Cytogenetic assessment of heterogeneous radiation doses in cancer patients treated with fractionated radiotherapy

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ABSTRACT. The purpose of this study was to evaluate the *in vivo* dose–response relation of chromosome aberration formation and distribution in a context of localised and fractionated radiotherapy. Cytogenetic analysis was applied to eight patients, all treated for the same tumour localisation; the same localisation was used to prevent the variability usually observed between patients treated with radiotherapy and to allow the corresponding roles of the size of irradiation field and of the dose rate to be studied. The yield of dicentrics, centric rings and fragments was measured in blood samples taken before treatment, during the course of radiotherapy and up to 6 months after. After the first fraction of radiotherapy, we observed that the whole-body dose estimated from the yield of dicentrics and rings was higher (0.35 ± 0.2 Gy) than the calculated equivalent whole-body dose (0.07 ± 0.04 Gy). By contrast, the partial-body dose derived from the Qdr (quotient of dicentrics and rings) model was estimated to be 2.2 ± 0.3 Gy, which agreed quite well with the dose delivered to the tumour (2.1 ± 0.1 Gy). We also found a correlation between the yield of induced chromosome aberrations and the target field size ($p=0.014$). *U*-value analysis showed that the distribution of dicentrics and rings was overdispersed, despite the fractionation of the exposure, and a positive correlation between the *U*-value and the dose rate was observed ($p=0.017$). Overall, these results suggest that the proportion of undamaged lymphocytes could increase with the dose rate.

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Quantification of chromosome aberrations in circulating lymphocytes is conventionally used to estimate the dose received by individuals accidentally exposed to ionising radiation. The observed frequency of dicentrics and centric rings is referred to a dose–response curve established *in vitro*, which provides the whole-body dose [1, 2]. When irradiation is heterogeneous over different parts of the body, only some lymphocytes are exposed. Because irradiated and unirradiated lymphocytes are mixed in the blood circulation, the dose received by the irradiated lymphocytes and consequently the dose delivered locally should be underestimated. Two mathematical models, Qdr and Dolphin's deviation from the Poisson distribution, have been developed to assess the dose according to the fraction of exposed lymphocytes [3, 4]. Both models have been validated *in vitro* by mixing irradiated and unirradiated blood in different proportions [5]. These approaches have also been tested *in vivo* in accidental situations, with promising results [6, 7]. Furthermore, recent *in vivo* studies of cancer patients who had received 8 Gy of radiotherapy in a single fraction have shown that the derived partial-body dose obtained with both Qdr and Dolphin methods are in

agreement with the doses estimated from the radiotherapy regimens [8].

Cytogenetic assessment of cancer patients undergoing fractionated therapeutic irradiation would also be useful to evaluate the impact of the treatment on the yield of chromosome aberrations and on their distribution within the lymphocyte population. This could allow the evaluation of damage induced by such treatment in the patient's circulating lymphocytes. Several studies have examined chromosome aberrations in blood samples from patients undergoing fractionated radiotherapy. These studies, which have used conventional cytogenetics, fluorescence *in situ* hybridisation (FISH) or both, showed a dose-dependent increase in the yields of aberrations, but with substantial interpatient variability [9–11]. The same variability in the rate of damage induced in lymphocytes during radiotherapy has been observed in other studies that used different measurements, such as premature chromosome condensation (PCC), micronuclei or γ -H2AX foci [12–14]. Explanations for these interpatient differences have suggested that damage yields after fractionated partial-body exposure do not depend only on the dose delivered locally, but may be also influenced by many other parameters, including individual variability in the response to ionising radiation, target field size or tumour localisation [9, 10, 12]. In fact, fractionated radiotherapy is a typical example of non-uniform exposure. During a session of radiotherapy, lymphocytes from the vascular pool may

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receive relatively small doses whereas those from the resident pool in the field may receive higher doses; both are ultimately mixed by the circulation in the following hours [15]. Furthermore, distribution of the lymphocyte pool may vary greatly within the human body; this implies that the geometry of irradiation may have a high impact on the proportion of vascular *vs* resident lymphocytes that may be irradiated. To measure the impact of radiotherapy treatment parameters on the aberration yields induced in peripheral lymphocytes, it is useful to study patients who have tumours in the same anatomical region. Therefore, to analyse the roles of the target field size and the dose rate relating to interpatient variability, we applied conventional cytogenetic methods to eight patients receiving fractionated radiotherapy for head and neck cancer. This anatomical region contains a high number of blood vessels and also a high number of lymph nodes. We evaluated the relation between the yield of dicentrics and centric rings measured in lymphocytes and the radiotherapy field size. We compared the dose estimate using cytogenetic methods with the physical doses obtained using calculations. Finally, we verified whether the test for deviation from Poisson distribution was able to detect the heterogeneity of the exposure after several fractions of radiotherapy.

Methods and materials

Patients

Blood samples from eight patients treated for head and neck cancer at the Institut Gustave Roussy, Villejuif, were collected in accordance with the French law regarding bioethics. Patients provided informed consent before enrolment. The patients' characteristics are detailed in Table 1. Tumour field size selection was based on simulator-CT scan images and radiographic film data [16]. This method used two-dimensional (2D) images and did not allow the entire dose-volume histograms to be calculated. The median total dose was 60 Gy (range 50–66 Gy). Blood samples for each patient were taken before starting the treatment and during the course of radiotherapy: after the first fraction (tumour dose=2 or 2.5 Gy), after five or six fractions (tumour dose=12 or 12.5 Gy) and at the end of treatment (final tumour dose, Table 1). Blood samples were taken during the hour following the radiotherapy session. Patients P3, P4, P6, P8 and P9 also provided samples 6 months after treatment ended, and patient P10, 4 months afterwards. None of the patients had chemotherapy during the course of radiotherapy, but patient P6 received 5-fluorouracil and cisplatin before radiotherapy began.

Cytogenetic analysis

Blood was cultured in RPMI 1640 medium (Invitrogen Carlsbad, CA, USA) with phytohaemagglutinin, antibiotics and 10% foetal calf serum (Invitrogen), as previously described [7]. To prevent the scoring of dicentrics in second-division cells, 5-bromodeoxyuridine (Sigma, St. Louis, HO, USA) was added to the culture. After 46 hours of culture at 37°C, colcemid (Karyomax, Invitrogen) was

Table 1. Description of patient pathologies and treatments

Patient number	Sex	Age (years)	Smoking habits	Tumour site	TNM classification	Treatment	Tumour dose (Gy)	Fractionation	Target area (cm ²)	SCF irradiation	Energy (MV)	Dose rate (Gy min ⁻¹)
P1	F	45	No	Hypopharynx posterior wall	pT4a N0M0	Surgery + RT	60	30 × 2 Gy	394.5	Yes	1.2 (⁶⁰ Co)	1
P3	M	68	Heavy	Right piriform sinus	T2N0M0	Surgery + RT	50	25 × 2 Gy	332.5	Yes	1.2 (⁶⁰ Co)	0.5
P4	M	63	Heavy but stopped 14 years before	Left piriform sinus	T3N2bM0	Surgery + RT	50	25 × 2 Gy	393	Yes	1.2 (⁶⁰ Co)	0.93
P6	M	79	Heavy but stopped 10 years before	Right piriform sinus	T3N3M0	CT + Surgery + RT	66	33 × 2 Gy	421.5	Yes	1.2 (⁶⁰ Co)	0.94
P8	F	47	Passive	Right vocal cord	T1aN0M0	RT	65	26 × 2.5 Gy	55	No	1.2 (⁶⁰ Co)	0.82
P9	F	52	Heavy	Right part of the oral floor	T2N0M0	Surgery + RT	64	32 × 2 Gy	286	Yes	4 (linear accelerator)	1.5
P10	F	45	Heavy	Right piriform sinus	T3N1M0	RT	50	25 × 2 Gy	332.5	Yes	1.2 (⁶⁰ Co)	0.91
P11	M	66	Heavy but stopped 16 years before	Right vocal cord	T1aN0M0	RT	65	26 × 2.5 Gy	62.7	No	1.2 (⁶⁰ Co)	0.64

TNM, international tumour nodes metastasis classification; CT, chemotherapy; RT, radiotherapy; total fields surface, sum of the areas of the irradiation fields; SCF, supraclavicular fossa.

added to the preparation for the final 2 h. The cells were harvested with a KCl (0.075 M) shock followed by three fixation steps (methanol–acetic acid, 3:1). The fluorescence plus Giemsa (FPG) technique was used as described in a previous publication [7]. Briefly, after washing, slides were stained with Giemsa 5%. Dicentrics, centric rings and fragments were scored only in first-division complete metaphases (46 chromosomes).

All dose estimates were obtained by reference to calibration curves established *in vitro* by exposing blood samples from healthy donors to γ -radiation from a cobalt-60 source at a dose rate of 0.5 Gy min⁻¹ [7].

Application of the Qdr method

The Qdr method was used to estimate the partial-body dose, that is the dose received by the exposed lymphocytes [3, 17]. The method has been described elsewhere [7]. Briefly, the Qdr method calculates the local radiation dose by taking into account the distribution of the frequency of dicentrics, centric rings and fragments in cells containing at least one chromosome aberration. Furthermore, the Qdr dose was used to calculate the fraction of the irradiated body as described in International Atomic Energy Agency Technica Report Series No. 405 [2].

Exposure heterogeneity

Papworth's extended *U*-test was applied to the distribution of chromosome aberrations to determine the heterogeneity of irradiation [18]. When exposure is heterogeneous, the distribution no longer follows a Poisson distribution, and the *U*-test quantifies the deviation of the results relative to Poisson's law. When the calculated *U*-value exceeds ± 1.96 , the distribution of the corresponding chromosome aberrations can be considered to be overdispersed with a 95% confidence interval.

Physical whole-body dose estimation

Each patient's dose was calculated to the whole body during the course of radiotherapy, that is the equivalent whole-body dose (EWBD) using the following formula [19]:

Integral dose =

$$\frac{1.44 \times D_0 \times d_{1/2} \times A \times [1 - \exp(-0.6938 d/d_{1/2})] \times (1 + 2.88 d_{1/2}/f)}{1000}$$

where D_0 = administered dose (Gy), A = field area (cm²), d = patient diameter (cm), $d_{1/2}$ = half-value thickness ($d_{1/2}$ of ⁶⁰Co γ -rays = 10.3 cm and $d_{1/2}$ of 4 MV X-rays = 12.6 cm) and f = source to skin surface distance (cm).

The formula was applied to each irradiation field in order to obtain a total integrated dose that was divided by the patient's weight to obtain the EWBD.

Statistical analysis

To test whether the spontaneous yield of dicentrics and rings in patients before treatment was significantly

higher than the control value of our laboratory, we applied a one-way test for the Poisson variable, which allows the comparison of a scored value with a reference value [20]. For a determined number of scored metaphases, this test calculates the number of chromosome aberrations significantly higher with a 95% confidence interval.

To analyse the relation between more than two variables, two-way analysis of variance (ANOVA) was used. A *t*-test was used to test the difference in the mean values of two groups. The correlation coefficient (r^2) was calculated using the Pearson test to analyse the relation between two variables. The corresponding two-tailed probability value (*p*) was obtained using a table of critical values for the Pearson test.

A *p*-value less than 0.05 was considered significant.

Results

Background and evolution of the yield of chromosome aberrations during the course of treatment

Table 2 shows the scores for dicentrics and centric rings during treatment and up to 6 months later. In three patients (P1, P8 and P11), the spontaneous chromosome aberration rate was in the range of control values in our laboratory (0–0.02) [21]. By contrast, for patients P3, P4, P6, P9 and P10, we observed an elevated spontaneous chromosome aberration rate significantly higher than the mean control value yield with a 95% confidence interval. Such a high yield may be explained for patient P6, whose head and neck cancer was treated with 5-fluorouracil and cisplatin before radiotherapy, and for patient P4, who suffered from cardiovascular diseases that needed numerous radiological examinations. In addition, patient P9 had a history of cervical neoplasia and may have been predisposed to chromosome instability. For patients P3 and P10, the higher yield of spontaneous chromosomal aberrations remains unexplained.

Overall, the yield of dicentrics and rings (dic + r) increased with the dose delivered to the tumour for all patients (Figure 1). However, the evolution of the yield of dic + r over the course of radiotherapy varied greatly between patients. For instance, at the end of treatment, tumour doses ranged from 50 to 66 Gy, but the yields of dic + r of patients P4 and P10, who had received 50 Gy, were much higher than those of patients P8 and P11, who had received 65 Gy (Figure 1).

When the influence of the treatment parameters was considered, a significant influence on the yield of dic + r was observed for the number of radiotherapy fractions as well as for the size of the irradiation field ($p=0.003$ and $p=0.014$, respectively) (Figure 2).

Comparison of physical doses with doses estimated by cytogenetic methods

Because the biological dose estimated by measuring the yield of dic + r can be considered as a mean whole-body dose, we compared this value obtained with the corresponding calculated EWBD (for calculation details

Table 2. Distribution and frequency of dicentrics and centric rings in lymphocytes of the eight head and neck cancer patients

	Number of cells scored	Total dic + r	Number of cells with aberrations (dic and/or r and/or Fg)	Frequency of dic + r	U-test	dic + r distribution											
						0	1	2	3	4	5	6	7	8	9	10	11
P1																	
T	570	1	4	0.002	–	569	1	0	0	0	0	0	0	0	0	0	0
2 Gy	536	10	19	0.019	–0.29	526	10	0	0	0	0	0	0	0	0	0	0
12 Gy	557	75	70	0.135	7.02 ^b	500	42	12	3	0	0	0	0	0	0	0	0
End of T	255	108	77	0.424	12.07 ^b	189	43	13	5	4	0	0	0	1	0	0	0
P3																	
T	513	2	5	0.004 ^a	–0.04	511	2	0	0	0	0	0	0	0	0	0	0
2 Gy	445	5	7	0.011	–0.15	440	5	0	0	0	0	0	0	0	0	0	0
12 Gy	450	46	46	0.102	3.10 ^b	411	32	7	0	0	0	0	0	0	0	0	0
6 months	209	117	64	0.560	12.94 ^b	152	24	14	16	1	1	0	0	1	0	0	0
P4																	
T	439	2	8	0.005 ^a	–0.05	437	2	0	0	0	0	0	0	0	0	0	0
2 Gy	409	9	15	0.022	0.3	400	9	0	0	0	0	0	0	0	0	0	0
12 Gy	399	98	83	0.246	8.57 ^b	331	47	14	5	2	0	0	0	0	0	0	0
End of T	241	83	60	0.344	7.70 ^b	189	32	10	9	1	0	0	0	0	0	0	0
6 months	353	100	131	0.283	21.12 ^b	303	25	12	6	3	3	1	0	0	0	0	0
P6																	
T	514	3	6	0.006 ^a	–0.08	511	3	0	0	0	0	0	0	0	0	0	0
2 Gy	557	27	33	0.048	–0.79	530	27	0	0	0	0	0	0	0	0	0	0
12 Gy	439	35	35	0.080	13.45 ^b	416	15	5	2	1	0	0	0	0	0	0	0
End of T	249	101	72	0.406	9.28 ^b	184	45	11	4	4	0	1	0	0	0	0	0
6 months	354	128	235	0.361	27.80 ^b	285	46	12	1	4	3	1	0	0	2	0	0
P8																	
T	557	1	1	0.002	–	556	1	0	0	0	0	0	0	0	0	0	0
2.5 Gy	488	3	4	0.006	–0.08	485	3	0	0	0	0	0	0	0	0	0	0
12.5 Gy	507	7	8	0.014	4.7 ^b	501	5	1	0	0	0	0	0	0	0	0	0
End of T	484	10	13	0.021	2.98 ^b	475	8	1	0	0	0	0	0	0	0	0	0
6 months	500	18	20	0.036	8.49 ^b	486	11	2	1	0	0	0	0	0	0	0	0
P9																	
T	538	5	7	0.009 ^a	7.20 ^b	534	3	1	0	0	0	0	0	0	0	0	0
12 Gy	728	41	57	0.056	21.57 ^b	703	16	3	5	1	0	0	0	0	0	0	0
End of T	418	85	68	0.203	13.18 ^b	362	37	14	2	2	0	1	0	0	0	0	0
6 months	318	102	61	0.320	24.09 ^b	263	34	6	12	1	1	0	0	0	0	0	1
P10																	
T	516	2	7	0.004 ^a	–0.04	514	2	0	0	0	0	0	0	0	0	0	0
2 Gy	503	21	22	0.041	–4.20 ^b	481	19	1	0	0	0	0	0	0	0	0	0
12 Gy	500	64	89	0.128	3.47 ^b	446	45	8	1	0	0	0	0	0	0	0	0
End of T	194	108	87	0.556	3.53 ^b	122	47	16	7	2	0	0	0	0	0	0	0
4 months	318	158	123	0.496	9.78 ^b	224	58	19	12	5	0	1	0	0	0	0	0
P11																	
T	470	0	3	0.000	–	470	0	0	0	0	0	0	0	0	0	0	0
2.5 Gy	570	7	9	0.012	–0.16	563	7	0	0	0	0	0	0	0	0	0	0
12.5 Gy	601	4	6	0.007	–0.04	597	4	0	0	0	0	0	0	0	0	0	0
End of T	507	9	15	0.018	–0.27	498	9	0	0	0	0	0	0	0	0	0	0

The U-test gives the deviation from the Poisson law.

^aSignificant higher yield with a 95% confidence interval.

^bSignificant U-value with a 95% confidence interval.

T, before treatment; end of T, end of treatment; dic, dicentric; r, centric ring; Fg, fragment.

see Methods and materials). This dose estimation was made only after the first 2 Gy session of radiotherapy in order to avoid a dilution effect of the fractionated irradiation. The mean dic + r whole-body dose value was 0.35 ± 0.2 Gy, whereas the mean EWBD was only 0.07 ± 0.04 Gy (Table 3). We then applied the Qdr method in order to calculate the local doses in the same conditions. The mean partial-body dose estimated using Qdr was 2.2 ± 0.7 Gy (Table 3). This is very close to the physical dose actually delivered to the tumour after the first session, which was 2.1 ± 0.2 Gy. Furthermore, we used the Qdr dose to estimate the fraction of the

irradiated body (data not shown). After comparison of the radiotherapy field size with the fraction of the irradiated body calculated using Qdr, a significant correlation was observed ($r^2 = 0.78$, $p < 0.05$).

Dispersion analysis of chromosome aberrations

The dispersion of dicentrics and rings among the lymphocyte population over the course of radiotherapy was analysed. After the first session, there was no significant deviation in dispersion, U-values were below

Figure 1. Dicentric and rings (dic+r) yield during the radiotherapy of 8 patients treated for head and neck tumours. At the end of the course of radiotherapy, doses to the tumour ranged from 50 Gy to 66 Gy. Error bars represent 95% confidence limits of the yield following the Poisson law.

± 1.96 in all patients, except for patient P10 (Table 2). After five or six sessions and until the end of treatment, U -values were >1.96 for all patients except for patient P11. Thus, exposure heterogeneity could be detected in seven of eight patients. During the months following treatment, U -values increased significantly ($p=0.035$), reflecting an increasing overdispersion of dicentric and rings among the lymphocyte population (Figure 3).

When the influence of the target field size on the dispersion was studied, no significant correlation with the U -value was observed. We then analysed the influence of

other radiotherapy parameters, such as the number of fractions and the dose rate. No influence of the number of fractions was observed, whereas a significant correlation between dose rate and U -value was noted ($p>0.5$ and $p=0.017$, respectively) (Figure 4).

Discussion

The present study was undertaken to quantify the relationship between *in vivo* chromosome aberration

Figure 2. Yield of dicentric and rings (dic+r) as a function of the target field size in individuals undergoing radiotherapy. Rings correspond to seven of the eight patients tested after the first session, diamonds correspond to the eight patients tested after the 12-Gy session and squares refer to seven of the same patients tested after the last session of radiotherapy. Error bars represent 95% confidence limits of the yield following the Poisson law. The lines represent the best fit with a linear function.

Table 3. Comparison of the doses estimated by cytogenetics with the physical doses after the first session of radiotherapy

Patient	First session Local dose		Whole-body dose	
	Tumour dose (Gy)	Qdr local dose (Gy) (95% CI)	Calculated EWBD (Gy)	dic + r whole-body dose (Gy) (95% CI)
P1	2.0	1.0 (0.0–2.5)	0.12	0.3 (0.2–0.5)
P3	2.0	2.2 (0.0–4.0)	0.07	0.2 (0.0–0.4)
P4	2.0	1.5 (0.0–3.0)	0.10	0.3 (0.1–0.6)
P6	2.0	2.7 (1.3–3.7)	0.11	0.6 (0.5–0.8)
P8	2.5	2.4 (0.0–4.6)	0.01	0.1 (0.0–0.3)
P9	2.0	ND	0.03	ND
P10	2.0	3.3 (1.6–4.3)	0.10	0.6 (0.4–0.8)
P11	2.5	2.5 (0.0–4.1)	0.01	0.2 (0.1–0.4)
Mean ($\pm$ SD)	2.1 ($\pm$ 0.2)	2.2 ($\pm$ 0.7)	0.07 ($\pm$ 0.04)	0.35 ($\pm$ 0.2)

CI, confidence interval; ND, not determined.

formation and distribution and heterogeneous irradiation by analysing data from cancer patients treated with different irradiation field and dose rates to the same site of the body. In addition, the biological doses were estimated in order to check their ability to assess accurately the physical dose in a context of heterogeneous irradiation.

In three of the eight patients, the value of the frequency of dicentric and rings before treatment was in the value range (0–0.02) usually observed in the laboratory. In contrast, five other patients had a significantly higher number of dicentric and rings in their non-exposed lymphocytes. Such a high yield can be explained for patient P9, who had a history of cervical neoplasia treated with surgery, suggesting a predisposition to cancer that could be linked to chromosome instability. In addition, patient P6 was treated with 5-fluorouracil and cisplatin before radiotherapy and patient P4 underwent multiple radiological examinations to diagnose several cardiovascular pathologies, which can explain their high rate of chromosomal aberrations before radiotherapy. For patients P3 and P10, this high yield of spontaneous chromosomal aberrations remains unexplained, although this could possibly be linked to head and neck cancer susceptibility [17]. These spontaneous frequencies of chromosome aberrations were,

however, much lower than the frequencies observed after irradiation.

We performed dose estimations only after the first session of radiotherapy in order to avoid any bias due to the fractionation of the exposure. After 2 Gy of radiotherapy, we observed that the mean whole-body dose estimated from the yield of dicentric and rings was higher than expected from the calculated EWBD value. This implies that the biological whole-body dose was overestimated compared with the physical whole-body dose. This discrepancy may be due to the high concentration of lymph nodes and blood vessels located in the head and neck region which had been irradiated. While the calculated EWBD does not take into account this parameter, the biological whole-body dose estimated from the chromosome aberrations scored in lymphocytes may inevitably be impacted. This hypothesis is supported by data from other studies indicating variations in the yield of chromosome aberrations according to the localisation of the exposure, even when corrections were made for the volume of exposed tissue [9, 10, 22]. Also, in cervical cancer patients treated with radiotherapy, the EWBD calculated with the same formula and the whole-body dose estimated by cytogenetic methods were quite similar [13]. As a consequence, when the distribution of the lymphocytes is homogeneous and is therefore representative of the global lymphocyte distribution of the human body, physical and biological whole-body doses should be similar. Altogether this suggests that, in case of partial-body exposure, the mean whole-body dose estimated from chromosome aberration yield may vary with the dose received locally but also with the anatomical localisation of the irradiated area, which may contain a variable density of lymphocyte nodes and blood vessels.

We applied the Qdr method to estimate the dose delivered locally and the fraction of the irradiated body. We showed that, after the first session of radiotherapy, the partial-body dose derived from the Qdr method agreed quite well with the dose actually delivered to the tumour. Furthermore, we observed that the fraction of the irradiated body estimated using the Qdr dose was significantly correlated with the size of the irradiation field. These results suggest that, in case of exposure of a part of the body containing a high lymphocyte density, it is possible to make a meaningful dose estimate for a

Figure 3. U-test values of overdistribution of dicentric and rings for the five patients tested at the end of treatment and 4–6 months after the end of radiotherapy (End T).

Figure 4. *U*-test values of overdistribution of dicentrics and rings as a function of dose rate for the eight patients undergoing radiotherapy. Diamonds correspond to the eight patients tested after the 12-Gy session and squares refer to seven of the same patients tested after the last session of radiotherapy. The line represents the best fit with a linear function.

single dose as low as 2 Gy. It has also been shown in previous publications that local doses estimated by the Dolphin and the Qdr methods agreed quite well with the doses delivered locally in patients treated by a hemi-body, single radiation dose of 8 Gy [8, 23].

The evolution of the yield of chromosome aberrations was studied during the course of radiotherapy treatment. The influence of treatment parameters on the yield of dicentrics and rings and on the distribution of these aberrations was also studied. As previously described, the yield of dicentrics and rings increased with the tumour dose for each patient but with substantial interindividual variability [24–26]. In agreement with other studies, a strong correlation between the size of irradiation field and the yield of chromosome aberrations was found [12, 26, 27]. This “volume effect” may result from an increasing number of lymph nodes and/or blood vessels included in the field of irradiation. By contrast, no significant correlation was observed between the size of irradiation field and the *U*-value corresponding to overdispersion of dicentrics and rings. We may have expected a negative relation between *U*-value and irradiation field size, since the dose heterogeneity increases when the exposed volume decreases. In fact, we observed a positive relation between the *U*-value and field size since for the same session of radiotherapy, *U*-values were systematically higher in patients treated with larger target areas (Tables 1 and 2). In patients with lower target areas without supraclavicular fossa irradiation, the dose fraction of 2 or 2.5 Gy delivered after each radiotherapy session could have been too low to obtain highly significant *U*-values. This observation is supported by an *in vitro* study in which, after one acute radiation exposure of 2 Gy, there was no significant detection of partial exposure using the *U*-value calculation when the fraction of irradiated blood was inferior to 50% [5]. Furthermore, we did not observe any significant *U*-value after the first fraction of radiotherapy, except for patient P9, who had received the radiotherapy treatment

with the highest dose rate, which may suggest an influence of the dose rate on the overdispersion of dicentrics and rings. In fact, when this influence was analysed during the course of radiotherapy, a positive correlation between *U*-value and dose rate was observed. These observations can give information about the respective contribution of circulating lymphocytes *vs* resident lymphocytes in the induction of chromosome aberrations during radiotherapy: the higher the dose rate, the shorter the duration of irradiation. Thus, for high instantaneous dose rates, few circulating lymphocytes are heavily exposed, leading to a greater number of lymphocytes containing no chromosome aberration and, consequently, to higher *U*-values. By contrast, for lower instantaneous dose rates, a higher number of circulating lymphocytes are weakly exposed, resulting in a more homogeneous dose. Altogether, this suggests significant involvement of the blood vessels within the target field rather than lymph nodes in the induction of chromosome aberrations.

During the months following the radiotherapy treatment, *U*-values increased significantly, reflecting an increasing overdispersion of dicentrics and rings among the lymphocyte population. This increase in *U*-values after radiotherapy ended is probably due to the “dilution” of the exposed subfraction of lymphocytes by undamaged cells. This may reflect repopulation of lymphocytes by new cells from the bone marrow or peripheral cell division.

Conclusion

Overall, using radiotherapy as an *in vivo* working model, our cytogenetic measurements were able to assess the outcome of radiotherapy regimens on the induction of chromosome aberrations in lymphocytes. We confirm the strong impact of the size of the radiotherapy target field on the yield of induced

chromosome aberrations. We show that in cases of exposure of a part of the body containing a high lymphocyte density, the mean whole-body dose tends to be overestimated whereas the partial-body dose can be assessed accurately using the Qdr method. After multiple fraction exposure, the dose heterogeneity can be detected. Our findings also emphasised that increasing the dose rate may lead to an increase in the proportion of undamaged circulating lymphocytes. The main purpose of cytogenetic assessment after radiotherapy might then be to determine late toxic effects on healthy lymphocytes.

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