survival and HR status. Our results demonstrated that patients with tumours having high ER or PR expression had better survival than those with low or no expression (p=0.002 for ER and p=0.005 for PR, log-rank).

Significance of cut-off point for staining positivity. The most significant difference was found between completely negative hormonal staining (J score 0) and positive staining (J score 1, 2 and 3). The cumulative number of deaths at five years were 8/27 and 9/28 in patients with tumour negative for ER and PR, respectively; only 4/35 and 3/32 patients had died at 5 years among patients with ER– and PR–positive tumours, respectively.

The significance of overall survival for a cut-off point at J score 2 was $p=0.03$ for ER and $p=0.02$ for PR, while that for a cut-off point at J score 3 was $p=0.056$ for ER and $p=0.02$ for PR (Figure 3).

Discussion

Distribution of ER and PR IHC in female breast cancer in Libyans. Both steroid HRs are present in breast cancer in the Libyan female population. The ER-positive rate in our series (57%) was higher than the PR-positive rate (52%). These results are in line with those reported by other authors on female European patients with breast carcinoma (28, 29). Data on breast cancer in Central Africa is not available. The corresponding percentage for African-Americans was about 39%, suggesting that the Central African population might also have a smaller fraction of ER and PR positivity than in the Libyan and European populations. We seem to be able to suggest this even though in clinical terms, breast cancer in Libya is much more like breast cancer in Central Africans.

Quantitative relationship between ER and PR. The values for the two types of steroid HR show good correlations. As values increase for one receptor, there is a corresponding increase in values for the other receptor. This has been previously described by Allred *et al*. (10).

HR expression related to clinicopathological features. There are conflicting data on the correlation of ER with age and menopausal status. This study showed that neither of the receptors correlated with age and this is in line with previous results of Thike *et al*. (30). However, Jalava *et al*. (29) noted that ER but not PR had a positive correlation with age. Some authors have reported the correlation of ER with menopausal status (29). They found that a higher ER value was seen in post-menopausal women. Our results lack such correlation, which is consistent with results of Thike *et al*. (30) and Pichon *et al*. (31). In the current study, no correlation was found between PR and ER receptors and tumour size. This lack of correlation has been previously described by Jalava *et al*. (29), Aaltomaa *et al*. (32), Thike *et al*. (30) and Blanco *et al*. (33). Although previous studies (29, 33, 34) reported a significant correlation between HR positivity and invasive lobular cancer type, Belkis *et al*. (28) reported a lack of correlation between HR status and histological type of carcinoma. Our results are in line with the latter findings. The expression of ER and PR correlates well with low histological grade (28, 30, 32, 33). Furthermore, positive HR status was more common in patients with low stage tumour, and in node-negative disease. These results are consistent with the findings of Belkis *et al*. (28), Blanco *et al*. (33), and Helin *et al*. (34), although not all investigators have obtained the same results (29).

HR expression related to nuclear size parameters. Studies of Larsimont *et al*. (24) and Giardina *et al*. (25) showed that HR-negative or weakly positive breast carcinomas possess cells with significantly larger nuclei than tumours with highly positive HRs (24, 25). Our results were consistent with these findings, and the MNA was significantly greater in ER- and PR-negative cases.

Prognostic value of ER and PR in female breast cancer. Our study is in line with studies of Arpino *et al*. (35), Ellis *et al*. (36), Blanco *et al*. (33) and Jalava *et al*. (29), all of which showed that positive expression of ER and PR correlate with better survival and response to estrogen antagonists such as tamoxifen, regardless of tumour size, stage, and age. The study of Jalava *et al*. (29) showed that the IHC ER score is associated with prognosis. However, cut-off points for defining the groups with good or worse prognosis may differ between LN– and LN+ cases. Our results suggested that the cut-off point for defining the groups with good or worse prognosis might be set low, between score 0 and 1 (weak positive), patients with ER- and PR-positive cancer had better overall survival than patients with HR-negative cancer. However, the LN status was a stronger prognostic factor (15).

Value of MNA and its prognostic role in female breast cancer in Libyans. There was considerable difference in MNA and other nuclear size parameters among the Libyan patients and those found for Finnish patients (18), and Nigerian patients (4, 18). The mean of MNA in the Finnish population was 38.6 μm^2 (SD=15.0 μm^2), which was within the range of previous European data [from 24.4 (SD 12.8) up to 67.8 (SD 18.35) μm^2] (19, 37). This was lower than the mean MNA value in Libyans 74.25 (SD=23.74) μm^2. The mean MNA value in Nigerians was still higher, 89.2 (SD=34.0) μm^2. These differences might be due to differences in the employed fixation techniques. However, the biological differences among the three populations could be another explanation for this variation.

The morphometric measurement of nuclear size was identified as a potential prognostic marker in breast carcinoma. A higher MNA correlated with increased tumour size, stage and tumour grade, and was related to a low survival rate. This is consistent with other studies such as the one of Tosi *et al*. who concluded that patients whose tumours have higher nuclear area values tend to have a poor prognosis (22).

Conclusion

The fraction of HR-positive cases among Libyan females with breast cancers is about the same as the fraction of positive cases in Europeans. The study also suggests that weak staining is of prognostic value. The prognostic significance may be associated with the practice of using

antihormonal therapy in HR-positive cases. Morphometric MNA is a prognostic factor in breast cancer in Libyans but at a lower level of significance than in Europeans.

Conflict of Interest

We declare that we have no conflict of interest.

Acknowledgments

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References

1 Curado MP: Breast cancer in the world: incidence and mortality. Salud publica de Mexico *53*: 372-384, 2011.
2 Singh R and Al-Sudani OE: Cancer mortality in Benghazi, Libyan Arab Jamahiriya, 1991-96. East Mediterr Health J *7*: 255-273, 2001.
3 Adesunkanmi AR, Lawal OO, Adelusola KA and Durosimi MA: The severity, outcome and challenges of breast cancer in Nigeria. Breast *15*: 399-409, 2006.
4 Ikpatt F: Nigerian breast cancer: Prognostic value of histomorpho- metry. Turku: Ann Univ Turku *487*: 1-128, 2002.
5 Abdalla F, Boder J, Buhmeida A, Hashmi H, Elzagheid A and Collan Y: Nuclear morphometry in FNABs of breast disease in Libyans. Anticancer Res *28*: 3985-3989, 2008.
6 Elzagheid A: Application of E-cadherin immunostaining, morphometry and DNA cytometry in the diagnosis and prognosis of the breast lesions. Turku: Ann Univ Turku *586*: 1-108, 2003.
7 Barbareschi M and Doglioni C: The immunohistochemical detection of steroid hormone receptors in breast cancer: open problems and new perspectives. Pathologica *94*: 115-120, 2002.
8 Rosenberg LU, Magnusson C, Lindstrom E, Wedren S, Hall P and Dickman PW: Menopausal hormone therapy and other breast cancer risk factors in relation to the risk of different histological subtypes of breast cancer: a case control study. Breast Cancer Res *8*: R11, 2006.
9 Kurosumi M: Immunohistochemical assessment of hormone receptor status using a new scoring system (J-Score) in breast cancer. Breast Cancer *14*: 189-193, 2007.
10 Allred DC, Harvey JM, Berardo M and Clark GM: Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol *11*: 155-168, 1998.
11 MacMahon B: Epidemiology and the causes of breast cancer. Int J Cancer *118*: 2373-2378, 2006.
12 Jobling MA, Hurles M and Tyler-Smith C: Human Evolutionary Genetics: Origin, Peoples and Disease. Garland Science, Taylor and Francis group: New York 2004.
13 Ikpatt F and Olopade O: Genetics of Breast cancer in women of African descent: An overview in: Breast Cancer in Women of African Descent. Wiliams C, Olopade O and Falkson C (eds.). Springer, p. 23-38, 2006.
14 Singletary SE: Rating the risk factors for breast cancer. Ann Surg *237*: 474-482, 2003.
15 Boder J, Abdalla F, Elfageih M, Abusaa A, Alfagieh M, Buhmeida A and Collan Y: Breast cancer patients in Libya: Comparison with European and central African patients. Oncol Lett *2*: 323-330, 2011.
16 Newman LA, Griffith KA, Jatoi I, Simon MS, Crowe JP and Colditz GA: Meta-analysis of survival in African American and white American patients with breast cancer: ethnicity compared with socioeconomic status. J Clin Oncol *24*: 1342-1349, 2006.
17 Whitworth A: New research suggests access, genetic differences play role in high minority cancer death rate. J Natl Cancer Inst *98*: 669, 2006.
18 Abdalla F, Boder J, Markus R, Hashmi H, Buhmeida A and Collan Y: Correlation of nuclear morphometry of breast cancer in histological sections with clinicopathological features and prognosis. Anticancer Res *29*: 1771-1776, 2009.
19 Baak JP, Van Dop H, Kurver PH and Hermans J: The value of morphometry to classic prognosticators in breast cancer. Cancer *56*: 374-382, 1985.
20 Elzagheid A, Kuopio T, Pyrhonen S and Collan Y: Lymph node status as a guide to selection of available prognostic markers in breast cancer: The clinical practice of the future? Diagn Pathol *1*: 41, 2006.
21 Zajdela A, De LaRiva LS and Ghossein NA: The relation of prognosis to the nuclear diameter of breast cancer cells obtained by cytologic aspiration. Acta Cytol *23*: 75-80, 1979.
22 Tosi P, Luzi P, Sforza V, Santopietro R, Bindi MTucci E, Barbini P and Baak JP: Correlation between morphometrical parameters and disease-free survival in ductal breast cancer treated only by surgery. Appl Pathol *4*: 33-42, 1986.
23 Kronqvist P, Kuopio T and Collan Y: Morphometric grading of invasive ductal breast cancer. I. Thresholds for nuclear grade. Br J Cancer *78*: 800-805, 1998.
24 Larsimont D, Kiss R, d'Olne D, de Launoit Y, Mattheiem W, Paridaens R, Pasteels JL and Gompel C: Correlation between nuclear cytomorphometric parameters and estrogen receptor levels in breast cancer. Cancer *63*: 2162-2168, 1989.
25 Giardina C, Serio G, Simone G, Pennella A, Vacca E, Ricco R, Scordari D and Pesce Delfino V: Nuclear morphometry and estrogen receptors in infiltrating ductal carcinoma of the breast. Boll Soc Ital Biol Sper *66*: 135-141, 1990.
26 Elston C. Grading of invasive carcinoma of the breast. In: Diagnostic histopathology of breast. Edinburgh London, Melbourne and New York: Churchill Livingstone; 1987.
27 Romppanen T and Collan Y: Practical guidelines for a morphometric study. Acta Stereol *2*: 274-297, 1983.
28 Belkis E, Langhammer H, Sonderhaus G and Bush R: Estrogen and progesterone receptor status in breast carcinoma: correlation with age and histopathology. Turk Cancer *2*: 49-53, 1991.
29 Jalava P, Kuopio T, Huovinen R, Laine J and Collan Y: Immunohistochemical staining of estrogen and progesterone receptors: aspects for evaluating positivity and defining the cut-off points. Anticancer Res *25*: 2535-2542, 2005.
30 Thike AA, Chng MJ, Fook-Chong S and Tan PH: Immunohistochemical expression of hormone receptors in invasive breast carcinoma: correlation of results of H-score with pathological parameters. Pathology *33*: 21-25, 2001.
31 Pichon MF, Pallud C, Brunet M and Milgrom E: Relationship of presence of progesterone receptors to prognosis in early breast cancer. Cancer Res *40*: 3357-3360, 1980.

32 Aaltomaa S, Lipponen P, Eskelinen M, Kosma VM, Marin S, Alhava E and Syrjänen K: Hormone receptors as prognostic factors in female breast cancer. Ann Med *23*: 643-648, 1991.
33 Blanco G, Alavaikko M, Ojala A, Collan Y, Heikkinen M, Hietanen T, Aine R and Taskinen PJ: Estrogen and progesterone receptors in breast cancer: relationships to tumour histopathology and survival of patients. Anticancer Res *4*: 383-389, 1984.
34 Helin HJ, Helle MJ, Kallioniemi OP and Isola JJ: Immunohistochemical determination of estrogen and progesterone receptors in human breast carcinoma. Correlation with histopathology and DNA flow cytometry. Cancer *63*: 1761-1767, 1989.
35 Arpino G, Green SJ, Allred DC, Lew D, Martino S, Osborne CK and Elledge RM: *HER-2* amplification, HER-1 expression, and tamoxifen response in estrogen receptor-positive metastatic breast cancer: a Southwest Oncology Group Study. Clin Cancer Res *10*: 5670-5676, 2004.
36 Ellis MJ, Coop A, Singh B, Mauriac L, Llombert-Cussac A, Jänicke F, Miller WR, Evans DB, Dugan M, Brady C, Quebe-Fehling E and Borgs M: Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ERBB-1- and/or ERBB-2-positive, estrogen receptor-positive primary breast cancer: evidence from a phase III randomized trial. J Clin Oncol *19*: 3808-3816, 2001.
37 Kronqvist P, Collan Y, Kuopio T and Kujari H: Nuclear morphometry in breast cancer: the influence of sampling rules and freezing of samples. Mod Pathol *8*: 187-192, 1995.

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