Predictive value of SCC-Ag, CYFRA 21-1 and selected acute phase proteins in radiotherapy of pharyngeal and laryngeal cancer. A preliminary report

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The diagnostic sensitivity and specificity of tumor markers in head and neck cancers is not satisfactory. It is a stimulus for search of other biochemical indicators, among others determinations of acute phase proteins, helpful in head and neck cancers diagnostics and prognosis. In a group of 33 patients with squamous cell carcinoma of the pharynx and larynx (T_{1-4} N_{0-3} M_0) CYFRA 21-1, SCC-Ag and acute phase proteins such as prealbumin, albumin, alpha-1 acid glycoprotein, alpha-1 antytrypsin, transferrin, ceruloplasmin and C-reactive protein (CRP) determinations were performed before radiotherapy. Significantly greater area under ROC curve for CYFRA 21-1 than for SCC-Ag was found. In T_{3-4} group, significantly lower albumin and higher AAG and CRP concentrations in comparison with T_{1-2} patients were observed. Multivariate analysis revealed that apart from tumor stage, elevated concentrations of SCC-Ag and/or CRP are independent unfavorable prognostic factors.

Key words: pharynx and larynx cancer, CYFRA 21-1, SCC-Ag, acute phase proteins, prognostic value

For many years, head and neck cancers have been counted among relatively frequent malignancies in men, whereas at present there an increase in newly registered cases in women is observed. In respect to histology, the most frequent type is squamous cell cancer [17, 22]. The marker of choice for head and neck cancer in respect to dominant histologic type, seems to be the squamous cell carcinoma antigen (SCC-Ag). Sensitivity of this marker ranges from 10 to 50% [4, 10, 12, 16, 25]. Such great differences in the estimation of this value might be significantly affected by selection of patients in the studied groups, because marker concentrations and frequency of its elevated results depend on stage and grade of malignancy. Recently, trials were undertaken for evaluation of usefulness of CYFRA 21-1, soluble fragment of cytokeratin 19 levels in head and neck cancer diagnostics. The results of its determinations are characterized by high diagnostic sensitivity in non-small cell lung cancer patients, especially in squamous cell cancer [9, 20, 23]. According to the data originating from many research centers, diagnostic sensitivity of this marker in head and neck cancer patients ranges from 15 to 60% [1, 2, 3]. Limited diagnostic usefulness of SCC-Ag and CYFRA 21-1 presents a stimulus for search of other biochemical indicators, the results of which would give additional information in head and neck cancer diagnostics as well as prognosis assessment [15, 26]. In this aspect many acute phase proteins seem to be interesting.

The aim of this study is to evaluate potential usefulness of CYFRA 21-1, SCC-Ag, and selected acute phase proteins as prognostic factors in head and neck cancer patients undergoing radiotherapy.

Material and methods

The determinations of circulating tumor markers CYFRA 21-1, and SCC-Ag and acute-phase proteins such as

Abbreviations: SCC-Ag – squamous cell carcinoma antigen, CYFRA 21-1 – soluble fragment of cytokeratin 19, PRE – prealbumin, ALB – albumin, AAG – alpha-1 acid glycoprotein, AAT – alpha-1 antitrypsin, TRF – transferrin, CER – ceruloplasmin, CRP – C-reactive protein, ECLIA – electrochemiluminescence immunoassay, MEIA – microparticle enzyme immunoassay, ROC – receiver operating characteristics, TPS – tissue polypeptide-specific antigen, Il – interleukin, TNF – tumor necrosis factor.

prealbumin (PRE), albumin (ALB), alpha-1 acid glycoprotein (AAG), alpha-1 antitrypsin (AAT), transferrin (TRF), ceruloplasmin (CER) and C-reactive protein (CRP) were performed before treatment in a selected group of 33 patients with histologically confirmed squamous cell carcinoma of the pharynx and larynx stage $T_{1-4}\,N_{0-3}\,M_0$, qualified to radical radiotherapy at the Center of Oncology in Cracow, between 1996 and 1998 (Tab. 1 and 2). Follow-up time was 19–83 months. Sex-, age-, and the percentage of smokers-matched group of 40 healthy subjects was a reference group.

Peripheral venous blood samples were obtained from all patients and controls by a standard procedure, between 8 and 9 a.m., in fasting state. The serum specimens obtained by centrifugation were divided into portions and stored at –25 °C until assayed. CYFRA 21-1 concentration was determined by electrochemiluminescence immunoassay (ECLIA), using the Roche Diagnostics reagent kits and Elecsys 2010 analyzer. The level of SCC-Ag was assayed by enzymeimmunological method in MEIA technology with commercial kit and IMx system from Abbott Laboratories. Serum PRE, AAG, AAT, TRF, CER and CRP concentrations were measured by immunonephelometric technique, using monoclonal antibody, calibrators and BN II nephelometer of Dade Behring.

The albumin levels were determined on the basis of total protein concentrations measured by a biuret method and the percentage of electrophoretical fractions calculated after separating proteins on agarose gel.

The statistical evaluation of differences between the results in the investigated groups was based on non-parametric Kruskal-Wallis test. In the analysis of relationships between concentrations of determined parameters the Pearson's correlation coefficient was calculated. The probability of patients survival related to the concentrations of analysed tumor markers and proteins before treatment, were estimated by Kaplan-Meier method using univariate analysis based on log-rank test. The Cox's proportional hazard model was used to determine the prognostic value of the investigated variables by multivariate analysis.

Table 1. Stage of disease according to TNM UICC 1997 classification

	N_0	N_{1-3}	M
T_1	7	1	0
T_2	11	0	0
T_3	3	1	0
T_4	4	6	0

Table 2. Treatment schedule

Technique	Laryngeal cancer	Pharyngeal cancer
2 oblique wedged fields 60 Gy/24 fr. (^{60}Co)	6	–
2 opposed lateral fields 60 Gy/30 fr. (^{60}Co) (boost to 70 Gy)	12	12
1 lateral mixed field* 60 Gy/30 fr.	3	–

*30 Gy given with ^{60}Co gamma rays, and 30 Gy with 15 MeV electrons

Results

Markedly higher concentrations of CYFRA 21-1 and SCC-Ag were found in squamous pharynx and larynx cancer patients, in comparison to the reference group (Tab. 3). Higher than cut off value (2.6 and 1.5 μg/L, respectively) concentrations of CYFRA 21-1 and SCC-Ag were found in 27.3% and 24.2% of patients. The area under ROC curves for results of CYFRA 21-1 in squamous cell pharynx and larynx cancer patients, was significantly greater than for SCC-Ag (Fig. 1).

With lack of differences between the reference group and pharynx and larynx cancer patients, for AAG, TRF, and CER levels, significantly higher AAT, CRP, lower PRE and ALB concentrations were observed. Whereas elevated concentrations of AAT and CRP were observed in 66.7%

Table 3. Concentrations of analyzed parameters in patients with pharynx and larynx squamous cell cancer and in the reference group

PARAMETERS			Pharynx and larynx cancer	Reference group	p value
CYFRA 21-1	[μg/L]	Me range	2.29 0.47–14.74	1.30 0.27–2.84	0.0000
SCC-Ag	[μg/L]	Me range	1.00 0.10–3.10	0.9 0.30–2.10	0.0377
PRE	[g/L]	Me range	0.23 0.02–0.42	0.29 0.21–0.49	0.0001
Albumin	[g/L]	Me range	38.6 27.1–46.1	43.2 34.6–49.0	0.0000
AAG	[g//L]	Me range	0.82 0.49–1.59	0.82 0.45–1.37	NS
AAT	[g/L]	Me range	2.09 0.51–5.56	1.28 0.88–1.74	0.0000
TRF	[g/L]	Me range	2.14 0.45–2.61	2.22 1.48–3.00	NS
CER	[g/L]	Me range	0.35 0.07–0.64	0.32 0.21–0.47	NS
CRP	[mg/L]	Me range	5.6 0.4–75.2	0.9 0.8–9.5	0.0000

Table 4. Concentrations of determined parameters in patients with pharynx and larynx squamous cell depending on tumor stage

PARAMETERS			T_{1-2}	T_{3-4}	p value
CYFRA 21-1	[μg/L]	Me	2.16	2.29	NS
		range	0.50–4.97	0.47–14.74	
SCC-Ag	[μg/L]	Me	1.10	0.95	NS
		range	0.10–3.10	0.70–2.90	
PRE	[g/L]	Me	0.18	0.24	NS
		range	0.02–0.38	0.02–0.42	
Albumin	[g/L]	Me	39.4	36.35	0.0038
		range	33.5–46.1	27.1–39.4	
AAG	[g//L]	Me	0.78	1.04	0.0034
		range	0.49–1.18	0.68–1.59	
AAT	[g/L]	Me	2.08	2.29	NS
		range	1.12–3.97	0.51–5.56	
TRF	[g/L]	Me	2.14	2.10	NS
		range	1.43–2.61	0.45–2.46	
CER	[g/L]	Me	0.33	0.45	NS
		range	0.07–0.56	0.08–0.64	
CRP	[mg/L]	Me	5.0	8.84	0.0445
		range	0.35–17.9	0.9–75.2	

Table 5. Concentrations of analyzed parameters in patients with pharynx and larynx squamous cell cancer depending on lymphatic nodes status

PARAMETERS			N_0	N_{1-3}	p value
CYFRA 21-1	[μg/L]	Me	2.16	2.31	NS
		range	0.47–4.97	0.91–14.74	
SCC-Ag	[μg/L]	Me	1.20	0.90	NS
		range	0.10–3.10	0.70–2.90	
PRE	[g/L]	Me	0.23	0.20	NS
		range	0.02–0.38	0.03–0.42	
Albumin	[g/L]	Me	38.9	34.8	0.0208
		range	31.5–46.1	27.1–39.4	
AAG	[g/L]	Me	0.82	0.97	NS
		range	0.49–1.59	0.58–1.17	
AAT	[g/L]	Me	2.08	2.35	NS
		range	0.51–4.71	1.59–5.56	
TRF	[g/L]	Me	2.14	2.02	NS
		range	0.45–2.61	1.54–2.46	
CER	[g/L]	Me	0.35	0.50	NS
		range	0.07–0.56	0.25–0.64	
CRP	[mg/L]	Me	5.4	11.1	NS
		range	0.9–75.2	0.35–41.6	

and 75.8% of patients, respectively, lower than 34.0 g/L ALB and lower than 0.20 g/L PRE were found in 24.2% and 45.5% of patients, respectively. In the studied group, no significant relationships were observed between tumor markers and acute phase proteins, however CRP concentration correlated with other acute phase proteins.

Analysing concentrations of the determined factors in respect to tumor stage (T_{1-2} vs. T_{3-4}), significantly lower ALB and higher AAG and CRP concentrations were found in more advanced group of patients, with lack of significant differences in CYFRA 21-1, SCC-Ag and remaining acute phase proteins (Tab. 4). The percentages of elevated CYFRA 21-1 and SCC-Ag concentrations in T_{1-2} and T_{3-4} groups were 31.6%, 26.3% and 21.4%, 21.4%, respectively while for AAG, AAT and CRP they were 5.3%, 63.2%, 68.4% and 21.4%, 71.4%, 85.7%. Hypoalbuminemia was observed in 10.5% of T_{1-2} and 42.9% of T_{3-4} patients, and PRE concentrations lower than 0.20 g/L in 52.6% and 35.7% of patients, respectively.

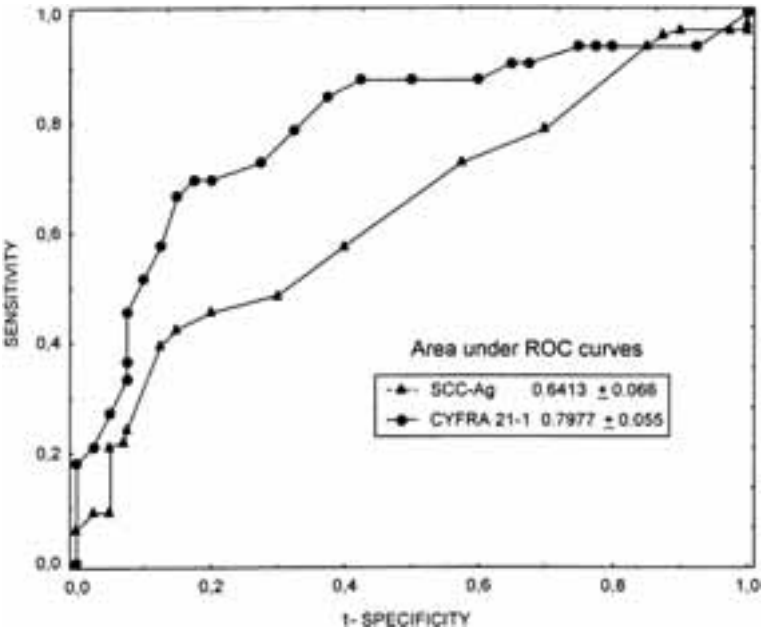

Figure 1. ROC curves for CYFRA 21-1 and SCC-Ag in patients with squamous cell carcinoma of pharynx and larynx.

Except for albumin, the concentration of which in patients with involved lymphatic nodes (N_{1-2}) was markedly lower than in those without (N_0), there were no significant differences in levels of remaining factors, between groups selected in respect of lymphatic glands status (Tab. 5). Per-

Table 6. Correlation between the analyzed biochemical/clinical parameters and 60 months survival

Parameter		Univariate analysis Variant	p value	Multivariate analysis RR	p value
CYFRA21-1	[μg/L]	<2.6/>2.6	NS	–	–
SCC-Ag	[g/L]	<1.5/>1.5	0.03551	6.83	0.002252
PRE	[g/L]	<0.20/>0.20	NS	–	–
Albumin	[g/L]	<35.0/>35.0	0.03302	–	NS
AAG	[g/L]	<1.2/>1.2	NS	–	–
AAT	[g/L]	<1.9/>1.9	NS	–	–
TRF	[g/L]	<2.0/>2.0	NS	–	–
CER	[g/L]	<0.58/>0.58	NS	–	–
CRP	[mg/L]	<18.0/>18.0	0.03263	–	NS
Tumor stage		T_{1-2}/T_{3-4}	0.03586	5.81	0.004853
Lymph nodes status		N_0/N_{1-2}	NS	–	–

Table 7. Correlation between the analyzed biochemical/clinical parameters and 60 months survival

Parameter	Univariate analysis Variant	p value	Multivariate analysis RR	p value
CYFRA 21-1 and SCC- Ag	Normal vs. Cyfra 21-1 elevated and/or SCC-Ag elevated	NS	–	NS
CYFRA 21-1 and CRP	Normal vs. Cyfra 21-1 elevated and/or CRP elevated	0.02509	–	NS
CYFRA 21-1 and albumin	Normal vs. Cyfra 21-1 elevated and/or hypoalbuminemia	NS	–	NS
SCC- Ag and albumin	Normal vs. SCC-Ag elevated and/or hypoalbuminemia	0.01839	–	NS
SCC-Ag and CRP	Normal vs. SCC-Ag elevated and/or CRP elevated	0.01366	8.06	0.000741
Tumor stage	T_{1-2} vs.T_{3-4}	0.03586	4.23	0.011348

centages of elevated CYFRA 21-1 and SCC-Ag concentrations were similar in both groups; 28.0%, 24.0% and 25.0%, 25.0%, respectively. Hypoalbuminemia was observed in 16.0% of N_0 and in 50.0% of the involved lymphatic nodes group, and PRE concentration lower than 0.20 μg/L, in 44.0% and 50.0% of patients, respectively.

Univariate analysis has shown significant relationships between survival and stage of disease, pretreatment SCC-Ag, albumin, and C reactive protein concentration (Tab. 6). Patients with stage T_{3-4} tumors, SCC-Ag higher than 1.5 g/L, hypoalbuminemia, and CRP concentration higher than 18.0 mg/L had significantly shorter survival (Fig. 2, 3, 4, 5). Multivariate analysis has shown that in patients with squamous cell pharyngeal and laryngeal cancer qualified for radical radiotherapy, apart from tumor stage, elevated SCC-Ag concentration is an independent unfavorable prognostic factor. The relationships between survival and complementary results of CYFRA 21-1 and SCC-Ag determinations, as well as of each of these markers, with albumin or CRP in complex, were analysed. It was found that patients with elevated concentrations of SCC-Ag and/or CRP, elevated SCC-Ag and/or hypoalbuminemia and elevated CYFRA 21-1 and/or CRP are characterized by significantly shorter survival than those with normal levels of these factors (Tab. 7). The median survival of patients with elevated SCC-Ag and/or CRP concentrations was 10 months, compared with that of the remaining patients, exceeding 60 months, whereas in the group with elevated SCC-Ag and/or hypoalbuminemia it was 26 months, and over 60 months in others. Multivariate analysis revealed that apart from tumor stage, elevated concentration of SCC-Ag and/or CRP are also independent, however non-specific, unfavorable prognostic factor.

Discussion

There are no markers with organ specificity and satisfactory diagnostic sensitivity and specificity for head and neck cancer. Big differences in values of diagnostic sensitivity, found in the relevant literature, resulted mostly from various clinical composition of different study groups, and different cut off values accepted for interpretation of results [1, 3, 16, 19]. Frequency of elevated markers concentrations usually stay in clear dependence on the stage of disease and is the highest in patients with metastases. It should be pointed out that our investigations were made in a selected group of patients, more than a half of which had stage T_{1-2} N_0 M_0 cancer. Therefore, the percentages of elevated SCC-Ag and CYFRA 21-1 concentrations were rather low i.e. 24.2% and 27.3%, respectively. Some investigators suggest that rather low markers concentrations in head and neck cancer patients may be due to relatively slow cell proliferation, others consider that the squamous cancer cells may have limited ability of producing tumor markers or – for anatomical reasons – only small amounts of markers may be released to the circulation [15, 26]. In the present study, the results of CYFRA 21-1 have higher diagnostic sensitivity than SCC-Ag. The under ROC curves area for CYFRA

21-1 was significantly greater than for SCC-Ag and was identical with that presented by BANAL et al [1]. In their study, however, more than a half of patients had stage IV disease. The opinions differ in relation to dependence of concentration and frequency of elevated markers results in respect of stage, tumor size, lymphatic nodes status, and histological grade [1, 16, 19]. In the studied group there was a tendency to higher CYFRA 21-1 concentrations in patients with stage T_{3-4} cancer though no statistical significance was found. No such a tendency for SCC-Ag results was also observed.

The information in relation to prognostic value of CYFRA 21-1 in head and neck cancer is rather rare, and it still should be a subject of verification, although some investigators underline the usefulness of this marker determinations in monitoring of treatment and recurrence detecting [1, 3]. In the studied group, no significant differences between pretreatment levels of CYFRA 21-1 and probability of survival were observed. Similarly, in the literature, there is little information in relation to SCC-Ag usefulness in prognosis of head and neck cancer patients. MOLINA et al [13] found correlation between elevated SCC-Ag concentration and shorter asymptomatic survival. They suggest usefulness of complementary to SCC-Ag determination of TPS (tissue polypeptide-specific antigen) for improvement of diagnostic efficacy, especially for recurrence detecting. Our preliminary results seem to confirm the usefulness of SCC-Ag in prognosis assessment for patients with elevated SCC-Ag concentrations who have a shorter survival. Elevated SCC-Ag and/or CRP concentrations appeared to be strong negative prognostic factors in radiotherapy of patients with laryngeal and pharyngeal cancer. One of the fields focusing interest in aspect of improvement of biochemical diagnostic efficacy of head and neck cancer, are the disturbances of host homeostasis connected with the response to cancer. Cancer is a traumatic stimulus which apart from infection, mechanical or thermal trauma, causes a complex of non-specific reactions, which by many mediators lead to the systemic reaction. One of the characteristic symptoms for this status are severe changes in specific proteins levels - positive or negative reactants of acute phase [6, 7, 11, 18, 21, 24]. Although these changes are non specific for head and neck cancer, their direction and intensity shows strong connection with the stage of disease and immunological status of patients. Significant role in the mechanisms causing

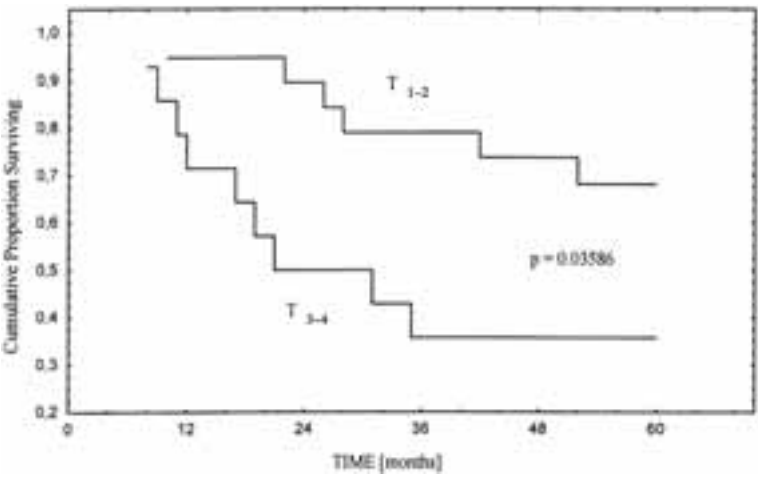

Figure 2. Survival probability of pharyngeal and laryngeal cancer patients according to tumor stage.

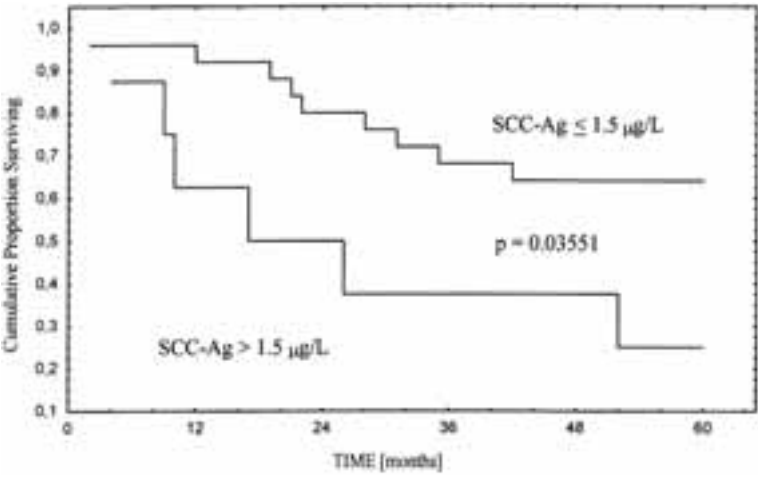

Figure 3. Survival probability of pharyngeal and laryngeal cancer patients according to the pretreatment concentration of SCC-Ag.

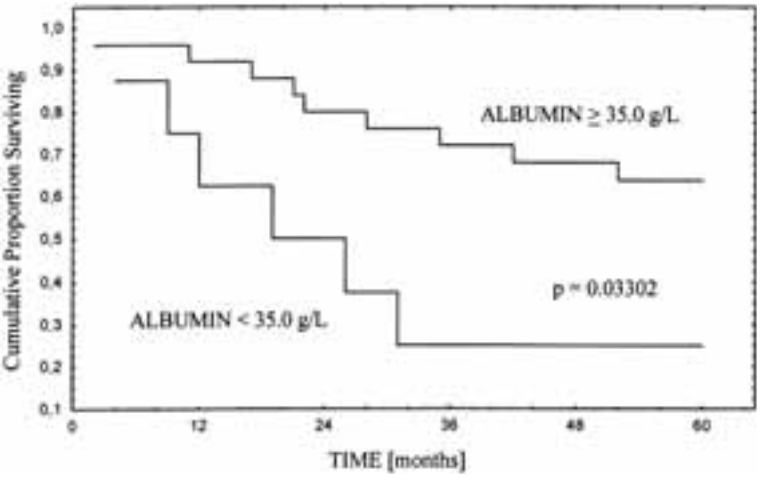

Figure 4. Survival probability of pharyngeal and laryngeal cancer patients according to the pretreatment concentration of albumin.

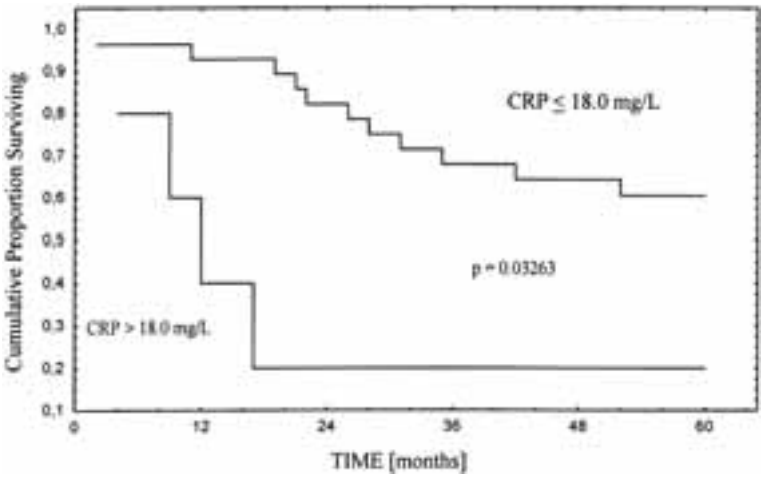

Figure 5. Survival probability of pharyngeal and laryngeal cancer patients according to the pretreatment CRP level.

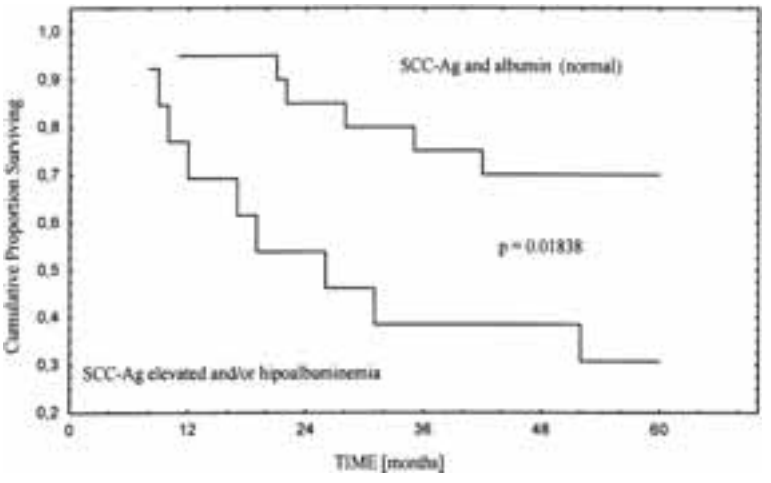

Figure 6. Survival probability of pharyngeal and laryngeal cancer patients according to the pretreatment SCC-Ag level and/or hypoalbuminemia.

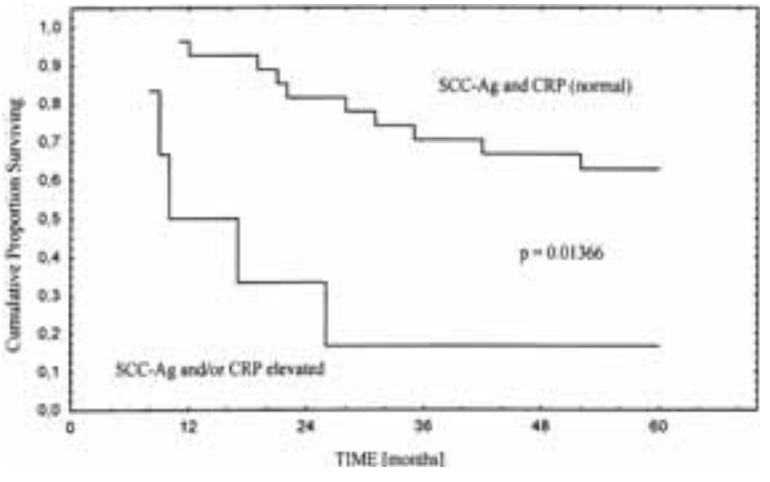

Figure 7. Survival of pharyngeal and laryngeal cancer patients according to the pretreatment SCC-Ag and/or CRP levels.

these changes is attributed to some proinflammatory cytokins e.g. Il-1, Il-6, TNF-α. In head and neck cancer patients, a strong correlation between Il-6 concentration and CRP level was found [5]. Elevated concentration of this protein was also observed in different types of cancer, underlining the significance of this status for patients prognosis. In a considerable percentage of the studied group, elevated CRP concentration was observed revealing significant connection with the total time of [6, 18]. Elevated AAG concentration is recognized as an index of cell proliferation and aggressiveness of cancer [14]. Considering that in only 9.1% of patients elevated AAG concentration was observed it may confirm an opinion suggested by some investigators, that in head and neck cancer there is low cell proliferation.

Prealbumin and albumin are recognized as markers of nutrition status. It is underlined that relatively long half life time of albumin made changes of its concentrations be less effective in nutrition assessment [6, 8, 18]. In the opinion of some researchers the frequency of lowered prealbumin and albumin concentrations in head and neck cancer do not depend on the stage of disease but nutrient intake [26]. Our own observations are not in agreement with these opinions. Hypoalbuminemia was found in 24% of patients, frequency of its occurrence was depended on tumor stage, and status of the lymphatic nodes. Low prealbumin concentration was observed in almost a half of patients, and percentage of lowered levels showed increasing trends in dependence on tumor stage and lymphatic nodes status.

Conclusion

Complementary to SCC-Ag determination of CRP, and albumin give additional information regarding prognosis of squamous cell laryngeal and pharyngeal cancer patients qualified for radiotherapy. Elevated SCC-Ag and/or CRP concentrations are negative prognostic factors in radiotherapy of squamous cell laryngeal and pharyngeal cancer patients.

References

[1] BANAL A, HACENE K, BERTHELOT-RUFF E, MAHE E, FONTANA X, PICHON MF. Comparison of Cyfra 21-1 and SCC assays in head and neck tumors. Tumor Biol 2001; 22: 27–35.

[2] BONGERS V, BRAAKHUIS BJM, SNOW GB. Circulating fragments of cytokeratin 19 in patients with head and neck squamous cell carcinoma. Clin Otolaryngol 1995; 20: 479–482.

[3] DOWECK I, BARAK M, GREENBERG E. The prognostic value of the tumour marker Cyfra 21-1 in carcinoma of the head and neck and its role in early detection of recurrent disease. Br J Cancer 2000; 83: 1696–1701.

[4] FICHBACH N, MEYER T, BARTHEL K. Squamous cell carcinoma antigen in the diagnosis and treatment follow-up of oral and facial squamous cell carcinoma. Cancer 1990; 65: 1321–1324.

[5] GALLO O, GORI AM, ATTANASIO M, MARTINI F, GIUSTI B et al. Interleukin-6 and acute-phase proteins in head and neck cancer. Eur Arch Otorhinolaryngol 1995; 252: 159–162.

[6] GORANSSON J, JONSSON S, LASSON A. Pre-operative plasma levels of C-reactive protein, albumin and various plasma protease inhibitors for the pre-operative assessment of operability and recurrence in cancer surgery. Eur J Surg Oncol 1996; 22: 607–617.

[7] HANNISDAL E, TVEIT KV, THEODORSEN L, HOST H. Host markers and prognosis in recurrent rectal carcinomas treated with radiotherapy. Acta Oncologica 1994; 33: 415–421.

[8] JAMES WPT, HAY AM. Albumin metabolism: Effect of the nutritional state and the dietary protein intake. J Clin Invest 1968; 47: 1958–1971.

[9] KULPA J, WÓJCIK E, REINFUSS M, KOLODZIEJSKI L. Carcinoembryonic antigen, squamous cell carcinoma antigen, CYFRA 21-1, and neuron-specific enolase in squamous cell lung cancer patients. Clin Chem 2002; 48(11): 1931–1937.

[10] LARA PC, CUYAS JM. The role of squamous cell carcinoma antigen in the management of laryngeal and hypopharyngeal cancer. Cancer 1995; 76: 758–764.

[11] LJUNGBERG B, GRANKVIST K, RASMUSON T. Serum acute phase reactants and prognosis in renal cell carcinoma. Cancer 1995; 76: 1435–1439.

[12] MOLINA R, FILELLA X, TORRES MD, BALLESTA AM, MENGUAL P et al. SCC antigen measured in malignant and nonmalignant diseases. Clin Chem 1990; 36: 251–254.

[13] MOLINA R, TORRES MD, MORAGAS M, FILELLA X, JO J et al. Prognostic value of TPS in patients with head and neck malignancies: comparison with SCC. Anticancer Res 1995; 15: 479–484.

[14] MULLER T, MARSHALL RJ, COOPER EH, WATSON DA, WALKER DA, MEARNS AJ. The role of serum tumour markers to aid the selection of lung cancer patients for surgery and the assessment of prognosis. Eur J Cancer Clin Oncol 1985; 21: 1461–1466.

[15] NAKASHIMA T, TANAKA M, OKAMURA S-I. Survey of immunosuppressive acidic protein and other immunological parameters in head and neck cancer patients. J Laryngol Otol 1991; 105: 939–945.

[16] OGAWA T, TSURUSAKO Y, KIMURA N, NISHIOKA S, AKAGI H et al. Comparison of tumor markers in patients with squamous cell carcinoma of the head and neck. Acta Otolaryngol 1999; Suppl 540: 72–76.

[17] OVERGAARD J, HANSEN HS, JORGENSEN K, HANSEN MH. Primary radiotherapy of larynx and pharynx carcinoma-an analysis of some factors influencing local control and survival. Int J Radiat Oncol Biol Phys 1986; 12(4): 515–521.

[18] PINILLA JC, HAYES P, LAVERTY W, ARNOLD C, LAXDAL V. The C-reactive protein to prealbumin ratio correlate with the severity of multiple organ dysfunction. Surgery 1998; 124: 799–806.

[19] PRADIER O, HILLE A, SCHMIBERGER H, HESS CF. Monitoring of therapy in head and neck patients during the radiotherapy by measurement of Cyfra 21-1. Cancer/Radiother 2002; 6: 15–21.

[20] PUJOL JL, GRENIER J, DAURES JP, DAVER A, PUJOL H, MICHEL FB. Serum fragment of cytokeratin 19 measured by Cyfra 21-1 immunoradiometric assay as a marker of lung cancer. Cancer Res 1993; 53: 61–66.

[21] SNYDER S, ASHWELL G. Quantitation of specific serum glycoprotein in malignancy. Clin Chimica 1971; 34: 449–455.

[22] SPITZ MR. Epidemiology and risk factors for head and neck cancer. Semin Oncol 1994; 21(3): 281–288.

[23] STIEBER P, HASHOLZNER U, BODENMULLER H, NAGEL D, SUNDER-LASSMANN L et al. Cyfra 21-1 a new marker in lung cancer. Cancer 1993; 72: 707–713.

[24] VELTRI RW, RODMAN SM, MAXIM PE, BASELER MW, SPRINKLE PM. Immune complexes, serum proteins, cell-mediated immunity, and immune regulation in patients with squamous cell carcinoma of the head and neck. Cancer 1986; 57: 2295–2308.

[25] WALTHER EK, DAHLMANN N, GORGULLA HT. Tumour markers in the diagnosis and follow-up of head and neck cancer: role of CEA, CA 19-9, SCC, TK, and DTTPASE. Head & Neck 1993; 15: 230–235.

[26] WOLF GT, CHRIETIEN PB, ELIES EG, MAKUCH RW, BASKIES AM et al. Serum glycoproteins in head and neck squamous carcinoma. Correlations with tumor extent, clinical tumor stage and T-cell levels during chemotherapy. Am J Surg 1979; 138: 489–500.