Clinicopathological Significance and Prognostic Importance of Circulating Plasma DNA Expression in Advanced Non-Small Cell Lung Cancer and its Efficacy as a Diagnostic Tool

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Abstract: Background/Aim: Lung cancer is one of the commonest neoplasms. So, there is a continuous need for the development and search for new prognostic markers which will aid in diagnosis and therapy. Circulating plasma DNA levels is over-expressed in many human cancers, including lung. The aim of this work is to study the expression of circulating plasma DNA in NSCLC and assessment of its utility as a diagnostic marker, and in evaluating its impact on therapeutic efficacy as well as correlation of these data with clinicopathologic findings and patient survival to assess its prognostic significance. Patients and Methods: The amount of plasma DNA was determined through the use of real-time quantitative polymerase chain reaction (PCR) amplification of the human telomerase reverse transcriptase gene (*hTERT*) in 41 patients with advanced non-small cell lung cancer (NSCLC) and 38 age-matched controls. All of the 41 patients with advanced NSCLC received platinum-based chemotherapy. The regimen was Gemcitabine 1000 mg/m^2 (day 1, 8) and platinol 70 mg/m^2 (day 1), the cycle was repeated at interval of 21 days for at least 3 cycles. About 3 to 4 weeks after chemotherapy, response was evaluated by restaging- computed tomography. Circulating plasma DNA levels was correlated with established clinicopathologic factors, response to therapy, progression free and overall survival, and lactate dehydrogenase (LDH) levels. Results: There was a significant correlation between circulating plasma DNA levels and stage (p=0.001), LDH levels (p=0.001), smoking status (p=0.02) as well as tumor status (p=0.004). Circulating plasma DNA levels were significantly inversely correlated with treatment response (p<0.001). There was no statistical significant correlation when looking at the effect of age (p = 0.103), sex (p = 0.164), performance status (p = 0.267), pathological subtype (p = 0.26), and nodal status (p = 0.278) on the circulating plasma DNA levels. There was borderline statistical significant correlation between circulating plasma DNA levels and presence of distant metastases (p = 0.058). Circulating plasma DNA levels had also a highly significant relationship with shorter duration of PFS (p<0.001) and OS (p=0.0014).The mean circulating plasma DNA levels were 141.9 ng/mL (±56.3SD) in NSCLC patients and 69.9 ng/mL (±13.3SD) in controls, the difference being highly significant (p < 0.001). Conclusion: our results show that circulating plasma DNA levels is frequently over-expressed in primary NSCLC, and appears to be potentially useful marker for diagnosis. Overall, circulating plasma DNA levels was a significant predictor of survival and response to therapy. Circulating plasma DNA might be used as a new marker to stratify NSCLC patients for more optimal treatment modalities.

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Key words: Circulating plasma DNA, non-small cell lung cancer (NSCLC), diagnosis, clinicopathologic Study, prognosis, survival.

1. Introduction

Lung cancer is the leading cause of cancer death worldwide and NSCLC accounts for 80% of the cases[(1)]. The average 5-year survival in Europe is 10%, not much better than the 8.9% observed in developing countries[(2)]. The poor outcome is attributable to the absence of early detection plans, the frequency of metastases at diagnosis[(3)], and poor responsiveness to radiation therapy and chemotherapy[(4)]. However, survival of patients undergoing lung resection for small intrapulmonary cancers is greater than 80%[(5)]. As a consequence, there is a need to develop new tests that may facilitate earlier diagnosis and more effective treatment.

Diagnostic assays based on blood sample analysis are attractive because of the simplicity of sample collection. Accurate analysis of tumor markers in blood from cancer patients could have significant impact in facilitating the screening, diagnosis, and monitoring for disease recurrence after initial therapy[(6)].

With the introduction of PCR-based technologies in 1980s and refinements thereof, numerous molecular and biological markers on lung cancer tissues and exfoliated cancer cells have been

investigated[7]. The finding that tumors are capable of shedding nucleic acids (DNA or RNA) into the blood stream, which can be recovered from both serum and plasma and used as surrogate source of tumor DNA, has opened new areas in cancer diagnosis and prognosis in the past decade[8].

It is believed that plasma/serum DNA is of tumor origin because the genetic alterations are similar to those found in the corresponding primary tumors[9-11]. Thus, quantification of cell-free DNA in plasma/serum and characterization of specific molecular changes could be very useful in the management and screening of lung cancer.

To achieve maximum specificity and sensitivity, it is necessary to have a DNA concentration that does not overlap with the concentrations in control groups. It is clear that explicit cutoff values for DNA concentrations cannot be established at present because most of the published studies differ in the assays used. Three studies used real-time PCR for defining explicit DNA cutoff values[4,6,12], but all used different genes for the amplification. It was found that higher cutoff values increased the specificity of the assay but at the cost of sensitivity and vice versa. In a study by **Leon *et al.***[13], 61% of lung cancer patients had higher circulating DNA concentrations [above the cutoff value of 50 µg/L; mean (SE), 164 ± 44 µg/ml]; DNA concentrations decreased in 75% of these patients after therapy.

Sozzi *et al.*[14], demonstrated in their analysis of circulating tumor DNA in plasma at diagnosis and during follow-up of lung cancer patients that, DNA concentration in the follow-up plasma samples (mean, 34 µg/L) was significantly lower than before surgery (mean, 345 µg/L) and was comparable to the concentration detectable in the control group[14]. Total DNA was increased in patients with untreated cancer and in those with disease recurrence, with a sensitivity of 75% and specificity of 86%[14]. From these studies it can be concluded that no explicit cutoff has been established that can serve as a valuable tool for the diagnosis and follow-up of individuals.

In the current study, we evaluated the circulating plasma DNA levels in newly diagnosed, advanced stage NSCLC and assessing its utility as a diagnostic marker when compared with age-matched controls. In addition, evaluating its impact on therapeutic efficacy and correlating these data with clinicopathologic findings and patient survival to assess its prognostic significance.

2. Patients and Methods

Patient Characteristics & Inclusion Criteria:

A total of 41 patients with newly diagnosed, histologically confirmed advanced stage NSCLC and 38 age-matched controls (patients with benign pulmonary diseases, including 15 chronic obstructive pulmonary disease, 10 interstitial lung disease, 7 pulmonary tuberculosis, 2 sarcoidosis, and 4 bronchiectasis) treated at Clinical Oncology Department and Chest Department, Faculty of Medicine, Tanta University Hospital between May 2008 and March 2011 were studied.

All NSCLC patients were required to have advanced stage NSCLC, age less than 75 years and greater than 18 years, Eastern Cooperative Oncology Group performance status (ECOG) of 0 to 2, adequate cardiac function (EF > 60%), adequate bone marrow reserve, adequate renal and hepatic functions. Patients with NSCLC with non-malignant systemic disease that precluded them from receiving systemic chemotherapy (e.g. active infection, any clinically significant cardiac arrhythmia, or congestive heart failure) or patients who were pregnant were not eligible.

The following parameters were assessed at baseline: circulating plasma DNA levels, lactate dehydrogenase (LDH) level, bronchoscopy, ECOG performance status, weight, chest and abdomino-pelvic computed tomography (CT) scan, isotopic bone scan, ECG, echocardiography, and CT or magnetic resonance imaging (MRI) scan of the brain (if indicated), blood counts (Total leukocyte counts, hemoglobin, granulocytes, and platelets), and blood chemistry (renal and liver function tests).

Sample Collection and DNA Isolation:

A 7.5-mL sample of peripheral blood was collected in tubes containing EDTA, from patients at time of study entry as well as 6 months after the end of treatment from responders during follow-up period and from controls at the time of spiral CT examination, and stored at deep freeze Plasma separation and DNA extraction were performed as previously reported by **Chang *et al.***[15]. The DNA purified from 1 mL of plasma was eluted in a final volume of 50 mL of water. Testing of plasma DNA was performed by technicians with no knowledge of the patient or control status.

DNA Quantification in Plasma:

To quantify the circulating DNA in plasma, we used a real-time quantitative PCR approach based on the 5' nucleotide method. This methodology is based on continuous monitoring of a progressive fluorogenic PCR by an optical system. The PCR system uses two amplification primers and an additional amplicon-specific and fluorogenic hybridization probe, the target sequence of which is

located within the amplicon. The probe is labeled with two fluorescent dyes. One serves as a reporter on the 5' end (VIC dye; Applied Biosystems, Foster City, CA). The emission spectrum of the dye is quenched by a second fluorescent dye at the 3' end (TAMRA; Applied Biosystems). If amplification occurs, the 5' to 3' exonuclease activity of the AmpliTaq (Applied Biosystems) DNA polymerase cleaves the reporter from the probe during the extension phase, thus releasing it from the quencher. The resulting increase in fluorescent emission of the reporter dye is monitored during the PCR process.

Primers and probes were designed to specifically amplify the ubiquitous gene of interest, the *hTERT* single copy gene mapped on 5p15.33. The amplicon size of the *hTERT* gene was 98 bp (position 13059 to 13156, GenBank accession number AF128893). The sequences of the primers and of the probe were the following: primer forward, 5'-GGC ACA CGT GGC TTT TCG-3'; primer reverse, 5'-GGT GAA CCT CGT AAG TTT ATG CAA-3'; probe, VIC5'-TCA GGA CGT CGA GTG GAC ACG GTG-3' TAMRA.

Fluorogenic PCRs were carried out in a reaction volume of 50 mL on a GeneAmp 5700 Sequence Detection System (Applied Biosystems). Fluorogenic probe and primers were custom synthesized by Applied Biosystems. Each PCR reaction mixture consisted of 25 µL of TaqMan Universal Master Mix (Applied Biosystems), 0.67 µL of probe (15 mmol/L), 0.45 µL of primer forward (10 mmol/L), 0.45 µL of primer reverse (10 mmol/L), and 18.43 µL of sterile water. DNA solution (5 µL) was used in each real-time PCR reaction. Thermal cycling was initiated with a first denaturation step of 50°C for 2 minutes and then 95°C for 10 minutes. The thermal profile for the PCR was 95°C for 15 seconds and 60°C for 1 minute. Data obtained during 50 cycles of amplification were analyzed.

Amplifications were carried out in 96-well plates in a GeneAmp 5700 Sequence Detection System. Each plate consisted of patient samples in triplicates and multiple water blanks as negative control. For construction of the calibration curve on each plate, we used a standard TaqMan Control Human Genomic DNA (Applied Biosystems) at 10 ng/µL with appropriate serial dilutions at 50, 5, 2.5, and 0.5 ng, and 250, 50, and 10 pg. Linear amplification down to the last dilution point representing 10 pg of target DNA was obtained in each experiment (correlation coefficient, 0.999 to 0.995; slope, 3.25 to 3.35).

All of the data were analyzed using the Sequence Detection System software (Applied Biosystems) to interpolate the standard amplification curve of DNA at a known quantity with amplification cycle threshold of the unknown target sample, thus obtaining the relative amount of DNA in the experimental sample

Treatment

All of the 41 NSCLC patients had received systemic chemotherapy. Chemotherapy was applied in the form of GC regimen which consisted of a 60-120 minute intravenous infusion of gemcitabine (1000 mg/m^2, day 1 and 8), and platinol (70 mg/m^2, days 1), by intravenous infusion over 6 hrs and the cycle was repeated every 3 weeks and continued for 6 cycles unless there was evidence of disease progression or unacceptable toxicity. Patients were pre-medicated with 8 mg of dexamethasone, 50 mg of diphenhydramine, and 50 mg of ranitidine given intravenously. In addition, pre- and post-chemotherapy hydration was applied with platinol to avoid cisplatin-induced nephrotoxicity. Prophylactic use of growth factors was not recommended.

Supportive care included blood transfusions, growth factors and the administration of antiemetics and analgesics, as appropriate. The protocol provided for a decrease in Gemcitabine and platinol dose in patients experiencing grade 4 hematological toxicity or grade 3 non-hematological toxicities. G-CSF support was allowed in case of prolonged leucopenia (> 7 days) or febrile neutropenia in the prior cycle.

Evaluation of Treatment Response

Tumor response assessments were performed after 3 cycles. Response to therapy was classified according to the RECIST guidelines[16]. Evaluation was done using chest computed tomography (CT) owing to its convenient diagnosis of target lesion progress and identification of emerging new lesions.

Toxicity Evaluation:

Toxicities were graded according to the National Cancer Institute-Common Toxicity Criteria (NCI-CTCAE ver. 2.0). Treatment period was defined as the period from the initiation of therapy to 3 weeks after the last day of administration of Gemcitabine and platinol.

Follow-up Evaluation:

Basically, CT evaluations were performed every 3 months. Assessment of blood counts, blood chemistry, weight, performance status, toxicity, and chest examination was done every 3 months. Follow-up visits were scheduled every 3 months in the first 2 years after cessation of treatment and every 6 months thereafter.

Statistical analysis:

Patients were followed up until October 2011.

At the time of analysis, the mean follow-up for the entire group was 11 months (range, 3.00 to 36 months). Descriptive statistics were used to summarize patient characteristics and statistical analysis of the results was performed using SPSS version 12.0. Overall-survival (OS) rates were calculated from the time of initial treatment to the time of the last follow-up visit or death using the Kaplan-Meier method[17]. Mean and standard deviation were estimates of quantitative data. Chi-square/ Fischer exact were tests of proportion independence. Kaplan-Meier method was used for estimating survival and log rank to compare curves[17].

3. Results

Patient characteristics:

The study included A total of 41 patients with newly diagnosed, histologically confirmed advanced stage NSCLC and 38 age-matched controls (patients with benign pulmonary diseases, including 15 chronic obstructive pulmonary disease, 10 interstitial lung disease, 7 pulmonary tuberculosis, 2 sarcoidosis, and 4 bronchiectasis) treated at Clinical Oncology Department and Chest Department, Faculty of Medicine, Tanta University Hospital between years 2008 and 2011. The age of patients with NSCLC ranging from 36 to 70 years at the time of diagnosis (mean age 55.3±7.5 years) while the mean age of the controls at the time of study entry was 57.3±7.6 years (range 37-72 years). They showed positive history of smoking in 34 (82.9%) patients with NSCLC and in 26 cases (68.4%) of the controls. The majority of cases (63.4%) of NSCLC were T3 or greater, and node positive. The demographic data of the patients and controls and their relation to circulating plasma DNA levels were summarized in table (1). No statistically significant difference between the demographic characteristics of NSCLC patients and controls as regard to sex, smoking status, and age.

Baseline DNA concentrations were measured in plasma of 41 NSCLC patients and 38 age-matched controls. The mean circulating plasma DNA levels were 141.9 ±56.3 ng/mL in NSCLC patients and 69.9±13.3 ng/mL in controls, the difference being highly significant ($P < 0.001$). The mean DNA concentration was 2-fold higher in plasma from patients with NSCLC compared with age-matched controls (Table 1). Among the latter group, 37 (97.4%) of 38 age-matched controls presented DNA concentrations less than 104.5 ng/mL in plasma. Therefore, we defined these values as cutoff levels to differentiate between normal and elevated DNA. As determined by the Mann-Whitney rank sum test, plasma DNA concentrations were significantly higher ($P < 0.001$) in NSCLC patients than in age-matched controls. In plasma, Cox proportional hazards regression test revealed a significant trend ($P = 0.004$) towards higher DNA concentrations at advanced tumor stages.

Table (1): Demographic characteristics of patients and controls and their relation to cDNA expression

Characteristics		NSCLC group (41)		Control Group (38)		P Value
		No.	%	No.	%	
Sex						
	Male	37	90.2	30	78.9	0.166
	Female	4	9.8	8	21.1	
Smoking						
	Smoker	34	82.9	26	68.4	0.135
	Non Smoker	7	17.1	12	31.6	
Age in years						
	Mean	55.3		57.3		
	Median	55		56		
	Std. Deviation	7.5		7.6		0.346
	Range	36-70		37-72		
C- DNA levels(ng/mL)						
	Mean	141.9		69.9		
	Median	120.0		74.0		0.0001
	Std. Deviation	56.3		13.3		
	Range	40.8 - 235.6		40.8 – 105		

Circulating plasma DNA levels in correlation with clinico-pathological factors in NSCLC:

Table (2) summarizes the relation of circulating plasma DNA levels to the patient and tumor characteristics. There was a significant correlation between circulating plasma DNA levels and stage, with a higher frequency of stage IV cancers had elevated Circulating plasma DNA levels (P =0.001). There were also positive correlations between Circulating plasma DNA levels and smoking status (P =0.02), LDH level (P =0.001), as well as tumor status (P =0.004). There was no statistical significant

correlation when looking at the effect of age (P = 0.103), sex (P= 0.164), performance status (P = 0.267), pathological subtype (P = 0.26), and nodal status (P = 0.278) on the circulating plasma DNA levels. There was borderline statistical significant correlation between circulating plasma DNA levels and presence of distant metastases (P = 0.058).

Table (2): Circulating plasma DNA levels in relation to patient and tumor characteristics

Characteristics	No. (41)	Circulating plasma DNA levels (ng/mL)			
		Range	Mean	Median	P value
Age in years					
>60	23	40.8 – 235.6	129.3	105.6	0.103
<60	18	96.4 – 225.6	158.3	131.1	
Sex					
Male	37	56.2 – 235.5	137.9	119.7	0.164
Female	4	40.8 – 235.6	179.5	220.7	
Smoking Status					
Smoker	34	56.2 – 235.6	151.1	122.9	0.02
Non Smoker	7	40.8 – 119.7	97.8	105.6	
ECOG Performance Status					
≤2	26	40.8 – 235.6	134.5	107.3	0.267
>2	15	115.1 – 225.6	155	126.5	
Histopathology					
Adenocarcinoma	10	40.8 – 235.3	124.8	113.2	0.26
Squamous cell carcinoma	31	56.2 – 235.6	174.6	122.2	
Stage					
III	24	40.8 – 235.6	119.3	104.6	0.001
IV	17	115.1 – 235.3	174.1	193.5	
T-stage					
T1 -T2	15	40.8 – 215.8	110.1	105.6	0.004
T3 -T4	26	93.3 – 235.6	160.4	131.1	
N-Stage					
N0-N1	16	40.8 – 235.3	129.9	113.2	0.278
N2-N3	25	56.2 – 235.6	149.7	123.1	
M-Stage					
M0	32	40.8 – 235.6	133.2	115.2	0.058
M1	9	120 – 225.6	173.3	196.6	
LDH Level					
<240	18	40.8-215.8	110.4	112	0.001
>240	23	58.1- 235.6	166.8	193.5	

Relationships between Circulating Plasma DNA Levels and Response to Treatment:

Overall treatment response rate for patients with NSCLC was 39% (16/41), and tumor control rate (overall response and stable disease) was 73.2% (30/41) according to the RECIST criteria (Table 3). Complete response was observed in 3 patients (7.3%). All objective responses were confirmed at least 4 weeks after first observation. Circulating plasma DNA levels were significantly inversely correlated with treatment response (P < 0.001).

Overall, the median DNA concentration of responders during follow-up (75 ng/mL) showed a clear trend toward decreases.

Table (3): Relationships between Circulating Plasma DNA Levels and Response to Treatment

Response	No. (%)	Circulating plasma DNA levels (ng/mL)			
		Range	Mean	Median	P value
Complete response (CR)	3 (7.3%)	40.8 –117.5	94.3	103.5	
Partial response (PR)	13 (31.7%)				
Stable disease (SD)	14 (34.1%)	95.7-235.6	169.5	148	
Progressive disease (PD)	11 (26.8%)				
Objective response (CR+PR)	***16 (39%)***	***40.8 –117.5***	***94.3***	***103.5***	***0.00001***
No response (SD+PD)	***25 (61%)***	***95.7-235.6***	***169.5***	***148***	

Relationships to survival:

Median PFS and OS times for all patients with NSCLC were 8 months (95% confidence interval, 9.99 - 14.01; SE: 1.02) and 12.00 months (95% confidence interval, 6.43 – 9.57; SE: 0.8), respectively, (Figures 1, 2).

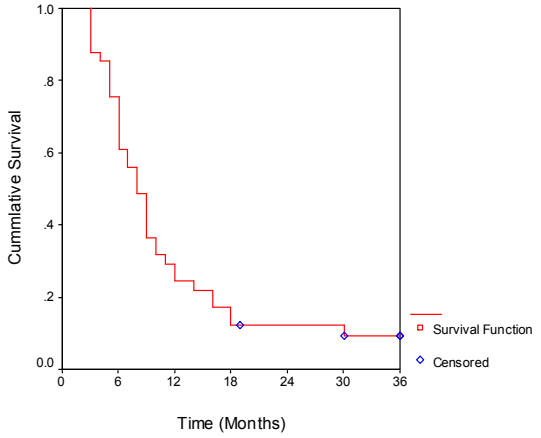

Figure 1. Kaplan–Meier curve of progression-free survival for all patients with NSCLC

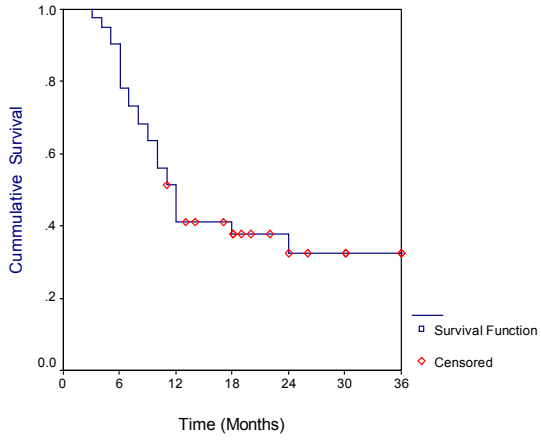

Figure 2. Kaplan–Meier curve of overall survival for all patients with NSCLC

To evaluate the prognostic significance of circulating plasma DNA levels, circulating plasma DNA levels were analyzed in relation to PFS and OS.

Circulating plasma DNA levels were significantly associated with a shortened PFS. Two-year PFS was 23.5% for patients with circulating plasma DNA levels ≤ 104.5 ng/mL (we defined these values as cutoff levels to differentiate between normal and elevated circulating plasma DNA levels) versus 4.2 % for patients with circulating plasma DNA levels > 104.5 ng/mL ($P <$ 0.001) (Figure 3).

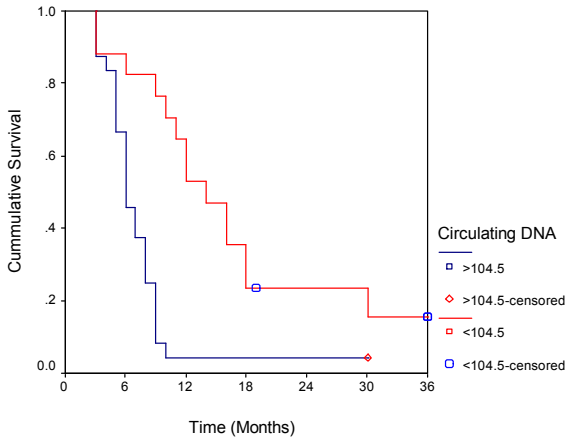

Figure 3. Progression free survival according to circulating plasma DNA levels

In terms of OS, The Kaplan–Meier survival curves demonstrate the better prognosis with circulating plasma DNA levels ≤ 104.5 ng/mL. Two-year OS was 58.4% for patients with circulating plasma DNA levels ≤ 104.5 ng/mL versus 14.3% for patients with circulating plasma DNA levels > 104.5 ng/mL ($P = 0.0014$) (Figure 4).

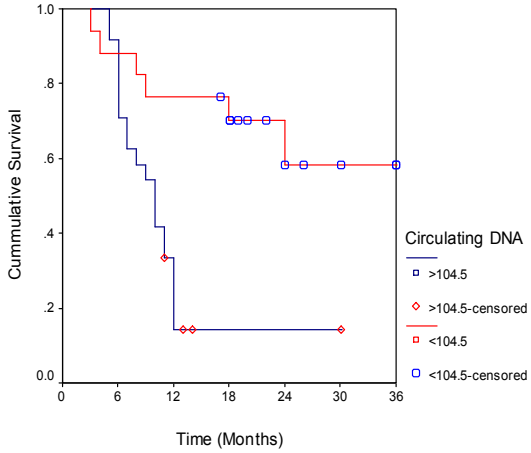

Figure 4. Overall survival according to circulating plasma DNA levels

4. Discussion

It is well recognized that tumor markers are not only of significance to the researcher in understanding tumor biology, but also to the clinician in treating patients with cancer[18]. Previous studies have reported significantly higher concentrations of circulating DNA in patients with various types of cancers, and have suggested the use of circulating DNA in cancer patients as a prognostic tool to monitor the effect of cancer therapy[13,19].

By using a simple colorimetric assay in a

representative series of lung cancer patients and controls, we have demonstrated that a quantitative plasma DNA test is a valuable diagnostic tool to discriminate patients from age-matched controls. **Chang *et al.*** [15] in their study performed in a group of miscellaneous tumors confirmed these results[15].

Our results show that, mean circulating plasma DNA levels were 141.9 ±56.3 ng/mL in NSCLC patients and 69.9 ±13.3 ng/mL in controls, the difference being highly significant ($p < 0.001$). The mean DNA concentration was almost 2-fold higher in plasma from patients with NSCLC compared with age-matched controls. Among the latter group, 37 (97.4%) of 38 age-matched controls presented DNA concentrations less than 104.5 ng/mL in plasma. Therefore, we defined these values as cutoff levels to differentiate between normal and elevated DNA. Similar values were reported previously by **Kumar *et al.*** [20] in their study and could be of substantial benefit in clinical practice.

Our results showed that circulating DNA concentrations, using the 104.5 ng/mL as cutoff levels, was significantly associated with stage, smoking status, LDH level, as well as tumor status. There was no statistical significant correlation when looking at the effect of age, sex, performance status, pathological subtype, and nodal status on the circulating plasma DNA levels. There was borderline statistical significant correlation between circulating plasma DNA levels and presence of distant metastases. On the other hand, an inverse relationship was found between circulating plasma DNA levels and response to chemotherapy. Circulating plasma DNA levels had also a highly significant relationship with shorter duration of PFS and OS.

Studies in patients with NSCLC have shown conflicting data about the prognostic significance of circulating plasma DNA levels, ranging from no prognostic significance, to adverse outcome. Disparity also exists with regard to variables such as clinical staging. In studies by **Fournie *et al.*** [21] and **Xie *et al.*** [22], plasma DNA was highest in patients with stage IV disease, whereas in other studies there was no such

association[4,6,12-14,23]. An association with age was reported in one study[4] but not in the other studies[6,12-14, 22,23,]. **Sozzi *et al.*** [4] found that no significant correlation was observed between plasma DNA concentrations and smoking intensity[4]. Similarly, no correlation has been established with histologic subtypes. **Xie *et al.*** [22] reported higher amounts of circulating DNA in NSCLC compared with SCLC, results in contrast to those reported by **Beau-Faller *et al.*** [23].

There are conflicting reports correlating the concentration of circulating DNA with survival. Some authors have reported no correlation between plasma DNA concentrations and PFS or OS[14,23], whereas other authors reported an association of plasma DNA with survival, lactate dehydrogenase[12,21], for a mixed group of SCLC and NSCLC patients[21], and for NSCLC patients only[12].

Overall, the median DNA concentration in our NSCLC responder patients during follow-up (75 ng/mL) showed a clear trend toward decreases, suggesting that quantification of plasma DNA might represent an approach to assess the efficacy of chemo-/radiotherapy[4]. **Gautschi *et al.*** [12] reported that tumor progression after chemotherapy was significantly associated with increasing plasma DNA concentrations.

In Conclusion, the presence of circulating tumor DNA in the plasma of lung cancer patients has sparked great interest because conventional diagnostic tests tend to be imperfect and more invasive, posing logistic difficulties for serial tumor sampling. Less-invasive techniques, such as blood tests, are attractive for screening, diagnosis, prognosis, surveillance for occult disease progression, identification of potential therapeutic targets, monitoring of tumor responses, and evaluation of disease pathophysiology and biology. Moreover, levels of plasma DNA could help identify high-risk individuals for chemoprevention trials, and could be tested as a potential intermediate biomarker of the efficacy of intervention.

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REFERENCES

1- Sher Y-P, Shih J-Y, Yang P-C, *et al*.(2011): Prognosis of Non–Small Cell Lung Cancer Patients by Detecting Circulating Cancer Cells in the Peripheral Blood with Multiple Marker Genes. Downloaded from clincancerres.aacrjournals.org on May 13, 2011.
2- Parkin DM, Bray F, Ferlay J, *et al*.(2005): Global cancer statistics, 2002. CA Can J Clin; 55:74–108.
3- Brambilla C, Fievet F, Jeanmart M, *et al*.(2003): Early detection of lung cancer: role of biomarkers. Eur Respir J; 21(39): 36s–44s.
4- Sozzi G, Conte D, Leon M, *et al*.(2003): Quantification of free circulating DNA as a diagnostic marker in lung cancer. J Clin Oncol.; 21: 3902–8.
5- Patz E JR, Rossi S, Harpole DH Jr, *et al*.(2000):

Correlation of tumor size and survival in patients with stage IA non-small cell lung cancer. Chest; 117:1568-571.
6- Herrera LJ, Raja S, Gooding WE, *et al*.(2005): Quantitative analysis of circulating plasma DNA as a tumor marker in thoracic malignancies. Clin Chem.; 51 (1):113–8.
7- Lacroix J, Becker HD, Woerner SM, *et al*.(2001): Sensitive detection of rare cancer cells in sputum and peripheral blood samples of patients with lung cancer by preproGRP-specific RT-PCR. Int J Cancer; 92:1–8.
8- Pathak AK, Bhutani M, Kumar S, *et al*.(2006): Circulating Cell-Free DNA in Plasma/Serum of Lung Cancer Patients as a Potential Screening and Prognostic Tool. Clin Chem.; 52 (10): 1833–42.
9- Chen XQ, Stroun M, Magnenat JL, *et al*.(1996): Microsatellite alterations in plasma DNA of small-cell lung cancer patients. Nat Med.; 2:1033–5.
10- Sozzi G, Musso K, Ratcliffe C, *et al*.(1999): Detection of microsatellite alterations in plasma DNA of non-small cell lung cancer patients: a prospect for early diagnosis. Clin Cancer Res.; 5: 2689–2.
11- Esteller M, Sanchez-Cespedes M, Rosell R, *et al*.(1999): Detection of aberrant promoter hypermethylation of tumor suppressor genes in serum DNA from non-small cell lung cancer patients. Cancer Res.; 59:67–70.
12- Gautschi O, Bigosch C, Huegli B, *et al*.(2004): Circulating deoxyribonucleic acid as a prognostic marker in non-small cell lung cancer patients undergoing chemotherapy. J Clin Oncol.; 22:4157–64.
13- Leon SA, Shapiro B, Sklaroff DM, *et al*.(1977): Free DNA in the serum of cancer patients and the effect of therapy. Cancer Res.; 37: 646–50.
14- Sozzi G, Conte D, Mariani L, *et al*.(2001): Analysis of circulating tumor DNA in plasma at diagnosis and during follow-up of lung cancer patients. Cancer Res.; 61: 4675–8.
15- Chang HW, Lee SM, Goodman SN, *et al*.(2002): Assessment of plasma DNA levels, allelic imbalance, and CA 125 as diagnostic tests for cancer. J Natl Cancer Inst.; 94:1697-703.
16- Therasse P, Arbuck SG, Eisenhauer EA, *et al*.(2000): New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst.; 92:205–16.
17- Kaplan EL, Meier P.(1958): Nonparametric estimation from incomplete observations. J Am Stat Assoc.; 53: 457-81.
18- Mumbarkar PP, Raste AS and Ghadge MS.(2006): Significance of tumor markers in lung cancer. Indian Journal of Clinical Biochemistry; 21 (1): 173-6.
19- Foss AJ, Guille MJ, Occleston NL, *et al*.(1995): The detection of melanoma cells in peripheral blood by reverse transcription-polymerase chain reaction. Br J Cancer; 72: 155–9.
20- Kumar S, Guleria R, Singh V, *et al*.(2010): Efficacy of circulating plasma DNA as a diagnostic tool for advanced non-small cell lung cancer and its predictive utility for survival and response to chemotherapy. Lung Cancer; 70: 211–7.
21- Fournie GJ, Courtin JP and Laval F.(1995): Plasma DNA as a marker of cancerous cell death: investigation in patients suffering from lung cancer and in nude mice bearing human tumour. Cancer Lett.; 2: 221–7.
22- Xie GS, Hou AR, Li LY, *et al*.(2004): Quantification of plasma DNA as a screening tool for lung cancer. Chin Med J; 117: 1485–8.
23- Beau-Faller M, Gaub MP, Schneider A, *et al*.(2003): Plasma DNA microsatellite panel as sensitive and tumor-specific marker in lung cancer Patients. Int J Cancer; 105:361–70

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