Featured Article

Circulating Nucleosomes Predict the Response to Chemotherapy in Patients with Advanced Non–Small Cell Lung Cancer

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ABSTRACT

Purpose: **We investigated the potential of circulating, nucleosomal DNA for the early prediction of the efficacy of chemotherapy in patients with advanced lung cancer.**

Experimental Design: **In serum of 212 patients with newly diagnosed non–small cell lung cancer (stages III and IV) undergoing chemotherapy, nucleosomes (ELISA, Roche) were measured at days 1, 3, 5, and 8 of the first cycle and before each new therapeutic cycle. Additionally, carcinoembryonic antigen and cytokeratin 19 fragments (CYFRA 21–1; Elecsys, Roche) were determined before each cycle. The therapeutic success was classified by computed tomography before start of the third cycle according to the World Health Organization criteria.**

Results: **In univariate analysis, responders (patients with remission) showed significantly ($P < 0.05$) lower values for the area under the curve of days 1 to 8 (AUC 1–8) of nucleosomes, the pretherapeutic baseline values of cycle 2 (BV2) and cycle 3 (BV3) of nucleosomes, and higher decreases of the baseline values from cycle 1 to 2 (BV1–2) and from cycle 1 to 3 (BV1–3) compared with nonresponders (patients with stable or progressive disease). Additionally, CYFRA 21–1 (BV1, BV2, BV3, BV1–2, BV1–3) and carcinoembryonic antigen (BV1–2) discriminated significantly between both groups.**

In multivariate analysis including all parameters available until end of the first therapeutic cycle, nucleosomes (AUC 1–8), CYFRA 21–1 (BV1), stage, and age were independent predictors of therapy response with nucleosomes (AUC 1–8) having the strongest impact.

Conclusion: **Circulating nucleosomes in combination with oncological biomarkers are valuable for the early estimation of the efficacy of chemotherapy in patients with lung cancer.**

INTRODUCTION

Lung cancer is worldwide one of the most frequent tumors in men and has an increasing prevalence in women (1, 2). Often it is detected accidentally and in advanced stages. In these situations, the therapeutic options are limited to chemo- and radiotherapy, which are associated with insufficient or only temporary success in many cases (3). However, there are many efforts to improve the therapeutic modalities by the introduction of new antibiotic drugs and combinations of chemo- and radiotherapeutic strategies.

The efficacy of the therapy applied is mostly controlled by imaging techniques and can be estimated after various cycles of chemotherapy or at the end of the therapy by macroscopic alterations of the tumor volume or diameter (4). However, it would be useful to know earlier whether a tumor responds to the treatment, to adapt the procedure individually and to change the regimen in time. One potential approach is an intense monitoring of the initial treatment phase by biochemical parameters that correlate with the number of the killed tumor cells or with the aggressiveness of the tumor.

Typical products of cell death are nucleosomes, complexes that are formed from a core particle of several histone components and DNA on the outside (5, 6). Linker DNA connects the nucleosomes to a chain-like structure. During cell death, endonucleases bind preferentially to these easy accessible linking sites between the nucleosomes and cut the chromatin into multiple mono- and oligonucleosomes (7, 8). In cases of enhanced cell death, as during chemotherapy, they are also released into the circulation and can be detected in elevated amounts in serum or plasma (9, 10).

Previous investigations into the spontaneous concentrations of nucleosomes in serum showed low levels in healthy individuals. In contrast, high amounts were found in patients with various malignant tumors but also in patients with benign pathologic conditions like severe inflammations. Among various tumor types, lung cancer was associated with the highest values of circulating nucleosomes (11, 12).

Focusing on the kinetics of nucleosomes in the serum of patients with malignant tumors during chemotherapy, a typical course could be observed. In the first days of the therapy, the levels of the nucleosomes increased rapidly, followed by a decrease in the treatment-free period and similar peaks in the following cycles. The baseline values, which were determined before each new cycle, increased typically in many patients with macroscopic progression and decreased in patients with remission of disease (12, 13). This approach is comparable with the

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use of other biochemical markers like tumor-associated antigens (14).

Because of the short half-time of nucleosomes in circulation (15) and their correlation with the extent of cell death (16), the course of nucleosomes during the first cycles of chemotherapy could already contain valuable information about the response of the tumor to the treatment applied. Therefore we concentrated in a further step on the first two cycles of chemotherapy, which was applied on patients with advanced lung cancer, to enlighten the potential use of nucleosomes for the early prediction of the therapeutic efficacy and to compare its power with well established biochemical parameters.

PATIENTS AND METHODS

Study Population. Two hundred and twelve patients with inoperable non–small-cell lung cancer (NSCLC; stages III and IV) under the care of the Department of Oncology of the Asklepios Clinics Gauting were included in our study. All patients were investigated initially by whole body computed tomography, bone scan, and bronchoscopy. Histology was available in all cases and confirmed 60 patients having squamous cell carcinoma, 84 patients having adenocarcinoma, and 68 patients having NSCLC without any further specification.

All patients received first-line chemotherapy regimens containing alternatively carboplatin area under the curve (AUC) 5 (day 1), 8 mg/m^2 mitomycin c (day 1) and 6 mg/m^2 vinblastin (day 1) or 8 mg/m^2 mitomycin c (day 1) and 25 mg/m^2 vinorelbin (days 1 and 8) or 1,250 mg/m^2 gemcitabine (days 1 and 8) and 80 mg/m^2 cisplatin (day 1), each followed by the next cycle at day 22.

Generally, the study was done prospectively. All patients with newly diagnosed lung cancer who received first-line chemotherapy were included without any selection criteria.

Classification of Response to Therapy. Before start of the third cycle of chemotherapy, staging investigations were done consisting of clinical examination, whole body computed tomography, and laboratory examinations. The response to therapy was classified according to the World Health Organization classifications defining "remission" as reduction of the tumor volume $\geq$50%, "progression" as increase of the tumor volume $\geq$25% or appearance of new tumor manifestations, and "no change" as reduction of the tumor volume $<$50% or increase $<$25%. In our study population, 83 patients reached remission (39.2%), 69 suffered from progression (32.5%), and 60 had no change of disease (28.3%)

Because the approach of the first-line therapies was a clear reduction of the tumor volume, the patients with progression and no change were joined to the group of nonresponders to therapy (129 patients, 60.8%). Patients with remission were classified as responders to therapy (Table 1).

Table 1 Characteristics of the patients investigated

	Median	Range
Age (y)	61	25–81
	Number	Percentage
Gender		
Female	60	(28.3)
Male	152	(71.7)
Stage		
III A	8	(3.7)
III B	61	(28.8)
IV	141	(66.5)
Histology		
Adenocarcinoma	84	(39.6)
Squamous carcinoma	60	(28.3)
Not classified carcinoma	68	(32.1)
Mode of therapy		
CMV	114	(53.8)
MV	25	(11.8)
GC	65	(30.7)
Others	8	(3.7)
Therapy response		
Remission	83	(39.2)
No change	60	(28.3)
Progression	69	(32.5)
Response groups		
Responders	83	(39.2)
Nonresponders	129	(60.8)

NOTE. Characteristics of the sample investigated. Patients received therapy protocols containing CMV or MV or GC. Patients with progression and no change were joined into the group of nonresponders; patients with remission were considered as responders.

Abbreviations: GC, gemcitabine + cisplatin; CMV, carboplatin + mitomycin c + vinblastin; MV, mitomycin c + vinorelbin.

Sample Collection and Assays. Blood samples were collected prospectively before the first, second, and third cycle of therapy for determination of baseline values (BV1, BV2, and BV3) and the kinetics of these baseline values. Additionally, samples were collected during the first week of the first cycle, ideally at days 1 (before start of the therapy), 3, 5, and 8 for the intensified monitoring of the initial phase of therapy.

The samples were centrifuged at 3000 $\times$ *g* for 15 minutes and treated with 10 mmol/L EDTA (pH 8) immediately after centrifugation. Subsequently, they were stored at $-$70°C and analyzed in batches containing all samples of a single patient. The details of the preanalytic handling are described in Holdenrieder *et al.* (17).

Nucleosomes were determined by the Cell Death Detection-ELISA^{plus} of Roche Diagnostics (Mannheim, Germany). This assay was modified for its use in serum matrix as specified in Holdenrieder *et al.* (17). Nucleosomes were quantified in arbitrary units (AU).

Additionally, the baseline values of tumor-associated antigens were determined before each therapeutic cycle (BV1, BV2, and BV3) by routine methods at the day of sample collection without any storage procedures: carcinoembryonic antigen (CEA) and cytokeratin 19 fragments (CYFRA 21–1; Elecsys 2010, Roche Diagnostics).

Statistical Analysis. The following values of nucleosomes were considered for the statistical analysis:

- the baseline values before the first, second, and third cycle (BV1, BV2, BV3);
- the changes between the baseline values 1 and 2 (BV1–2) and 1 and 3 (BV1–3);
- the area under the curve from days 1 to 8 (AUC 1–8). For the calculation of AUC 1–8, the nucleosome values of the days 1 and 8 and at least one of the days 3 and 5 were required.

Although the baseline values expressed the spontaneous

concentration of nucleosomes in serum, AUC 1–8 reflected the changes that were induced by therapy. Additionally, the baseline values (BV1, BV2, and BV3) and their kinetics (BV1–2 and BV1–3) of the tumor-associated antigens CEA and CYFRA 21–1 were included in the statistical analysis.

To evaluate all variables in a similar way, cutoffs were determined according to a defined procedure and tested for their power to discriminate between responders and nonresponders, as follows:

- the median of all patients investigated was defined as cutoff,
- in case of substantial discrimination between responders and nonresponders by the median, the quartiles (25th and 75th quantiles) of all patients were also tested to determine whether they could enhance the grade of significance.

Univariate analysis was done by χ^2 test. All parameters that showed substantial discriminating power in univariate analysis were included in a multivariate logistical regression analysis to evaluate the independent predictive factors for the response to therapy. In this model, interaction between mode of therapy, histology, and markers were also tested. A P value of $P < 0.05$ was considered statistically significant. All statistical analyses were done with SAS software (version 8.1, SAS Institute Inc. Cary, NC).

RESULTS

When the median of all patients investigated was used as cutoff for the discrimination between responders and nonresponders, the pretherapeutic levels of circulating nucleosomes (baseline value 1 = BV1) could not distinguish significantly between both groups (median = 388 AU; $P = 0.169$). However, the baseline values of nucleosomes before start of the second cycle (BV2; 216 AU; $P = 0.0001$) as well as before start of the third cycle of therapy (BV3; 184 AU; $P < 0.0001$) were significantly lower for patients with response than for those with no response to therapy. Consistently, patients with response showed significantly higher decreases from baseline value 1 to 2 ($P = 0.046$) and from baseline value 1 to 3 ($P = 0.036$). Interestingly the course during the first week of therapy already revealed significant differences between both groups: Patients with response to therapy exhibited only slight increases (pretherapeutic value, 344 AU; maximum value, 436 AU) followed by notable decreases until day 8 (140 AU). In patients with no response, the nucleosome values rose higher (pretherapeutic value, 442 AU; maximum value, 632 AU) and dropped less efficiently until day 8 (290 AU). When the area under the curve of all values available during the first week (AUC 1–8) was calculated, it was significantly lower for responders than for nonresponders ($P = 0.005$; Fig. 1).

Concerning other established oncological biomarkers in lung cancer, CEA did not show any significant difference between both groups for the pretherapeutic BV1 ($P = 0.273$), the baseline value before start of the second cycle (BV2; $P = 0.111$), and the baseline value before start of the third cycle (BV3; $P = 0.385$). However, the kinetics of the baseline values of CEA were different in both groups: Whereas most of the patients with response to therapy showed decreasing baseline values, they mainly increased in patients with no response. This difference was significant for the kinetics from cycle 1 to 2 ($P = 0.004$) but not for the kinetics from cycle 1 to 3 ($P = 0.172$).

With regard to CYFRA 21–1, responders had markedly lower values for all time points investigated compared with nonresponders. Already the pretherapeutic BV1 showed a strong tendency to discriminate between both groups ($P = 0.059$), and the difference was highly significant for the baseline value before start of the second cycle (BV2; $P < 0.0001$) and the baseline value before start of the third cycle (BV3; $P < 0.0001$). Furthermore, we observed in patients with response, a significantly stronger decrease of the baseline values from cycle 1 to 2 ($P = 0.0006$) and from cycle 1 to 3 ($P < 0.0001$) compared with those without response to therapy (Fig. 2).

By also testing all variables that discriminate significantly between responders and nonresponders with the 25th percentile (quartile 1 = Q1) and the 75th percentile (quartile 3 = Q3) of the whole sample as further cutoffs, in some cases the grade of significance could still be enhanced: such as for the baseline value 2 of nucleosomes (Q3), the decrease from baseline value 1 to 2 of nucleosomes (Q1), the decrease from baseline value 1 to 3 of nucleosomes (Q3), the AUC 1–8 of nucleosomes (Q1), the baseline value 1 of CYFRA 21–1 (Q1), and the decrease from baseline value 1 to 2 of CYFRA 21–1 (Q3). All other

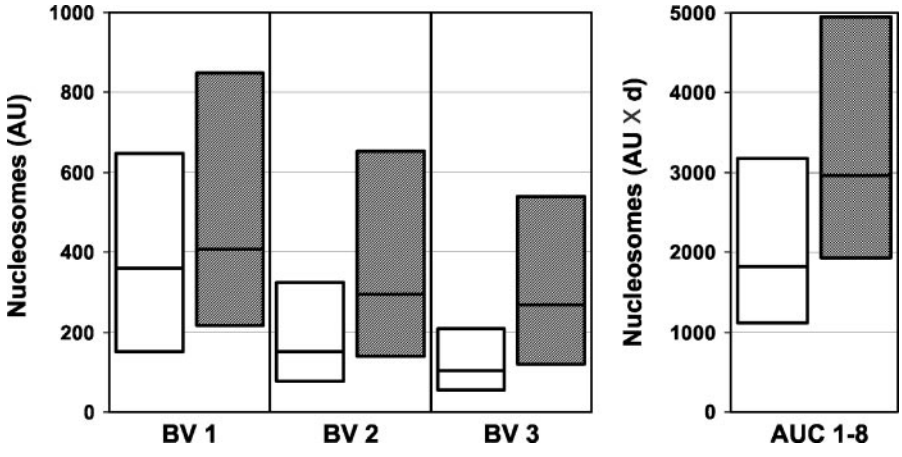

Fig. 1 Discrimination between responders □ and nonresponders to chemotherapy ▨ by nucleosomes. *Bars* indicate 25th percentile, median, and 75th percentile of the baseline values before therapy cycles 1 (BV1), 2 (BV2), 3 (BV3), and AUC 1–8 of the first treatment cycle.

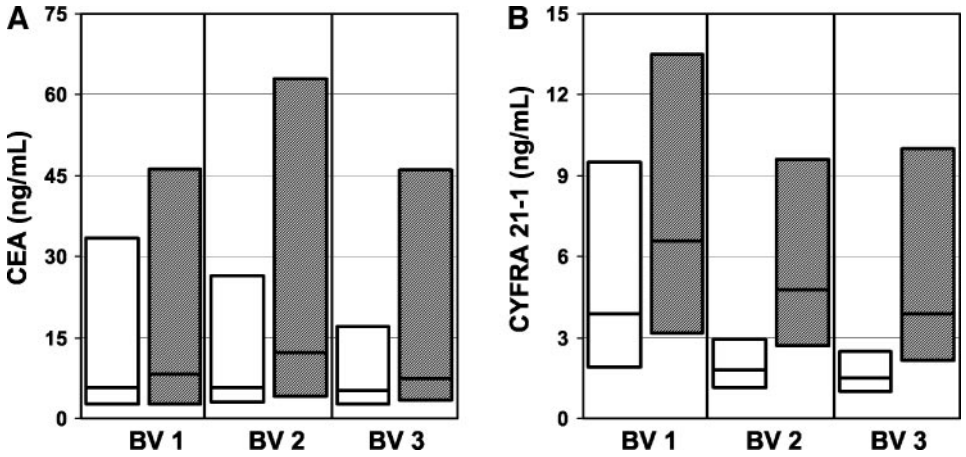

Fig. 2 Discrimination between responders □ and nonresponders to chemotherapy ▨ by CEA (*A*) and CYFRA 21–1 (*B*). *Bars* indicate 25th percentile, median, and 75th percentile of the baseline values before therapy cycles 1 (BV1), 2 (BV2), and 3 (BV3).

variables discriminated best when the median was used as cutoff (Table 2).

Concerning clinical parameters, patients with response and no response to therapy exhibited significant differences for age, stage, histology, and mode of therapy; however, not for gender; patients younger than 52 years (Q1) had a significantly poorer response to therapy than older ones ($P = 0.038$). Furthermore, patients with distant metastases showed a poorer response than those with stage 3 disease ($P < 0.0001$). In patients with adenocarcinoma or not further classified NSCLC less sufficient response was observed than in those with squamous cell carcinoma ($P = 0.008$). And patients receiving schedules containing carboplatin, mitomycin c, and vinblastin or mitomycin c and vinorelbin were correlated with worse response than patients treated by gemcitabine and cisplatin ($P = 0.0003$).

In multivariate analysis, all univariately significant parameters were included that were available until start of the second cycle of therapy. Among them, staging, mode of therapy, baseline value 2 of nucleosomes, and baseline value 2 of CYFRA 21–1 proved to be independent predictive factors for therapy outcome. When the time frame was restricted to the first cycle, staging, and age, AUC 1–8 of nucleosomes and baseline value 1 of CYFRA 21–1 remained as independent predictors of the therapy outcome with AUC 1–8 of nucleosomes having the strongest impact. In these models, no interactions among mode of therapy, markers, and therapy response nor among histology, markers, and therapy response were found. Table 3

DISCUSSION

Many studies dealing with circulating nucleic acids in serum and plasma revealed their high potential for diagnosis of cancer disease (18–20). Techniques like the PCR and mass spectroscopy contribute to a high diagnostic sensitivity (21). Characteristic mutations, alterations of microsatellites, and epigenetic changes provide a high specificity for the detection of cancerous DNA (22–26). Already at time of diagnosis, these parameters also contain valuable prognostic information for many cancers (22, 23). Besides the qualitative aspects of circulating DNA, their quantity was also shown to be elevated in the serum and plasma of cancer patients (11, 12, 27–31). However,

Table 2 Results of univariate analysis indicating the potential of all parameters investigated for discrimination between responders and nonresponders to therapy

Parameter	Median	χ^2 median	χ^2 25th percentile	χ^2 75th percentile
Nucleosomes				
BV1	388 AU	0.169		
BV2	216 AU	0.0001	0.020	**0.0001**
BV3	184 AU	**<0.0001**	0.0002	0.001
BV1–2	Dec 40.5%	0.046	**0.038**	0.039
BV1–3	Dec 45.3%	0.036	0.139	**0.0007**
AUC 1–8	2,722 AU × day	0.005	**0.0003**	0.015
CEA				
BV1	6.7 ng/ml	0.273		
BV2	9.0 ng/ml	0.111		
BV3	6.6 ng/ml	0.385		
BV1–2	Dec 2.7%	**0.004**	0.012	0.013
BV1–3	Dec 0.6%	0.173		
CYFRA 21–1				
BV1	5.2 ng/ml	0.059	**0.003**	0.211
BV2	3.2 ng/ml	**<0.0001**	<0.0001	<0.0001
BV3	2.5 ng/ml	**<0.0001**	0.001	<0.0001
BV1–2	Dec 29.7%	0.0006	0.218	**<0.0001**
BV1–3	Dec 36.7%	**<0.0001**	0.084	<0.0001
Clinical parameters				
Age	61 yrs	0.917	**0.038**	0.782
Gender		0.874		
Stage III–IV		**<0.0001**		
Histology				
SC–(AC + NCC)		**0.008**		
Therapy				
GC–CMV/MV		**0.0003**		

NOTE. Summary of all parameters investigated with the corresponding median of all patients and the *P* value (χ^2). In case of significant discrimination by the median, the quartiles (25th percentile and 75th percentile) were additionally tested as cutoffs on their discriminating power. The best discriminating cutoff is indicated by bold letters.

Abbreviations: AU, arbitrary units; Dec, decrease; GC, gemcitabine + cisplatin; CMV, carboplatin + mitomycin c + vinblastin; MV, mitomycin c + vinorelbin; SC, squamous cell carcinoma; AC, adenocarcinoma; NCC, not classified carcinoma.

Table 3 Results of multivariate analysis indicating independent predictive factors for response to therapy

	Cutoff	*P*	Odds ratio	Confidence interval
Parameters available until start of the second cycle of chemotherapy				
Stage	III–IV	0.029	2.46	1.10–5.50
Mode of therapy	GC–CMV/MV	0.028	2.58	1.11–6.02
Nucleosomes BV2	75th percentile	0.021	3.40	1.21–9.56
CYFRA 21–1 BV2	Median	<0.001	7.01	3.12–15.77
Parameters available until end of the first cycle of chemotherapy				
Stage	III–IV	0.004	3.73	1.53–9.12
Age	25th percentile	0.030	3.10	1.11–8.62
Nucleosomes AUC 1–8	25th percentile	<0.001	6.58	2.33–18.59
CYFRA 21–1 BV1	25th percentile	0.049	2.84	1.00–8.02

NOTE. Multivariate analysis including all significantly discriminating parameters available (*a*) until start of the second cycle of chemotherapy and (*b*) until end of the first cycle of chemotherapy with the corresponding cutoffs chosen, *P* values, odds-ratios, and confidence intervals.

Abbreviations: GC, gemcitabine + cisplatin; CMV, carboplatin + mitomycin c + vinblastin; MV, mitomycin c + vinorelbin.

because of the overlap with some benign diseases that are critical for the differential diagnosis of cancer, the diagnostic value of DNA concentration is limited (12). Nevertheless several studies have shown its prognostic value for various cancer types (32–34). Because the determination of these qualitative and quantitative parameters can be done in serum and plasma that is easily obtainable, these methods could also be used in serial measurements; in patients with EBV-associated cancers, the response to radiotherapy correlated well with the kinetics of EBV-DNA quantity in plasma (35, 36).

Most circulating DNA is likely to be bound to histones that conserve DNA from endonucleatic digestion in serum and plasma and to exist in form of mono- and oligonucleosomes (37–39). These nucleosomes can be detected directly by an ELISA that is easy to handle and cost effective (17). First investigations with this method confirmed the elevated nucleosome values in cancer patients compared with healthy persons and the correlation of the nucleosome kinetics with the therapeutic efficacy during radio- and chemotherapy (12, 13). As an important mechanism by which nucleosomes are released into circulation is apoptotic cell death (9, 11, 16), which is induced by these therapies most effectively at the initial phase, and as the half-life of nucleosomes in circulation is very short (15), we focused particularly on the first cycles of chemotherapy and investigated whether nucleosomes might be an early prediction of the response to therapy. If so, this would open the appealing possibility to modify and tune the therapeutic regimen at an early time point or even to change it completely.

In univariate analysis, patients with response to therapy tended to have lower pretherapeutic nucleosome levels than nonresponders. Because these values were not influenced by treatment, they would have rather prognostic than predictive impact. More related to therapy were the precyclic values before cycle 2 and 3 and the kinetics from cycle 1 to 2 as well as from cycle 1 to 3. Because response to therapy was clearly correlated to low absolute values and strong decreases, the balance of continuous release and elimination of nucleosomes in circulation was shown to be modulated effectively by successful treatment. In contrast, patients with no response to therapy continued to have higher rates of release and/or lower rates of elimination of circulating nucleosomes.

The direct therapeutic effect would be reflected most evidently by the values during the first week of cycle 1 of chemotherapy. Because antitumor therapy generally aims at the effective reduction of tumor cells, response to therapy was expected to be linked to an initial high release of nucleosomes. However, patients responsive to therapy showed lower maximum values during the first week as well as lower levels at day 8 compared with nonresponders. When all values during the first week were integrated in the AUC 1–8, responders exhibited highly significant lower values than nonresponders. This observation was surprising but could be attributed to various reasons. Patients with no response to therapy seem to have more aggressive tumor disease related to (*a*) a high spontaneous turnover of cell death and proliferation, (*b*) a high number of undifferentiated cells prone to be killed by antitumor therapy, and (*c*) dysfunctional DNA-repair mechanisms unable to save cells after substantial damages by therapy. Because patients with metastasized disease showed a poorer response, (*d*) the access to blood circulation of nucleosomes released by dying cells might also contribute to the more pronounced peak in nonresponders. Finally, (*e*) the elimination of nucleosomes from circulation by renal, hepatic, or intraplasmatic processes seems to be impaired in patients with insufficient response, which is also illustrated by the significantly higher value 1 week after start of the therapy.

There are several studies that confirm the rapid increase of circulating nucleosomes, DNA or EBV-DNA followed by a decrease in the first week during chemo- and radiotherapy and also after surgery. Lo *et al.* (36) could show that EBV-DNA values in patients with nasopharyngeal cancer rose immediately after start of radiotherapy (median time to the peak 3.0 days) and declined afterward with a median half-life of 3.8 days. Kuroi *et al.* (40) found a specific peak of circulating nucleosomes 48 to 72 hours after start of chemotherapy in patients with breast cancer. Trejo-Becerril *et al.* (41) confirmed these results in patients with cervical cancer undergoing chemotherapy. After surgery of patients with nasopharyngeal cancer, the initial peak of EBV-DNA followed by a rapid decrease could also be observed. The median half-life of the decay was calculated as 139 minutes (42). Failure of complete and rapid elimination of EBV-DNA was correlated to disease recurrence later on.

We have shown recently the typical kinetic patterns of circulating nucleosomes in serum during chemo- and radiotherapy in patients with various tumor types (12, 13). There we showed that response to chemotherapy was correlated with decreasing precyclic baseline values whereas progression was linked with increasing or constantly high values. However, this study is to our knowledge the first one revealing the potential of the course of circulating nucleosomes during the very initial phase of chemotherapy for the prediction of therapy response.

Concerning oncological biomarkers in NSCLC, CEA and CYFRA 21–1 are used often for differential diagnosis of

NSCLC (43, 44). Here we could show that CEA and particularly CYFRA 21–1 have great potential for monitoring therapy and predicting of therapy response. This was confirmed recently for the kinetics of CYFRA 21–1 in a smaller study including 58 patients with NSCLC (45). Because CYFRA 21–1 is located at least partly inside epithelial tumor cells and is released during or after cell death (46), the correlation of therapy response and decreasing CYFRA 21–1 levels could be explained by an effective reduction of the tumor cell number.

An essential point for the outcome of a study is the definition of appropriate cutoffs for the parameters investigated. Because the aim of the study was to evaluate all parameters in a comparable way, the median of all the patients was chosen as rationale for defining the cutoffs. In case of significant discrimination between responders and nonresponders by the median, the quartiles (25th and 75th quantiles) of the sample were additionally tested for whether they could enhance the grade of significance. Choosing this procedure, we took into account that some markers have a better power of discrimination at low or high levels. Although the results for each variable could have been better by optimization of the cutoff, a rationale for the definition of the cutoffs avoided the overfitting of the results to this special setting of patients and enabled the transfer to other samples.

The early prediction of therapy response by clinical and biochemical parameters would offer the possibility of having this important information before staging investigations and of modulating the treatment already at the initial phase. Among the parameters that were available until start of the second cycle of chemotherapy, stage and mode of therapy proved to be independent predictive factors in multivariate analysis as well as the baseline value 2 of nucleosomes and CYFRA 21–1. Concerning the time frame restricted to the first cycle, nucleosomes (AUC 1–8) and CYFRA 21–1 (baseline value 1) remained independent predictive parameters in addition to the clinical parameters stage and age. In the latter setting, the AUC 1–8 of nucleosomes was the only variable that was directly influenced by the therapy and that had the strongest predictive impact. These results indicate the power of circulating nucleosomes (if determined in a close meshed manner during the initial phase of chemotherapy) and CYFRA 21–1 in addition to well known clinical prognostic factors for the early prediction of the therapy response. After confirmation by multicenter trials, these results encourage the introduction of nucleosomes and CYFRA 21–1 in new therapeutic study protocols.

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REFERENCES

1. Greenlee RT, Murray T, Bolden S, Wingo PA. Cancer Statistics 2000. CA Cancer J Clin 2000;50:7–33.

2. Tychzynski JE, Bray F, Parkin DM. Lung cancer in Europe in 2000: epidemiology, prevention, and early detection. Lancet Oncol 2003;4: 45–55.

3. Gridelli C, Rossi A, Maione P. Treatment of non-small-cell lung cancer: state of the art and development of new biologic agents. Oncogene 2003;22:6629–38.

4. Werner-Wasik M, Xiao Y, Pequignot E, Curran WJ, Hauck W. Assessment of lung cancer response after nonoperative therapy: tumor diameter, bidimensional product, and volume. A serial CT scan-based study. Int J Radiat Oncol Biol Phys 2001;51:56–61.

5. Kornberg R, Lorch Y. Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. Cell 1999;98:285–94.

6. Luger K, Maeder AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature (Lond) 1997;389:251–60.

7. Kerr JF, Winterford CM, Harmon BV. Apoptosis. Its significance in cancer and cancer therapy. Cancer (Phila) 1994;73:2013–26.

8. Wyllie AH, Arends MJ, Morris RG, Walker SW, Evan G. The apoptosis endonuclease and its regulation. Semin Immunol 1992;4:389–97.

9. Lichtenstein AV, Melkonyan HS, Tomei LD, Umansky SR. Circulating nucleic acids and apoptosis. Ann N Y Acad Sci 2001;945: 239–49.

10. Stroun M, Maurice P, Vasioukhin V, et al. The origin and mechanism of circulating DNA. Ann N Y Acad Sci 2000;906:161–8.

11. Holdenrieder S, Stieber P, Foerg T, et al. Apoptosis in serum of patients with solid tumours. Anticancer Res 1999;19:2721–4.

12. Holdenrieder S, Stieber P, Bodenmueller H, et al. Nucleosomes in serum of patients with benign and malignant diseases. Int J Cancer 2001;95:114–20.

13. Holdenrieder S, Stieber P, Bodenmueller H, et al. Circulating nucleosomes in serum. Ann N Y Acad Sci 2001;945:93–102.

14. Stieber P. Sensible use of tumor markers. J Lab Med 2001;25:327–36.

15. Gauthier VJ, Tyler LN, Mannik M. Blood clearance kinetics and liver uptake of mononucleosomes in mice. J Immunol 1996;156:1151–6.

16. Jahr S, Hentze H, Englisch S, et al. DNA fragments in the blood plasma of cancer patients: Quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer Res 2001;61:1659–65.

17. Holdenrieder S, Stieber P, Bodenmueller H, et al. Nucleosomes in serum as a marker for cell death. Clin Chem Lab Med 2001;39: 596–605.

18. Anker P, Mulcahy H, Stroun M. Circulating nucleic acids in plasma and serum as a noninvasive investigation for cancer: time for large-scale clinical studies? Int J Cancer 2003;103:149–52.

19. Ziegler A, Zangemeister-Wittke U, Stahel RA. Circulating DNA: a new diagnostic gold mine? Cancer Treat Rev 2002;28:255–71.

20. Sozzi G, Conte D, Leon M, et al. Quantification of free circulating DNA as a diagnostic marker in lung cancer. J Clin Oncol 2003;21: 3902–8.

21. Johnson PJ. A framework for the molecular classification of circulating tumor markers. Ann N Y Acad Sci 2001;945:8–21.

22. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med 2003;349:2042–54.

23. Sidransky D. Emerging molecular markers of cancer. Nat Rev Cancer 2002;2:210–9.

24. Chen XQ, Stroun M, Magnenat JL, et al. Microsatellite alterations in plasma DNA of small cell lung cancer patients. Nat Med 1996;2: 972–4.

25. Nawroz H, Koch W, Anker P, Stroun M, Sidransky D. Microsatellite alterations in plasma DNA of head and neck cancer patients. Nat Med 1996;2:1035–7.

26. Andriani F, Conte D, Mastrangelo T, et al. Detecting lung cancer in plasma with the use of multiple genetic markers. Int J Cancer 2004;108: 91–6.

27. Leon SA, Shapiro B, Sklaroff DM, Yaros MJ. Free DNA in the serum of cancer patients and the effect of therapy. Cancer Res 1977; 37:646–50.

28. Shapiro B, Chakrabarty M, Cohn EM, Leon SA. Determination of circulating DNA levels in patients with benign or malignant gastrointestinal disease. Cancer (Phila) 1983;51:2116–20.

29. Maebo A. Plasma DNA level as a tumor marker in primary lung cancer. Nihon Kyobu Shikkan Gakkai Zasshi 1990;28:1085–91.

30. Kuroi K, Tanaka C, Toi M. Plasma nucleosome levels in node-negative breast cancer patients. Breast Cancer 1999;6:361–4.

31. Lo YMD, Chan LY, Lo KW, et al. Quantitative analysis of cell-free Epstein-Barr virus DNA in plasma of patients with nasopharyngeal carcinoma. Cancer Res 1999;59:1188–91.

32. Lo YMD, Chan AT, Chan LY, et al. Molecular prognostication of nasopharyngeal carcinoma by quantitative analysis of circulating Epstein-Barr virus DNA. Cancer Res 2000;60:6878–81.

33. Silva JM, Silva J, Sanchez A, et al. Tumor DNA in plasma at diagnosis of breast cancer patients is a valuable predictor of disease-free survival. Clin Cancer Res 2002;8:3761–6.

34. Lei KI, Chan LY, Chan WY, Johnson PJ, Lo YMD. Diagnostic and prognostic implications of circulating cell-free Epstein-Barr virus DNA in natural killer/T-cell lymphoma. Clin Cancer Res 2002;8:29–34.

35. Lo YMD, Chan LY, Chan AT, et al. Quantitative and temporal correlation between circulating cell-free Epstein-Barr virus DNA and tumor recurrence in nasopharyngeal carcinoma. Cancer Res 1999;59: 5452–5.

36. Lo YMD, Leung SF, Chan LY, et al. Kinetics of plasma Epstein-Barr virus DNA during radiation therapy for nasopharyngeal carcinoma. Cancer Res 2000;60:2351–5.

37. Rumore P, Muralidhar B, Lin M, Lai C, Steinman CR. Hemodialysis as a model for studying endogenous plasma DNA: oligonucleosome-like structure and clearance. Clin Exp Immunol 1992;90: 56–62.

38. Burlingame RW, Volzer MA, Harris J, Du Clos TW. The effect of acute phase proteins on clearance of chromatin from the circulation of normal mice. J Immunol 1996;156:4783–8.

39. Chan KCA, Zhang J, Chan ATC, et al. Molecular characterization of circulating EBV-DNA in the plasma of nasopharyngeal carcinoma and lymphoma patients. Cancer Res 2003;63:2028–32.

40. Kuroi K, Tanaka C, Toi M. Clinical significance of plasma nucleosomes levels in cancer patients. Int J Oncol 2001;19:143–8.

41. Trejo-Becerril C, Perez-Cardenas E, Trevino-Cuevas H, et al. Circulating nucleosomes and response to chemotherapy: an in vitro, in vivo and clinical study on cervical cancer patients. Int J Cancer 2003;104:663–8.

42. To EWH, Chan KCA, Leung SF, et al. Rapid clearance of plasma Epstein-Barr virus DNA after surgical treatment of nasopharyngeal carcinoma. Clin Cancer Res 2003;9:3254–9.

43. Stieber P, Hasholzner U, Bodenmueller H, et al. CYFRA 21–1 –a new marker in lung cancer. Cancer (Phila) 1993;72:707–13.

44. Schalhorn A, Fuerst H, Stieber P. Tumor markers in lung cancer. J Lab Med 2001;25:353–61.

45. Vollmer RT, Govindan R, Graziano SL, et al. Serum CYFRA 21–1 in advanced stage non-small cell lung cancer: an early measure of response. Clin Cancer Res 2003;9:1728–33.

46. Sheard MA, Vojtesek B, Simickova M, Valik D. Release of cytokeratin-18 and -19 fragments (TPS and CYFRA 21–1) into the extracellular space during apoptosis. J Cell Biochem 2002;85:670–7.