Clinical Relevance of Thymidine Kinase for the Diagnosis, Therapy Monitoring and Prognosis of Non-operable Lung Cancer

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Abstract. *Background: Whether thymidine kinase (TK) is considered a new diagnostic biomarker in lung cancer depends on it being superior to or adding further information to already established tumor markers. Here, we investigated its relevance in diagnosis, therapy monitoring and prognosis of patients with diverse forms of lung cancer. Patients and Methods: Pretherapeutic TK concentrations were analyzed by radioimmunoassay in serum of 181 patients with advanced lung cancer (53 small cell lung cancer (SCLC), 128 non-small cell lung cancer (NSCLC)), 40 with benign lung diseases, 44 with benign non-lung-related diseases and 29 healthy controls. Diagnostic power of TK was compared with that of established lung cancer markers carcinoembryonic antigen (CEA), cytokeratin 19-fragments (CYFRA 21-1), neuron-specific enolase (NSE) and progastrin-releasing peptide (ProGRP). Furthermore, TK courses of 29 NSCLC patients during cytotoxic chemotherapy were recorded and prognostic relevance of pretherapeutic TK levels was tested in 128 NSCLC patients. Results: While healthy controls had low TK serum levels (median 2.5 U/l, 95th percentile 8.8 U/l), they were significantly higher in patients with lung cancer (median 4.2 U/l, p=0.014) and also in patients with benign lung diseases (median 5.7 U/l; p=0.002). Patients with lung cancer and benign lung diseases could not be separated by TK values. No noticeable difference of TK concentrations was further found in NSCLC (median 4.3 U/l) as compared with SCLC patients (median 3.7 U/l) neither in adeno cell carcinomas (median 5.4 U/l) and squamous cell carcinomas (median 3.0 U/l). In NSCLC, the best diagnostic capacity versus benign lung diseases was found for CYFRA 21-1 (AUC 88.2%), NSE (AUC 86.4%), and CEA (AUC 82.9%), while TK reached only an AUC of 45.7%. The best diagnostic profile in SCLC versus benign lung diseases was observed for NSE (AUC 93.9%) and ProGRP (AUC 85.4%), while TK did not have any diagnostic power (AUC 46.6%). Concerning therapy monitoring, TK was unable to discriminate between the various response groups, neither pretherapeutically, nor before therapy cycles 2 and 3. However, pretherapeutic TK levels showed high prognostic value for overall survival in NSCLC patients: While median survival in patients with TK levels ≥20 U/l was only 3.1 months, it was 9.0 months in patients with TK levels <20 U/l. In multivariate analyses, TK remained an independent prognostic marker, along with the clinical variables stage and performance score. Conclusion: Although the performance of serum TK for diagnosis and therapy monitoring of advanced lung cancer was poor, it has a promising prognostic relevance which will have to be further validated.*

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Lung cancer is the most frequent type of cancer in the world, both in terms of incidence (1.2 million new cases or 12.3% of the world total cancer incidence) and mortality (1.1 million deaths or 17.8% of the total cancer mortality) (1). It is subdivided into two major histological types, non-small cell and small cell lung cancer (NSCLC and SCLC), due to differences in tumor biology and clinical consequences. NSCLC accounts for 75-85% of lung cancer patients and can be further classified into squamous cell, adeno cell, large cell carcinoma and some rare subtypes. In early stages, NSCLC can be treated by surgery and has an acceptable overall prognosis with a 5-year survival of 60-70% in stage I disease and 40-50% in stage II (2-3). However, about 70% of NSCLC patients are diagnosed at advanced stages when prognosis is poor (<5% overall 5-year survival rate in stages IIIB and IV) and systemic chemo- and/or radiotherapy are

the currently available therapy options (2-5). Similarly, SCLC patients, who account for 15-25% of all lung cancer patients, receive mainly systemic therapies which differ in the type and dose of the drugs used compared to NSCLC. Therefore, it is highly relevant in cases of suspicious lung masses to diagnose not only the grade of malignancy, but also the exact histological subtype in order to stratify the patients for the recommended therapies (2, 5-7).

Nowadays, pathology is still the golden standard for histological diagnosis of lung tumors. However, there are several obstacles that may impair the diagnosis in some cases. Even if biopsies are performed, it is not always possible to obtain material from the tumor due to the distant localization of the masses or due to technical means. Moreover, some patients presenting with advanced tumor disease are in poor condition which may hinder performance of bronchoscopy and biopsy. Finally, it is known that lung tumors often consist of heterogenous histological parts, and as biopsy is taken only at some localizations, an important information about a small-cell component of the tumor might be missed, leading to an incorrect treatment stratification of the patients (2, 4, 8).

Therefore, new diagnostic tools are needed to yield better diagnostic information. As biochemical properties of the tumor are mirrored not only by the tumor cells themselves but also by secreted or released molecules, circulating biomarkers in serum and plasma seem to be promising candidates indicating the presence and type of the tumor in blood investigations (9-12). To date, several biomarkers have shown high sensitivities and specificities for the detection and staging of specific lung cancer subtypes which are summarized in the new guidelines of the National Academy of Clinical Biochemistry (NACB; 12). Several studies have confirmed that in NSCLC, the most informative biomarkers are cytokeratin 19-fragments (CYFRA 21-1) and carcinoembryonic antigen (CEA; 9-10, 13-15). CYFRA 21-1 showed the highest sensitivity in squamous cell lung cancer, while CEA yielded the best rates of sensitivity in adeno cell lung cancer. However, it should be pointed out that both markers are released in other histologies of lung cancer, too (9-10, 16). In contrast, squamous cell cancer antigen (SCCA) is released very specifically by squamous cell lung cancer, even if it does not reach the high sensitivity of CYFRA 21-1 (10). In SCLC, neuron-specific enolase (NSE) and progastrin-related peptide (ProGRP) have demonstrated high sensitivities of 50-70% at a specificity of 95% *versus* benign lung diseases. Most importantly, both markers show an additive diagnostic sensitivity, detecting about 80% of SCLC patients with 95% specificity (17-21).

Among the biomarkers which have recently been proposed as new diagnostic tools for lung cancer, thymidine kinase (TK) has been reported to be a promising candidate (22-25). As is already known from lymphatic diseases, TK indicates the proliferative characteristics of neoplastic cells (26) and as many lung tumors proliferate very rapidly, the pathophysiological background suggests a high relevance of this biomarker in lung cancer (22-25). Thymidine kinase is an enzyme present in most living cells. It is active as two isoforms, TK I and TK II, which show chemical and biological differences. TK I was first found in fetal tissue, where it is cell-cycle dependently localized in cytoplasm in anticipation of cell division. In contrast, TK II is abundantly present in adult tissue and shows activity in mitochondria independently from the cell cycle (27).

For detection and estimation of prognosis in cancer, the TK I isoform is most relevant and can be measured by radio- or enzyme-immunological techniques. TK I is known as a salvage enzyme which appears only in the G_1 and S phase of cell division while it is absent in resting cells. Already two decades ago, the usefulness of TK I for the estimation of prognosis in patients with non-Hodgkin's lymphoma and plasmocytoma was described (26). Recently, it was proposed as a diagnostic and/or prognostic marker in various types of solid tumors such as prostate cancer and lung cancer (22-26). However, the diagnostic capacity of TK for lung cancer *versus* differential diagnostically relevant lung diseases, and in comparison with already established lung cancer biomarkers, has not been demonstrated yet. The present study was undertaken to investigate the relevance of TK concerning diagnosis of lung cancer and its histological subtypes in relation to CEA, CYFRA 21-1, NSE and ProGRP, and to identify potential influences of various non-lung diseases on TK metabolism relevant for the interpretation of serum TK levels. In addition, the relevance of TK for the estimation of prognosis and for the monitoring of disease during chemotherapeutic treatment was tested.

Patients and Methods

Patients. In total 294 patients were included in the present study. Among them were 181 patients with inoperable lung cancer (53 SCLC and 128 NSCLC) and 40 patients with benign lung diseases (pneumonia, sarcoidosis, fibrosis, tuberculosis, respiratory insufficiency) who were in the care of the Asklepios Clinic Gauting from 2000 to 2003. Samples from 29 healthy controls and 44 patients with non-lung diseases (15 bacterial infections, 21 renal insufficencies, 8 cholestatic liver diseases) were obtained from the University Hospital Munich-Grosshadern. All samples were obtained at the time of active disease; in cancer patients they were taken before start of chemotherapy.

Lung cancer diagnostic and staging investigations consisted of whole-body computed tomography, bone-scan, and bronchoscopy with biopsy. Histopathological classification was evaluated in accordance with the revised World Health Organization (WHO) classification of lung tumors. Performance score was defined according to Eastern Conference Oncology Group (ECOG) criteria. The median age of the patients was 62 years (range 25 to 86 years) and the male-to-female ratio was 127 to 54.

Potentially influencing diseases were selected according to laboratory indicators, *e.g.* C-reactive protein was higher than 1.5 mg/dl in all patients with bacterial infections, creatinine was higher than 2.5 mg/dl in all patients with renal insufficiency, and bilirubin was higher than 2.5 mg/dl in all patients with cholestatic liver disease.

For evaluation of TK relevance in monitoring the disease during cytotoxic treatment, 29 patients with advanced NSCLC receiving first-line chemotherapy with platin-containing regimens were observed. To objectify the response to therapy, staging investigations were conducted in all patients before the start of the third cycle of chemotherapy, including clinical examination, whole-body computed tomography and laboratory examinations. The response to therapy was classified according to the World Health Organization classifications defining "partial remission" (R) as tumor reduction by ≥50%, "progression" (P) as tumor increase by ≥25% or appearance of new tumor manifestations, and "no change" (NC=stable disease) as tumor reduction by <50% or increase by <25% (28).

For evaluation of TK relevance in estimating the prognosis, all 128 patients with non-operable NSCLC undergoing first-line chemotherapy were enrolled in the study. Pretherapeutical clinical parameters and TK values were tested for their power to predict overall survival in these patients.

Methods. Blood samples were centrifuged at 3000 ×g for 15 minutes and lung cancer biomarkers and clinical chemistry parameters were quantified subsequently. For TK determination, samples were stored at –70˚C and were analyzed in batches later on by radio-enzyme immunoassay (REA).

TK REA (IM1948) from Immunotech, Czech Republic, was based on the transformation of 5-[^{125}I]-deoxyuridine to 5-[^{125}I]-deoxyuridinemonophosphate (^{125}I d-UMP) by TK contained in the sample. Using adsorption on ion exchange resin (separating reagent), ^{125}I-d-UMP was separated from the reaction mixture. After washing of the resin, its radioactivity was determined on a gamma counter. In detail, to 25 µl of the sample, 500 µl tracer were added and incubated for 3.5 hours at 37˚C. After pipetting 300 µl of separating reagent, the sample was incubated again for 30 minutes and then washed with acetone. Finally, radioactivity was measured in counts per minute using a gammacounter.

The oncological biomarkers CEA, CYFRA 21-1 and NSE were determined by an automatied Elecsys 2010 System from Roche Diagnostics, Germany. ProGRP was quantified by ELISA from ALSI, Japan/IBL, Germany.

Statistics. Means, medians, ranges and percentiles are listed in Tables in the various subgroups investigated. Distribution of values is demonstrated in histograms and dot plot graphics. Differences between the various groups were calculated by Wilcoxon test. Diagnostic power of the single markers is shown by receiver operating characteristic (ROC) curves. Areas under the curves (AUC) and sensitivities at 95% specificity *versus* control groups were calculated.

For monitoring purposes, the TK baseline values before the first, second and third cycle (BV1, BV2 and BV3), and the percentage changes (BV1-2, BV1-3) were considered for statistical analysis.

Prognostic relevance of TK was univariately tested by log-rank test and are illustrated by Kaplan-Meier curves using a TK cutoff of 20 U/l. Multivariate analysis also including clinical factors was carried out using Cox regression analysis.

Generally, a *p*-value <0.05 was considered statistically significant. All calculations were performed with SAS software (version 9.1; SAS Institute Inc., Cary, NC, USA).

Table I. *Means, medians, ranges and 95th percentiles for TK in sera of healthy individuals, patients with benign lung diseases, with lung cancer and with non-lung-related benign diseases.*

Diagnosis	N	Mean (U/l)	Median (U/l)	Range (U/l)	95th Perc. (U/l)
Healthy individuals	29	3.9	2.5	2.5-9.8	8.8
Benign lung diseases	40	8.3	5.7	2.5-32.7	26.0
All lung cancer	181	9.5	4.2	2.5-202.4	23.7
All NSCLC	128	9.3	4.3	2.5-202.4	23.0
Squamous cell carcinoma	42	7.4	3.0	2.5-23.4	19.4
Adenocarcinoma	52	12.4	5.4	2.5-202.4	26.2
Other NSCLC	34	7.0	3.7	2.5-30.7	23.0
All SCLC	53	10.0	3.7	2.5-60.1	40.5
Limited disease	29	10.3	4.2	2.5-60.1	44.9
Extended disease	24	9.6	3.7	2.5-40.5	35.0
Non-lung-related benign diseases	44	6.9	3.3	2.5-21.5	20.6
Renal insufficiency	21	6.2	2.5	2.5-20.6	12.5
Acute infection	15	9.5	8.1	2.5-21.5	21.5
Cholestatic liver disease	8	4.0	2.7	2.5-10.1	10.1

Results

Healthy individuals had low TK values (median 2.5 U/l, 95th percentile 8.8 U/l), patients with lung cancer at initial diagnosis had significantly higher values (median 4.2 U/l, 95th percentile 23.7 U/l) than healthy controls (p=0.014). TK levels of individuals suffering from benign lung diseases (median 5.7 U/l, 95th percentile 26.0 U/l), and benign non-lung diseases (median 3.3 U/l, 95th percentile 20.6 U/l) were also often elevated. There was no significant difference between lung cancer and benign lung diseases (p=0.412; Table I, Figures 1 and 2).

No noticeable difference in TK concentrations was found in sera for differential diagnosis of NSCLC (median 4.3 U/l, 95th percentile 23.0 U/l) compared with SCLC (median 3.7 U/l, 95th percentile 40.5 U/l; p=0.828), nor between the histological subtypes adenocarcinoma (median 5.4 U/l, 95th percentile 26.2 U/l), squamous cell carcinoma (median 3.0 U/l, 95th percentile 19.4 U/l) and NSCLC without further subclassification (median 3.7 U/l, 95th percentile 23.0 U/l; p=0.479; Figure 1). There was also no significant difference in TK values concerning stage in NSCLC (M0 *versus* M1; p=0.492) and SCLC (median limited disease 4.2 U/l; extensive disease 3.7 U/l; p=0.926). While the AUC of ROC curves of TK for the discrimination between lung cancer patients and healthy controls was 63.7% and sensitivity at 95% specificity was 35%, the AUC only reached 46.0% and sensitivity at 95% specificity was 5% when patients with benign lung diseases were used as the control group (Figure 3A). Comparison with other lung cancer biomarkers revealed best discriminating performance between lung cancer and benign lung diseases for NSE (AUC 88.6%), and CYFRA 21-1 (AUC 81.7%; Table II, Figure 3B).

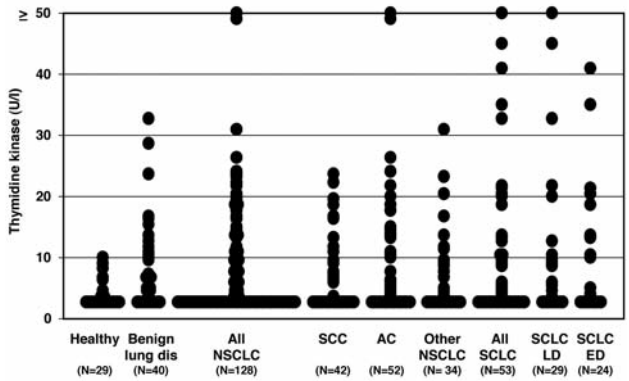

Figure 1. *Distribution of TK values in sera of healthy controls, patients with benign lung diseases and patients with various subgroups of lung cancer (SCC, squamous cell cancer; AC, adenocarcinoma; LD, limited disease; ED, extended disease).*

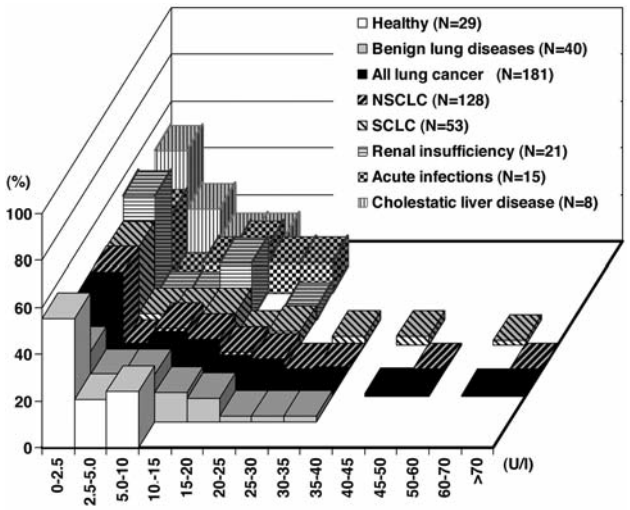

Figure 2. *Frequency distribution of TK in sera of healthy controls, patients with benign lung diseases, patients with NSCLC and SCLC, as well as patients with non-lung related benign diseases involved in TK metabolism.*

In NSCLC *versus* benign lung diseases, diagnostic capacity was best for CYFRA 21-1 (AUC 88.2%, 63% sensitivity always at 95% specificity), CEA (AUC 82.9%, 51% sensitivity) and NSE (AUC 86.4%, 47% sensitivity), while it was significantly lower for TK (AUC 45.7%, 2% sensitivity; Table II). Best diagnostic profiles in SCLC *versus* benign lung diseases were found for NSE (AUC 93.9%, 83% sensitivity always at 95% specificity) and ProGRP (AUC 85.4%, 70% sensitivity). In contrast, AUC of TK reached only 46.6% and sensitivity was only 9% (Table II).

Investigating the influence of benign non-lung diseases on TK concentrations revealed significantly higher TK levels in serum of patients with acute infections (median 8.1 U/l, range 2.5-21.5 U/l; p=0.016) as compared to healthy controls. Although median TK values of patients with renal insufficiency (2.5 U/l) and cholestatic liver diseases (2.7 U/l) were similar to the median of healthy controls (2.5 U/l), individual patients with renal insufficiency had considerably elevated levels up to 20 U/l (Figure 2).

Table II. *Areas under the curve (AUC) and sensitivities for lung cancer detection in various comparisons at 95% specificity versus benign lung diseases for TK and other lung cancer biomarkers.*

Comparison	AUC (%)	Confidence interval (%)	Sensitivity (%) at 95% specificity *vs*. control group
All lung cancer *vs*. benign lung diseases			
TK	46.0	36.7-55.3	5
CEA	79.2	72.5-86.0	43
CYFRA 21-1	81.7	75.3-88.3	51
NSE	88.6	83.3-93.9	58
ProGRP	63.3	54.5-71.9	34
NSCLC *vs*. benign lung diseases			
TK	45.7	35.9-55.6	2
CEA	82.9	76.6-89.1	51
CYFRA 21-1	88.2	82.6-94.0	63
NSE	86.4	79.9-93.0	47
ProGRP	54.2	43.6-64.6	19
SCLC *vs*. benign lung diseases			
TK	46.6	34.8-58.4	9
CEA	70.5	59.8-81.2	23
CYFRA 21-1	65.9	54.8-77.0	23
NSE	93.9	89.3-98.6	83
ProGRP	85.4	77.5-93.2	70
SCLC *vs*. NSCLC			
TK	51.0	41.6-60.5	9
CEA	33.3	24.9-41.7	2
CYFRA 21-1	25.6	17.3-33.9	2
CEA inv	66.8	58.4-75.2	12
CYFRA 21-1 inv	74.5	66.2-82.8	14
NSE	81.1	72.7-89.5	59
ProGRP	82.1	74.0-90.3	60

Concerning monitoring the disease in 29 patients with non-operable NSCLC during cytotoxic treatment, 10 patients had remission, 7 stable disease and 12 progressive disease at the time of staging investigations before the start of the third cycle of chemotherapy. Baseline values of TK before the first, second and third cycle (BV1, BV2 and BV3) were quite similar in the various response groups, with a tendency to lower values in patients with remission (medians: BV1: 5.7 U/l; BV2: 11.0 U/l; BV3: 7.9 U/l) when compared to patients with stable disease (BV1: 10.7 U/l; BV2: 15.5 U/l; BV3: 14.3 U/l) and progressive disease (BV1: 9.7 U/l; BV2: 15.4 U/l; BV3: 15.0 U/l). However, the variation of the values within the various groups was considerable (Figure 4A) and no significant results were obtained. The same heterogeneous picture was obtained for the

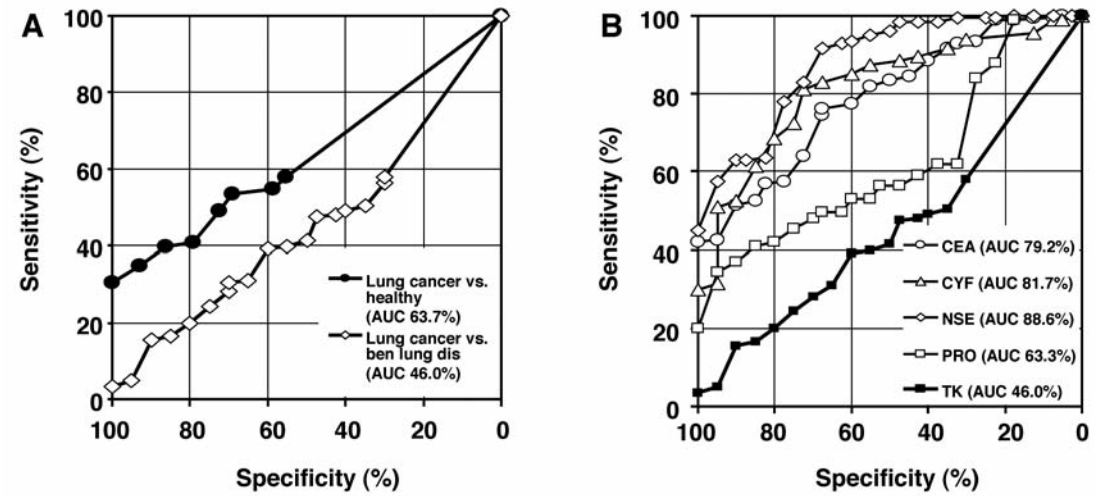

Figure 3. *A: ROC curves for the discrimination between patients with lung cancer versus healthy controls and between patients with lung cancer versus patients with benign lung diseases on the basis of TK. B: ROC curves for the discrimination between patients with lung cancer versus patients with benign lung diseases using TK, CEA, CYFRA 21-1, NSE, and ProGRP.*

Table III. *Multivariate analysis of TK and clinical variables for the overall survival of patients with non-operable NSCLC (p<0.0001).*

Variable	Chi-square	*P*-value	Hazard ratio	95% Confidence interval
Stage (metastasis other than lung)	11.4	0.0007	2.34	1.43-3.83
Performance score (ECOG 1 *vs*. 2 and ECOG 2 *vs*. 3)	18.4	<0.0001	2.25	1.55-3.25
Weight loss (>5% of body weight in 6 months)	0.5	0.4809	1.20	0.73-1.96
Age	<0.1	0.8310	1.00	0.97-1.03
Gender	0.3	0.6032	0.86	0.48-1.52
Thymidine kinase (>20 U/l)	13.1	0.0003	4.27	1.95-9.38

percentage changes between the various therapy cycles (BV1-2, BV1-3). Individual TK courses did not show characteristics for any of the response groups either. Interestingly, 7 out of 10 patients with remission showed an increase of TK values from cycle 1 to 2, and also 70% an increase of the values from cycle 1 to 3; in the group of stable disease, 6 out of 7 patients had increasing values for both comparisons. In the progressive group, TK increases were seen in only 6 and 5 out of 12 patients, respectively. However, in some cases, the increases were very pronounced (Figure 4B).

Prognostic relevance of TK was investigated in 128 patients with non-operable NSCLC receiving first-line chemotherapy. At a cut-off of 20 U/l, pretherapeutic TK levels showed a high prognostic discrimination concerning the overall survival as shown by Kaplan-Meier curves and log-rank analysis: While median survival in patients with TK levels ≥20 U/l was only 3.1 months (95% confidence interval 2.0-6.1), it was 9.0 months (7.7-11.1) in patients with TK levels <20 U/l ($p<0.0001$; Figure 5). In multivariate analysis including TK and the clinical factors stage, performance score, weight loss, age and gender, TK was still an independent prognostic marker, together with performance score and stage. Hazard ratios were 4.27 (95% confidence interval 1.95-9.38) for TK, 2.34 (1.43-3.83) for stage and 2.25 (1.55-3.25) for performance score (Table III).

Discussion

Lung cancer is still an immense medical challenge as it accounts for most deaths among all cancer types and has high incidence rates which are mainly due to the high nicotine consumption all over the world (1). Despite its often only being diagnosed in late stages, it is essential to determine the exact histological subtype to stratify the patients for the various therapies which are specific for NSCLC and SCLC patients (2-7).

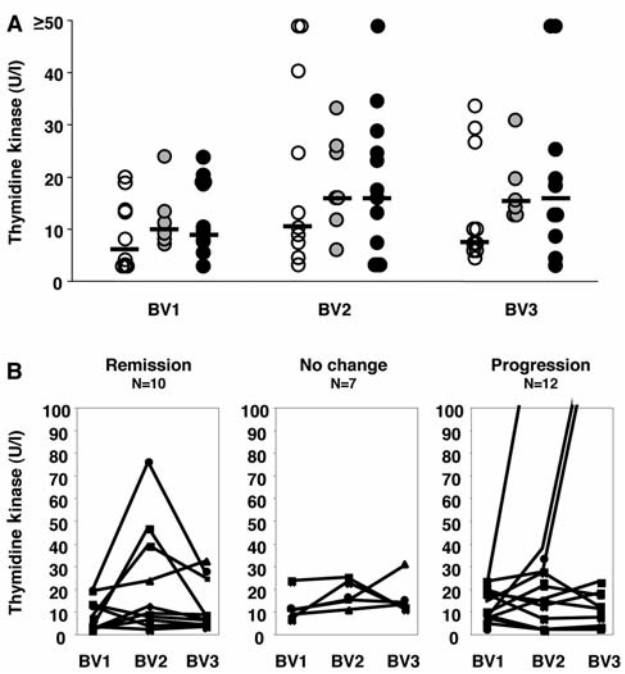

Figure 4. *A: Distribution of TK baseline values and medians in NSCLC patients before the start of the first three cycles of chemotherapy (BV1, BV2, BV3) for all response groups (○ remission, ● no change, ● progression). B: Individual TK courses of the patients in the various response groups, showing a considerable heterogeneity of the kinetics.*

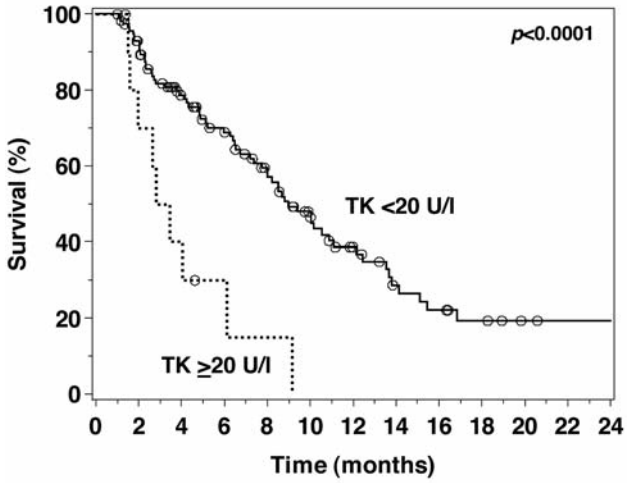

Figure 5. *Kaplan-Meier curves for overall survival in NSCLC patients receiving primary chemotherapy according to pretherapeutic TK values, showing significantly better survival for patients with TK values <20U/l as compared with those having higher TK values (○ censored patients).*

Although pathology is considered as the golden standard in determining diagnosis, it faces several limitations because it is not available in all cases due to multimorbidity of the patients and has to deal with the heterogenous nature of lung cancer (2-3). As biochemical properties of the tumor are mirrored by released molecules, circulating biomarkers in serum have been shown to be promising candidates for the improvement of diagnosis, histological differentiation and staging of lung cancer (9-12). Thus, several studies and guidelines recommend the use of CYFRA 21-1, CEA and SCCA in NSCLC, while NSE and ProGRP have been demonstrated as valuable markers in SCLC with high sensitivies of 50-70% at 95% specificity *versus* benign lung diseases (9-21).

As a further biomarker, TK, known as a valuable prognostic marker in non-Hodgkin's lymphoma and plasmocytoma, was recently proposed as a diagnostic and/or prognostic marker in solid tumors such as lung cancer (22-27). However, as recommended by the European Group on Tumor Markers (EGTM), a new biomarker has to show better or additive diagnostic sensitivity to the established ones if it is to be included in the diagnostic algorithm (29). Therefore, the aim of the present study was to investigate the relevance of TK for diagnosis of lung cancer and its histological subtypes in relation to CEA, CYFRA 21-1, NSE and ProGRP, and to identify potential influences of various non-lung diseases on TK metabolism relevant for interpretation of serum TK levels.

As observed for many biomarkers, TK levels in healthy individuals were very low, while patients with lung cancer had significantly higher values (22, 24). However, patients with benign lung diseases also had considerably elevated TK levels, thus limiting its diagnostic value. With regard to the pathophysiological background of many benign lung diseases, these results can be explained very well because infectious, granulomatous or allergic disease are massive stimuli for the proliferation of immune cells to fight against the abnormal condition. Therefore, the proliferation marker TK is also produced and released at higher frequency and amounts in these disorders. This observation is in line with other studies reporting on the release of TK in situations of immune stimulation, such as in viral infections by cytomegaly virus, varizella zoster virus and Epstein-Barr virus, but also in vitamin B12 deficiency and pernicious anaemia (26). As a potential source of elevated TK levels, hyperplastic and immature bone marrow and intramedullary destruction of immature proliferating cells have been discussed (26). As our results on patients with benign non-lung disease demonstrate, bacterial infections as well as renal insufficiencies in some cases are related to high TK release or confined elimination from the blood and should be taken into account when interpreting serum TK levels.

Although the diagnostic discrimination between lung cancer and healthy controls was promising, there was no diagnostic value when lung cancer was compared with

benign lung diseases, which are the relevant control group for differential diagnosis. The positive results on the diagnostic capacity of TK for lung cancer reported by Gronowitz et al. as well as by Li et al. based on the comparison with healthy controls are in line with our results (23, 24). However, in these studies, no comparison with benign lung diseases was made. Nevertheless, it should be pointed out that an association of TK levels with disease stage, prognosis and therapy response was reported (22-25). In our investigations, we were unable to confirm the relation with stage, both in NSCLC and in SCLC. However, it should be mentioned that we only focused on non-operable stages of lung cancer in this study. This may also be an important point why we did not find differences of TK levels in various lung cancer histologies. In addition, the discriminating power of TK in NSCLC and SCLC patients was similarly poor when tested against benign lung diseases.

As shown by many former studies, the biomarkers CYFRA 21-1 and CEA performed best for discriminating NSCLC from benign lung diseases, and NSE and ProGRP for discriminating SCLC from benign lung diseases (9-21). The unexpected good performance of NSE in the comparison of all lung cancer types and benign lung diseases, as well as of SCLC with benign lung diseases in our setting may be caused by the very low NSE levels in sera of our patients with benign lung diseases and the high proportion of SCLC patients among all lung cancer patients. TK did not add to the diagnostic sensitivity of the established markers in any case.

Because TK is frequently considered a proliferation marker, mirroring the activity of a tumor, it was expected that TK might correlate with the reduction of tumor volume and/or activity in patients who were successfully treated by cytotoxic therapies. However, the courses of TK levels, as well as the absolute TK baseline values before the start of the therapy cycles and during the first week of the treatment, were very heterogeneous in all patient groups. This observation was surprising as the patient group was quite homogeneous, consisting only of individuals with non-operable NSCLC receiving platin-based first-line chemotherapies. However, side-effects, infections and/or diverse stimulation of non-tumor cells might have contributed to the additional or reduced release of TK into the blood. For the monitoring of the therapy efficacy in advanced NSCLC patients, however, the use of TK at least seems to be critical.

Concerning the prognostic relevance of TK, we confirm and extend earlier results reported in several types of cancer. In univariate and multivariate analyses including clinical factors, pretherapeutic levels of TK showed clear prognostic relevance for advanced NSCLC patients during cytotoxic therapies. Of course, these results will have to be validated in further prognostic studies to show whether the cutoff at 20 U/l is appropriate, whether the prognostic relevance of TK is confirmed in another patient setting, and whether TK remains an independent prognostic marker when other strong prognostic parameters such as C-reactive protein, lactate dehydrogenase and CYFRA 21-1 are included in multivariate analyses.

Conclusion

Standard recommendations for the use of serum biomarkers in lung cancer such as the recently published guidelines of the NACB summarized the results of the abundant literature on biomarkers in this field and suggested CYFRA 21-1 and CEA, as well as NSE and ProGRP, as the most valuable markers for NSCLC and SCLC, respectively (12). In the present study, the diagnostic relevance of these markers was confirmed strikingly. The proliferation biomarker TK was found to be non-specifically elevated in lung cancer, benign lung diseases and non-lung diseases and did not demonstrate additive diagnostic power to the established biomarker panel. In addition, TK did not show any relevance for the monitoring of cytotoxic therapy, at least in this patient setting. However, the pretherapeutic TK levels showed a promising prognostic value for the overall survival in univariate, as well as in multivariate analyses, and deserve further evaluations in prospective studies.

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