Eur J Cancer, Vol. 27, No. 4, pp. 491–495, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00

Oestrogen and Progesterone Receptors in Primary Breast Cancer: a Population Study

Stjepan Gamulin and Ranka Romić-Stojković

The results of a single centre study on cytoplasmic oestrogen (ER) and progesterone (PR) receptors and their combinations in primary breast cancer of 1957 patients from various parts of Croatia are presented. The frequency of ER+ tumours and tumour mean ER concentration were higher in patients over 50 years of age, while PR frequency and concentration were similar in patients over and under 50. The ER concentration was positively correlated with the age of patients, but the age-related increase in ER concentration appeared between 50 and 70 years of age. The pattern of receptor coexistence was age related. The frequency of ER+PR+ and ER+PR− increased and that of ER−PR+ and ER−PR− tumours decreased with the age of patients. The concentrations of ER and PR were higher in ER+PR+ than in ER+PR− or ER−PR+ tumours, respectively. When the patients were divided into groups under and over 50 years of age these differences appeared only in the latter group, while in the former the concentrations of ER were similar in ER+PR+ and in ER+PR− tumours, and the concentration of PR was higher in ER+PR+ than in ER−PR+ tumours. These data suggest a biological difference between breast cancers with various receptor combinations, as well as a difference in pathogenesis of the receptor negative and positive breast cancer.
Eur J Cancer, Vol. 27, No. 4, pp. 491–495, 1991

INTRODUCTION

GEOGRAPHIC VARIATIONS in breast cancer incidence and mortality are well known. These variations are attributed to the influence of non-genetic factors [1]. Although these factors have not been identified, a number of them probably act through steroid sex hormones [2]. So the population studies of oestrogen (ER) and progesterone (PR) receptors in breast cancer might help to understand the aetiopathogenesis of the disease. That population differences in ER and PR status of breast cancer exist is indicated by a higher frequency of ER+ and PR+ tumours in postmenopausal American versus Japanese [3] and in white versus black or Asian patients [4].

The results presented in this paper are from a single centre study on a large number of patients and fulfill the criteria [5] as being representative of the population of Croatia.

The ER and PR were analysed on tumour specimens collected from 1956 patients from various parts of the country, but the assays were performed in the laboratory of the department, 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.

MATERIALS AND METHODS

Patients

The breast cancer patients included in this study were treated in hospitals in a number of towns of Croatia. The age distribution of the patients is shown on Table 1.

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 one point assay using the dextran-coated charcoal method based on that of Horwitz and McGuire [6], modified as described previously [7]. Tumour cytosol containing 1–2 mg/ml protein was incubated in triplicate at 4°C for 18 h 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 a 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 assays for both ER and PR were in good correlation with multipoint assays [8–12].

The tumours with a binding capacity lower than 5 fmol/mg cytosol protein for oestradiol and lower than 10 fmol/mg cytosol proteins for R5020 were considered receptor negative.

Table 1. Age distribution of primary breast cancer patients

Age	No. of patients (%)
20–29	23 (1)
30–39	219 (11)
40–49	525 (27)
50–59	508 (26)
60–69	414 (21)
70–79	244 (13)
80 and above	27 (1)
Total	1956

Correspondence to S. Gamulin; reprint requests to R. Romić-Stojković. The authors are at the Department of Pathophysiology, Medical Faculty, Clinical Hospital Centre, Rebro, 41000 Zagreb, Yugoslavia.
Revised 23 Nov. 1990; accepted 10 Jan. 1991.

Table 2. Frequency of ER, PR and their combinations in primary breast cancer

	Under 50*			Above 50*			All patients		
	PR+	PR−	Total	PR+	PR−	Total	PR+	PR−	Total
ER+	310 (38)	74 (9)	384 (47)	564 (49)	217 (19)	781 (68)	874 (45)	291 (15)	1165 (60)
ER−	167 (21)	258 (32)	425 (53)	105 (9)	261 (23)	366 (32)	272 (14)	519 (26)	791 (40)
Total	477 (59)	332 (41)	809 (100)	669 (58)	478 (42)	1147 (100)	1146 (59)	810 (41)	1956 (100)

* The distributions of various combinations of ER and PR in the age groups were significantly different ($P < 0.01$, χ^2 test)

RESULTS

The percentages of tumours containing ER, PR or their combinations and tumour receptor concentrations for all the patients and in groups under and above 50 years of age are shown in Tables 2 and 3.

The frequency of ER+ tumours and the mean ER concentrations were higher in the older than in the younger group, while there was no difference either in frequency of PR+ tumours or in the PR concentration between these two groups of patients.

The percentages of tumours with various combinations of ER and PR were different in these two groups of patients. The more frequent combinations in the older group were ER+PR+ and ER+PR−, and in the younger group ER−PR+ and ER−PR−.

The distribution of ER+ and PR+ tumours in patients divided into decade of age groups are shown on Fig. 1. The frequency of ER+ tumours increased with the age of patients, the highest rate of increase being between the ages 50 and 70. The frequency of PR+ tumours was similar in all age groups. The changes in frequency of ER and PR combinations dependent in the age of patients are displayed in Fig. 2, showing an increase of ER+PR+ and ER+PR−, and a decrease of ER−PR+ and ER−PR− tumours. The mean concentrations of ER and PR in tumours of patients divided into groups according to various combinations of receptor coexistence are shown in Table 4. The concentrations of both ER and PR were higher in ER+PR+ tumours than in ER+PR− or ER−PR+ tumours.

When the age of patients was taken into account additional differences in the receptor concentrations became evident. ER concentrations were higher in both ER+PR+ and ER+PR− tumours in the above 50 group. However, a comparison within the age groups showed that ER concentrations in ER+PR+ and ER+PR− tumours were similar in younger patients and dissimilar in older patients, being higher in ER+PR+ than in ER+PR− tumours.

The comparison of concentrations of PR in ER+PR+ and in ER−PR+ tumours within the age groups showed a higher concentration of the receptors in the former than in the latter tumours for both age groups.

Table 3. Concentrations of ER and PR in primary breast cancer

Age	ER+	PR+
< 50	31 (2)*	76 (6)
50 ≥	83 (4)*	79 (2)

Mean (S.E.)
* Significant difference ($P < 0.01$, Student's t test)

Correlation analysis of the age of patients and the tumour concentrations of ER and PR is shown in Table 5. There was a weak though significant correlation for ER concentrations, whilst there was no correlation between PR concentration and the age of patients. However, when correlation analysis between tumour ER concentrations and the age of patients was made separately for three age groups, under 50, 50–70 and above 70 it became evident that there was a positive correlation only in the second age group. These findings indicate that the increase of the ER concentrations with the age of patients is not continuous but stepwise occuring between the ages 50 and 70. This is clearly shown on Fig. 3 where the relationship between ER concentrations and the decade age of patients is displaced. Correlation analysis between the concentrations of ER and PR in ER+PR+ group shows a weak but highly significant association between these two variables ($n = 874$, correlation coefficient 0.196, $P < 0.001$).

DISCUSSION

Although there have been a number of reports on ER distribution in human breast cancer, there have been only a few of those analysing both ER and PR and their coexistence in a large sample of population. Single centre studies on demographic characteristics of breast cancer covering both ER and PR performed on upward of 1000 patients are those for North America [13], Denmark [5] and Sweden [14]. In the first study ER and PR were analysed separately without reference to their coexistence.

In comparing our results with these studies some methodological differences should be taken into account. The concentrations discriminating receptor positive and receptor negative tumours for ER and PR were 5 and 5 [13], 10 and 10 [5], and 5 and 10 fmol/mg cytosolic proteins in this study. Wilking *et al.* [14] expressed their results in fmol/μg DNA, setting the cut-off point between positive and negative results at 0.05 fmol/μg DNA for both ER and PR. The average receptor concentrations

Table 4. ER and PR concentrations in primary breast cancer with various receptor combinations

Age	ER+PR+		ER+PR−	ER−PR+
	ER	PR	ER	PR
< 50	34 (2.9)*	91 (9.2)§	32 (6.5)▼	32 (2.4)§
50 ≥	91 (4.5)*†	73 (4.7)‖	59 (4.7)▼†	28 (3.2)‖
Total	72 (3.1)‡	79 (4.4)▲	53 (3.9)‡	31 (2.0)▲

Mean (S.E.)
The pairs of symbols denote significant difference ($P < 0.01$, Student's t test).

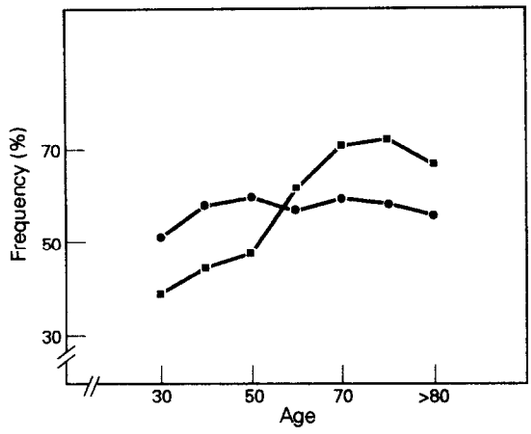

Fig. 1. Relation between frequency of ER+ (■) and PR+ (●) primary breast cancer and patient age.

were expressed either as medians [5, 13] or as means (this study and ref. 14).

A number of authors found a higher frequency and higher concentrations of ER in tumours of postmenopausal than in premenopausal patients, but there were no such differences for PR [13–16]. Assuming the age of 50 as the setpoint of menopause, our overall findings are consistent with this general pattern of ER and PR distribution in breast cancer. On the other hand Thorpe [5] found a higher frequency and higher concentration of PR tumours in premenopausal than in postmenopausal patients.

It is not clear whether the difference in ER frequency and content between premenopausal and postmenopausal women is due to age or to menopausal status. A number of researchers have found a positive correlation between ER concentrations and the age of patients, with a continuous increase of average ER concentrations [5, 13, 14]. Our results, on the contrary, suggest that the age-related increase of ER concentration occurs discontinuously between the ages 50 and 70.

Analysing the effects of both age and menopausal status on the ER concentrations, some investigators have found a dominant effect of age [5, 13, 16, 17]. Allegra *et al.* [18], however, found a lack of correlation between ER concentrations and the age within premenopausal and postmenopausal groups,

Table 5. Relations between ER and PR concentrations and the age of patients

Age	ER			PR		
	n	*r*	*P*	*n*	*r*	*P*
< 50	361	−0.093	NS	456	−0.09	NS
50–70	608	0.240	< 0.001	533	0.08	NS
> 70	196	−0.049	NS	157	−0.06	NS
Total	1165	0.274	< 0.001	1146	0.03	NS

r = correlation coefficient; NS = not significant ($P > 0.01$).

and they attributed the higher ER concentration in the latter group to association of ER with menopausal status.

Although there were positive correlations between ER concentrations and the age of patients and ER and PR concentrations in ER+PR+ tumours, we found no correlation between PR concentration and the age of patients. The association between ER and PR is rather weak, probably reflecting a dependency of PR on nuclear ER (ERn) [7]. Since we found no correlation between the age of patients and the ERn concentration [7], the same might be expected for PR. Separate analyses of premenopausal and postmenopausal patients have shown a positive correlation between the age and concentration of PR in postmenopausal patients [16], or in both premenopausal and postmenopausal; however, there was a with a higher concentration of the receptors in the former group for patients of the same ages [13, 14].

The analysis of coexistence of ER and PR reveals additional differences between premenopausal and postmenopausal patients. Thorpe [5] and Vihko *et al.* [16] found a higher frequency of ER−PR+ and ER−PR− tumours in the former, and that of ER+PR− in the latter group of patients, while the frequency of ER+PR+ was similar in both groups. Contrary to those results, we found a higher frequency of ER+PR+ tumours in patients older than 50. These findings are in agreement with the relation of frequencies of receptor combinations and the age of patients, with the increase of ER+PR+ and ER+PR−, and a decrease of ER−PR+ and ER−PR− tumours [5, 14] (Fig. 2).

The differences in breast cancer ER and PR frequency and content between premenopausal and postmenopausal patients might be due to host hormonal differences or to biological

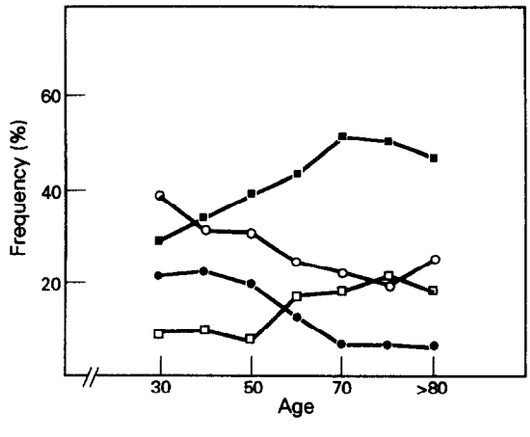

Fig. 2. Relation between frequency of primary breast cancer with various ER and PR combinations and patient ages. ER+PR+ (■) ER+PR− (□) ER−PR+ (●) ER−PR− (○).

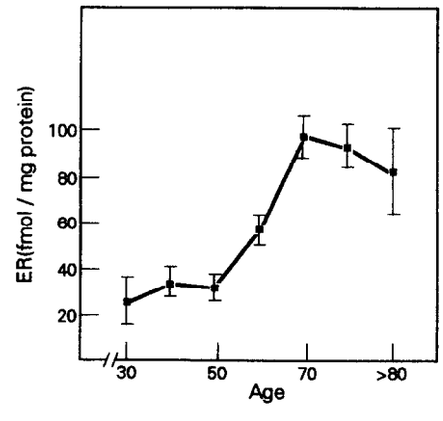

Fig. 3. Relation between patient age and primary breast cancer ER concentrations. Results are means (S.E.)

differences in tumours predominantly occurring in these two groups of patients.

The high frequency of ER+ tumours and high concentration of receptors in postmenopausal patients was attributed to low plasma oestrogen [16, 19] and progesterone [19] concentrations and consequent high concentrations of free ER. The high frequency of ER+PR− and ER+PR+ in this group of patients supported and conflicted with, respectively, this hypothesis. The former receptor combination might be due to insufficient oestrogen effects, while the latter is a consequence of sufficient oestrogen stimulation.

Although it was found that administration of oestrogens [20] could convert ER+PR− to ER+PR+, the differences between tumours with these receptor combinations are probably not solely due to host hormonal milieu. The ER+PR+ group of tumour have a higher mean ER concentration (Table 3) and a higher rate of response to hormonal therapy [21] than the ER+PR− group, indicating different biological features of these tumours.

A higher proportion of ER−PR+ tumours in premenopausal than in postmenopausal patients might be due to higher concentration of oestrogens in the former with consequent saturation of the ER, and therefore a great majority [7, 22] of ER−PR+ tumours are ERn+. Assuming that 83% of ER−PR+ are "false ER−" [7] and functionally belong to the ER+PR+ group, after the data in Table 1 have been corrected, the differences in frequencies of ER+PR+ between premenopausal and postmenopausal patients disappear (55% and 56%, respectively), and the frequency of ER−PR+ tumours become very low (4% and 2%, respectively). The biological similarities of ER+PR+ and ER−PR+ tumours are indicated by a similar rate of recurrence [23]. However, functional differences between these tumours probably exist, which is indicated by a higher mean PR (Table 3) concentration and a higher rate of response to hormone therapy [21] in the former than in the latter group of tumours.

There is sufficient evidence to suggest that differences in ER and PR frequencies and content between breast cancer in premenopausal and postmenopausal women are primarily due to biological differences of tumours with modulating hormonal effects. The differences probably are age dependent rather than related to menopausal status. The presence of ER and PR is associated with a high degree of differentiation [24–26], hormonal sensitivity [21], low growth rate [27], and a low level of oncogene activation [28] and expression [29]. Probably both ER− and ER+ tumours are initiated in premenopause, but the growth of the latter is slower and dependent on oestrogen stimulation, resulting in a positive selection of ER+ tumours with age-related increase in ER frequency and content [30]. Our results showing age-specific increase of ER frequency and content in breast cancer patients between 50 and 70 years of age support this hypothesis.

Assuming that variations in breast cancer incidence in various populations are due mostly to environmental factors [1] acting through oestrogen stimulation [2], the lack of age dependent positive selection of ER+ tumours might be expected in a population with low incidence of breast cancer. Consequently a similar frequency of ER+ tumours in Japanese premenopausal and postmenopausal breast cancer patients [3], as well as the similarity of the curve of total breast cancer incidence against age of the Japanese population, and that of ER− tumours of a western population, likewise support the hypothesis of positive selection of ER+ tumours by an influence of environmental factors, particularly in populations with high incidence of breast cancer [17]. A detailed analysis of epidemiological data of breast cancer characteristics including steroid receptors could help to understand the aetiopathogenesis of the disease. Further population studies of breast cancer characteristics including ER and PR should therefore be encouraged.

1. Willett W. The search for the causes of breast and colon cancer. *Nature* 1989, **338**, 389–394.
2. Key TJA, Pike MC. The role of oestrogens and progestagens in the epidemiology and prevention of breast cancer. *Eur J Cancer Clin Oncol* 1988, **24**, 29–43.
3. Nomura Y, Kobayashi S, Takatani O, Sugano H, Matsumoto K, McGuire WL. Estrogen receptor and endocrine responsiveness in Japanese versus American breast cancer patients. *Cancer Res* 1977, **37**, 106–110.
4. Pegoraro RJ, Nirmul D, Reinach SG, Jordaan JP, Joubert SM. Breast cancer prognosis in three different racial groups in relation to steroid hormone receptor status. *Breast Cancer Res Treat* 1986, **7**, 111–118.
5. Thorpe SM. Estrogen and progesterone receptor determinations in breast cancer. *Acta Oncol* 1988, **27**, 1–19.
6. Horwitz KB, McGuire WL. Progesterone and progesterone receptors in experimental breast cancer. *Cancer Res* 1977, **37**, 1733–1738.
7. Romić-Stojković R, Gamulin S. Relationship of cytoplasmic and nuclear receptors and progesterone receptors in human breast cancer. *Cancer Res* 1980, **40**, 4821–4825.
8. McGuire WL, De La Garza M, Chamness CC. Evaluation of estrogen receptor assays in human breast cancer tissue. *Cancer Res* 1977, **37**, 637–639.
9. Mulder J, Verhaar MAT. A comparative study on estradiol receptor assays in human breast cancer tissue. *Clin Chim Acta* 1979, **99**, 129–134.
10. Nicolò G, Carbone A, Esposito M, Santi L. Sampling and storage of breast cancer tissue for steroid receptor assays. In: Leclercq G, Toma S, Paridaens R, Heuson JC, eds. *Recent Results in Cancer Research*. Berlin, Springer, 1984, Vol. 91, 3–11.
11. Pichon MF, Milgrom E. Characterization and assay of progesterone receptor in human mammary carcinoma. *Cancer Res* 1977, **37**, 464–471.
12. Powell B, Garola RE, Chamnes GC, McGuire WL. Measurement of progesterone receptor in human breast cancer biopsies. *Cancer Res* 1979, **39**, 1678–1682.
13. Clark GM, Osborne CK, McGuire WL. Correlations between estrogen receptor, progesterone receptor, and patient characteristics in human breast cancer. *J Clin Oncol* 1984, **2**, 1102–1109.
14. Wilking N, Rutqvist LE, Nordenskjöld B, Skoog L. Steroid receptor levels in breast cancer. *Acta Oncol* 1989, **28**, 807–810.
15. Martin PM, Rolland PH, Jacquemier J, Rolland AM, Toga M. Multiple steroid receptors in human breast cancer. *Cancer Chemother Pharmacol* 1979, **2**, 107–113.
16. Vihko R, Jänne O, Kontula K, Syrjälä P. Female sex steroid receptor satus in primary and metastatic breast carcinoma and its relationship to serum steroid and peptide hormone levels. *Int J Cancer* 1980, **26**, 13–21,
17. Elwood JM, Godolphin W. Oestrogen receptors in breast tumours: associations with age, menopausal status and epidemiological and clinical features in 735 patients. *Br J Cancer* 1980, **42**, 635–644.
18. Allegra JC, Lippman ME, Thompson EB, *et al.* Distribution, frequency, and quantitative analysis of estrogen, progesterone, androgen and glucocorticoid receptors in human breast cancer. *Cancer Res* 1979, **39**, 1447–1454.
19. Saez S, Martin PM, Chouvet CD. Estradiol and progesterone receptor levels in human breast adenocarcinoma in relation to plasma estrogen and progesterone levels. *Cancer Res* 1978, **38**, 3488–3473.
20. Degenshein GA, Bloom N, Tobin E. The value of progesterone receptor assays in the menagement of advanced breast cancer. *Cancer* 1980, **46**, 2789–2793.
21. Wittliff JL. Steroid-hormone receptors in breast cancer. *Cancer* 1984, **53**, 630–643.
22. Thorpe SM. Immunological quantitation of nuclear receptors in human breast cancer: relation to cytosolic estrogen and progesterone receptors. *Cancer Res* 1987, **47**, 1830–1835.

23. Mason BH, Holdaway IM, Mullins PR, Yee LH, Kay RG. Progesterone and estrogen receptors as prognostic variables in breast cancer. *Cancer Res* 1983, **43**, 2985–2990.
24. McCarty KS, Barton TK, Fetter BF, *et al.* Correlation of estrogen and progesterone receptors with histologic differentiation in mammary carcinoma. *Cancer* 1980, **46**, 2851–2858.
25. Mohammed RH, Lakatua DJ, Haus E, Yasmineh WJ. Estrogen and progesterone receptors in human breast cancer. Correlation with histologic subtype and degree of differentiation. *Cancer* 1986, **58**, 1076–1081.
26. Coulson PB, Thornthwaite JT, Woolley TW, Sugarbaker EV, Seckinger D. Prognostic indicators including DNA histogram type, receptor concent, and staging related to human breast cancer patient survival. *Cancer Res* 1984, **44**, 4187–4196.
27. Vollmer G, Gerdes J, Knuppen R. Relationship of cytosolic estrogen and progesterone receptor content and the growth fraction in human mammary carcinomas. *Cancer Res* 1989, **49**, 4011–4014.
28. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987, **235**, 177–182.
29. Delaure JC, Friedman S, Mouriesse H, *et al.* Epidermal growth factor receptor in human breast cancers: correlation with estrogen and progesterone receptors. *Breast Cancer Res Treat* 1988, **11**, 173–178.
30. Simpson HW, Pauson AW, Griffiths K, Candlish W, McArdle CS, Small RG. Genesis of breast cancer in the premenopause. *Lancet* 1988, i, 74–76.

Acknowledgement—This work has been supported by Research Council of SR Croatia grant No. 1. 04.01.01.103.

Eur J Cancer, Vol. 27, No. 4, pp. 495–498, 1991.
Printed in Great Britain

0277-5379/91 $3.00 + 0.00

Pulmonary Tumour Embolism from Squamous Cell Carcinoma of the Oesophagus

Fernando A. Soares, Glaucia A. Magnani Landell and Jose A. Mello de Oliveira

Pulmonary tumour embolism and subacute "cor pulmonale" have been reported in association with tumours of different origins. Even though these features were first described in a patient with carcinoma of the oesophagus, the frequency and importance of oesophageal tumours as the source of pulmonary tumour embolism have not been studied. In the present investigation, the lungs of 16 autopsied patients with squamous cell carcinoma of the oesophagus were studied prospectively. The lungs were removed as a block and 15 sections (3 from each lobe) were analysed. Pulmonary tumour embolism was detected in 7 cases. The lymphatic vessels were involved in all of them, and were associated with arteries and arterioles in 2. 2 patients presented a classical picture of subacute cor pulmonale, and dyspnoea was present in 3 other cases. The present study permitted us to conclude that carcinomas of the oesophagus frequently evolve toward carcinomatous lymphangitis and that pulmonary tumour embolism should be included in the differential diagnosis of the dyspnoea presented by the patients.
Eur J Cancer, Vol. 27, No. 4, pp. 495–498, 1991

INTRODUCTION

PULMONARY TUMOUR embolism was first described in 1868 by Bristowe, who reported the development of secondary pulmonary arterial hypertension in a patient with carcinoma of the oesophagus [1]. The author correlated the clinical picture with the presence of intense carcinomatous lymphangitis. In 1937, Brill and Robertson developed the concept of subacute cor pulmonale as an entity characterised by the rapid development of symptoms of right congestive heart failure in patients with no previous history of cardiopulmonary disease or other conditions triggering right ventricular failure [2]. Several studies of pulmonary vessel involvement secondary to tumours have been conducted over the years, including large series in retrospective studies [3–6].

With respect to the primary tumour sites that most frequently cause the involvement of lymphatic and blood vessels of the lung, the literature always placed strong emphasis on the stomach [3, 4, 7–12]. Other organs have also been indicated, such as the breast [3, 6, 13, 14], the lung itself [5], the liver [3, 15, 16] and the kidney [3, 6, 17]. Isolated cases of development of subacute cor pulmonale from tumours originating in practically all body organs have been reported. To our knowledge, no studies or even case reports of pulmonary vascular involvement in carcinoma of the oesophagus have been published.

For this reason, in the present investigation we studied prospectively 16 consecutive autopsies of patients with primary squamous cell carcinoma of the oesophagus in terms of the development of pulmonary tumour embolism and its clinicopathological manifestations.

MATERIAL AND METHODS

Pulmonary vessel tumour involvement was studied in 16 consecutive autopsies performed between 1986 to 1989 in patients with squamous cell carcinoma of the oesophagus in the Department of Pathology, Faculty of Medicine of Ribeirão Preto, University of São Paulo.

Clinical data concerning the presence or absence of dyspnoea, cyanosis and right congestive heart failure and the modality of treatment were obtained from medical records of the patients.

Correspondence to F.A. Soares.
The authors are at the Department of Pathology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Av. Bandeirantes 3900, 14049 Ribeirão Preto SP, Brazil.
Revised 30 Nov. 1990; accepted 25 Jan. 1991.