National Academy of Clinical Biochemistry Guidelines for the Use of Tumor Markers in Lung Cancer

Petra Stieber[1*], Rudolf Hatz[2], Stefan Holdenrieder[1], Rafael Molina[4], Marius Nap[5], Joachim von Pawel[6], Andreas Schalhorn[3], Joachim Schneider[7], Ken Yamaguchi[8]

[1]Departments of Clinical Chemistry, [2]Surgery and [3]Medicine, Klinikum der Universität München - Großhadern-Marchioninistr., München, Germany; [4] Department of Clinical Biochemistry, Hospital Clin, Barcelona, Spain; [5] Department of Pathology, Atrium Medical Centre, Heerlen, Netherlands; [6]Asklepios Fachklinik für Pneumologie, Gauting, Germany; [7]Institute for Social Medicine, Justus-Liebig-Universitat Giessen, Giessen, Germany: [8]National Cancer Center Research Institute, Tokyo, Japan.

*Sub-Committee Chair, to whom all comments should be addressed via e-mail to Petra.Stieber@med.uni-muenchen.de, with copies to C.Sturgeon@ed.ac.uk and ediamandis@mtsinai.on.ca

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Abbreviations: AUC, area under the curve; LDH, lactate dehydrogenase; NSCLC, non-small cell lung cancer; NSE, neuron specific enolase; progastrin-releasing peptide, ProGRP; SCC, squamous cell carcinoma; SCCA, squamous cell carcinoma antigen

Introduction

Lung cancer is the most frequent cancer in the world, both in terms of incidence (1.2 million new cases or 12.3% of the world total) and mortality (1.1 million deaths or 17.8% of the total). Trends in lung cancer incidence and mortality reflect smoking habits and/or exposure to other environmental or occupational carcinogens. The incidence rate in men is 34.9 per 100,000 with highest rates observed in more developed countries although in countries in which male tobacco consumption has declined, incidence and mortality are now slowly decreasing. In women, incidence rates are lower (11.1 per 100.000) with the highest rates found in North America and Northeastern Europe, but there is a rising trend in incidence and mortality (1-3).

Reflecting different clinical behaviour and sensitivity to chemo- and radiotherapy, lung cancers can be grouped in two major histological types, i.e. non-small cell and small cell lung cancer (NSCLC and SCLC respectively). NSCLC accounts for 75-85% of lung cancer patients and consists of several subtypes, predominantly squamous cell carcinomas, adenocarcinomas and large cell carcinomas, which are treated in the same manner. Small cell lung cancer accounts for 15-25% of lung cancer patients, often has neuroendocrine components, and is primarily treated with chemotherapy and/or radiotherapy. Many lung cancers constitute histologically mixed tumor types consisting of non-small cell and small cell components (4-6). Histological differentiation and staging of lung cancer is mandatory for therapeutic stratification.

Patients with lung cancer often do not exhibit specific symptoms, particularly in early stage disease. Dyspnoea, cough and thoracic pain are early signs, while hemoptysis often indicates advanced disease. Relapsing infectious diseases of the respiratory system in combination with a smoking history suggest a need for further diagnostic investigations, including medical history and physical examination, laboratory tests, chest radiography, thoracic CT or MRI, bronchoscopy and biopsy. For staging according to UICC criteria, additional CT or MRI of the abdomen and the brain, bone scan, and eventually positron emission tomography are used (5,6). Serum tumor marker measurements also potentially have an important role in both diagnosis and staging.

For patients with NSCLC, particularly those with early stage disease (Stages I to IIIA), surgery is the mainstay of treatment. The additional application of adjuvant radio- or chemotherapy after tumor resection has previously been shown to have only limited benefit. However, more recent data indicate a considerable improvement in overall survival when modern adjuvant chemotherapies are applied (7,8). The use of neoadjuvant systemic therapies prior to surgery to provoke tumor shrinkage and early eradication of systemic micrometastases is still under discussion (4,5). Five-year-survival rates depend strongly on tumor stage, with 60%-70% five-year survival reported for patients with Stage I disease,

40%-50% for Stage II and 15-30% for Stage IIIA (4,9). Currently, few patients with non-resectable NSCLC in advanced stages (IIIB and IV) will be cured. Median survival for Stage IV disease patients has been stable for years at 8 to 10 months. Although response rates for chemo- and radiotherapy are low, several studies have demonstrated moderate beneficial effects concerning survival, time to disease progression, and quality of life, as compared with best supportive care (4).

Small cell lung cancer is characterized by its rapid doubling time and propensity for early metastases. In clinical practice only two stages of SCLC are distinguished: limited stage disease with tumor confined to one hemithorax only and extensive stage disease with metastases in the contralateral chest or at distant sites. Approximately 20 to 25% of patients have limited disease, treatable with curative intent. However 5-year survival rates are still low (15-25%, compared with <5% in extensive disease). In these patients, multimodal approaches of chemo- and radiotherapy are recommended followed by prophylactic cranial irradiation to prevent cerebral metastases. Optimal timing, dose and fractionation of radiotherapy treatment have yet to be defined (4-6). For extensive SCLC, the treatment of choice is combination chemotherapy, usually cis- or carboplatin and etoposide. Current approaches also include new drugs such as topoisomerase I inhibitors and taxans (4-6).

The intensive search for new therapeutic drugs in advanced lung cancer is highlighted in the 2003 ASCO guidelines for the treatment of NSCLC, which for patients with good performance score suggest second and third-line therapies that were not available when previous recommendations were made in 1997 (10).

With the prospect of more effective therapeutic options for advanced stage disease, current follow-up procedures for lung cancer should perhaps be reviewed. Tumor marker measurements, which potentially provide sensitive and cost-effective early detection of recurrence, may become increasingly important in assessing the efficacy of therapy. The aim of this article is to assess the current state of knowledge of the clinical use of serum-based tumor markers in lung cancer and to present new National Academy of Clinical Biochemistry (NACB) recommendations. Guidelines published by other Expert Panels on this topic are also summarised.

Currently Available Markers for Lung Cancer

Table 1 lists the mostly widely investigated tissue-based and serum-based tumors markers for lung cancer. Also listed is the phase of development of each marker as well as the level of evidence (LOE) for its clinical use. The levels of evidence grading system used is based on that described by Hayes *et al* (11) *[see Section 1]*.

Tumor Markers in Lung Cancer: NACB Recommendations

Table 2 presents a summary of recommendations from various Expert Panels on the use of tumor markers in lung cancer. This table also summarises the NACB guidelines for the use of markers in this malignancy.

Neuron specific enolase (NSE)

Screening and diagnosis. NSE does ot have the sensitivity or specificity necessary for use in screening, but numerous studies support its use as an aid in the diagnosis of small cell lung cancer. High serum levels of NSE (>100 μg/L) in patients with suspicion of malignancy suggest the presence of SCLC with high probability, with the differential diagnoses including neuroendocrine tumors of other localisations, liver cancer, lymphoma and seminoma. Moderate elevations of NSE are also found in patients with benign lung diseases as well as in some pancreatic, gastric, colorectal and breast cancers. Several groups have reported improved diagnostic discrimination when NSE is combined with measurement of prograstrin releasing peptide (ProGRP) (12-19).

Prognosis and monitoring. The prognostic value of NSE has been demonstrated in multivariate trials for both SCLC (15,20-23) and NSCLC (19,24-29). NSE has shown considerable potential for the monitoring of post-treatment SCLC (13,15,16,30-32) as well as for the detection of recurrent disease of SCLC after primary therapy (13,16).

Analytical concerns: As NSE is present in erythrocytes, plasma cells and platelets, serum or plasma must be separated from red cells within 60 minutes of venipuncture to avoid spuriously high results. Serum samples should be stored at +4°C (short term) and at -70°C (long term).

Carcinoembryonic antigen (CEA)

Screening and diagnosis. CEA concentrations are particularly high in adenocarcinoma and large cell lung cancer, but the elevated concentrations also found in various benign pathologies and other malignancies preclude its use in screening and limit its diagnostic use. However, CEA may be helpful in the differential diagnosis of non-small cell lung cancer, preferably in combination with CYFRA 21-1 (12,16,19,33,34).

Prognosis and monitoring. CEA may provide prognostic information in NSCLC, particularly in adenocarcinoma of the lung (19,29,34-42). Further it may have a role in monitoring therapy in advanced stages (16,43,44), and detecting recurrent disease of non-small cell adenocarcinoma (16,45).

Analytical concerns: Slightly higher CEA results may be observed in smokers.

Cytokeratin-19 fragments (CYFRA 21-1)

Screening and diagnosis. CYFRA 21-1 is the most sensitive tumor marker for NSCLC, particularly squamous cell tumors. Since CYFRA 21-1 determines only fragments of cytokeratin 19, the test shows a higher specificity than tissue polypeptide antigen (TPA), which determines a mixture of cytokeratins 8, 18 and 19. It is also elevated in urological, gastrointestinal and gynaecological cancers and in lower amounts in various benign diseases (34,46-48), precluding its use in screening and limiting its use in diagnosis. However, its measurement may be helpful in the differential diagnosis of suspicious lung masses, particularly if biopsy is not possible. Although a metaanalysis has not yet been performed, numerous authors have reported that in certain circumstances CYFRA 21-1 can aid in diagnosis (12,14,16,19,27,29,34,46,49,50).

Prognosis and monitoring. A recent analysis of pooled data from nine centers demonstrated CYFRA 21-1 to be an independent prognostic factor in both early and late stages of NSCLC (51), confirming earlier multivariate studies demonstrating its prognostic relevance (27,34,37,38,51-60). Other reports have suggested CYFRA 21-1 may also have prognostic value in SCLC (61-63).

CYFRA 21-1 has considerable potential for monitoring treatment of NSCLC in advanced disease (16,49,54,64-69) as well as for the detection of recurrent disease after primary therapy, particularly in squamous cell lung cancer (16,45,67,70-72). Recent reports suggest that in patients with advanced stages of NSCLC undergoing chemotherapy, trends in CYFRA 21-1 during the initial treatment phase predict the response to subsequent therapy (60,67,69).

Analytical concerns: When frozen samples are thawed for cytokeratin analysis, vigorous mixing of samples should be avoided, as cytokeratins may adhere to tube walls after extreme agitation. CYFRA 21-1 values may be significantly influenced by renal failure, in which higher results may be observed.

Progastrin-releasing peptide (ProGRP)

Screening and diagnosis. ProGRP is a reliable marker for SCLC, with good specificity and sensitivity (73-76), although in view of the incidence of SCLC in the general population these are not high enough to allow its use in screening. However it is rarely elevated in other malignancies, and if so, generally only mildly. Renal disease may cause elevations up to 300 ng/L, but raised concentrations are not seen in other benign diseases. ProGRP concentrations >200 ng/L are highly suspicious for lung cancer, and concentrations >300 ng/L for SCLC if renal function is not impaired (73,75-77).

ProGRP has shown to be helpful in differential diagnosis, particularly in distinguishing SCLC from other lung cancers. When used as a single marker, it is superior to NSE, while

combining both markers provides additional information. ProGRP is released in measurable amounts in early stage SCLC and does not correlate with tumor extent (14,16,18,73,75-81).

Prognosis and monitoring. Only one report supports the use of ProGRP in prognosis. (82). However, several studies suggest it may be useful in monitoring SCLC (16,32,73,78,79,82,83) or detecting recurrent disease after primary therapy (16,82-84).

Analytical concerns. Because of the instability of GRP in serum, the more stable recombinant ProGRP [31-98] was developed as serum parameter. ProGRP values may also be significantly elevated due to renal failure.

Squamous cell carcinoma antigen (SCCA)

Screening and diagnosis. Although significantly less sensitive in NSCLC than CYFRA 21-1, and not suitable for use in screening, SCCA has superior specificity for squamous cell cancer and can be used for histological subtyping. However it may be significantly raised in squamous tumors of the cervix, oesophagus, head, neck and lung, as well as in dermatological diseases. SCCA may be used in the differential diagnosis of NSCLC, particularly for squamous cell cancer, preferably in combination with CEA and CYFRA 21-1 (12,19,85,86).

Prognosis and monitoring. Potential prognostic utility of SCCA in NSCLC has been reported (34,87-89).

Analytical concerns. Preanalytical contamination (e.g. with skin or saliva) can result in significant elevations of SCCA, as can renal failure.

Role of Tumor Markers for Early Detection of Lung Cancer

Screening There are no reports demonstrating the usefulness of single markers or combinations of markers for the early diagnosis of lung cancer in asymptomatic populations or in specific high-risk groups such as smokers.

Diagnosis Tumor markers have considerable potential for differential diagnosis and histological subtyping, particularly in lung tumors of unknown origin. Despite the overlap with healthy controls and patients with benign diseases, highly elevated concentrations of CEA, CYFRA 21-1, NSE, SCCA, and ProGRP are suggestive of malignancy. Within the marker profile, the leading markers suggest the most probable histologies, as follows: in adenocarcinoma CEA; in squamous cell carcinoma CYFRA 21-1 and SCC; in large cell cancer CYFRA 21-1 and NSE; and in small cell lung cancer NSE and ProGRP. Most markers including CYFRA 21-1, CEA, NSE and SCC correlate clearly with tumor burden. Only ProGRP can reach high levels even in limited SCLC disease. However normal or only slightly increased marker concentrations never exclude any kind of tumor disease or

progression. Despite these limitations, the determination of tumor markers at the time of primary diagnosis may be helpful for the following reasons:

- The pattern of tumor marker release points to the histological background of the tumor and can reveal mixed histological components.
- Tumor markers expressed and released at the time of primary diagnosis are likely to be the most relevant markers for follow-up monitoring.
- CYFRA 21-1, CEA, NSE and lactate dehydrogenase (LDH) are independent prognostic factors of high significance in NSCLC, as are NSE, LDH, and CYFRA 21-1 in SCLC.
- The rate and extent of decrease of preoperatively released markers after surgery provide useful information about remaining tumor burden and the effectiveness of therapy.
- ProGRP at high levels reaches 100% specificity for small cell lung cancer and serves as valuable diagnostic tool for therapeutic stratification.

Determination of carcinoembryonic antigen (CEA), CYFRA 21.1, NSE, ProGRP and SCCA at the time of primary diagnosis may be performed as suggested in Table 3.

In all types of NSCLC including the squamous cancer cell subtype, highest diagnostic sensitivity has been reported for CYFRA 21-1 in a number of studies (12,19,27,30,33,46,49,54,76,86). Although SCCA had a lower sensitivity than CYFRA 21-1, its high specificity for squamous cell cancer is valuable for differential diagnosis. SCC concentrations >2 μg/L are associated with a 95% probability of having NSCLC and 80% probability of having a squamous tumor (19). If CEA is >10 μg/L and CA125 >100 U/mL, the presence of adenocarcinoma or large cell carcinoma is very likely (19). Due to the additive diagnostic sensitivity of CEA and CYFRA 21-1, the combined use of both markers may be helpful in NSCLC.

In SCLC, NSE and ProGRP are superior to CEA and CYFRA 21-1 concerning tumor and organ specificity (12). The diagnostic sensitivity of ProGRP was found to be higher than (15,76) or comparable to NSE (14,18). Because of the different pathophysiologic background, both markers show additive sensitivity and play a complementary role in the diagnosis of SCLC (14,18,76). As ProGRP reaches high levels already in limited stages of disease and as mildly elevated concentrations are observed only rarely in other benign and malignant diseases, ProGRP >500 ng/L is considered to be a diagnostic tool for SCLC. In receiver operating characteristic (ROC) comparisons of histological discrimination between NSCLC and SCLC, ProGRP reaches an area under the curve (AUC) of 0.85-0.97 and NSE an AUC of 0.81-0.95 (18,81).

Concerning the differentiation of suspicious lung masses, computed algorithms of marker combinations provide an additional increase in diagnostic sensitivity: by 10% compared with CYFRA 21-1 as best single marker when using a multiple regression analysis, and by 20% when using a fuzzy-logic based classification system (90,91).

Role of Tumor Markers for Prognosis

Numerous studies report the prognostic value of one or several tumor markers in conjunction with clinical and/or laboratory parameters. However, comparing these studies is frequently difficult due to a) the heterogeneity of the study populations (mixture of early and advanced stages, mixture of various histological types), b) the use of univariate and multivariate analyses, c) failure to compare single (often new) parameters with established prognostic parameters, particularly clinical variables, d) failure to define how ”optimized“ cut off levels were chosen and even occasionally using different methods to select cut off values within the same investigation, as well as other pitfalls. It would be highly desirable to compare all potential prognostic factors parameters in a single set of patients in order to identify the most useful ones (92,93).

Of those markers evaluated in NSCLC, CYFRA 21-1 appears to be the best prognostic marker in NSCLC patients, both in patients with early operable disease and in those with advanced disease, as recently demonstrated by a analysis of pooled data (51). In addition to CYFRA 21-1, LDH, albumin, calcium, NSE, CEA, CA125, TPS and DNA have shown independent prognostic value in various studies and should be integrated in future prognostic trials (24-29,35-42,51-55,57-59,71,88,89,94-96).

In SCLC, LDH, sodium, albumin and NSE have shown prognostic relevance in multivariate analyses (97). Recent work suggests that CYFRA 21-1 and Chromogranin A (62) and CYFRA 21-1 and NSE (63) may also be strong prognostic indicators.

Role of Tumor Markers for Patient Surveillance

Postoperative follow-up care, control of therapy efficacy and detection of recurrent disease are the main indications for tumor marker determinations in lung cancer.

Post-surgery follow up care The velocity and the completeness of tumor marker decrease after surgery is indicative of the further outcome of the patients. After a short-term increase immediately after therapeutic intervention, due to marker release from operatively damaged normal and tumor tissue, the decline depends on both biological marker half-life and residual tumor cells (98-100). Following curative resection, the levels of CYFRA 21-1, TPA, and SCC (half life 1.5-3 hours) are expected to decrease rapidly reaching the range of healthy persons within 1-2 days whereas CEA decrease occurs with some delay depending on the initial marker level [half life 1-4 days (98-102)]. If renal or liver dysfunction which can prolong the half life of tumor markers considerably (75,103,104) are excluded, a slowed marker clearance and/or an elevated plateau is indicative for the presence of residual tumor cells and predict early the recurrence of disease (98).

Control of systemic therapy When monitoring the efficacy of chemo- or radiotherapy by tumor markers, a substantial decrease often correlates with response to therapy whereas an

increase or an insufficient decrease are generally associated with progressive disease. In NSCLC, CYFRA 21-1 had the best concordance with tumor response [59% to 75%; (54,64,68)]. In the detection of progressive disease, specificity was 100% and sensitivity 52% respectively (54,68), whereas the concordance with remission was lower (42%). Early CYFRA 21-1 changes after one course of chemotherapy have been reported to be predictive for the further outcome (60,69), although another group did not observe this effect (67).
In SCLC, NSE and ProGRP reflect the clinical course and the response to therapy (32,83,105). During chemotherapy, their levels may increase temporarily 24 to 72 hours after therapy application as a result of tumor cytolysis (106) but then decrease rapidly to the individual baseline values (107). In contrast, failure of therapy is associated with persistently elevated or insufficiently decreasing marker concentrations. In cases with simultaneously elevated NSE and ProGPR, the combined determinations provide additional information (83).

Detection of recurrent disease

In the post-therapeutic surveillance situation tumor markers are sensitive indicators for recurrence of disease, often with a lead-time of several months as compared to imaging methods. In NSCLC, CYFRA 21-1 showed a sensitivity of 79% which increased further to 100% in patients with preoperative CYFRA 21-1 levels >3.3 µg/L. The lead-time was 2 to 15 months (108). As well as CYFRA 21-1 (70,107), TPS (109) and SCCA (110) have been reported to be potentially useful for the detection of recurrent disease in the squamous cancer cell subtype, while TPS and CEA were the best markers in adenocarcinoma (111).

In SCLC, NSE, ProGRP and CEA are relevant markers for detecting recurrent disease (83). Among them, ProGRP revealed the highest detection rate with a sensitivity of 67% (cf NSE, 20% and CEA, 38%), but there was a clear additive effect up to 79% sensitivity when ProGRP and NSE were combined. The median lead-time was 35 days for ProGRP, with no lead-time found for NSE (84). Similarly to the diagnostic approach, computed algorithms of marker combinations such as the fuzzy-logic based classification system provide an increased sensitivity for the detection of recurrent disease (32).

An absolute prerequisite for any kind of monitoring investigations is the maintainance of the same methods for tumor marker determinations. Changing methods should include one to two serial measurements with both methods in parallel.

Final Comments on the NACB Recommendation in Lung Cancer

The National Academy of Clinical Biochemistry, in making the recommendations for the use of serum tumor markers in lung cancer summarized in Tables 2 and 3, recognises that the routine use of these markers may, quite reasonably, not be widely implemented for clinical and/or financial reasons in the near future. However, in those centers already chosing to use

tumor markers in lung cancer patients, it is highly desirable that the appropriate markers are selected. It is for this reason that these early NACB recommendations have been developed.

While none of the tumor marker studies reviewed approach the highest level of evidence (11), the use of these markers in clinical practice is likely to increase as improved treatments become available. Future trials concerning prognosis, therapy monitoring and prediction of therapy response should be rigorous and should ideally be undertaken as part of prospective and randomized intervention trials. However, for diagnostic and differential diagnostic purposes, results from large retrospective or prospective studies conducted according to the current state of the art (112) do appear to demonstrate the value of specific markers or marker combinations.

Based on these pragmatic considerations the following recommendations can be made regarding the use of serum tumor markers in lung cancer:

1. Currently, single tumor markers, such as CYFRA 21-1, CEA, NSE, and ProGRP should not be used for screening purposes either in asymptomatic populations or in those at high risk for lung cancer (e.g. smokers).
2. Depending on histology, determination of CYFRA 21-1, CEA, NSE and/or ProGRP may be helpful in lung cancer patients prior to the first therapy. Where no histology can be obtained before surgery, measurement of all four markers is necessary to identify the leading marker (usually that present in highest concentration).
3. Where inoperable lung cancer is suspected but no histology is available, raised serum NSE and especially ProGRP are highly suggestive of small cell lung cancer while raised serum SCCA is suggestive of squamous cell cancer.
4. Follow-up of asymptomatic patients after primary therapy of lung cancer is controversial. However serial determinations of the appropriate tumor marker may help assess the completeness of tumor removal and provide early indication of recurrence.
5. CEA and CYFRA 21-1 can be measured during systemic treatment of non-small cell lung cancer, NSE and ProGRP during systemic treatment of small cell lung cancer to reflect response to therapy and to document progressive disease. Reliable criteria for “biochemical progression” are still required to initiate tumor marker-based intervention trials in future.
6. Careful attention to pre-analytical factors is essential. Specimens for NSE determination should be separated from the clot within 60 minutes of collection, and haemolysed samples should not be assayed. Vigorous mixing of serum samples after thawing should be avoided for cytokeratin measurements. Contamination of samples with skin or saliva must be avoided for SCCA measurements. Samples may be stored at +4°C (short term) and at -70°C (long term).

7. Serial measurements should be performed using the same tumour marker test, which should be indicated on the laboratory report and documented in the patient's medical records.

Table 1. **Useful and potentially useful markers for lung cancer.**

Cancer Marker	Proposed Use/Uses	**Phase of Development**	**LOE**[1]	**Ref**
NSE	Differential diagnosis of lung masses when biopsy is not available: in high levels high specificity for small cell carcinoma; in SCLC, additive information to ProGRP	In clinical use, but value not validated in a high-level evidence study	III	12-19
	Assessing prognosis. High levels predict adverse outcome in SCLC	In clinical use, but value not validated in a high-level evidence study	II-III	15,20-23
	Assessing prognosis. High levels predict adverse outcome in NSCLC	In clinical use, but value not validated in a high-level evidence study	II-III	19,24-29
	Monitoring therapy in SCLC	In clinical use, but value not validated in a high-level evidence study	III	13,15,16,30-32
	Monitoring therapy in advanced disease (NSCLC)	Not in clinical use	IV-V	44
	Detection of recurrent disease. Increasing kinetics indicate progressive disease in SCLC	In clinical use, but value not validated in a high-level evidence study	IV	13,16
CEA	Differential diagnosis of lung masses when biopsy is not available; in high levels high specificity for adenocarcinoma; in NSCLC, additive information to CYFRA 21-1	In clinical use, but value not validated in a high-level evidence study.	III	12,16,19,33,34
	Assessing prognosis. High levels predict adverse outcome in early and advanced stage NSCLC	Not in clinical use	III-IV	19,29,34-42
	Monitoring therapy in advanced disease (NSCLC and SCLC)	Not in clinical use	IV	16,43,44
	Detection of recurrent disease. Increasing kinetics indicate progressive disease in NSCLC, part. in adeno cancer.	Not in clinical use	III-IV	16,45

CYFRA 21-1	Differential diagnosis of lung masses when biopsy is not available: in high levels high specificity for squamous cell carcinoma; best marker for NSCLC	In clinical use, but value not validated in a high-level evidence study.	III	12,14,16,19,27,29,34,46,49,50
	Assessing prognosis. High levels predict adverse outcome in early and advanced NSCLC	Recommended for clinical use	I-II	27,34,37,38,51-60
	Assessing prognosis. High levels predict adverse outcome in SCLC	Not in clinical use	III	61,62
	Monitoring therapy in advanced disease (NSCLC)	In clinical use, but value not validated in a high-level evidence study.	II-III	16,49,54,64-69
	Early prediction of therapy response in advanced disease (NSCLC)	Not in clinical use yet, undergoing further validation	II-III	60,67,69
	Detection of recurrent disease. Increasing kinetics indicate progressive disease in NSCLC, part in squamous cell cancer.	In clinical use, but value not validated in a high-level evidence study.	III	16,45,67,70-72
ProGRP	Differential diagnosis of lung masses when biopsy is not available: in high levels high specificity for small cell carcinoma; best marker for SCLC; additive information to NSE	In clinical use, but value not validated in a high-level evidence study.	III	14,16,18,73,75-81
	Assessing prognosis. High levels predict adverse outcome in SCLC	Not in clinical use	IV	82
	Monitoring therapy in SCLC	In clinical use, but value not validated in a high-level evidence study.	III	16,32,73,78,79,82,83
	Detection of recurrent disease. Increasing kinetics indicate progressive disease in SCLC.	In clinical use, but value not validated in a high-level evidence study.	III	16,82-84
SCCA	Differential diagnosis of lung masses when biopsy is not available: in high levels high specificity for squamous cell carcinoma; in SQC additive information to CYFRA 21-1 Abnormal levels are associated with a high probability of NSCLC, mainly squamous tumors	In clinical use, but value not validated in a high-level evidence study.	III	12,19,85,86
	Assessing prognosis. High levels predict adverse outcome in NSCLC	Not in clinical use	III-IV	34,87-89
CA 125	Differential diagnosis of lung masses when biopsy is not available; in high levels relative specificity for	Not in clinical use	III	19

	adenocarcinoma, large cell carcinoma			
	Assessing prognosis in NSCLC. High levels predict adverse outcome in NSCLC	Not in clinical use	IV	35,59,94,113
	Monitoring therapy in advanced disease (NSCLC)	Not in clinical use	IV	43
	Early prediction of therapy response in advanced disease (NSCLC)	Not in clinical use	IV	94
Chromogranin A	Differential diagnosis of lung masses when biopsy is not available; particularly for neuroendocrine tumors	In clinical use, but value not validated in a high-level evidence study.	III	81,114-116
	Assessing prognosis. High levels predict adverse outcome in SCLC and in neuroendocrine tumors	Not in clinical use	III-IV	62,117
	Monitoring therapy in neuroendocrine tumors	Not in clinical use	IV	116
HER2-neu	Not appropriate for differential diagnosis	Not in clinical use	III	118,119
	Assessing prognosis. High levels predict adverse outcome in advanced NSCLC: conflicting data	Not in clinical use	IV	119,120
	Monitoring therapy in NSCLC not possible	Not in clinical use	IV	120
DNA fragments	Assessing diagnosis; correlation with stage	Not in clinical use yet, undergoing further validation	III	95,121,122
	Assessing prognosis. High levels predict adverse outcome	Not in clinical use yet, undergoing further validation	II-IV	95,96
	Monitoring therapy in advanced disease (NSCLC)	Not in clinical use yet, undergoing further validation	II-IV	69,95
	Early prediction of therapy response in advanced disease (NSCLC)	Not in clinical use yet, undergoing further validation	II-III	69
	Detection of recurrent disease. Increasing kinetics indicate progressive disease in NSCLC	Not in clinical use	III	121,122
TPA	Differential diagnosis of lung masses when biopsy is not available	Not in clinical use, undergoing further validation	III	50,57
	Assessing prognosis. High preoperative levels predict adverse outcome in NSCLC	Not in clinical use	III-IV	34,56,88
TPS	Assessing diagnosis (inferior to CYFRA 21-1 and TPA); correlation with stage	Not in clinical use	IV	54,57,123-125

	Assessing prognosis. High levels predict adverse outcome in NSCLC	Not in clinical use	III-IV	34,54,57
	Assessing prognosis. High levels predict adverse outcome in SCLC		III-IV	53,61
	Monitoring therapy in advanced disease (NSCLC)	Not in clinical use	III	54
	Early prediction of therapy response in SCLC	Not in clinical use	III-IV	53
	Detection of recurrent disease. Increasing kinetics indicate progressive disease in NSCLC.	Not in clinical use	III	109,111
TU M2-PK	Assessing diagnosis; inconsistent data are available	Not in clinical use	IV	126,127
	Monitoring therapy in NSCLC and SCLC	Not in clinical use	IV	128
	Detection of recurrent disease. Increasing kinetics indicate progressive disease in NSCLC and SCLC	Not in clinical use	IV	128

CEA, carcinoembryonic antigen; CYFRA 21-1, cytokeratin 19 fragments; HER2-neu; shed form of Her2-receptor; LOE, level of evidence; NSE, neuron specific enolase; ProGRP, progastrin-releasing peptide; SCCA; squamous cancer cell antigen; TPA, tissue polypeptide antigen, cytokeratin fragments 8, 18, and 19; TPS, tissue polypeptide specific-antigen; cytokeratin fragments 18; TU M2-PK, tumor M2 pyruvate kinase;

Table 2. Recommendations for use of markers in lung cancer by different expert groups.* [See Final Comments, p.9 for discussion of caveats relating to the recommendations in this Table.]

Marker	Application	EGTM 1999	NACB 2005
NSE	For differential diagnosis	Yes, for SCLC	Yes, for SCLC
	For prognosis	None published	None published
	For post-operative surveillance	Yes, in SCLC	Yes, in SCLC
	For monitoring therapy in advanced disease	Yes, in SCLC	Yes, in SCLC
	For detection of recurrent disease	Yes, in SCLC	Yes, in SCLC
CEA	For differential diagnosis	Yes, for NSCLC	Yes, for NSCLC
	For prognosis	None published	None published
	For post-operative surveillance	Yes, in adenocarcinoma	Yes, in NSCLC
	For monitoring therapy in advanced disease	Yes, in adenocarcinoma	Yes, in NSCLC
	For detection of recurrent disease	Yes, in adenocarcinoma	Yes, in NSCLC
CYFRA 21-1	For differential diagnosis	Yes, for NSCLC	Yes, for NSCLC
	For prognosis	None published	Yes, in NSCLC
	For post-operative surveillance	Yes, in all NSCLC and SCLC	Yes, in NSCLC
	For monitoring therapy in advanced disease	Yes, in all NSCLC and SCLC	Yes, in NSCLC
	For detection of recurrent disease	Yes, in all NSCLC and SCLC	Yes, in NSCLC
ProGRP	For differential diagnosis	None published	Yes, for SCLC
	For prognosis	None published	None published
	For post-operative surveillance	None published	Yes, in SCLC
	For monitoring therapy in advanced disease	None published	Yes, in SCLC
	For detection of recurrent disease	None published	Yes, in SCLC

*The American Cancer Society (ACS) and the American Society of Clinical Oncology (ASCO) have not as yet published recommendations relating to the use of tumor markers in lung cancer.

Abbreviations: EGTM, European Group on Tumor Markers; NACB, National Academy of Clinical Biochemistry; NSE, neuron specific enolase; CEA, carcinoembryonic antigen; CYFRA 21-1, cytokeratin 19 fragments; ProGRP, progastrin-releasing peptide; SCLC; small cell lung cancer; NSCLC; non-small cell lung cancer.

Table 3. Recommendations for use of markers according to histologies of lung cancer and application forms.

Histology	**Before therapy**	**Post-therapy follow-up**
Unknown	CYFRA 21-1, CEA, NSE, ProGRP	After surgery: following histology In advanced disease: using the leading marker
Adenocarcinoma	CYFRA 21-1 and CEA	CYFRA 21-1 and/or CEA
Squamous cell carcinoma	CYFRA 21-1 and CEA (and SCCA)	CYFRA 21-1 and/or CEA (and/or SCCA)
Large cell carcinoma	CYFRA 21-1 and CEA	CYFRA 21-1 and/or CEA
Small cell carcinoma	NSE and ProGRP	NSE and/or ProGRP

CEA, carcino embryonic antigen; CYFRA 21-1, cytokeratin 19 fragments; NSE, neuron specific enolase; ProGRP, progastrin-releasing peptide; SCCA, squamous cell carcinoma antigen

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