Review

Clinical Value of Using Serological Cytokeratins as Therapeutic Markers in Thoracic Malignancies

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Abstract. *In recent years, there has been an increasing awareness among physicians of the value of therapeutic interventions in patients suffering from lung cancer and mesothelioma. A search for an optimal approach using surgery, irradiation and chemotherapy in different settings of the tumour disease, including curatively aimed adjuvant chemotherapy after locoregional surgery or radiotherapy, has resulted in gradually improved survival rates. Still, early detection is crucial if there is to be a possibility of curing patients or prolonging life in cases of relapsed disease. Several studies have been initiated in which surrogate markers are evaluated in comparison to chest X-rays and computer tomography. The present review focuses on the predictive and prognostic value of using serological cytokeratins as tumour markers for patients suffering from thoracic malignancies.*

Lung cancer kills more than 1,200,000 people every year worldwide (1). Lung cancer is divided into two entities, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC represents 80% of all lung cancer cases, and at the time of diagnosis, approximately 20-30% (stages 1 and 2) are candidates for surgical intervention, resulting in a 5-year survival rate in cases of small tumours less than 4 cm (T1 and T2 tumours) of approximately 50-70% (2). The value of neoadjuvant chemotherapy is still highly controversial, hence the results of ongoing phase III studies are eagerly awaited. Adjuvant chemotherapy for patients with NSCLC is today accepted as mandatory for patients with operable tumours and survival rates are similar to those in breast and ovarian cancer (3). Patients with localized, inoperable, non-small cell lung cancer are treated with radiotherapy. A recently updated literature review indicates that concurrent radiochemotherapy is better than radiotherapy alone, with regards to locoregional control, progression-free survival and relative mortality risk at 2 years' follow-up (4). Treatment for advanced-stage NSCLC generally includes the use of systemic chemotherapy as well as biological 'targeted therapy' at later stages of the disease. Several treatment regimes are available and today a vast majority of patients are treated with both second- as well as third-line treatments.

Surgical management of small cell lung cancer generally yields little benefit, since these tumours disseminate early to regional lymph nodes and distant sites. However, this issue has not yet been fully elucidated and some authors advocate that patients with very early-stage tumours should be considered for a combined treatment modality including surgery and chemotherapy (5). Chemotherapy is the backbone of the treatment of SCLC. The role of chest irradiation is well documented, especially in limited-stage disease. Patients in complete remission should receive prophylactic cranial irradiation to reduce the risk of brain metastases (6).

Mesothelioma, still a relatively rare disease, displays an increasing incidence (7). Patients who are surgically fit are offered pleurectomy or extrapleural pneumonectomy (8, 9). Radiation therapy has been advocated as a suitable treatment, but, as stated in a recent review, needs further evaluation (10). Patients with advanced disease are treated with chemotherapy and new combinations with pemetrexed/cisplatin have resulted in increased survival (11).

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Thus, today several potentially effective treatment options exist for at least significant subpopulations of patients suffering from thoracic malignancies. There is a real need to predict the identity of these patient subpopulations who may benefit from a toxic treatment, including detection of relapse/progressive disease at an early stage. The diagnostic tools most commonly used today are various X-ray techniques, including chest X-ray, computer tomography and PET investigations. These methods are reliable, but other more easily available applied techniques, such as analysing serum/blood samples, may be of interest as complementary tools in managing thoracic cancer patients. This review presents a compilation of the current literature, focusing on the predictive and prognostic implications of the use of serological cytokeratin tumour markers in managing thoracic malignancies.

Cytokeratins: General Background

Cytokeratins are a family of more than 20 intermediate filament proteins expressed in cells of epithelial origin (12). Their subdivision into two groups (cytokeratins 1-8 , type II group, 53-68 kDa, neutral to basic proteins; and cytokeratins 9–20, type I group, 40-56 kDa, acidic proteins) reflects the functionally very important heterodimeric nature of cytokeratins. Cytokeratins assemble into obligate non-covalent structures of one type I and one type II cytokeratin protein, respectively; gradually, they become organized into larger filamentous polymeric structures (13).

The most abundant cytokeratins are 8, 18 and 19, and a common example of the heteropolymer complex is the combination of cytokeratins 8 and 18 (14). In knockout mice, it has been demonstrated that cytokeratin 18 can be replaced by cytokeratin 19 and together with cytokeratin 8 provide a normal cytoskeleton (15). Exactly which cytokeratins are expressed varies with epithelial cell type, extent of differentiation and development of the tissue (16). Commonly, the cytokeratin expression profile is stable, even during malignant transformation (17). This biological feature is utilized in routine pathology, in which cytokeratin antibodies are used in immunohistochemistry to distinguish, for example, lung carcinomas from metastatic carcinomas of the lung (18, 19). Cytokeratins are detected either as partially degraded single-protein fragments, as small complexes or as large polymeric protein complexes when present in the circulation (20). In healthy individuals, the levels of cytokeratins are low in the circulation, but rise significantly in patients with epithelial cell-associated carcinomas. The exact process leading to the release is not yet completely understood, but is likely the result of multiple pathways including spill-over from rapidly proliferating tumour cells, abnormal mitosis, neo-vascularization and/or apoptosis.

Apoptosis involves the activation of numerous downstream targets and effectors, most of which include activation of caspases (21). Interestingly, most type I cytokeratins bear motifs that make them likely substrates for caspase degradation, with subsequent release during the intermediate apoptotic events. Of course, this makes cytokeratins important reflectors of ongoing tumour cell death, partly explaining their role as markers of tumour activity (22).

An important question is what the increased levels of cytokeratins in serum represent. An in vitro study, in which fragments of cytokeratin 8 and 18 were measured in the supernatants of cells exposed to irradiation, found a trend towards increased amounts of cytokeratin fragments (23). Furthermore, patients subjected to surgical intervention for gastrointestinal or lung cancer were shown to have increased amounts of cytokeratins in their circulation during the first weeks after surgery (24). These studies thus suggest that increased amounts of circulating cytokeratins are related to released proteolytic debris, which in turn reflects cell death.

Cytokeratin Assays

Several monoclonal anti-cytokeratin antibodies are available that react with the most abundantly found cytokeratins, i.e. 8, 18 and 19. The immunoreactivity patterns of thirty of these antibodies have previously been characterized elsewhere using a variety of methods (12). According to the literature, several cytokeratin tumour marker assays are commercially available. Many of them are available in both manual formats (mainly based on open-system microtitre plate analysis, but also as radiometric bead assays) and on automated random-access instruments. Assays in manual formats are best suited for sample batch analysis, which can be completed quickly at larger clinics or, for example, by means of daily or weekly based sample collection prior to simultaneous analysis. Manual formats might, however, cause difficulties for laboratories wishing to provide short turn-around times. For cancer patient monitoring this is often not as crucial as with STAT testing, as the patients are treated according to predefined schemes. In most cases, it is thus sufficient that the test result be available within a couple of weeks. However, for diagnostic purposes it is often important to have rapid test results. Currently, CYFRA 21-1 (Modular, Roche Diagnostics, Mannheim, Germany), TPA (Liaison®, Diasorin Spa, Saluggia, Italy) and TPS (Immulite, DPC, Los Angeles, CA, USA) are available on random-access instruments, thus reducing the time required to make the results available. All of these tests are also available in manual formats. The recently introduced cytokeratin marker with a focus on non-small cell lung cancer, MonoTotal (IDL Biotech, Bromma, Sweden), is available in a manual format only (25).

Cytokeratins as Serum Tumour Markers

Being generally characterized as so-called activity tumour markers, cytokeratins have their main clinical value in patient management for the early detection of recurrence and in the prompt assessment of therapeutic response (26).

A limiting factor for the diagnostic use of cytokeratin markers is that they are not organ specific, but rather can be used for a number of epithelial cancers (26). The three most commonly applied cytokeratin markers overall are TPA, TPS and CYFRA 21-1. TPA is a broad-spectrum test that measures cytokeratins 8, 18 and 19, while TPS and CYFRA 21-1 measure cytokeratins 18 and 19, respectively. Recently, a new broad-spectrum cytokeratin assay has been introduced, MonoTotal, which also measures cytokeratins 8, 18 and 19, but using a different combination of antibodies (25). Although based on detection of the same type of proteins in serum, individual cytokeratin assays may give different reactivity profiles, reflecting the uniqueness of each assay. This is due to both the different detector antibodies employed in the individual assays and to the actual release of different cytokeratins into the circulation, a process that may differ between the cytokeratins. Thus, as with many other types of tumour markers, cytokeratin tumour markers are not simply interchangeable and their performance should not be assumed to be similar (26).

Cytokeratin Assays in Non-small Cell Lung Cancer

Cytokeratins may be considered as markers of epithelial cell turnover, and their potential uses in the diagnosis, prognosis and monitoring of carcinomas have been discussed. Table I summarizes most of the published studies in which different cytokeratin assays have been evaluated as tumour markers in NSCLC.

Increased serum levels of cytokeratins, especially the cytokeratin fragment 19 (CYFRA 21-1), have been demonstrated in patients with carcinomas of the lung. As early as 1994, Niklinski *et al.* (27) demonstrated elevated levels of CYFRA 21-1 in NSCLC patients compared to controls and that levels were associated with the clinical stages. These results were later confirmed by several other authors (Table I) (28). Thus, cytokeratins may provide useful information to the clinician. The issue is, of course, how to interpret these levels. Also in this respect CYFRA 21-1 has been most studied and elevated levels of this marker at diagnosis are clearly associated with a poor prognosis and reduced survival (29-31). In addition, some studies have indicated that increased cytokeratin serum levels are negatively/inversely associated with survival (32, 33). Post-treatment monitoring of CYFRA 21-1 for the early detection of recurrent disease has been suggested as a

clinically valuable option, since evaluations of the marker could demonstrate that serially increasing values were associated with progressive disease (34) and recurrence after surgery (35). Typically, CYFRA 21-1 levels decrease within 2 weeks of surgery but remain elevated or increase over the follow-up period (12-18 months) in patients with recurrent disease (36-38).

Cytokeratins other than CYFRA 21-1 have received relatively little attention in this regard. In a small study, increased circulating levels of cytokeratin 8 and 18 were found to be associated with advanced disease (39); more recently, levels of circulating cytokeratins, analyzed using MonoTotal, have been shown to correlate with progressive disease (25).

Compared to other tumour markers (Table I), most studies suggest that cytokeratins, CYFRA 21-1 in particular, provide an important adjunct to the clinical staging system and may help in better assessing prognosis (30). In many cases, CYFRA 21-1 compares favourably or similarly to other alternatives, such as CEA, and in some cases the markers may be considered complementary. One of the first and largest evaluations of CYFRA 21-1 in a clinical setting was a European multicentre study; here, a predefined cut-off level displayed 57% sensitivity, at 96% specificity, for SCC, which was higher than for all the other included markers, *i.e.* squamous cell carcinoma marker (SCC), carcinoembryonic antigen (CEA) and TPA (40). The study supports the notion that increasing CYFRA 21-1 levels may be of value for the clinician in indicating the early discontinuation of, or change in, therapy for patients with recurrent or progressive disease.

Cytokeratin Assays in Small Cell Lung Cancer

Several published studies evaluate different cytokeratin assays in SCLC, indicating a more controversial role of cytokeratins alone in SCLC (Table II). Significantly elevated levels of CYFRA 21-1 have been demonstrated in SCLC compared to those in healthy subjects or in cases of benign lung disease (41-43) and CYFRA 21-1 has also been shown to be useful for the differential diagnosis of SCLC and NSCLC (49). Moreover, Takei *et al.* (44) suggested a significant correlation between survival and pre-treatment levels of CYFRA 21-1 ($p=0.0036$). On the other hand, data from Pujol *et al.* (45) indicate a negative prognostic effect of CYFRA 21-1, significant only in squamous cell carcinoma, but not in SCLC or other subtypes of NSCLC. Serum neuron-specific enolase (NSE) seems to be more useful in SCLC (46), and the combined use of CYFRA 21-1 and NSE has been shown to be an interesting combination in the diagnosis of SCLC (50). Paone *et al.* (47) found NSE and CYFRA 21-1 to provide good discrimination between SCLC and NSCLC. The combination further significantly

Table I. *A survey of literature concerning cytokeratins in sera in NSCLC.*

Authors (ref.)	Cytokeratin	No of patients	Results
			Diagnostics
Niklinski *et al.* (27)	CYFRA 21-1	115	Increased levels of CYFRA 21-1 at diagnosis compared to controls; levels correlate with clinical staging.
Pavicevic *et al.* (28)	CYFRA 21-1	250	CYFRA 21-1 significantly ($p<0.001$) higher in NSCLC patients than in controls.
Chantapet *et al.* (56)	CYFRA 21-1 and CEA	51	CYFRA 21-1 and CEA are useful serum markers for the diagnosis of NSCLC, with accuracy of approx. 70%.
Kim *et al.* (57)	CYFRA 21-1 and SCC	124	CYFRA 21-1 is superior to SCC in the diagnosis of squamous cell carcinoma of the lung.
Oremek *et al.* (58)	CYFRA 21-1	134	The high sensitivity and specificity of CYFRA 21-1 for the differential diagnosis of malignant and non-malignant pulmonary diseases as well as of SCLC and NSCLC.
			Prognostic value – impact on survival
Pujol *et al.* (29)	CYFRA 21-1	2063	Meta-analysis of 2063 patients proving CYFRA21-1 to be a putative co-variable in analyzing NSCLC outcome.
Niklinski *et al.* (59)	CYFRA 21-1	94	Pre-operative CYFRA 21-1 has prognostic value.
Fukunaga *et al.* (32)	Cytokeratin 8	8	CK8 ↑ associated with poor prognosis.
Bergqvist *et al.* (33)	TPAcyk	69	TPAcyk ↑ associated with poor prognosis.
			Monitoring of lung cancer
Yeh *et al.* (60)	CYFRA 21-1	48	CYFRA 21-1 ↑ is an early predictor of recurrence after surgery.
Ebert *et al.* (61)	CYFRA 21-1	48	CYFRA 21-1 allowed early detection of progressive disease in non-operable patients.
Lai *et al.* (34)	CYFRA 21-1	164	Serially increasing CYFRA 21-1 associated with progressive disease.
Niklinski *et al.* (35)	CYFRA 21-1	57	Increasing post-operative CYFRA 21-1 preceded or coincided with tumour recurrence.
Pendleton *et al.* (39)	CK8 and CK18	24	CK8 and CK18 ↑ associated with advanced disease.
Ericsson *et al.* (25)	MonoTotal	45	Levels measured correlate with progression.
			Comparison with other tumour markers
Giovanella *et al.* (48)	CEA, NSE, TPS and CYFRA 21.1	169	In patients with suspected lung cancer, the serum NSE and CYFRA 21.1 assay presents a suitable association to confirm the clinical hypothesis.
Kasimir-Bauer *et al.* (31)	TPA and CYFRA 21-1	80	The detection of CK+ cells should be added to routine pathology and for tumour marker determination; studies should focus on CYFRA 21-1 and TPA.
Pujol *et al.* (45)	CYFRA 21-1	621	The prognostic information provided by a high serum CYFRA 21-1 level is independent of other well-known variables, such as performance status and disease stage, and is perennial throughout an extended follow-up period.
Nisman *et al.* (62)	TPA, CYFRA 21-1 and CEA	94	CYFRA 21-1 and TPS are significant prognostic factors and effective monitors of therapy.
Moro *et al.* (63)	CEA, SCC and CYFRA21-1	105	The study suggests using a combination of CEA and CYFRA 21-1 in the clinical care of NSCLC.
Wieskopf *et al.* (64)	CEA, NSE, SCC and CYFRA 21-1	161	CYFRA 21-1 is a sensitive and specific tumour marker of NSCLC, especially of the squamous cell subtype.
Takada *et al.* (65)	CEA, SCC NSE and CYFRA 21-1	185	CYFRA 21-1 appeared to have the most discriminatory power of the markers tested in the diagnosis of lung cancer.
Koga *et al.* (66)	CYFRA 21-1, CEA and SCC	137	It is concluded that CYFRA 21-1 could replace SCC for diagnosing squamous cell carcinoma of the lung.
Molina *et al.* (41)	CEA, CA 125, SCC and NSE	189	There was a clear relationship between CYFRA 21-1 and tumour extension.
Stieber *et al.* (49)	CYFRA 21-1, TPA and TPS	218	With single determinations, CYFRA 21-1 proved to have the highest general sensitivity for lung cancer.

Table I. *continued*

Authors (ref.)	Cytokeratin	No of patients	Results
van der Gaast *et al.* (67)	CEA, SCC,TPA and CYFRA 21-1	212	CYFRA 21-1 is a useful serum marker for patients with NSCLC, especially for the disease monitoring of patients with squamous cell carcinoma during and after chemotherapy.
Rastel *et al.* (40)	SCC, CEA, TPA and CYFRA 21-1	2250	CYFRA 21-1 is a sensitive tumour marker for NSCLC, especially squamous cell lung cancer.
Miedouge *et al.* (68)	CEA, SCC, CYFRA 21-1 and epidermis-type proteins (1, 2, 10/11, 14 and filaggrin)	138	The authors confirmed the high diagnostic sensitivity of CYFRA 21-1 (55.6%), but were unable to detect significant levels of epidermis-type cytokeratins or filaggrin.

Carcinoembryonic antigen (CEA), squamous cell carcinoma antigen (SCC), neuron-specific enolase (NSE), tissue polypeptide antigen (TPA), tissue polypeptide specific antigen (TPS), cytokeratin 19 (CYFRA 21-1), cytokeratin 8 and 18 fragments (TPAcyk). ↑ means that the investigated marker was associated with survival.

improves the diagnostic sensitivity and accuracy in SCLC patients (48, 49).

Giovanella *et al.* (48) did demonstrate CYFRA 21-1 to be strongly linked to patient outcome, independent of both clinical prognostic factors and NSE levels, while expression seems to be relatively independent of tumour volume modifications. They suggest the combined use of NSE and CYFRA 21-1 in pre-therapeutic assessment and therapy planning.

A study by Plebani *et al.* (43) found that SCLC patients with extensive disease had high levels of several of the tumour markers studied, including the cytokeratin markers CYFRA 21-1, TPA, TPA-M and TPS; squamous cell carcinoma antigen (SCC) was not found to be altered in SCLC.

Cytokeratin and Mesothelioma

A large number of markers used in immunohistochemistry can facilitate the distinction between epithelial pleural mesotheliomas and pulmonary peripheral adenocarcinomas. Antibodies against cytokeratins are strongly positive in mesotheliomas, but not in adenocarcinomas, where CEA or Leu-M1 instead are detected. There are no established serum tumour markers for mesothelioma, including the cytokeratin serum assays summarized in Table III. Several groups have followed cytokeratin assays over the course of the disease, but have included only a limited number of patients (50-53). The trend seems to be that all cytokeratin markers tend to rise as disease progresses, though further study is needed to establish the real significance of these results. Their value in mesothelioma is limited as the specificity of such tests is too low (Table III). An exception could be that of a population heavily exposed to asbestos, where both CYFRA 21-1 and TPA demonstrate good positive prognostic values for mesothelioma (54) and TPA at least has been shown to increase before clinical signs of disease (55).

Conclusion

Over the last ten years, treatment for patients with thoracic malignancies has changed dramatically and scientific interest in the condition has virtually exploded. Though initially regarded as resistant to all available therapeutics, today these malignancies are treated with a broad therapeutic arsenal. Several clinical studies have demonstrated that the combined use of radiation, chemotherapy and novel biological agents improves survival. Patients with advanced thoracic malignancies are now offered both second- and third-line treatments and several research protocols are available. Since different treatment options now exist for this patient category, the role of follow-up for patients being treated successfully has gradually attracted greater interest.

This review has focused on the potential of circulating cytokeratins as disease markers of thoracic malignancies. Since these serological markers have relatively high sensitivity and specificity, the overall findings of the present review imply that various cytokeratin assays may be of value for the follow-up of patients with thoracic malignancies who received curatively intended treatment, especially for predicting early relapse in non-mesothelioma tumours. However, these cytokeratin assays should not replace the standard follow-up modalities used for these patients, *i.e.* various X-ray techniques, but could, in conjunction with clinical data, be used as complementary tools in the everyday working situation.

References

1 Minna JD, Fong K, Zochbauer-Muller S and Gazdar AF: Molecular pathogenesis of lung cancer and potential translational applications. Cancer J *8*: 41-46, 2002.

2 Luketich JD: Lungcancer, Principles and Practice. *In*: J Mitchell, Turrisi: Lungcancer, Principles and Practice. Lippincott-Raven; 1996.

Table II. *A survey of literature concerning cytokeratins in sera in SCLC.*

Authors	Cytokeratin	No. of patients	Results
			Survival
Takei *et al.* (44)	CYFRA 21-1	87	CYFRA 21-1 might be useful as a possible indicator of survival and therapeutic effect for lung cancer.
Pujol *et al.* (31)	TPS and CYFRA 21-1	405	In both small cell and non-small cell lung cancers, univariate survival analyses demonstrated that either a CYFRA 21-1 level over 3.6 ng/mL or a TPS level over 140 U/L significantly indicated a poor survival rate.
Pujol *et al.* (60)	CYFRA 21-1	148	High serum CYFRA 21-1 and CgA levels in SCLC are both prognostic determinants of prognosis.
Ando *et al.* (60)	CYFRA 21-1, NSE	57	The group of patients positive for both the NSE and CYFRA 21-1 markers had a worse prognosis than the group positive for only NSE.
Pujol *et al.* (60)	CYFRA 21-1	165	The negative prognostic effect of CYFRA 21-1 was highly significant in squamous cell carcinoma, whereas it was nonsignificant for the other histologies, including SCLC.
			Monitoring of lung cancer
Boher *et al.* (60)	CYFRA 21-1, TPS	52	Lack of a true reversible property of the cytokeratin markers
			Diagnostics
Fukunaga *et al.* (28)	CK8	70	The level of serum CK8 in patients with NSCLC was significantly higher than in those with SCLC ($p<0.05$).
Bombardieri *et al.* (56)	CYFRA 21-1	584	In patients with SCLC the global sensitivity of CYFRA 21-1 was 52.3%.
Molina *et al.* (41)	CYFRA 21-1, CEA, CA 125, SCC and NSE	189	Abnormal level of CYFRA 21-1 in 30% of patients with SCLC ($p<0.0001$)
Oremek *et al.* (58)	CYFRA 21-1	134	CYFRA 21-1 has high sensitivity and specificity for the differential diagnosis of malignant and non-malignant pulmonary diseases as well as of SCLC and NSCLC.
Paone *et al.* (56)	CYFRA 21-1, NSE	67	NSE and CYFRA 21-1 provide good discrimination between SCLC and NSCLC.
Szturmowicz *et al.* (56)	CYFRA 21-1	116	Elevated CYFRA 21-1 values were found in 34% of small-cell lung cancer patients.
			Comparison with other tumour markers
Giovanella *et al.* (48)	CEA, NSE, TPS and CYFRA 21-1	169	In patients with suspected lung cancer, including SCLC, the serum NSE and CYFRA 21-1 assay presents a suitable association to confirm the clinical hypothesis.
Giovanella *et al.* (48)	NSE and CYFRA 21-1	62	An applicable model of biomarkers in SCLC could be the concurrent assay of NSE and CYFRA 21-1 in pre-therapeutic assessment and therapy planning.
Plebani *et al.* (56)	CYFRA 21-1, TPA, TPA-M, TPS, NSE, SCC, CEA,	124	In patients with SCLC, high levels of all markers except SCC were found when the disease was extensive. The combined use of CYFRA 21-1 and TPA-M may be useful for the diagnosis of lung tumours.
Takada *et al.* (65)	CEA, SCC NSE and CYFRA 21-1	185	CYFRA 21-1 appeared to have the most discriminatory power of the markers tested in the diagnosis of lung cancer.
Molina *et al.* (41)	CYFRA 21-1, CEA, CA 125, SCC and NSE	189	There was a clear relationship between CYFRA 21-1 and tumour extension.
Stieber *et al.* (49)	CYFRA 21-1, TPA and TPS	218	In small cell carcinomas a clear increase in sensitivity could be achieved with combined determinations of CYFRA 21-1 + NSE.

Carcinoembryonic antigen (CEA), squamous cell carcinoma antigen (SCC), neuron-specific enolase (NSE), tissue polypeptide antigen (TPA), tissue polypeptide specific antigen (TPS), cytokeratin 19 (CYFRA 21-1), cytokeratin 8 and 18 fragments (TPAcyk).

Table III. *A survey of literature concerning cytokeratins in sera in mesothelioma.*

Author	Cytokeratin	No. of patients	Results
			Survival
Hedman *et al.* (51)	TPA	11	High TPA corresponds to short survival duration.
Schouwink *et al.* (50)	TPA and CYFRA 21-1	52	Elevated TPA or CYFRA 21-1 are significant prognostic factors independent of other factors but not of each other.
			Monitoring of mesothelioma
Hedman *et al.* (51)	TPA	5	TPA level follows progression according to CT-scans and clinical status of the patients.
Schouwink *et al.* (50)	TPA and CYFRA 21-1	2	TPA and CYFRA 21-1 rose towards the end of life.
Marukawa (52)	CYFRA 21-1	5	CYFRA 21-1 concentration changed in proportion to disease activity in all cases.
Nisman *et al.* (53)	TPS and CYFRA 21-1	10	TPS and CYFRA 21-1 have similar patterns of reactivity; TPS better reflects clinical response.
			Diagnostics
Fuhrman *et al.* (69)	TPA and CYFRA 21-1	85	Both CYFRA 21-1 and TPA are significantly higher in sera from patients with malignant pleura effusion than in sera from controls.
Marukawa (52)	CYFRA 21-1	5	Sensitivity 40%.
Nisman *et al.* (53)	TPS	14	TPS levels significantly higher in MPM than in SQC
Ebert *et al.* (70)	CYFRA 21-1, TPA-M and TPS	33	Sensitivity 36.4% for CYFRA 21-1 and TPS; lower sensitivities for TPA-M, CEA and NSM.
Viallat *et al.* (54)	CYFRA 21-1 and TPA	41	Good sensitivity and specificity in discriminating between asbestosis and asbestos-induced cancer for both CYFRA 21-1 and TPA. Positive predictive value 0.95 respectively 0.91. TPA slightly better in ROC curve analysis.
Plebani *et al.* (43)	CYFRA 21-1, TPA, TPM, TPS	9	All are significantly higher in mesothelioma than in benign disease; TPM, TPA and CYFRA 21-1 are significantly higher than in SCLC and SQC, while TPM and TPA are significantly higher than in AC.
Pluygers (55)	TPA		TPA increases in asbestosis and even more in mesothelioma; the increase occurs early, before other signs.
			Comparison with other tumour markers
Hedman *et al.* (51)	TPA	11	TPA is associated with survival and better than the corresponding data for hyaluronan and CA 125.
Fuhrman *et al.* (69)	TPA and CYFRA 21-1	41	CEA is better than TPA or CYFRA 21-1 at distinguishing mesothelioma from other pleural malignancies.

Carcinoembryonic antigen (CEA), tissue polypeptide antigen (TPA), tissue polypeptide specific antigen (TPS), cytokeratin 19 (CYFRA 21-1).

3 Arriagada R, Bergman B, Dunant A, Le Chevalier T, Pignon JP and Vansteenkiste J: Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. N Engl J Med *350*: 351-360, 2004.

4 Rowell N and O'Rourke N: Concurrent chemoradiotherapy in non-small cell lung cancer. Cochrane Database Syst Rev 4, 2004.

5 Deslauriers J: Surgery for small cell lung cancer. Lung Cancer *17*: 91-98, 1997.

6 Osterlind K: Chemotherapy in small cell lung cancer. Eur Respir J *18*: 1026-1043, 2001.

7 Price B: Analysis of current trends in United States mesothelioma incidence. Am J Epidemiol *145*: 211-218, 1997.

8 Sugarbaker DJ, Norberto JJ and Swanson SJ: Extrapleural pneumonectomy in the setting of multimodality therapy for diffuse malignant pleural mesothelioma. Semin Thorac Cardiovasc Surg *9*: 373-382, 1997.

9 Sugarbaker DJ, Norberto JJ and Swanson SJ: Surgical staging and work-up of patients with diffuse malignant pleural mesothelioma. Semin Thorac Cardiovasc Surg *9*: 356-360, 1997.

10 Ung YC, Yu E, Falkson C, Haynes AE, Stys-Norman D and Evans WK: The role of radiation therapy in malignant pleural mesothelioma: a systematic review. Radiother Oncol *80*: 13-18, 2006.

11 Vogelzang NJ, Rusthoven JJ, Symanowski J, Denham C, Kaukel E, Ruffie P, Gatzemeier U, Boyer M, Emri S, Manegold C, Niyikiza C and Paoletti P: Phase III study of pemetrexed in combination with cisplatin *versus* cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol *21*: 2636-2644, 2003.

12 Stigbrand T, Andres C, Bellanger L, Bishr Omary M, Bodenmuller H, Bonfrer H, Brundell J, Einarsson R, Erlandsson A, Johansson A, Leca JF, Levi M, Meier T, Nap M, Nustad K, Seguin P, Sjodin A, Sundstrom B, van Dalen A, Wiebelhaus E,

Wiklund B, Arlestig L and Hilgers J: Epitope specificity of 30 monoclonal antibodies against cytokeratin antigens: the ISOBM TD5-1 Workshop. Tumour Biol 19: 132-152, 1998.

13 Stigbrand T: The versatility of cytokeratins as tumor markers. Tumour Biol 22: 1-3, 2001.

14 Nap M, van Wel T, Andres C, Bellanger L, Bodenmuller H, Bonfrer H, Brundell J, Einarsson R, Erlandsson A, Johansson A, Leca JF, Meier T, Seguin P, Sjodin A, Stigbrand T, van Dalen A, Wiebelhaus E, Wiklund B and Hilgers J: Immunohistochemical profiles of 30 monoclonal antibodies against cytokeratins 8, 18 and 19. Second report of the TD5 workshop. Tumour Biol 22: 4-10, 2001.

15 Magin TM, Schroder R, Leitgeb S, Wanninger F, Zatloukal K, Grund C and Melton DW: Lessons from keratin 18 knockout mice: formation of novel keratin filaments, secondary loss of keratin 7 and accumulation of liver-specific keratin 8-positive aggregates. J Cell Biol 140: 1441-1451, 1998.

16 Rye PD, Nustad K and Stigbrand T: Tumor marker workshops. Tumour Biol 24: 165-171, 2003.

17 Chu PG and Weiss LM: Keratin expression in human tissues and neoplasms. Histopathology 40: 403-439, 2002.

18 Scarpatetti M, Tsybrovskyy O and Popper HH: Cytokeratin typing as an aid in the differential diagnosis of primary versus metastatic lung carcinomas, and comparison with normal lung. Virchows Arch 440: 70-76, 2002.

19 Cai YC, Banner B, Glickman J and Odze RD: Cytokeratin 7 and 20 and thyroid transcription factor 1 can help distinguish pulmonary from gastrointestinal carcinoid and pancreatic endocrine tumors. Hum Pathol 32: 1087-1093, 2001.

20 Rydlander L, Ziegler E, Bergman T, Schoberl E, Steiner G, Bergman AC, Zetterberg A, Marberger M, Bjorklund P, Skern T, Einarsson R and Jornvall H: Molecular characterization of a tissue-polypeptide-specific-antigen epitope and its relationship to human cytokeratin 18. Eur J Biochem 241: 309-314, 1996.

21 Petak I and Houghton JA: Shared pathways: death receptors and cytotoxic drugs in cancer therapy. Pathol Oncol Res 7: 95-106, 2001.

22 Ku NO and Omary MB: Effect of mutation and phosphorylation of type I keratins on their caspase-mediated degradation. J Biol Chem 276: 26792-26798, 2001.

23 Silen A, Westlin JE, Letocha H, Wiklund B, Ekblom J and Nilsson S: Novel monoclonal antibodies reactive with cytokeratins 8 and 18. Immunocyt, Immunohist and Immunoscint, Antibody, Immunoconj Radiopharmaceut 7: 179-194, 1994.

24 Bauer T, Muhrer KH, Muller H and Grebe SF: Short-term and long-term monitoring of the serum level of TPA after radical resection of gastrointestinal or lung cancer. Nucl Med Commun 7: 121-127, 1986.

25 Eriksson P, Brattstrom D, Hesselius P, Larsson A, Bergstrom S, Ekman S, Goike H, Wagenius G, Brodin O and Bergqvist M: Role of circulating cytokeratin fragments and angiogenic factors in NSCLC patients stage IIIa-IIIb receiving curatively intended treatment. Neoplasma 53: 285-290, 2006.

26 Barak V, Goike H, Panaretakis KW and Einarsson R: Clinical utility of cytokeratins as tumor markers. Clin Biochem 37: 529-540, 2004.

27 Niklinski J, Furman M, Chyczewska E, Chyczewski L, Rogowski F, Jaroszewicz E and Laudanski J: Evaluation of CYFRA 21-1 as a new marker for non-small cell lung cancer. Eur J Cancer Prev 3: 227-230, 1994.

28 Pavicevic R, Milicic J, Bubanovic G and Supe S: Serum tumor marker CYFRA 21-1 in the diagnostics of NSCLC lung cancer. Coll Antropol 22: 629-635, 1998.

29 Pujol JL, Molinier O, Ebert W, Daures JP, Barlesi F, Buccheri G, Paesmans M, Quoix E, Moro-Sibilot D, Szturmowicz M, Brechot JM, Muley T and Grenier J: CYFRA 21-1 is a prognostic determinant in non-small-cell lung cancer: results of a meta-analysis in 2063 patients. Br J Cancer 90: 2097-2105, 2004.

30 Muley T, Dienemann H and Ebert W: Increased CYFRA 21-1 and CEA levels are negative predictors of outcome in p-stage I NSCLC. Anticancer Res 23: 4085-4093, 2003.

31 Kasimir-Bauer S, Schleucher N, Weber R, Neumann R and Seeber S: Evaluation of different markers in non-small cell lung cancer: Prognostic value of clinical staging, tumour cell detection and tumour marker analysis for tumour progression and overall survival. Oncol Rep 10: 475-482, 2003.

32 Fukunaga Y, Bandoh S, Fujita J, Yang Y, Ueda Y, Hojo S, Dohmoto K, Tojo Y, Takahara J and Ishida T: Expression of cytokeratin 8 in lung cancer cell lines and measurement of serum cytokeratin 8 in lung cancer patients. Lung Cancer 38: 31-38, 2002.

33 Bergqvist M, Brattstrom D, Hesselius P, Wiklund B, Silen A, Wagenius G and Brodin O: Cytokeratin 8 and 18 fragments measured in serum and their relation to survival in patients with non-small cell lung cancer. Anticancer Res 19: 1833-1836, 1999.

34 Lai RS, Hsu HK, Lu JY, Ger LP and Lai NS: CYFRA 21-1 enzyme-linked immunosorbent assay. Evaluation as a tumor marker in non-small cell lung cancer. Chest 109: 995-1000, 1996.

35 Niklinski J, Furman M, Rapellino M, Chyczewski L, Laudanski J, Oliaro A and Ruffini E: CYFRA 21-1 determination in patients with non-small cell lung cancer: clinical utility for the detection of recurrences. J Cardiovasc Surg 36: 501-504, 1995.

36 Tan Y, Zhang P and Zheng C: Usefulness of CYFRA21-1 as a tumor marker of non-small-cell lung cancer. Zhonghua Zhong Liu Za Zhi 21: 287-289, 1999.

37 Kao CH, Hsieh JF, Ho YJ and Ding HJ: Cytokeratin fragment 19 (CYFRA 21-1) and carcinoembryonic antigen for early prediction of recurrence of lung adenocarcinoma. Lung 177: 333-337, 1999.

38 Sun SS, Hsieh JF, Tsai SC, Ho YJ, Lee JK and Kao CH: Cytokeratin fragment 19 and squamous cell carcinoma antigen for early prediction of recurrence of squamous cell lung carcinoma. Am J Clin Oncol 23: 241-243, 2000.

39 Pendleton N, Occleston NL, Walshaw MJ, Littler JA, Jack CI, Myskow MW and Green JA: Simple cytokeratins in the serum of patients with lung cancer: relationship to cell death. Eur J Cancer 30: 93-96, 1994.

40 Rastel D, Ramaioli A, Cornillie F and Thirion B: CYFRA 21-1, a sensitive and specific new tumour marker for squamous cell lung cancer. Report of the first European multicentre evaluation. CYFRA 21-1 Multicentre Study Group. Eur J Cancer 30: 601-606, 1994.

41 Molina R, Agusti C, Mane JM, Filella X, Jo J, Joseph J, Gimenez N, Estape J and Ballesta AM: CYFRA 21-1 in lung cancer: comparison with CEA, CA 125, SCC and NSE serum levels. Int J Biol Markers 9: 96-101, 1994.

42 Szturmowicz M, Sakowicz A, Rudzinski P, Zych J, Wiatr E, Zaleska J and Rowinska-Zakrzewska E: The clinical value of Cyfra 21-1 estimation for lung cancer patients. Int J Biol Markers 11: 172-177, 1996.

43 Plebani M, Basso D, Navaglia F, De Paoli M, Tommasini A and Cipriani A: Clinical evaluation of seven tumour markers in lung cancer diagnosis: can any combination improve the results? Br J Cancer *72*: 170-173, 1995.

44 Takei Y, Minato K, Tsuchiya S, Takise A, Nakano H, Ezawa K, Fueki N, Hoshino H, Naruse I, Nomoto T, Makimoto T, Ishihara S, Saito R and Mori M: CYFRA 21-1: an indicator of survival and therapeutic effect in lung cancer. Oncology *54*: 43-47, 1997.

45 Pujol JL, Boher JM, Grenier J and Quantin X: Cyfra 21-1, neuron specific enolase and prognosis of non-small cell lung cancer: prospective study in 621 patients. Lung Cancer *31*: 221-231, 2001.

46 Bombardieri E, Seregni E, Bogni A, Ardit S, Belloli S, Busetto A, Caniello B, Castelli M, Cianetti A and Correale M: Evaluation of cytokeratin 19 serum fragments (CYFRA 21-1) in patients with lung cancer: results of a multicenter trial. Int J Biol Markers *9*: 89-95, 1994.

47 Paone G, De Angelis G, Munno R, Pallotta G, Bigioni D, Saltini C, Bisetti A and Ameglio F: Discriminant analysis on small cell lung cancer and non-small cell lung cancer by means of NSE and CYFRA-21.1. Eur Respir J *8*: 1136-1140, 1995.

48 Giovanella L, Ceriani L, Bandera M, Beghe B and Roncari G: Evaluation of the serum markers CEA, NSE, TPS and CYFRA 21.1 in lung cancer. Int J Biol Markers *10*: 156-160, 1995.

49 Stieber P, Dienemann H, Hasholzner U, Fabricius PG, Schambeck C, Weinzierl M, Poley S, Samtleben W, Hofmann K and Meier W: Comparison of CYFRA 21-1, TPA and TPS in lung cancer, urinary bladder cancer and benign diseases. Int J Biol Markers *9*: 82-88, 1994.

50 Schouwink H, Korse CM, Bonfrer JM, Hart AA and Baas P: Prognostic value of the serum tumour markers Cyfra 21-1 and tissue polypeptide antigen in malignant mesothelioma. Lung Cancer *25*: 25-32, 1999.

51 Hedman M, Arnberg H, Wernlund J, Riska H and Brodin O: Tissue polypeptide antigen (TPA), hyaluronan and CA 125 as serum markers in malignant mesothelioma. Anticancer Res 23: 531-536, 2003.

52 Marukawa M, Hiyama J, Shiota Y, Ono T, Sasaki N, Taniyama K and Mashiba H: The usefulness of CYFRA 21-1 in diagnosing and monitoring malignant pleural mesothelioma. Acta Med Okayama *52*: 119-123, 1998.

53 Nisman B, Barak V, Heching N, Kramer M Reinus C and Lafair J: Cytokeratin markers in malignant pleural mesothelioma. Cancer Detect Prev *22*: 416-421, 1998.

54 Viallat JR, Henri A, Sauvan R, Farisse P, Pasquier J, Hassoun J and Boutin C: Study of 2 markers, keratin and neuron-specific enolase, in bronchial cancers. Rev Pneumol Clin *42*: 119-124, 1986.

55 Pluygers E, Baldewyns P, Minette P, Beauduin M, Gourdin P and Robinet P: Biomarker assessments in asbestos-exposed workers as indicators for selective prevention of mesothelioma or bronchogenic carcinoma: rationale and practical implementations. Eur J Cancer Prev *1*: 129-138, 1992.

56 Chantapet P, Riantawan P, Lebnak P and Getngern P: Utility of serum cytokeratin 19 fragment (CYFRA 21-1) and carcinoembryonic antigen (CEA) as tumour markers for non-small cell lung cancer. J Med Assoc Thai *83*: 383-391, 2000.

57 Kim YC, Park KO, Choi IS, Kim HJ, Lim SC and Bom HS: A comparison of serum CYFRA 21-1 and SCC Ag in the diagnosis of squamous cell lung carcinoma. Korean J Intern Med *11*: 50-57, 1996.

58 Oremek GM, Seiffert UB, Siekmeier R and Kirsten R: Cyfra 21-1 – a new tumor marker of the cytokeratin series in differential diagnosis of lung diseases. Med Klin *90*: 23-26, 1995.

59 Niklinski J, Burzykowski T, Niklinska W, Laudanski J, Chyczewski L, Rapellino M and Furman M: Preoperative CYFRA 21-1 level as a prognostic indicator in resected nonsmall cell lung cancer. Eur Respir J *12*: 1424-1428, 1998.

60 Yeh JJ, Liu FY, Hsu WH, Wang JJ, Ho ST and Kao A: Monitoring cytokeratin fragment 19 (CYFRA 21-1) serum levels for early prediction of recurrence of adenocarcinoma and squamous cell carcinoma in the lung after surgical resection. Lung *180*: 273-279, 2002.

61 Ebert W and Muley T: CYFRA 21-1 in the follow-up of inoperable non-small cell lung cancer patients treated with chemotherapy. Anticancer Res *19*: 2669-2672, 1999.

62 Nisman B, Lafair J, Heching N, Lyass O, Baras M, Peretz T and Barak V: Evaluation of tissue polypeptide specific antigen, CYFRA 21-1, and carcinoembryonic antigen in nonsmall cell lung carcinoma: does the combined use of cytokeratin markers give any additional information? Cancer *82*: 1850-1859, 1998.

63 Moro D, Villemain D, Vuillez JP, Delord CA and Brambilla C: CEA, CYFRA21-1 and SCC in non-small cell lung cancer. Lung Cancer *13*: 169-176, 1995.

64 Wieskopf B, Demangeat C, Purohit A, Stenger R, Gries P, Kreisman H and Quoix E: Cyfra 21-1 as a biologic marker of non-small cell lung cancer. Evaluation of sensitivity, specificity, and prognostic role. Chest *108*: 163-169, 1995.

65 Takada M, Masuda N, Matsuura E, Kusunoki Y, Matui K, Nakagawa K, Yana T, Tuyuguchi I, Oohata I and Fukuoka M: Measurement of cytokeratin 19 fragments as a marker of lung cancer by CYFRA 21-1 enzyme immunoassay. Br J Cancer *71*: 160-165, 1995.

66 Koga H, Eguchi K, Shinkai T, Tamura T, Ohe Y, Oshita F, Saijo N, Kondo H, Oki K and Okura H: Preliminary evaluation of the new tumor marker, CYFRA 21-1, in lung cancer patients. Jpn J Clin Oncol *24*: 263-268, 1994.

67 Van der Gaast A, Schoenmakers CH, Kok TC, Blijenberg BG, Cornillie F and Splinter TA: Evaluation of a new tumour marker in patients with non-small-cell lung cancer: Cyfra 21.1. Br J Cancer *69*: 525-528, 1994.

68 Miedouge M, Devys A, Simon M, Rouzaud P, Salama G, Reyre J, Pujazon M, Carles P and Serre G: High levels of cytokeratin 19 fragments but no evidence of cytokeratins 1, 2, 10/11, 14 or filaggrin in the serum of squamous cell lung carcinoma patients. Tumour Biol *22*: 19-26, 2001.

69 Fuhrman C, Duche JC, Chouaid C, Abd Alsamad I, Atassi K, Monnet I, Tillement JP and Housset B: Use of tumor markers for differential diagnosis of mesothelioma and secondary pleural malignancies. Clin Biochem *33*: 405-410, 2000.

70 Ebert W, Hoppe M, Muley T and Drings P: Monitoring of therapy in inoperable lung cancer patients by measurement of CYFRA 21-1, TPA- TP CEA, and NSE. Anticancer Res *17*: 2875-2878, 1997.