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Original scientific paper

Estrogen and Progesterone Receptor Status in Primary Breast Cancer – A Study of 11,273 Patients from the Year 1990 to 2002

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A B S T R A C T

The aim of this study was to gain insight of the breast cancer hormone receptor status of our patients, its stratification according to age as well as its changes during the period of 13 years. 11,273 patients with primary breast cancer from several towns in Croatia were included in this study. Patients' tumour specimens were collected from 1990 to 2002 and were analysed on estrogen (ER) and progesterone (PR) receptors in the Laboratory of the Department of Medical Oncology, University Hospital Centre Zagreb. More than half of our breast cancer patients had ER positive tumours (54.3%). We observed ER+ tumours increased with age continuously, with highest percentage in the age group of 70 to 79 years (68.1%). Similarly, proportion of PR+ tumours was higher in the older age groups, being the highest between 40 and 49 years (55.9%). During 13 years of the study, the increase in frequency and proportion of ER+ tumours was observed (from 52% in 1990 to 62% in 2002) and decrease of PR+ tumours (56% to 53%). We confirm previous findings that the risk of hormone dependent breast cancer increases with aging. Risk of ER+ breast cancer increased for 10% from 1990 to 2002 and PR+ tumours decreased for 3.5% in the same period.

Key words: *estrogen receptor, progesterone receptor, breast cancer*

Introduction

Breast cancer hormone receptor status suggests different biological and clinical profiles. ER positive tumours are more likely to be histological well-differentiated, diploid and have a lower S phase fraction. ER positivity is also associated with increasing age, postmenopausal status and is an independent factor of better prognosis.

In our study we analysed distribution of breast cancer hormone receptor status according to age groups of patients as well as overall and age stratified hormone receptor status of our patients from the year 1990 to 2002.

The results presented in this paper are from a single centre study on 11,273 patients with primary breast cancer treated in hospitals in several towns in Croatia. The tumour specimens were collected from the year 1990 to 2002 and analysed on ER and PR in the Laboratory of the Department of Medical Oncology, University Hospital Centre Zagreb, thus eliminating the variations in the results due to interlaboratory methodological differences. The results are comparable to other European centres, as the Laboratory is included in the EORTC receptor study group.

Patients and Methods

Patients

The breast cancer patients included in this study were treated in hospitals in several towns of Croatia. The age distribution of patients was as follows: 28% of patients were of 60 to 69 years of age, 24% were aged 50 to 59, 19% 40 to 49 as well as 70 to 79 years, and in the age group of 20 to 39 years there were 6% of patients, while 4% of patients were older than 80 years.

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Methods

Tumour tissue specimens were placed on ice immediately after excision during biopsy or mastectomy and within 30 minutes were frozen in liquid nitrogen. The specimens were then sent to the laboratory and stored until assayed, for not longer than 3 weeks.

The ER and PR were measured by a point assay using the dextran- coated charcoal method based on that of Horwitz and McGuire[1], modified as described previously[2]. Tumour cytosol containing 1–2 mg/ml protein (measured by Lowry's method) was incubated in triplicate at 4°C for 18 hours with appropriate radioactively labelled ligands, with or without »cold» competitors, as follows: for ER with ^{3}H- oestradiol in final concentration of 0.8 nmol/l without and with 100 fold excess of diethylstilbestrol; for PR with ^{3}H-promegestone (R5020) in final concentration of 8.0 nmol/l in the presence of a 100 fold excess of cortisol and without or with a 100 fold excess of »cold« R5020. Cortisol was added to prevent the binding of R5020 to glucocorticoid receptors. It was found that in these conditions (protein, ligand and competitor concentrations) one point assay for both ER and PR were in good correlation with multipoint assays[3–7].

The tumours with a binding capacity higher than 10 fmol/mg cytosol protein for estradiol and R5020 were considered receptor positive.

Results

In the study we observed that more than half of our breast cancer patients had estrogen receptor positive tumours. The most frequent subtype of tumour was ER+ PR+ involving 34.3% of all cases, followed by ER-PR- subtype with 27.8%, ER+PR- with 20.2%, and ER-PR+ with 17.7%. These results are comparable to those of the US study on 5,993 breast cancer patients[8].

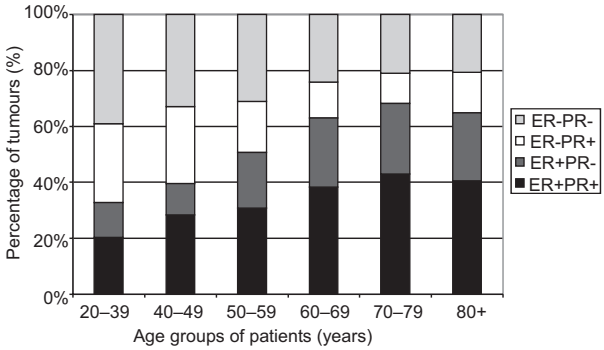

Fig. 1. Proportion of different breast cancer subtypes (according to hormone receptor status) in different age groups. The risk of hormone dependent breast cancer increases with age. ER – estrogen receptor, PR – progesterone receptor.

Comparing estrogen receptor status of tumours to patients' age showed that the frequency of ER+ tumours (ER+ PR+ and ER+ PR-) increased with age continually with a decrease around age of 80. Highest percentage of ER + tumours was in the age of 70 to 79 – with 68.1%, and the age group of 80 years and older followed with 64.8% of ER+ tumours. Patients with 69 years and younger had progressively lower percentage of ER+ tumours. Similarly, proportion of PR+ tumours was higher in older age groups, being the highest between 40 and 49 years of age. As concerns ER- tumours the frequency of ER- PR+ subtype decreased with age prior to age 80 and afterwards increased. Subtype of ER- PR- tumours decreased continually with age (Figure 1).

These findings are similar to the results of comparable studies of ER PR age distribution among 3,359 Danish[9] and 110,010 US[10] breast cancer patients.

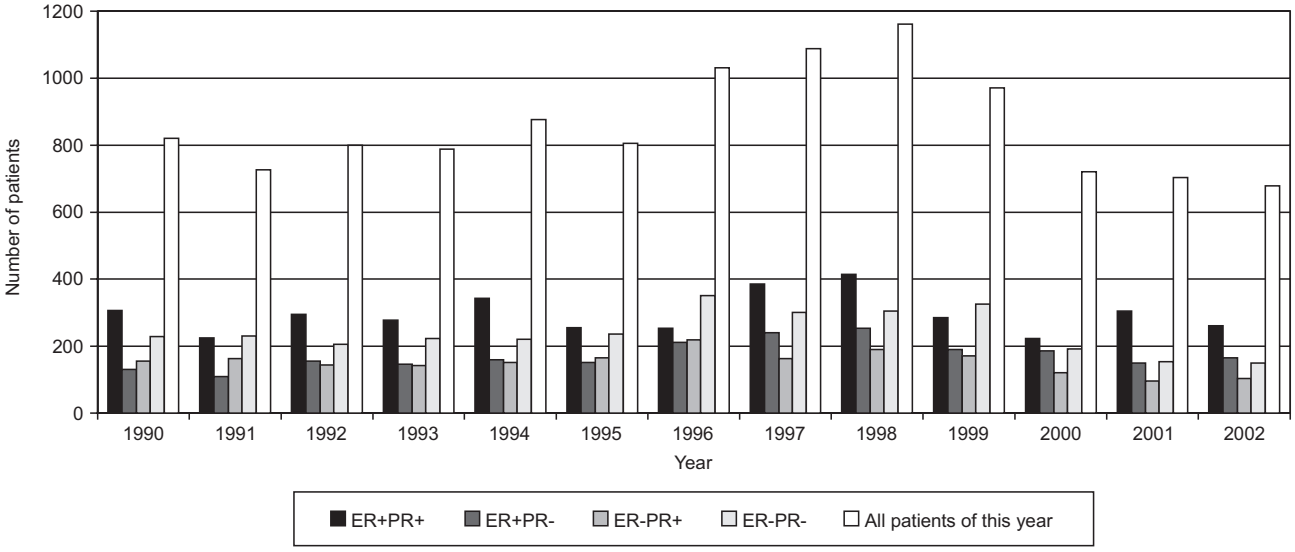

Fig. 2. Diagram illustrating number of patients with different tumour subtypes and total number of breast cancer patients in each year of the study. ER – estrogen receptor, PR – progesterone receptor.

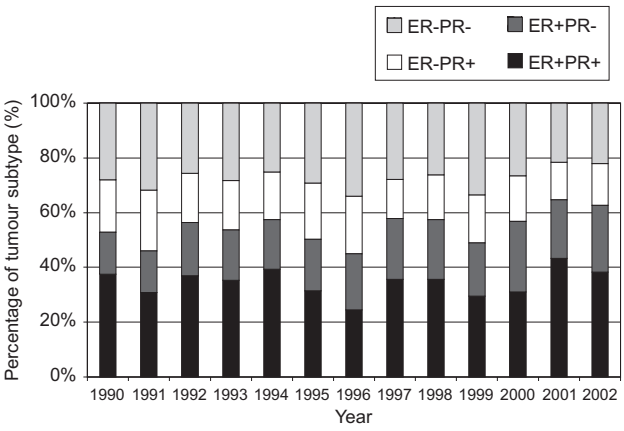

Fig. 3. Diagram showing how distribution of breast cancer subtypes (percentage) changes over the period of 13 years. ER – estrogen receptor, PR – progesterone receptor.

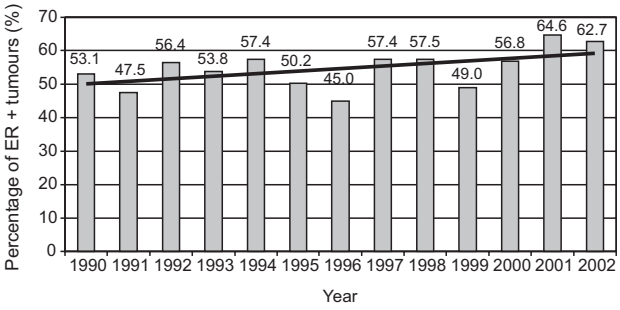

Fig. 4. Changes of proportion of estrogen receptor positive breast cancer during the period of study (13 years). ER – estrogen receptor, PR – progesterone receptor.

During the period of 13 years (1990–2002) we followed the frequency and proportion of ER+ and ER-, PR+ and PR- tumours as well as their combinations (Figures 2 and 3). We observed an overall increase of ER+ tumours among breast cancer patients. More precisely the proportion of ER+ tumours declined from year 1990 (52%) to 1996 (44%) and then had a constant increase until 2002 (62%) (Figure 4), alternatively proportion of ER negative tumours changed correspondingly. As concerns frequency of ER+ tumours, after an increase in number of cases in the first 7 years of follow up, it declined below the initial number. Stratifying ER+ breast cancer patients in different age groups and following their proportions during 13 years we found that trend of increase of ER+ tumours was present in all age groups (Table 1 and 2).

Overall, the proportion of women diagnosed with PR positive tumours, as well as their number, decreased over the study period. Initially 56% of tumours were PR+, in 1996 45% and 2002 53% (Figure 5). Stratifying these results into age groups we found the decline of PR+ tumours was present in age groups 20 to 39 and older than 80. In other age groups the proportion of PR+ tumours remained constant during 3 years (Table 1 and 3).

Analysing different subtypes of breast cancer according to ER and PR status we observed overall increase in proportion of ER+PR+ and ER+ PR- tumours from 37% to 38% and 15% to 24%, respectively over 13 years. Alternatively percentage of ER-PR- and ER-PR+ tumour subtypes decreased during the same period from 28% to 22% and 19% to 15%, respectively (Figure 3). Differences by age were observed, as ER+PR+ tumours increased only among 20- to 49-year-olds and aged 80 and older in the period of 13 years (Table 1). Also, ER+PR- tumours increased in all age groups but 40 to 49-year-olds (Table 2). ER-PR+ tumours decreased among every age group but

TABLE 1
PROPORTION OF BREAST CANCER PATIENTS WITH ESTROGEN AND PROGESTERONE RECEPTOR POSITIVE TUMOURS STRATIFIED IN DIFFERENT AGE GROUPS IN THE PERIOD OF 13 YEARS

Year	Age groups of ER+PR+ breast cancer patients						
	<20	20–39	40–49	50–59	60–69	70–79	80+
1990	0%	24.4%	28.8%	36.1%	41.0%	52.6%	36.0%
1991	0%	21.1%	25.0%	34.2%	38.7%	38.0%	34.8%
1992	0%	31.3%	29.0%	31.4%	41.3%	50.4%	53.9%
1993	0%	25.8%	30.0%	34.6%	39.0%	44.8%	35.1%
1994	0%	23.9%	27.1%	33.3%	44.6%	51.2%	45.5%
1995	0%	20.5%	29.4%	28.6%	33.6%	36.8%	47.4%
1996	0%	4.7%	18.4%	23.1%	27.1%	35.9%	40.0%
1997	0%	24.5%	29.3%	28.5%	41.5%	42.2%	50.0%
1998	0%	23.5%	32.0%	29.4%	41.9%	42.3%	35.8%
1999	0%	11.3%	22.7%	28.6%	29.3%	39.8%	39.6%
2000	0%	19.4%	27.5%	26.8%	34.4%	36.8%	39.3%
2001	100%	29.3%	42.4%	42.9%	46.3%	48.3%	28.1%
2002	0%	27.6%	34.7%	25.3%	42.0%	48.0%	42.2%

ER – estrogen receptor, PR – progesterone receptor

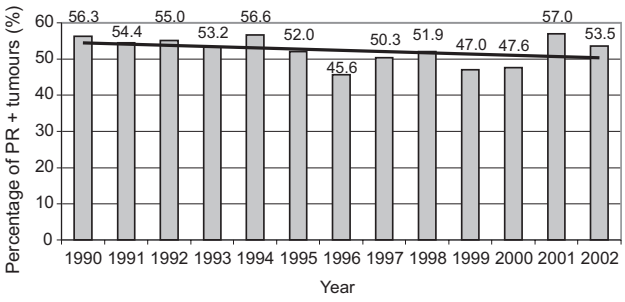

Fig. 5. Changes of proportion of progesterone receptor positive breast cancer during the period of study (13 years). ER – estrogen receptor, PR – progesterone receptor.

70 to 79 (Table 3). Decrease of ER-PR- tumours over time was limited to women aged 50 and older (Table 4).

We should mention that there were no changes in methodology of hormone receptor status determination, which could influence our findings and effects we observed.

Discussion

Characterisation of breast tumours on both ER[11] and PR status suggests different biological and clinical profiles. ER positive tumours are more likely to be histological well-differentiated, diploid and have a lower S phase fraction[12]. ER positivity is also associated with increasing age and postmenopausal status, and is negatively correlated with tumour size[13,14]. ER status remains an independent factor of prognosis. Therefore presence of functional ER serves as a marker of differentiation and a predictor of disease-free survival in node-negative and node positive breast cancer patients[14,15]. Apart from biological and clinical characteristics of breast cancer ER and PR status may define fundamental types of breast tumours[16] with different risk factors and therefore different aetiologies. ER+PR+ tumours were associated with risk factors such as parity, age at first birth, age at menarche, body mass index and body fat distribution. ER- PR- and ER+PR- breast cancers showed inverted pattern of associations with several risk factors of ER+PR+ tumours – parity and waist to hip ratio for ER-PR-, history of bilateral oophorectomy and oral contraceptive use for ER+PR-, body mass index for ER+PR- and ER--PR-[17]. Considering the facts on the role of breast cancer hormone dependency in its aetiology and biology we examined whether age-specific risk of breast cancer, especially its change at menopause, differs by oestrogen and progesterone receptor status.

Our study showed stratification of ER PR breast cancer status by age. The risk of hormone dependent breast cancer increased with aging. Before menopause (age of 50) the majority of tumours were estrogen receptor negative (67% of all tumours in the younger age group (20–39 years) and 60% in the older (40–49 years)). Increasing risk of ER positive tumours started around menopause – at the age of 50 and later, when they made 50% to 68% of all cases, the highest frequency between the ages 70 to 79. These results match those of two studies – US[10] and Danish[9] which revealed substantially higher risk of ER+PR+ tumour subtype with increasing age.

Further comparison of our findings to Danish[9], US white[10] and Japanese[18] female populations showed following results. Substantially higher risk of ER+ PR+ subtype was evident in US (64.2% of all cases) and Danish (62.9%) compared to Croatian (34.34%) and Japanese (29%) female population. The risk difference appeared to increase with

TABLE 2
PROPORTION OF BREAST CANCER PATIENTS WITH ESTROGEN RECEPTOR POSITIVE AND PROGESTERONE RECEPTOR NEGATIVE TUMOURS STRATIFIED IN DIFFERENT AGE GROUPS IN THE PERIOD OF 13 YEARS

Year	Age groups of ER+PR- breast cancer patients						
	<20	20–39	40–49	50–59	60–69	70–79	80+
1990	0%	4.4%	14.1%	14.6%	20.1%	15.5%	12.0%
1991	0%	9.9%	10.3%	15.2%	17.3%	18.0%	21.7%
1992	0%	9.4%	13.8%	19.1%	24.4%	21.0%	15.4%
1993	0%	9.7%	11.3%	14.8%	25.4%	32.4%	10.8%
1994	0%	8.7%	9.7%	15.9%	22.5%	23.3%	33.3%
1995	0%	15.9%	6.7%	20.2%	24.9%	18.4%	23.7%
1996	0%	14.1%	11.4%	20.6%	27.1%	13.8%	23.3%
1997	0%	15.1%	14.4%	23.2%	24.4%	29.2%	25.0%
1998	0%	7.8%	13.6%	23.1%	24.0%	31.4%	11.3%
1999	0%	12.9%	7.2%	19.1%	27.3%	22.8%	27.1%
2000	0%	12.9%	13.0%	29.3%	29.6%	31.0%	25.0%
2001	0%	31.0%	6.8%	18.0%	23.4%	25.5%	53.1%
2002	0%	10.3%	10.5%	27.4%	26.0%	28.0%	31.1%

ER – estrogen receptor, PR – progesterone receptor

TABLE 3
PROPORTION OF BREAST CANCER PATIENTS WITH ESTROGEN RECEPTOR NEGATIVE AND PROGESTERONE RECEPTOR POSITIVE TUMOURS STRATIFIED IN DIFFERENT AGE GROUPS IN THE PERIOD OF 13 YEARS

Year	Age groups of ER-PR+ breast cancer patients						
	<20	20–39	40–49	50–59	60–69	70–79	80+
1990	0%	37.8%	26.0%	19.0%	15.0%	7.8%	24.0%
1991	0%	31.0%	31.4%	20.3%	13.3%	18.0%	30.3%
1992	0%	32.8%	26.9%	19.1%	12.9%	8.4%	7.7%
1993	0%	16.1%	30.0%	19.2%	10.7%	9.5%	18.9%
1994	0%	28.3%	32.6%	16.9%	13.1%	10.9%	3.0%
1995	0%	34.1%	33.6%	17.3%	17.0%	12.5%	15.8%
1996	0%	32.8%	31.4%	23.1%	14.7%	9.4%	16.7%
1997	100%	20.8%	21.8%	14.2%	12.0%	10.9%	14.6%
1998	0%	23.5%	27.7%	16.7%	10.4%	7.3%	26.4%
1999	0%	33.9%	28.2%	16.6%	13.3%	11.7%	6.3%
2000	0%	29.0%	26.0%	17.7%	12.7%	11.0%	10.7%
2001	0%	7.1%	20.3%	15.5%	11.7%	10.7%	9.4%
2002	0%	20.7%	19.0%	20.6%	12.2%	12.0%	8.9%

ER – estrogen receptor, PR – progesterone receptor

TABLE 4
PROPORTION OF BREAST CANCER PATIENTS WITH ESTROGEN AND PROGESTERONE RECEPTOR NEGATIVE TUMOURS STRATIFIED IN DIFFERENT AGE GROUPS IN THE PERIOD OF 13 YEARS

Year	Age groups of ER-PR- breast cancer patients						
	<20	20–39	40–49	50–59	60–69	70–79	80+
1990	0%	33.3%	31.1%	30.2%	23.5%	24.1%	28.0%
1991	0%	38.0%	33.3%	30.4%	30.6%	26.0%	13.0%
1992	0%	26.6%	30.3%	30.4%	21.3%	20.2%	23.1%
1993	0%	48.4%	28.7%	31.3%	24.9%	13.3%	35.1%
1994	0%	39.1%	30.6%	33.9%	19.8%	14.7%	18.2%
1995	0%	29.6%	30.3%	33.9%	24.5%	32.4%	13.2%
1996	0%	48.4%	38.9%	33.3%	31.1%	24.3%	20.0%
1997	0%	39.6%	34.6%	34.2%	22.2%	17.7%	10.4%
1998	0%	45.1%	26.7%	30.9%	23.7%	19.1%	26.4%
1999	0%	41.9%	42.0%	35.7%	30.1%	25.7%	27.1%
2000	0%	38.7%	33.6%	26.2%	23.3%	21.3%	25.0%
2001	0%	33.3%	30.5%	23.6%	18.6%	15.4%	9.4%
2002	0%	41.4%	35.8%	26.7%	19.9%	12.0%	17.8%

ER – estrogen receptor, PR – progesterone receptor

age in former three populations. Age differences across populations did not explain the differences in ER PR distribution: ER+PR+ subtype in the Danish case-series stratified by menopausal status was 62% in premenopausal, 57% in perimenopause and 64% in postmenopausal women, all of which were higher than the 29% in the Japanese case-series and Croatian (where this subtype was distributed into 24%, 30% and 40% respectively).

Our findings showed that proportion of ER+ PR+ and ER+ PR- tumours had continuous rise towards older age with slowing rate at the age of 80. The opposite is true for ER-PR- tumours (Figure 1). Compared to our study, the large Danish dataset showed incidence rates of the ER+PR+ subtype had constant rate of increase with age, similar to that of ER+PR- subtype which had rapid increase in perimenopausal period, while those of ER-PR+ and ER-PR- decreased or remained unchanged after menopause. In the US study for both white and black women the age-specific rates of ER- breast cancer ceased increasing after 50 years of age, but age-specific rates of ER+ breast cancer continue to increase after 50 years of age.

From 1990 to 2002 ER+ tumours in our population of patients increased for 10% (in all age groups), and PR+ decreased 3.5% (in age groups 20 to 49 years of age and 80

and older). Similar rise of ER+ tumours proportion was presented in the US study[19] following patients from 1992 to 1998. The former study also reported increase of PR+ tumours. This increase of hormone receptor positive tumours seemed to be limited to women 40 to 69 years of age. It also showed that incidence rates rose for ER+ and PR+, but remained constant for ER- and PR- tumours. Therefore the overall rise in breast cancer incidence rates in the United States[20] seemed to be primarily the result of increase in the incidence of hormone receptor positive tumours. Hormonal factors may have accounted for this trend, according to their study. It was connected to higher use of hormone replacement therapy, mean BMI increasing, the mean age of menarche decreasing, and increasing number of women in the US who are nulliparous and older when they have their first live birth.

Conclusion

In our study we observed ER+ tumours increased with age continuously, with highest percentage in the age group of 70 to 79 years. Similarly, proportion of PR+ tumours was higher in the older age groups, being the highest between 40 and 49 years. These results match those of two studies – US[10] and Danish[9] which revealed substantially higher risk of ER+PR+ tumour subtype with increasing age.

During 13 years of the study, we detected the trend of increase in frequency and proportion of ER+ tumours (for 10%) and the decrease of PR+ tumours (for 3,5%). The trend of ER+ tumours was comparable to that of the US study[19].

REFERENCES

1. HORWITZ KB, MCGUIRE WL, Cancer Res, 37 (1977) 1733. — 2. ROMIĆ-STOJKOVIĆ R, GAMULIN S, Cancer Res, 40 (1980) 4821. — 3. MCGUIRE WL, DE LA GARZA M, CHAMNESS CC, Cancer Res, 37 (1977) 637. — 4. MULDER J, VERHAAR MAT, Clin Chim Acta, 99 (1979) 129. — 5. NICOLO G, CARBONE A, ESPOSITO M, SANTI L, Sampling and storage of breast cancer tissue for steroid receptor assays. In: LECLERQ G, TOMA S, PARIDAENS R, HEUSON JC (Eds), Recent Results in Cancer Research (Springer, Berlin, 1984). — 6. PICHON MF, MILGROM E, Cancer Res, 37 (1977) 464. — 7. POWELL B, GAROLA RE, CHAMNES GC, MCGUIRE WL, Cancer Res, 39 (1979) 1678. — 8. NADJI M, GOMEZ-FERNANDEZ C, GANJEI-AZAR P, MORALES AR, Am J Clin Pathol, 123 (2005) 21. — 9. YASUI Y, POTTER JD, Cancer Causes Control, 10 (1999) 431. — 10. TARONE RE, CHU KC, Cancer Causes Control, 13 (2002) 7. — 11. GUREL I, LIVSHITS G, Coll. Antropol, 27 (2003) 599. — 12. MCGUIRE WL, CHAMNESS GC, FUQUA SAW, Mol Endocrinol, 5 (1991) 1571. — 13. THORPE SM, ROSE C, PERDERSEN BV, RASMUSSEN BB, Breast Cancer Res Treat, 3 (1983) 103. — 14. CLARK GC, OSBORNE CK, MCGUIRE WL, J Clin Oncol, 2 (1984) 1102. — 15. OSBORNE CK, YOCHMOWITZ MG, KNIGHT WA, MCGUIRE WL, Cancer, 12 (1980) 2884. — 16. SHEIKH MS, GARCIA M, PUJOL P, FONTANA JA, ROCHEFORT H, Invasion Metastasis, 14 (1994) 329. — 17. COLDITZ GA, ROSNER BA, CHEN WY, HOLMES MD, HANKINSON SE, J Natl Cancer Inst, 96 (2004) 218. — 18. NOMURA Y, MIURA S, KOYAMA H, Cancer, 69 (1992) 153. — 19. LI CI, DALING JR, MALONE KE, J Clin Oncol, 21 (2003) 28. — 20. HOWE HL, WINGO PA, THUM MJ, J Natl Cancer Inst, 93 (2001) 824.

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STATUS ESTROGENSKIH I PROGESTERONSKIH RECEPTORA KOD PRIMARNOG KACINOMA DOJKE: ISTRAŽIVANJE NA 11 273 PACIJENTICE OD 1990. DO 2002. GODINE

SAŽETAK

Cilj ovog rada je istražiti status hormonskih receptora karcinoma dojke kod naših pacijentica, stratificirati ih prema dobi, te utvrditi mijenja li se status receptora tijekom 13 godina praćenja pacijentica. U istraživanje je bilo uključeno 11 273 pacijentica s primarnim karcinomom dojke, iz nekoliko gradova Hrvatske. Uzorci tumora prikupljani su od 1990. do 2002. godine, te je učinjena analiza statusa estrogenskih i progesteronskih receptora u Laboratoriju Zavoda za patofiziologiju, Kliničkog bolničkog centra Zagreb. Većina pacijentica imala je pozitivne estrogenske receptore u tumoru (54,3%). Uočili smo kontinuirani porast učestalosti estrogen pozitivnih tumora s dobi, s najvišom učestalosti u dobnoj skupini od 70 do 79 godina (68,1%). Slično, udio progesteron pozitivnih tumora raste s dobi, te je najviši između 40 i 49 godina (55,9%). Tijekom 13 godina istraživanja, uočen je porast broja i postotka estrogen pozitivnih tumora (s 52% 1990.g. na 62% 2002.g.) te smanjenje progesteron pozitivnih tumora (s 56% na 53%). Ovim istraživanjem potvrđeni su rezultati ranijih studija kojima je utvrđen porast hormon ovisnih tumora s dobi. Rizik estrogen pozitivnih karcinoma dojke porastao je od 1990.g. do 2002.g. za 10%, dok se rizik progesteron pozitivnih smanjio za 3,5%.