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NUCLEOSOMES IN SERUM OF PATIENTS WITH BENIGN AND MALIGNANT DISEASES

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High quantities of mono- and oligonucleosomes circulate in the blood of patients with malignant tumors. For their direct quantification in serum, we modified the Cell Death Detection^{plus}-ELISA for its application in liquid materials. We examined sera samples from 590 persons, including 418 patients with malignant tumors, 109 patients with benign diseases and 63 healthy persons. We also observed the kinetics of the concentration of nucleosomes in serum samples from 20 patients undergoing chemotherapy and from 16 patients undergoing radiotherapy. Sera of patients with malignant tumors contained considerably higher concentrations of nucleosomes (mean = 350 arbitrary units [AU], median = 190 AU) compared with those of healthy persons (mean = 36 AU, median = 24 AU; $p = 0.0001$) and patients with benign diseases (mean = 264 AU, median = 146 AU; $p = 0.072$). Concerning the follow-up investigations, the concentration of nucleosomes in serum increased 24–72 hr after the first application of chemotherapy and 6–24 hr after the start of radiotherapy. A subsequent decrease was often correlated with regression of the tumor. In patients undergoing chemotherapy, an increase in the baseline values of circulating nucleosomes >50%, which were determined before each new therapeutic cycle, was correlated with progression of disease; all patients with disease regression showed a decrease >50% of the baseline values. In patients undergoing radiotherapy, an early decrease of the nucleosomal concentration (≤1 day after the initial peak during therapy) to low minimum levels (≤100 AU) correlated with good clinical outcome; a late decrease (>1 day) to higher minimum levels (>100 AU) was associated with a worse clinical outcome. Thus, the concentration of nucleosomes in serum might be a useful tool for monitoring the biochemical response during antitumor therapy, especially for the early estimation of therapeutic efficacy.

Key words: *cell death; apoptosis; nucleosomes; serum; cancer; therapy*

Growth of solid tumors is generally characterized by an imbalanced homeostasis of cell proliferation and cell death.[1] Often, the regulation and the mechanisms of programmed cell death are impaired. However, in many cases, the incidence of cell death is enhanced in order to counter-regulate the effects of the hyperproliferating tumor cells.[2,3] Whereas cells in the center of solid tumors mainly die *via* oncosis (formerly known as necrosis), cells at the margins are preferentially eliminated by apoptosis.[4] During this active, energy- requiring process, various specific, intracellular endonucleases are activated. They cleave chromatin at the internucleosomal linker regions to mononucleosomes and oligonucleosomes.[5,6] These are complexes formed from DNA and histones and are multiples of 180 bp in size.[7,8] Under physiological conditions, these nucleosomes are packed into apoptotic bodies and engulfed by macrophages and neighboring cells.[9] However, at high rates of apoptosis, these phagocytosing mechanisms are saturated, leading to elevated concentrations of nucleosomes in the circulating blood.[10]

It is well known that high levels of free DNA circulate in patients with various malignant tumors[11–16] and in patients with systemic lupus erythematosus (SLE).[17,18] Most of the DNA in the serum and plasma exist in the form of oligonucleosomes and mononucleosomes.[19–21] We modified a test system that was originally created for cytoplasmic detection of nucleosomes (the Cell Death Detection ^{plus}-ELISA; Roche Diagnostics, Penzberg, Germany) in order to directly quantify nucleosomes in liquid materials, particularly in serum.[22]

Many tests have been developed to quantify the rate of proliferation or cell death for estimation of tumor activity.[23,24] Most of them require tumor tissue as matrix, are invasive and might only yield information about the time of first diagnosis. If the concentration of nucleosomes in the serum reflects tumor activity or correlates with the death of tumor cells, their quantification *via* the Cell Death Detection ^{plus}-ELISA would be an almost non-invasive and easy-to-perform method. It could be applied in daily routine and would be suited particularly for kinetic measurements in patients during or after antitumor therapy.

As nucleosomes are released from cells immediately after disintegration of the plasma membrane[25,26] and as their half life in serum is short,[21,27] the concentration of nucleosomes in serum might reveal a snapshot of the rate of cell death at a defined time. Thus, the spontaneous rate of cell death in sera of patients before therapy as well as the induced rate of cell death during and after therapy might contain important information about tumor activity and its sensitivity to therapy.

MATERIAL AND METHODS

The Cell Death Detection^{plus}-ELISA is based on a quantitative sandwich EIA principle. Mouse MAbs directed against DNA (single-strand [ss] and double-strand [ds] DNA) and histones (H1, H2a, H2b, H3 and H4) from mouse clones M-CA-33 and H11-4, respectively, detect specifically mononuclesomes and oligonucleosomes deriving from eukaryotic cells.

Whereas the anti-histone antibodies are biotinylated and fix the complexes to the microtiter plate, the anti-DNA antibodies are associated with a peroxidase label that reacts with the substrate ABTS (2,2'-Azino-di (3-ethylbenzthiazolin-sulfonat)). The resulting color development, which is proportional to the amount of nucleosomes captured in the antibody sandwich, is measured pho-

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tometrically at 405 nm. Whereas determination of the electrophoretic DNA ladder requires the content of at least 10^6 cells, the Cell Death Detection^{plus}-ELISA already allows detection of 10^3 cells.

In order to enable direct quantification of the nucleosomes and to improve the intraassay and interassay comparability of the ELISA system, we established reference material according to the following procedure. From 3 healthy donors, equal volumes of EDTA-stabilized whole blood samples were mixed and incubated for 3 days at 37°C (5% CO_2). After centrifugation, the supernatant, which contained high concentrations of nucleosomes, was used to produce the standards. In the ELISA, the material was diluted with incubation buffer [IP] (1:24, 1:32, 1:48, 1:64, 1:96 and only IP), ensuring that the highest standard constantly reached values of about 2,500 arbitrary units (AU) after 30 min of ABTS color development.

As matrix, we used serum because of its better stability compared with plasma and the planned automatization of the test. The blood samples were centrifuged within 1–2 hr after blood was taken. Subsequently, we added 10 mM EDTA (pH 8) to stabilize the nucleosomes in the serum and stored the samples at -20°C. Under these conditions, the blood samples remained stable for at least 6 months.[22] Immediately before measurement of the nucleosomes in the ELISA, the samples were homogenized and diluted 1:4 with incubation buffer.

Patients

We investigated serum samples from 590 persons, including 63 healthy persons, 109 patients with benign diseases and 418 patients with malignant tumors. Serum samples were obtained for all patients at time of acute disease and before start of the recommended therapy. Of the 109 patients with benign diseases, 38 suffered from benign gastrointestinal diseases (colitis, pancreatitis, cholecystolithiasis, subileus and others), 13 from benign pulmonary diseases (emphysema, pneumonia a.o.), 37 from benign gynecological diseases (ovarian cysts, endometriosis, uterus myomatosus a.o.) and 21 from other benign diseases (abscesses, nodular goiter, coronary heart disease). Among these 109 patients were 50 patients with acute inflammatory diseases. According to C- reactive protein (CRP), we classified these patients into 5 groups of 10 patients each: I: CRP $\leq$1 ng/ml; II: 1 ng/ml $<$ CRP $\leq$ 5 ng/ml; III: 5 ng/ml $<$ CRP $\leq$ 10 ng/ml; IV: 10 ng/ml $<$ CRP $\leq$ 20 ng/ml; V: 20 ng/ml $<$ CRP. Of the 418 patients with malignant tumors, 60 suffered from lung cancer, 79 from colorectal cancer, 49 from other gastrointestinal cancers, 61 from breast cancer, 45 from ovarian cancer, 20 from other gynecological cancers, 40 from lymphoma, 20 from renal cancer, 17 from prostate cancer and 27 from other carcinoma.

Of these 418 cancer patients, we observed additionally the course of 16 patients (6 with lung cancer, 4 with head and neck cancer, 4 with lymphoma and 2 with colorectal cancer) during radiotherapy. We also observed 20 patients (8 with lymphoma, 6 with colorectal cancer, 2 with pancreatic cancer, 2 with sarcoma, 2 with lung cancer) during chemotherapy for a period of 1–12 months.

Radiotherapy was applied in daily fractions of 1.6–2.0 Gy, 5 days a week for 4–6 weeks according to the radiation regimen of the respective tumor diseases. Blood was taken before radiation and at 3 and 6 hr, 1 day (immediately before the second fraction was given), 4 and 7 days after initiation of therapy and additionally weekly before the first fraction of the week.

Chemotherapy was applied in cycles of 1–5 days according to the therapeutic regimen, followed by a therapy-free interval of 3–4 weeks. Blood was taken at the first, second and fourth day of each cycle immediately before chemotherapy was administered.

We correlated the pre-therapeutic nucleosomal level in the serum samples, the increase in values after initiation of therapy, the maximum value during therapy, the delay and completeness of decrease of the values, the minimum value between the cycles and at the end of therapy, respectively, and the kinetics of the baseline values of circulating nucleosomes — which were determined before each new therapeutic cycle — with the clinical outcome.

RESULTS

Distribution of the values

Of the 63 healthy persons, 60 had very low concentrations of nucleosomes in serum below 100 AU. The median concentration was 24 AU and the mean concentration was 36 AU (Fig. 1, Table I). Age, sex and lifestyle (particularly smoking and drinking alcohol) did not cause any significant differences. We calculated the 95th percentile for healthy persons at 98 AU.

In the sera of patients with malignant tumors, we found nucleosomal levels that ranged from 1 to more than 1,500 AU. A median of 190 AU and a mean of 350 AU were markedly higher than levels found in the sera of healthy persons (Fig. 1, Table I). Among the various tumor types, sera of patients with lung cancer showed the highest values with a median of 417 AU and a mean of 569 AU. The sera of patients with prostate cancer had the lowest values with a median of 9 AU and a mean of only 31 AU. The sera of patients with other tumor entities showed intermediate values (Fig. 2, Table I).

We also observed a wide range (from 1 to more than 1,000 AU) of the measured values in the sera of patients with benign diseases. The median of 146 AU (mean 264 AU) was considerably lower than that found in the sera of tumor patients (Fig. 1, Table I). Among these patients, those with acute inflammations showed a clear correlation between the level of CRP and circulating nucleosomes (Fig. 3).

The discrimination between healthy persons and patients with malignant diseases as well as between healthy persons and patients with benign diseases was highly significant ($p = 0.0001$ Wilcoxon test). The difference between the groups of patients with benign and malignant diseases did not reach statistical significance ($p = 0.072$, Wilcoxon test; Fig. 1).

Follow-up

In the follow-up of patients with acute inflammations, we found a correlation among the concentration of nucleosomes, CRP and the clinical state of the patient. In acute stages of disease, we observed a high CRP and nucleosomal levels; during reconvalescence, both parameters declined simultaneously (Fig. 4).

During chemotherapy, most of the patients showed a rapid increase of the concentration of nucleosomes with a peak between 24–72 hr after initiation of therapy, followed by a slow decrease to almost normal values. The peak of the second cycle was generally

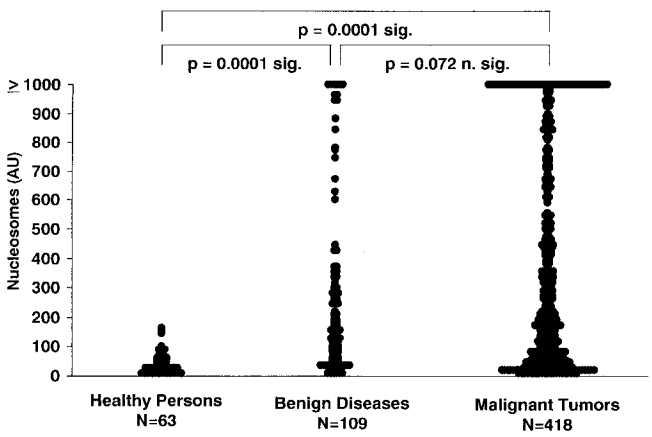

FIGURE 1 – Distribution of the spontaneous concentration of nucleosomes in serum (AU) for healthy persons, patients with benign diseases and patients with malignant tumors .

TABLE I – DISTRIBUTION OF THE SPONTANEOUS CONCENTRATIONS OF NUCLEOSOMES IN SERUM BEFORE START OF THE THERAPY

	Number	Concentration of nucleosomes in serum (AU)				
		Mean	Median	75%	95%	Range
Healthy persons	63	36	24	50	98	1–156
Patients with malignant diseases	418	350	190	528	1,084	1–1,812
Lung cancer	60	569	416	984	1,195	3–1,812
Colorectal cancer	79	343	187	505	1,074	1–1,191
Other gastrointestinal cancers	49	334	175	550	1,051	12–1,252
Breast cancer	61	330	185	432	1,032	13–1,260
Ovarian cancer	45	391	216	670	1,086	12–1,347
Other gynecological cancers	20	347	232	477	1,264	16–1,424
Lymphoma	40	324	136	572	1,012	1–1,137
Renal cancer	20	194	60	299	716	4–767
Prostatic cancer	17	31	9	44	110	1–167
Others	27	249	151	404	651	5–1,032
Patients with benign diseases	109	264	146	330	988	7–1,248
Benign pulmonary diseases	13	273	149	349	906	11–1,006
Benign gastrointestinal diseases	38	265	93	279	1,022	7–1,248
Benign gynecological diseases	37	244	177	336	806	7–1,026
Other benign diseases	21	293	139	291	960	9–1,138

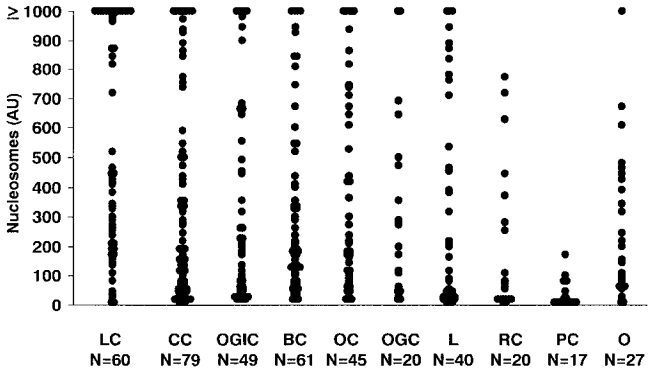

FIGURE 2 – Distribution of the spontaneous concentration of nucleosomes in serum (AU) for patients with malignant tumor diseases: lung cancer (LC), colorectal cancer (CC), other gastrointestinal cancers (OGIC), breast cancer (BC), ovarian cancer (OC), other gynecological cancers (OGC), lymphoma (L), renal cancer (RC), prostatic cancer (PC) and other cancers (O).

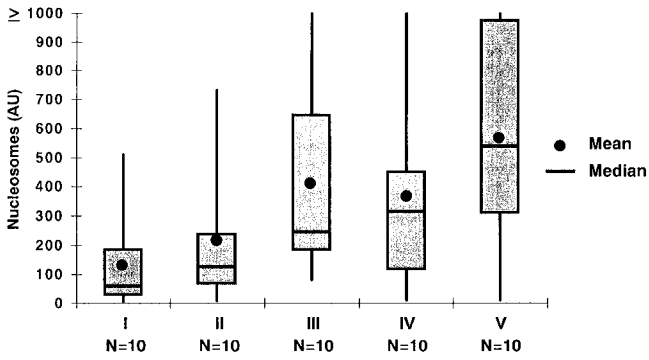

FIGURE 3 – Mean, median, 25th percentile, 75th percentile and range of the concentration of nucleosomes in serum (AU) for patients with acute inflammations concerning the level of CRP: I: CRP ≤1 ng/ml; II: 1 ng/ml < CRP ≤ 5 ng/ml; III: 5 ng/ml < CRP ≤ 10 ng/ml; IV: 10 ng/ml < CRP ≤ 20 ng/ml; V: 20 ng/ml < CRP.

lower than that of the first one. Infections and other side effects led to a temporary elevation of the nucleosomes in the serum (Figs. 5, 6).

In patients undergoing radiotherapy, we observed an even quicker increase of the concentration of nucleosomes in the serum about 6–24 hr after inititation of therapy. In some of the patients,

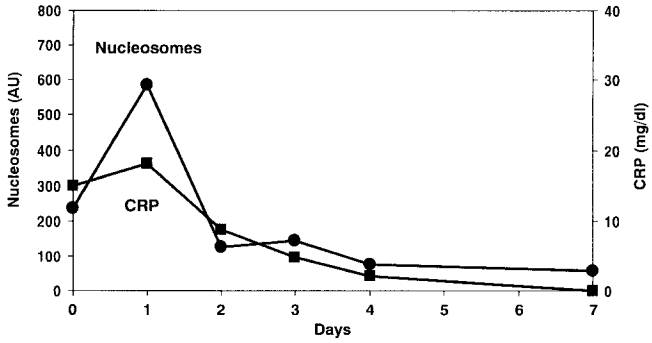

FIGURE 4 – Course of the concentration of nucleosomes in serum and CRP in a patient suffering from acute cholangitis with cholestasis. During antibiotic therapy (starting at day 1), the concentration of nucleosomes in the serum, CRP and other signs of inflammation normalized subsequently.

we found a temporary decline of the values after 3 or 6 hr followed by a rapid increase. During the course of therapy, the concentration of nucleosomes often decreased constantly, correlating with tumor regression. This was documented by imaging techniques (Figs. 7, 8).

Correlation to the clinical outcome

In chemotherapy patients, there was no correlation of pre-therapeutic values, increase rate and maximum values with the clinical outcome. The kinetics of the baseline values of circulating nucleosomes were determined before starting each new therapeutic cycle. These values correlated well with the clinical outcome. In all 8 patients with partial or complete remission of disease (UICC criteria), we observed a decrease >50% of the baseline values. An increase >50% of the baseline values (N = 6) was only seen in patients with disease progression (Table II).

During radiotherapy, we found a correlation between the pre-therapeutic concentration of nucleosomes and the maximum concentration of nucleosomes during therapy. The nucleosomal concentrations increased <50% during therapy in 9 of 11 patients with pre-therapeutic values higher than 100 AU. Increases more than 50% were observed in 5 patients, all of whom had pre-therapeutic values less than 100 AU. This means that moderate changes in absolute numbers could provoke considerable percentual increases. However, there was neither a correlation of the pre-therapeutic nucleosomal levels nor of the maximum values during therapy with the clinical outcome.

However, clinical outcome correlated with the start of decline of the maximum nucleosomal concentration and with the minimum

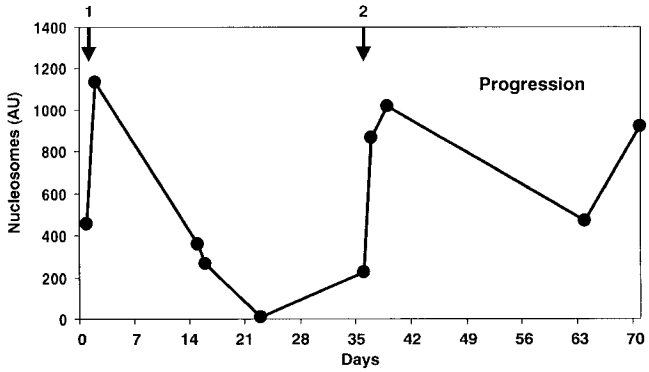

FIGURE 5 – Course during chemotherapy in a patient with pancreatic carcinoma T4 N1 M1, treated with gemcitabine 1,000 mg/m^2 (days 1, 8, 15) and cisplatin 50 mg/m^2 (days 1 and 15) (1). The concentration of nucleosomes in serum increased within 3 days and declined gradually afterward. Subsequently, therapy was changed to folinic acid 300 mg/m^2 bolus and 5-fluorouracil 500 mg/m^2/2 hr (days 1 to 5) (2) because of disease progression. The concentration of the circulating nucleosomes peaked again but decreased only incompletely. In parallel with a further progression of the metastatic disease and a deterioration of the general condition, the spontaneous concentration of nucleosomes in serum increased again.

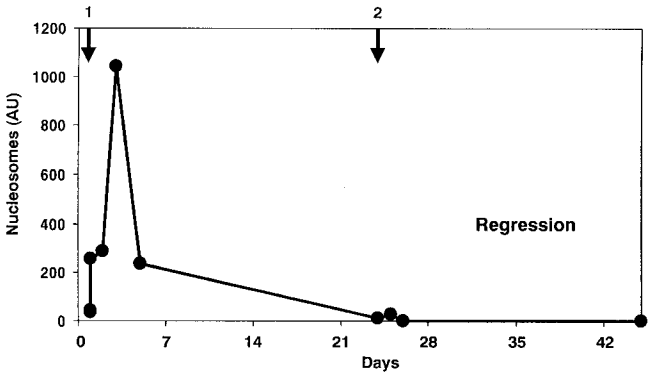

FIGURE 6 – Course during chemotherapy in a patient with small-cell lung cancer (extended disease), treated with cyclophosphamide 1,400 mg (day 1), doxorubicin 60 mg (day 1) and etoposide 100 mg (day 1 to 3) (1). After the first cycle of chemotherapy, the clinical situation of the patient improved markedly. Tumor regression was documented by ultrasound and later almost complete remission of hepatic and bone metastases was found by CT and scintigraphy. In accordance with this high efficacy of chemotherapy, the concentration of nucleosomes in serum decreased rapidly. The second cycle of chemotherapy produced only little changes (2).

concentration after therapy (Table III). In 9 of 10 patients, we observed a partial or complete remission of the tumor when the nucleosomal levels started to decrease within 24 hr after the initial peak. In patients with disease progression, the decline of the nucleosomal levels started only after more than 7 days (3 patients), between 2 and 7 days (1 patient) and only once within the first day after the initial peak. Furthermore, in 9 of 10 patients with remission, the minimum concentration of nucleosomes was lower than 100 AU; in 4 of 5 patients with disease progression, the concentration was higher than 100 AU.

DISCUSSION

Distribution of the values

As in many other studies[11–14] based on circulating DNA in plasma or serum, our results showed healthy persons as a homogeneous group with very low concentrations of nucleosomes in the serum (<100 AU). Patients with malignant tumors or benign diseases had nucleosomal concentrations that varied considerably. In serum samples from patients with malignant tumors, we detected high levels of nucleosomes. However, there were also patients with advanced tumor disease with very low levels of nucleosomes, overlapping with those of healthy persons. Many of the patients with benign diseases had only slightly elevated con-

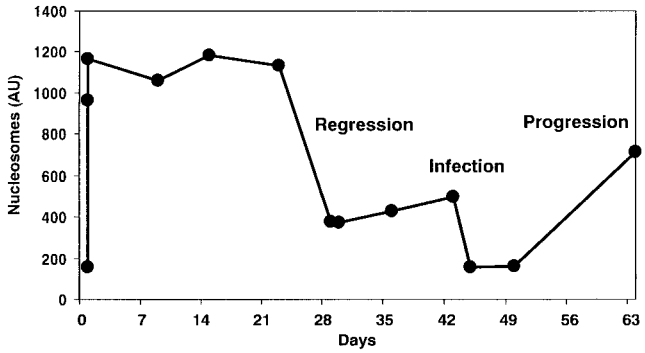

FIGURE 7 – Course during radiotherapy in a patient with metastatic carcinoma of the lung, irradiated with a total dose of 60 Gy (30 daily fractions with 2.0 Gy each, volume 9.9l). After a temporary decline, the concentration of nucleosomes in serum increased enormously at the first day of therapy and remained high over weeks. Corresponding to the decline of the level of circulating nucleosomes, metastases showed X-ray documented regression. Because of pericardial empyema, the values increased slightly at day 40 and returned to the basic level when antibiotic treatment was initiated. Subsequently, the patient developed multiple metastases and an increase in the concentration of nucleosomes in serum.

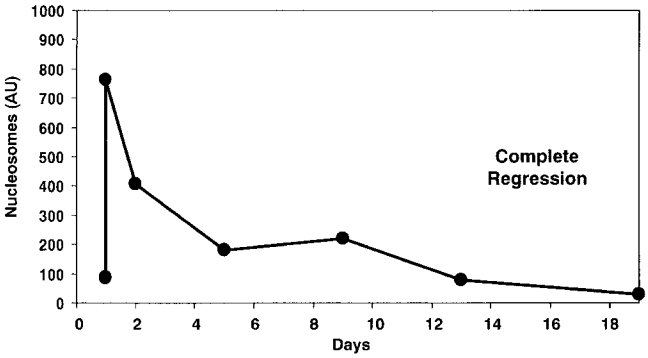

FIGURE 8 – Course during radiotherapy in a patient with head and neck cancer, treated with a total dose of 33.6 Gy (16 daily fractions with 2.1 Gy each, volume 3.8l) and concomitant cisplatin infusions (6 mg/m^2/day). The concentration of nucleosomes in serum peaked at the first day followed by a complete decrease starting immediately after having reached the maximum. Regression of the tumor was documented by imaging techniques.

TABLE II – CORRELATIONS BETWEEN BASELINE VALUES OF CIRCULATING NUCLEOSOMES IN SERUM DURING CHEMOTHERAPY AND CLINICAL OUTCOME

	Remission (N = 8)	No change (N = 1)	Progression (N = 11)
Baseline values of circulating nucleosomes			
Decrease >50%	8	1	4
No change	0	0	1
Increase >50%	0	0	6

TABLE III – CORRELATIONS BETWEEN CHANGES OF THE CONCENTRATION OF NUCLEOSOMES IN SERUM DURING RADIOTHERAPY AND CLINICAL OUTCOME

	Remission (N = 10)	No change (N = 1)	Progression (N = 5)
Start of decrease of the concentration of nucleosomes in serum			
>7 Days	0	0	3
2–7 Days	1	0	1
≤1 Days	9	1	1
Minimum values of the concentration of nucleosomes in serum			
>100 AU	1	0	4
≤100 AU	9	1	1

centrations of nucleosomes, whereas we observed extremely high levels of more than 1,000 AU in other patients with benign disease. Although the discrimination between the groups of patients with malignant tumors and benign diseases almost reached the level of significance, the concentration of nucleosomes in serum cannot predict malignancy of diseases for a specific person, due to inflammatory processes. High CRP levels indicate that acute stages of inflammations delay the removal of nucleosomes from circulation by binding to the histone component.[28]

In patients with malignant tumors, the intratumor and intertumor heterogeneity of spontaneous and induced apoptosis is a well known feature,[29,30] which explains our findings of widely varying levels of nucleosomes in serum. Interindividual differences regarding the relation of free DNA and nucleosome-bound DNA may also contribute to this phenomenon.

Additionally, tumor stage and activity, proliferation rate and perfusion grade may also influence the concentration of nucleosomes. As we have shown, higher concentrations of circulating nucleosomes occur in tumor entities that are highly active or that are detected at advanced stages (*e.g.*, lung cancer). In contrast, cancers of the prostate give rise to low concentrations of nucleosomes in serum, possibly due to the fact that they are mostly detected at earlier stages. Although advanced stages were characterized by higher amounts of nucleosomes than lower stages, the concentration of circulating nucleosomes cannot replace the classic staging investigation at the single patient's level. Similar data were published previously for patients with lung cancer,[14] gastrointestinal tumors[13] and with various tumors[12] with respect to DNA in circulation.

Follow-up

Regarding the follow-up investigations in patients undergoing chemotherapy and radiotherapy, the rapid increase of the concentration of nucleosomes in serum confirmed our hypothesis that spontaneous and induced cell death leads to elevated levels of nucleosomes in serum. Depending on the tumor type, sensitivity and medication, many chemotherapeutic agents,[5,31–34] as well as radiotherapy in therapeutic doses up to 2.0 Gy per fraction,[5,29,30,35,36] cause apoptotic cell death. Thus, the nucleosomes in serum we measured derived at least partly from the apoptotic death of tumor cells.

However, many normal cell populations with a high proliferation rate are also damaged by chemotherapy and radiotherapy (*e.g.*, cells of the hematopoetic system, lymphocytes, epithelial cells and cells during spermatogenesis[37]) and contribute to the measured amounts of circulating nucleosomes. In addition to apoptosis, oncosis or a mixture of both types of cell death[4,38] could lead to an increase of the concentration of nucleosomes in serum.

If better specification of the degraded cells is required, quantification of the nucleosomes in serum can be combined with tumor-associated antigens, such as neuron specific enolase (NSE),[14] prostate specific antigen (PSA) or others. Qualitative aspects of the circulating nucleosomes could be enlightened by molecular and genetic investigations.[39,40] For many tumor entities, point mutations at the ras gene that were detected in tumor DNA were also found in DNA circulating in plasma or serum (*e.g.*, for pancreatic cancer,[41,42] colorectal cancer,[43] other gastrointestinal malignancies[44] and for hematopoetic diseases as well as for various solid tumors[45]). In addition, detection of microsatellite instability of circulating DNA might provide insights as to their tumor origin and could possibly be used in the future for early diagnosis of many malignancies,[46,47] as shown for patients with breast cancer,[48–50] lung cancer,[51,52] renal cancer,[53] head and neck cancer[54] and melanoma.[55]

Regarding the course of the nucleosomal concentration during therapy, the delay of the increase was considerably shorter after radiotherapy than after chemotherapy. This is due to direct and synchronical damage of DNA by irradiation and the indirect and often cell cycle-specific effect of chemotherapeutic drugs.[35,56] As shown by Stephens *et al.*,[57] Meyn *et al.*[58] and Mirkovic *et al.*,[56] the maximum induction of morphological apoptotic alterations was observed 4 hr after application of radiotherapy and 24–48 hr after treatment with chemotherapy. Adding the time for disintegration of the plasma membrane, release of the cellular content and distribution into the circulation, our findings (maximum values of circulating nucleosomes 6–12 hr after radiotherapy and 24–72 hr after chemotherapy) agree well with the results of these studies.

Correlations to the clinical outcome

In patients undergoing chemotherapy, the baseline values of the concentration of circulating nucleosomes, which were determined before each new cycle, corresponded to the spontaneous apoptotic rate of the remaining active tumor tissue. This explains the increase of the baseline values in 6 of 11 patients with disease progression and the decrease of the baseline values in all patients with disease regression. However, some patients had a decrease of their baseline values in spite of disease progression. This was possibly caused by mutations of cell death-regulating genes, such as p53 or bcl2, which made the tumor cells resistant to apoptosis and led to a higher aggressiveness of the cancer.[59,60]

During radiotherapy, the delay time of decline and the level of the minimum concentration of nucleosomes in serum are probably explained by the sensitivity to radiation. Most cells die directly after initiation of therapy because of the effective and extended damage of the DNA,[57] which obviously leads to the early peak of circulating nucleosomes as observed in most of the irradiated patients. An early and rapid decline in the course of therapy correlated with disease regression, which was due to the effective elimination of radiosensitive tumor cells. Thus, a rapid decline to low minimum values indicates high radiosensitivity of the tumor and high efficacy of the therapy, as was seen in the serum samples in 9 of 10 patients.

In contrast, constantly high concentrations of nucleosomes during therapy and a late decline are explained by extended tumor volume and a high proliferation rate of the tumor. Additionally, in the periods between the applications of radiotherapy, new subpopulations of tumor cells are primed to become apoptosis sensitive, as reported by Thames *et al.*[60] and Meyn *et al.*.[61] This combination leads to high cell death rates during the following therapeutic fractions and to prolonged high levels of the measured nucleosomes in the circulation. Therefore, higher minimum levels of nucleosomes may be explained by high activity of tumors associated with inefficient therapy.

The spontaneous and radiation-induced apoptotic rate might have prognostic relevance. Although the data of our kinetic investigations are based on a small number of patients, they indicate a correlation of the spontaneous pre-therapeutic concentration of nucleosomes in serum and the radiation-induced maximum value determined after the first fraction of radiotherapy. However, nei-

ther the spontaneous nor the radiation-induced level of nucleosomes correlated with the clinical outcome of the patient.

CONCLUSION

The modified version of the Cell Death Detection^{plus}- ELISA offers the possibility to measure quickly and quantitatively the concentration of nucleosomes in serum. High amounts of nucleosomes indicate the presence of disease, whether benign or malignant cannot be distinguished.

The course of the concentration of nucleosomes in serum might be useful for therapeutic monitoring of patients with malignant tumors during chemotherapy or radiotherapy. During chemotherapy, an increase in baseline values of circulating nucleosomes indicates disease progression, which would lead to a change of the therapeutic regimen. Decrease of the baseline values is an indicator of disease regression. During radiotherapy, an early decrease of the concentration of nucleosomes to low minimum levels indicates a good sensitivity to irradiation, whereas a late decrease to higher minimum levels is associated with less radiosensitivity. These results suggest that the measurement of the concentration of nucleosomes in serum is a useful tool to estimate the efficacy of radiotherapy.

Further prospective studies with more patients with defined tumor entities and therapeutic regimens are necessary to validate our preliminary results of the concentration of nucleosomes in serum during chemotherapy or radiotherapy.

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