

REVIEW

Serum tumour markers in lung cancer: history, biology and clinical applications

D. Ferrigno*, G. Buccheri*, A. Biggi**

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ABSTRACT: The association of biological markers with cancer has been recognized for many decades. Current interest in markers for cancer arose in the mid 1960s, with the discoveries of alpha-fetoprotein and carcinoembryonic antigen. They were called oncofetal proteins, because of their presence in high concentrations during embryonic development, their virtual disappearance in the neonatal period, and their reappearance with cancers of specific cell types. Essentially, any molecular species may be produced in abnormal amounts or under abnormal circumstances by a tumour, and thereby become useful as a tumour marker.

Several tumour markers have been studied in lung cancer. Unfortunately, none of these appear to be sufficiently sensitive and specific to be reliable for screening and diagnostic purposes. However, there is a body of evidence which proves that at least some of these substances may be useful in the evaluation of the course and prognosis of the disease.

This review presents data concerning the most studied and interesting tumour markers in lung cancer.

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Tumour markers (TMs) are substances, usually of peptide nature, secreted by tumour cells. These substances are normally absent in the serum (or present in very low concentrations), since they are not secreted (or are secreted in very small amounts) by normal cells. COOMBS and co-workers [1, 2] defined the potential clinical applications of a tumour marker. It should: 1) facilitate the early diagnosis of tumour; 2) offer a guide for the evaluation of prognosis; 3) help in selecting patients for adjuvant chemotherapy; and 4) help in assessing the response to therapy and in diagnosing early relapses.

Moreover, the serum level of an ideal marker should: 1) increase pathologically in the presence of a neoplasm (high sensitivity); 2) not increase in the absence of neoplasms (high specificity); 3) relate to tumour burden and metastatic spread; 4) change in accordance with the clinical evolution, reflecting the current status of disease; or, better, 5) anticipate clinical changes, e.g. indicating the presence of relapse before it becomes obvious at a clinical level.

List of abbreviations: ACTH=adrenocorticotrophic hormone; ADH=antidiuretic hormone; BN=bombesin; CA-19-9=carbohydrate antigenic determinant 19-9; CEA=carcinoembryonic antigen; CPK-BB=creatine phosphokinase-BB; CT=calcitonin; ED=extensive disease; GRP=gastrin releasing peptide; IGF=insulin-like growth factors; IL-2=interleukin-2; LD=limited disease; NNE=nonneuronal enolase; NSCLC: non small cell lung cancer; NSE=neurone-specific enolase; POMC=pro-opiomelanocortin; SCLC: small cell lung cancer; SCC: squamous cell carcinoma; SCC-ag=squamous cell carcinoma antigen; sIL-2R=soluble interleukin-2 receptor; TM=tumour marker; TPA=tissue polypeptide antigen.

*Dept of Respiratory Medicine, A. Carle Hospital, Cuneo, Italy. **Dept of Nuclear Medicine, S. Croce General Hospital, Cuneo, Italy.

Correspondence: D. Ferrigno
Dept of Respiratory Medicine
A. Carle Hospital
12100 Cuneo
Italy

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The TM should at least: 1) possess constant serum and/or urinary levels (no major fluctuation); and 2) be of easy and cheap determination.

The initial evaluation of a TM concerns its expression in patients with tumour and in normal subjects in order to define sensitivity and specificity. The sensitivity of a test is defined as the proportion of patients with a disease having a positive test; the specificity is the proportion of patients without tumour who have a negative or normal test.

A problem in the diagnosis of cancer is that nonmalignant diseases can be associated with abnormal marker elevations. For example, even when a marker has a positive predictive value of 95%, 5% of patients with abnormal tests will have no cancer.

Many serum TMs have been singly evaluated as a tool for cancer diagnosis, staging and treatment monitoring. In several instances, TMs have also been evaluated as prognostic factors, either alone, or in combination with other histopathological, biochemical and clinical variables. This review presents data concerning the most studied and interesting markers for lung cancer. A list is shown in table 1.

Tumour-associated antigens

Carcinoembryonic antigen

Carcinoembryonic antigens (CEAs) represents a heterogeneous group of glycoproteins, with common antigenic determinants [3]. The single polypeptide chain of the protein

Table 1. – List of reviewed tumour markers in lung cancer

Tumour-associated antigens
Carcinoembryonic antigen (CEA)
Tissue polypeptide antigen (TPA)
Squamous carcinoma antigen (SCC-ag)
Other polypeptide antigens
Ferritin
Soluble interleukin-2 receptors (sIL-2r)
Chromagranin A
Enzymes
Neuron-specific enolase (NSE)
Creatine phosphokinase-BB (CPK-BB)
Glycosyl-transferases
Hormones
Bombesin/gastrin releasing peptide (BN/GRP)
Adrenocorticotropin (ACTH)
Antidiuretic hormone (ADH)
Calcitonin (CT)
Insulin-like growth factor (IGF-I and II)

portion appears to account for only about one third of the molecule, the remaining two thirds being carbohydrate [4]. The ratio of protein to carbohydrate may vary from 1:1 to 1:5 in CEAs derived from different tumours [5]. CEA is produced by the secretory cells of the normal adult gastrointestinal tract [6]. An elevation of the concentration of CEA in blood and other body fluids is due to a combination of factors: increase in the number of cells producing CEA, increased synthetic rate in malignant cells, and decreased ability to use normal pathways of excretion from the body. The clearance of CEAs is accomplished primarily in the liver [7], and the highest concentrations of the marker are found in patients with liver metastases from carcinoma of the colon. Nonmalignant diseases of the liver, especially those producing extrahepatic biliary obstruction or intrahepatic cholestasis, will also produce a rise of circulating CEA. The level will decrease when the disease process subsides, or is relieved [8, 9].

CEA was first identified by GOLD and FREEDMAN [10], in 1965 as an antigen specific for adenocarcinomas of the digestive tract. Early studies showed that serum concentrations of CEA were higher in healthy cigarette smokers than in nonsmokers [11, 12]. This was suggested to be the result of epithelial damage in some smokers, with an increased release of the antigen from the lung into the serum [12]. CEA might be an indicator of the small proportion of smokers who are "susceptible" to the effects of cigarette smoke and, thus, to lung diseases [13]. Several nonmalignant disorders may be associated with high values of CEA: alcoholic cirrhosis [14], hepatitis [15], obstructive jaundice [9], ulcerative colitis [16], bronchitis and emphysema [11]. These conditions usually produce transient and only modestly elevated CEA levels, rarely above 10 ng·ml⁻¹, that decrease as the condition improves [17]. The list of malignancies associated with abnormal CEA levels includes: colorectal cancer, pancreatic cancer, breast cancer, prostatic cancer, bladder cancer, gastric cancer, ovarian cancer, neuroblastoma, biliary tract cancer, and osteosarcoma [11, 18–22].

In a large study, partially supported by autopsy material, VINCENT *et al.* [23] concluded that the level of CEA in

lung cancer is not related selectively to the tumour volume, the site of metastasis, or the number of organs involved. This conclusion, however, was at variance with several other studies, which reported increased CEA values in advanced bronchogenic cancers of various histological types [24–29]. A good relationship between CEA levels and treatment response has been demonstrated both in small cell lung cancer (SCLC) and non small cell lung cancer (NSCLC) [23, 24, 27, 29]. Generally, CEA levels vary in accordance with obvious changes in disease status, or may precede their clinical recognition. CEA has been studied as a predictor of survival in both SCLC [23–25, 27, 28, 30–32] and NSCLC [23–25, 27, 32, 33]. Most studies using univariate methods, showed a significant relationship between CEA and prognosis [23–25, 27, 28, 30]. Conflicting results were obtained in studies using multivariate methods [25, 31, 32, 34].

In conclusion, CEA assays are moderately useful in lung cancer clinical management, as stated at the 1980 consensus conference of the National Institutes of Health at Bethesda [35].

Tissue polypeptide antigen

Tissue polypeptide antigen (TPA) is a chemically well-defined substance identified by BJÖRKLUND and BJÖRKLUND [36], in 1957. It consists of four protein subunits (A1, B1, B2, C) with molecular weights between 20,000–45,000 Da. The main subunit B1, has been found in foetal tissues at 10, 17, and 24 weeks [37], and with higher concentration in liver, lung, stomach, intestine, kidney and meconium [38]. Increased serum levels of TPA may be present in several nonmalignant diseases: bacterial and viral infections [39], acute hepatitis [40], pregnancy (with particularly high values in the placenta and amniotic fluid [41, 42]), and autoimmune disorders [43]. High levels of TPA have been reported in serum and urine of patients with several tumours, such as carcinoma of the breast, lung, stomach and colon-rectum, pancreas, bladder, uterus, prostate, melanoma and lymphoma [44]. TPA is synthesized during the S- to M-phase of the cell cycle and released upon proliferation into the blood stream [45]. Thus, the concentration of the antigen is an indicator of the rate of cell division and tumour aggressiveness, and, therefore, of the host survival.

MIZUSHIMA *et al.* [46], assaying a panel of diverse tumour markers (carbohydrate antigenic determinant 19-9 (CA-19-9), CEA, neuron-specific enolase (NSE), squamous cell carcinoma antigen (SCC-ag), and TPA), showed that TPA had a fairly high sensitivity and good diagnostic accuracy (66% and 82%, respectively). Incidentally, this value was the highest observed by the authors. In lung cancer patients, the pretreatment serum levels of TPA have been shown, by our team, to correlate with both the primary tumour characteristics (defined as T1, T2 and T3), the nodal involvement (N0, N1 and N2), the metastatic status (M0 and M1), and the classification into 4 stages of disease [24, 47–52]. In general, the greater the tumour bulk at diagnosis, the more elevated the serum concentration of the marker [48]. Post-treatment TPA assays may vary in accordance with obvious changes in disease status [24, 47], and may sometimes

precede them [48] (fig. 1 and table 2). TPA may also be helpful in predicting the course of disease [24, 47, 48, 52, 53]. GRONOWITZ *et al.* [34] evaluated five tumour markers (*i.e.* lactate dehydrogenase (LDH), CEA, serum thymidine kinase (S-TK), NSE and TPA) for assessment of prognosis in 125 patients with SCLC. Tissue polypeptide antigen was found to be the most powerful independent prognostic determinant. In a study of 563 untreated patients with lung cancer [54], we confirmed that raised values of this

marker are very often associated with shortened survival. In the latter study, a multivariate analysis, including all major prognostic factors, selected TPA as the fourth independent survival predictor. Both univariate and multivariate analyses indicated that the usefulness of this marker is maximum in adenocarcinomas, large cell and small cell carcinomas [54]. In a more recent study [32], focusing on 360 patients with squamous cell carcinoma, we further confirmed the prognostic value of TPA.

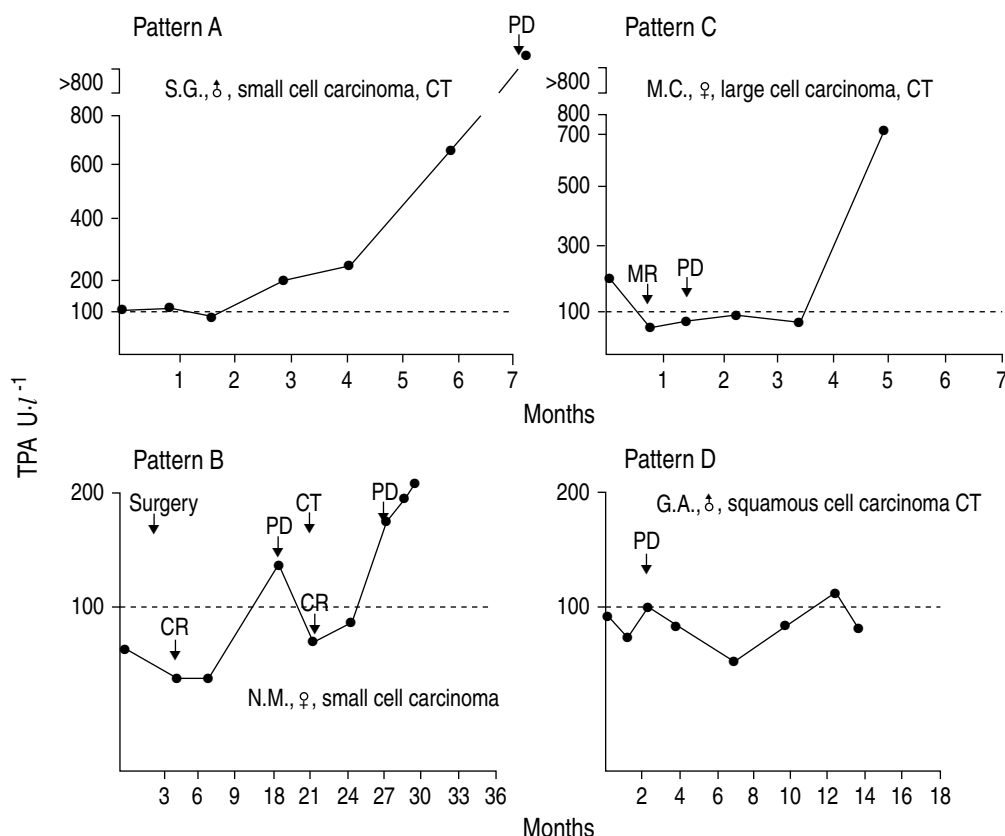


Fig. 1. – Paradigmatic patterns of serial tissue polypeptide antigen (TPA) measurements in four patients. Concordant changes of TPA values may precede (Pattern A), be concurrent with (Pattern B) or follow (Pattern C) the clinical recognition of disease status change (PD). In the last example (Pattern D), there is no rise of TPA after disease progression. CT: chemotherapy; RT: radiation therapy; CR: complete response; MR: minor regression; PD: progressive disease. (From [48]).

Table 2. – Relationships between changes in disease status and TPA serum levels

Histology	Pattern							
	A		B		C		D	
	n	%	n	%	n	%	n	%
Squamous cell carcinoma	17	21	26	33	5	6	32	40
Adenocarcinoma	3	27	3	27	1	9	4	36
Small cell carcinoma	8	28	14	48	5	17	2	7
Large cell carcinoma	1	7	8	53	1	7	5	33
Total	29	21	51	38	12	9	43	32

Changes in tissue polypeptide antigen (TPA) concentrations were considered only if at least three consecutive assays showed the same trend or if, in the case of less numerous tests, there was at least a single change from the baseline level of $\geq 35\%$. As in figure 1, consistent modifications in TPA profiles were grouped on the basis of whether they preceded (Pattern A), were concurrent to (Pattern B), or followed (Pattern C) the clinical recognition of concordant disease status changes, or were totally absent (Pattern D). (From [48]).

Squamous cell carcinoma-related antigen

Squamous cell carcinoma-related antigen (SCC-ag), a purified subfraction of the tumour antigen 4, with a molecular weight of 45,000 Da, is a new tumour marker that appears to be very promising in the evaluation of cancers of the uterine cervix [55–57]. Elevated serum SCC-ag levels were also found in patients with squamous cell carcinoma (SCC) of the bronchus and nasopharynx, and occasionally in healthy volunteers and patients with tumours of other histological origin [58].

In 1988, MINO *et al.* [59] found high serum levels of SCC-ag in 59% of 76 patients with SCC of the lung, and in only 20% of patients with other cell types. The mean levels of the marker was 3.6 times higher in patients with SCC of the lung, than in healthy subjects and patients suffering from other types of lung cancer or benign pulmonary diseases. Preliminary data suggest that serum SCC-ag may be useful in evaluating therapeutic effectiveness in SCC. In

a recent study on 291 patients (129 with SCLC and 162 with NSCLC, including 36 with SCC), BODY *et al.* [60] measured both CEA and SCC-ag, and reached the following conclusions: 1) the use of SCC-ag is inappropriate for lung cancer screening in asymptomatic patients; 2) high values can be observed in patients with benign pulmonary disease; 3) SCC-ag serum levels, in contrast to CEA, are not determined by smoking habits; 4) SCC-ag has lower sensitivity but higher specificity than CEA in SCC; and 5) high pretreatment levels are indicative of a negative prognosis. Another study by MIZUSHIMA *et al.* [46] confirmed these results.

Other polypeptide antigens

Ferritin

Ferritin is an iron-storage protein with a molecular weight of approximately 450,000 Da. Trace amounts of ferritin are normally present in the serum and other body fluids [61, 62]. Ferritin is present in high concentration in the cytoplasm of reticuloendothelial cells, liver cells, spleen cells, and developing precursors of red cells in bone marrow. Extracts from various tissues have different isoferritin distribution [62–64]. Increased ferritin concentration in the serum or in the cerebrospinal fluid has been found in several malignancies, such as lymphoma [65, 66], acute leukaemia [67], multiple myeloma [68], breast cancer [69], and testicular cancer [70]. Several mechanisms are responsible for the increased concentration of ferritin in malignant disease. They include augmented synthesis in tumour-associated inflammations [71], increased secretion by malignant cells [72], and hepatocellular necrosis caused by liver metastases.

Conflicting results have been obtained concerning the possible clinical utility of this substance in lung cancer. GROPP *et al.* [73], demonstrated that ferritin levels were significantly higher in metastatic disease, irrespective of the histological type. In their study, serial measurements of ferritin were useful in evaluating the effects of therapy. Another study, by COX *et al.* [74], showed that ferritin was considerably higher in 39 SCLC patients than in normal individuals, but no relationship was found with the disease extent and the clinical course. In the same study, patients with low pretreatment levels of ferritin had a significantly longer median survival time. From 1988 until 1990, we performed 169 pretreatment and 31 post-treatment assays of serum ferritin in lung cancer patients [75, 76]. The combined prognostic significance of 31 other clinical or biological pretreatment characteristics, including the serum concentrations of CEA and TPA, was studied. We were, however, unable to show any significant relationship between serum levels of ferritin and histological type, clinical stage of disease, or response to treatment. Patients with ferritin levels below the median (236 ng·ml⁻¹) had a more favourable outcome, in accordance with the findings by COX *et al.* [74]. In addition, ferritin was selected as an independent variable in a multivariate survival model, including the most important prognostic factors, other than tumour burden-related variables [75, 76].

In conclusion, ferritin may have some prognostic significance in lung cancer, but does not appear to be useful for staging and monitoring.

Soluble interleukin-2 receptor

Interleukin-2 (IL-2) is a well-characterized cytokine, with various immunological functions, the most important one being the capacity to initiate the proliferation of activated T-cells [77]. This property has stimulated the recent renewal of interest in immunotherapy of cancer [78]. IL-2 acts upon a specific surface receptor [IL-2R], absent in resting T cells but appearing within hours of activation [79]. Activated lymphocytes produce and release into the circulation a soluble form of the same receptor (sIL-2R), that retains the capability of binding the lymphokine [80]. Serum levels of sIL-2R may be elevated in patients with virus infections [81, 82], sarcoidosis [83], Grave's disease [84], organ transplants [85, 86], lymphoproliferative disorders [87, 88], and solid tumours [89, 90].

MARINO *et al.* [91] reported increased levels of sIL-2R in serum samples of patients with untreated lung cancer. In various human malignancies, a relationship has been observed between sIL-2R and some clinical parameters, such as tumour burden and treatment response [89, 90]. Recently, our group performed 326 sIL-2R serum assays in 126 lung cancer patients, 112 patients with pulmonary benign disease, and 63 healthy volunteers [92]. We found increased concentrations of sIL-2R in lung cancer, compared to controls and noninflammatory pulmonary benign disease. Pretreatment sIL-2R correlated neither with the stage of disease nor with the cell type [92]. On the contrary, post-treatment levels of the receptor correlated significantly with the status of disease, particularly in nonsurgical patients. Raised pretreatment values of sIL-2R were associated with a shortened survival [92]. GINNS *et al.* [93] confirmed that patients with lung cancer (squamous cell carcinoma (SCC) or adenocarcinoma (AC)) have high values of sIL-2R. In patients with AC of the lung, the level of sIL-2R did not correlate with the extent of the disease, as in our study. In SCC patients, however, the peripheral blood concentration of sIL-2R appeared to be inversely correlated with the bulk of disease. In fact, the highest levels were found in patients with asymptomatic stage I disease.

Soluble interleukin-2 receptor may be an important marker of the immune alterations associated with lung cancer. Activation of the immune response, reflected by an elevated concentration of sIL-2R, may either enhance host defences or promote tumour development through various growth factors.

Chromogranin A

Chromogranin A is a 68,000 Da protein, that has been demonstrated in serum of patients with lung cancer and, by immunohistochemical techniques, in sections from lung tumours [94, 95]. O'CONNOR and DEFTOS [96] observed chromogranin A in a wide variety of neuroendocrine tumours. NAKAJAMA *et al.* [94] found a positive reaction to

chromogranin A in 5 out of 29 biopsy specimens from SCLC examined [94], whilst SAID *et al.* [95] failed to demonstrate any positive histochemical reaction in 12 cases of SCLC [35]. SOBOL *et al.* [97] assayed the sera of 46 patients with SCLC: 52% of those patients with limited disease (LD) and 72% of those with extensive disease (ED) had elevated levels of chromogranin A. In the same study, four patients with elevated chromogranin A levels, originally classified as NSCLC, were eventually found to have a mixed SCLC and NSCLC histology [97].

Enzymes

Neuron-specific enolase

Enolase molecules in mammalian tissues are dimers composed of three immunologically distinct subunits [98, 99]. The α subunit of enolase (α -enolase) is widely distributed in various tissues. The β subunit (β -enolase) is found mainly in the heart and other striated muscles. The γ subunit (γ -enolase), which has been designated as neuron-specific enolase (NSE), is highly concentrated in neurons, neuroendocrine cells [100], and in neurogenic tumours [101, 102]. Significant levels of γ -enolase have also been found in smooth muscle tissues [103, 104], blood platelets and lymphocytes [105]. Recently, HAIMOTO *et al.* [106] have found γ -enolase in other nonneuron cells and in nonneuroendocrine tissues, such as epithelial cells of the loops of Henle, macula densa cells of the kidney, the conducting system of the heart, bronchial epithelial cells, and type II pneumocytes.

Several studies have determined serum NSE levels in patients with bronchogenic carcinoma. Elevated serum concentrations of NSE have been found in approximately 70% of 450 cumulated patients with SCLC [107–112], and in only 14% of 190 NSCLC patients [108, 110, 112]. High pretreatment values of NSE were noted in 38–71% of SCLC patients with LD, and in 83–98% of those with ED (table 3) [107–112]. Like few other markers, NSE levels may fall with the clinical response to chemotherapy, and rise again during tumour progression or relapse. Sequential measurements of this marker may anticipate the clinical detection of relapse [109, 111]. Daily serum determinations of NSE, performed immediately before and after cytotoxic therapy, may also be predictive of the overall response. Usually, transient elevations of NSE are seen immediately

Table 3. – Raised levels of NSE in serum of untreated SCLC patients

Study	[Ref. No.]	LD	ED
CARNEY <i>et al.</i>	[107]	15/38	49/56
ARIYOSHI <i>et al.</i>	[108]	6/13	24/27
COOPER <i>et al.</i>	[109]	25/38	34/39
ESSCHER <i>et al.</i>	[110]	34/48	54/55
JOHNSON <i>et al.</i>	[111]	23/39	45/54
AKOUN <i>et al.</i>	[112]	6/16	22/27

NSE: neuron-specific enolase; SCLC: small cell lung cancer; LD: limited disease; ED: extensive disease.

after chemotherapy, followed by a subsequent decline to lower or normal values [110, 112, 113]. Several reports have assessed the prognostic capability of NSE. A significant inverse correlation between NSE levels and survival has been found at univariate analysis [30, 34, 114–116]. However, less unequivocal results were reported by JORGENSEN *et al.* [114] and GRONOWITZ *et al.* [34], using multivariate analyses.

Recently, VIALARD *et al.* [117] suggested that the NSE/nonneuron enolase (NNE) ratio increases the ability of the test to separate SCLC from NSCLC patients. In fact, the NSE/NNE ratio misclassified only three out of 57 NSCLC (5.3% of false positive). Its sensitivity was 76 and 100% in the diagnosis of SCLC (for LD and ED, respectively). This index could represent a better approach for diagnosis, assessment of therapeutic effect, and detection of SCLC relapse.

Creatine phosphokinase-BB

In 1981, GAZDAR *et al.* [118] found abnormal concentrations of the isoenzyme BB of creatine phosphokinase (CPK-BB) in most extracts and in the supernatants of SCLC growing in tissue culture. Creatine phosphokinase is an enzyme which reversibly catalyses the transfer of a high energy phosphate from creatine phosphate to adenosine diphosphate (ADP). CPK levels are 10–100 times higher in clinical specimens and in established cell lines of SCLC than in normal lung tissue, in NSCLC, and in normal cell cultures [119]. Interestingly, "variant" SCLC cell lines that lose L-Dopa decarboxylase activity retain high levels of CPK-BB [120]. In two recent clinical studies, CPK-BB was elevated in about 41% of untreated patients with extensive SCLC, but in only 2% of patients with LD [118, 121]. A direct correlation between the number of metastatic sites and the serum level of the isoenzyme, as well as an inverse correlation with survival were also described [118, 121]. BORK *et al.* [122] reported the most frequent elevations of serum CPK-BB, 82 and 50% in untreated SCLC patients with ED and LD, respectively. High concentrations of the isoenzyme in cerebrospinal fluid were useful to distinguish between meningeal spread and parenchymal cerebral metastases [123].

Glycosyltransferases

The glycosyltransferases constitute a group of enzymes which catalyses the transfer of individual sugars from nucleotide-sugar precursor molecules into appropriate acceptors [124]. An increase in serum glycosyltransferase activity has been observed in various malignancies [125–128]. The α -(1-3)-L-fucosyltransferase is one of the glycosyltransferases thought to be responsible for the synthesis of tumour-associated antigens [128–130]. The accumulation of tumour-associated antigens, such as carcinoembryonic antigen and sialyl Lewis χ -i, has frequently been observed in the sera of patients with lung cancer [131–133]. Recently, ASAO *et al.* [134] performed a clinical evaluation of α -(1-3)-L-fucosyltransferase. They observed higher serum levels of

the enzyme in patients with lung cancer, compared to patients with benign pulmonary diseases and healthy controls. The elevation of enzyme activity correlated significantly with the clinical stage and with the size of the primary tumour, whereas there were no differences in enzyme activity among the histological types [134]. Follow-up studies showed that, in some patients, glycosyltransferase measurements were helpful in monitoring the clinical course of disease [134]. The utility of the enzyme as a diagnostic marker was obvious when its levels were compared with those of CEA and sialyl Lewis χ -i antigen [134].

Hormones

Bombesin/gastrin releasing peptide

Bombesin (BN) is an amphibian skin peptide of 14 amino acids [135]. Gastrin releasing peptide (GRP), the mammalian equivalent of BN, consists of 27 amino acids, and is able to release gastrointestinal hormones [136]. Cells containing GRP are rare in the normal adult lung, but are regularly found in the foetal and infantile epithelium of the lung [137]. A bombesin-like immunoreactivity has been demonstrated in normal human brain tissue, peripheral nerves, and neuroendocrine bronchial cells [136, 138, 139]. Notably, SCLC is often equipped with receptors for GRP [140], which has a growth promoting activity in SCLC [141]. Unfortunately, increased concentrations of GRP in the serum are rare, because of the very short half-life [142–144]. For the same reason, high concentrations of the hormone may be found in neoplastic tissues, without corresponding serum increase [139]. Recently, a radioimmunoassay for a GRP precursor has been developed; using this assay, elevated plasma concentrations of pro-GRP have been found in 72% of 71 SCLC patients [145]. In a study by PEDERSEN *et al.* [146], high plasma levels of BN/GRP were demonstrated in the fluid of patients with meningeal carcinomatosis from SCLC, independent of a positive cytology. Combining calcitonin (CT) and BN findings increased the overall detection rate of patients with central nervous system (CNS) metastases to 67%. Importantly, 93% of patients with increased BN or calcitonin had CNS metastases. However, with regard to meningeal carcinomatosis, BN alone was just as sensitive and specific as the combined CT and BN analysis [146].

Adrenocorticotrophic hormone and related molecules

Ectopic secretion of adrenocorticotrophic hormone (ACTH) was initially observed in 1928 by BROWN [147] in a patient with Cushing's syndrome and small cell carcinoma of the lung (SCLC). Ectopic ACTH production has been described in association with many malignancies, *i.e.* ovarian tumours, thymoma, islet cell cancer of the pancreas, medullary cancer of the thyroid, and carcinoid tumour [148, 149].

Elevated serum levels of ACTH have been reported in 25–30% of patients with SCLC [150–152]. The clinical picture of ectopic Cushing's syndrome is characterized by

oedema, hypokalaemia or impaired glucose tolerance without obesity, striae and osteoporosis. Its frequency ranged 1–5% of 346 SCLC patients in five consecutive series [153–157]. Conflicting results have been reported concerning a possible correlation between serum levels of the hormone and stage of disease, treatment response, and survival of patients with lung cancer [150, 151, 155, 158].

The common precursor to ACTH is pro-opiomelanocortin (POMC), which has been shown to be abnormally elevated in the plasma of lung cancer patients with epidemoid, adeno- and small cell type [159]. A significant difference between pulmonary vein and artery concentrations provides evidence that POMC is synthesized by the bronchogenic tumour [159]. The NH₂ terminal portion of this molecule is easier to assay than ACTH, and may be the more appropriate antigen to use as tumour marker.

Summing up, ACTH seems to be neither a specific nor a sensitive indicator of lung malignancy, nor does it appear to be reliable in monitoring the response to therapy, or in predicting relapses.

Antidiuretic hormone

Hyponatraemia was first observed in association with cancer in 1938 [160]. In 1957, SCHWARTZ *et al.* [161] postulated inappropriate secretion of vasopressin, also known as antidiuretic hormone (ADH), as the cause of persistent hyponatraemia in two patients with bronchogenic carcinoma. Subsequently, GEORGE *et al.* [162] demonstrated *in vitro* biosynthesis of ADH in short-term culture of lung cancer. Other malignancies with ectopic secretion of ADH include: bronchial carcinoid tumours, adenocarcinoma of the pancreas, Hodgkin's disease, bladder carcinoma, thymoma, mesothelioma, leiomyosarcoma of the stomach, prostatic carcinoma, and adrenocortical cancer [163].

Inappropriate ADH secretion has been demonstrated in SCLC patients, with rates depending on the method used for the hormone identification [164]. The concentration of ADH was elevated in 35% of 279 patients with SCLC [151, 165–168], whilst the frequency of the clinical syndrome was quite low (approximately 10% of 596 patients in six different studies [153–157, 169]). Elevated serum levels of ADH did not correlate with the stage of disease [151], or with the response to treatment [158]. Tumour-produced ADH may be bound to neurophysin, as in the posterior pituitary. MAURER *et al.* [170] found that plasma concentrations of ADH-neurophysin were elevated in 65% of 103 patients with SCLC. In patients with initially high values, concentrations were related to the response to therapy, whilst initially normal levels were of no use in disclosing subsequent relapses [168].

Calcitonin

Calcitonin (CT) is a 32 amino acid peptide, with a molecular weight of 3,419 Da synthesized by the thyroid C cells [171]. Normally, CT is secreted by the thyroid in response to increased plasma calcium concentration, or following the stimulation of certain gastrointestinal hormones. The

hormone inhibits the release of calcium and phosphate from bone [172].

Marked elevations of serum calcitonin are usually found in familial medullary thyroid carcinoma [173]. Elevated levels have also been reported in other malignancies, including breast cancer, carcinoid tumour, hepatoma, renal cell carcinoma, and gastrointestinal cancer [174].

Calcitonin was elevated in 59% of 425 SCLC patients [151, 175–180]. Elevated concentrations are rare in other types of lung cancer [177, 181]. Serum concentrations of CT do not seem to be correlated with stage of disease. However, merging the data from three studies, an increase of calcitonin has been found in 80% of 81 patients with ED, and in only 56% of 71 patients with LD [151, 175, 181]. Conflicting reports about the use of calcitonin in monitoring treatment response have so far been published. WALLACH *et al.* [180] reported a decrease of CT serum levels in SCLC patients responding to chemotherapy, and an increase in progressive diseases. MULDER *et al.* [182], on the contrary, showed no such effect.

In conclusion, the assay of serum calcitonin seems to be a general indicator of the course of SCLC disease, but it is not sufficiently reliable for evaluating the response to treatment.

Insulin-like growth factors

The insulin-like growth factors [IGF], or somatomedin, are polypeptides of about 7.5 kDa, having a structural similarity to pro-insulin [183]. Insulin-like growth factors I and II (IGF-I, IGF-II) share a 62% sequence homology. Their action is mediated through 2 distinct receptors with different characteristics [184, 185]. IGF-I is mitogenic for both classic and variant SCLC cell lines [186], and this suggests that IGF-I may be an autocrine growth factor for human SCLC.

There are contrasting reports concerning the utility of IGF as a tumour marker. MACAULY *et al.* [187] measured serum IGF-I concentrations in 42 SCLC patients, and concluded that IGF-I levels do not correlate with the tumour bulk, or with the therapeutic responsiveness of SCLC. REEVE *et al.* [188] found elevated levels of these proteins in the serum of patients with both SCLC and NSCLC, and a good correlation between circulating levels and clinical course. Further studies are needed for a better evaluation of these substances.

Conclusion

Lung cancer is the most common and lethal malignant neoplasm in the Western world, and is also becoming one of the major health problems in undeveloped countries [189]. Its devastating incidence and clinical seriousness have stimulated innumerable research studies, with any possible approach.

Historically, NSCLC and SCLC were considered to have different origins (ectodermal and endodermal, respectively), thus, accounting for the many differences observed in their clinical behaviour. However, in more recent years, it has become clear that considerable overlaps exist between SCLC

and NSCLC. Several data suggest a common stem for all lung cancers, supporting the concept that individual tumours may spontaneously differentiate into other cell types. Histological evaluation of specimens of untreated lung cancers reveals that about 15% of SCLC tumours contains NSCLC subtypes. In 13–28% of autopsy specimens from patients treated with cytotoxic therapy for SCLC, a mixed histology was proved [190–194]. TERASAKI *et al.* [195] reported that changes of the culture medium may, in some cases, induce changes in SCLC morphology from small cell to squamous cell and *vice versa*. The expression of neuroendocrine biomarkers is detected in 15% of NSCLC cell lines [196, 197].

The above data on the clonal heterogeneity of lung cancer may explain the several limitations in the clinical use of serum tumour markers. Indeed, none of the serum components proposed, so far, seems to be sufficiently sensitive and specific to be reliable in the screening and diagnosis of lung cancer. The most fruitful application at present is monitoring of tumour activity. During active therapy, a marker can give an accurate estimate of the effectiveness of treatment. Earlier detection of relapse may allow a modification of therapy at a time that might precede the normal clinical evidence of tumour recurrence by several weeks. TPA, CEA, and SCC-ag seem to be the most fruitful in NSCLC; NSE, BN/GRP and CPK-BB in SCLC patients. Other TMs are worthy of further investigations, whilst peptide hormones have been almost definitively omitted.

The study of these substances may lead to a better understanding of the biological characteristics of bronchogenic carcinoma as occurred recently, when SCLC was reclassified according to its biological characteristics [198], and ultimately to new therapeutic strategies for each tumour histotype.

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