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Analysis of chromosome aberrations by FISH and Giemsa assays in lymphocytes of cancer patients undergoing whole-body irradiation: comparison of *in vivo* and *in vitro* irradiation

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Abstract.

Purpose: To study the cytogenetic effects of fractionated radiotherapy in peripheral blood lymphocytes of five cancer patients. *In vitro* experiments were performed in parallel using the same dose range and a comparison was made of the induced frequencies of stable and unstable chromosome aberrations. The object was to clarify the use of an *in vitro* calibration curve for immediate and retrospective dosimetry in cases of radiation accidents.
Materials and methods: Patients were exposed to ^{60}Co γ-rays at a single dose of 11.5 cGy each day up to a total dose of 57.5 cGy, given in 5 days. For measurement of chromosome aberrations, blood was collected from patients before irradiation and after each exposure. Blood taken before treatment was used as a control and for *in vitro* irradiation experiments in the dose range 8–50 cGy. Chromosome aberration frequency (stable as well as unstable) was determined using fluorescence *in situ* hybridization (FISH) assay with specific DNA libraries for chromosomes 1, 4 and 8 and a pancentromertic probe for the whole genome. Giemsa-stained preparations were used to score unstable aberrations following *in vivo* and *in vitro* exposure.
Results: A linear dose–response curve was determined for both dicentrics and translocations. The *in vivo* frequency of translocations was higher than for dicentrics. Dose–response curves generated for translocations following *in vivo* and *in vitro* irradiation yielded similar frequencies. In contrast, for dicentrics, *in vitro* irradiation yielded a higher frequency when compared with data generated following *in vivo* exposure.
Conclusions: For dose reconstruction purposes, translocations frequency seems to be a more adequate end-point than the scoring of dicentrics. The established *in vitro* calibration curve for dicentrics may underestimate absorbed radiation dose in cases of protracted exposure.

1. Introduction

Radiation dose in nuclear accidents is often heterogeneous both between individuals and anatomically. Studies of the frequencies of chromosome exchange aberrations in peripheral lymphocytes provide useful biodosimetric information (IAEA 1986, Darroudi 2000). For individual dose estimation, a calibration dose–response curve constructed for human lymphocytes irradiated *in vitro* is often used. Wider applicability of the *in vivo* calibration dose–response curve requires further studies in the low-dose region and under conditions of protracted irradiation.

The objective of the present study was to compare *in vivo* and *in vitro* dose–responses for both stable and unstable chromosome exchanges detected by fluorescence *in situ* hybridization (FISH; Pinkel *et al.* 1986) and fluorescence plus Giemsa (FPG; Perry and Wolff 1974) methods in lymphocytes of cancer patients undergoing protracted whole-body irradiation at low doses before local radiotherapy at high dose.

2. Materials and methods

2.1. *Subjects*

The study was performed on five patients aged 23–70 years, one woman and four men, with advanced cancers and distant metastases.

- Patient B10: female aged 43. One month before whole-body irradiation, the patient underwent nephrectomy for kidney cancer. She received whole-body irradiation for a haemangiopericytoma. The patient was a non-smoker and had no occupational exposure; her mother died from breast cancer.
- Patient B13: male aged 70. Whole-body irradiation was given for cancer of the pancreatic and distant skeletal metastases. Patient smoked a pack of cigarettes per day; his occupation was connected with high-voltage electricity.
- Patient B16: male aged 60. Cancer of the kidney with skeletal metastases. Smoked half a pack of cigarettes per day. His occupation was connected

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with high-frequency wave radiolocators in the Arctic and Antarctic.

- Patient B17: male aged 53. Prostate cancer with bone metastases; smoked about half a pack of cigarettes per day; occupation a driver.
- Patient B18: male age 23. Had undergone nephrectomy for cancer of the kidney and metastases in bones; non-smoker; medical student.

The patients received no chemotherapy before or during the period of whole-body irradiation.

2.2. *Irradiation and dosimetry*

Whole-body irradiation of patients was performed using a ^{60}Co unit at a dose-rate of 1.3 cGy min^{-1}. The patient laying prone and then spine was irradiated with a source-central axial distance of 475 cm by horizontal beam, 5 min in each position. To minimize inhomogeniety of irradiation due to body relief as well as variation in the tissue density and to compensate the dose-rate variation from head and feet to the middle of the body, configured paraffin blocks were used. Doses were calculated for 4000–5000 points of a standard anthropomorphic phantom by a computer program, which took account of weight, height and pelvis size (Ermakov and Cherviakov 1997). The average single whole-body dose was estimated to be 11.5 cGy. Dose variation between patients did not exceed 2%, the dose-rate variation along the body was ~10%. Whole-body irradiation was usually performed each day to a total dose of 57.5 cGy.

In vitro irradiation of blood collected before whole-body exposure was carried out with the same γ-ray source at doses of 0, 8, 16, 24, 32 and 40 cGy for all patients except B18 (0, 10, 20, 30, 40 and 50 cGy). Ten Eppendorf tubes supported in paraffin blocks were given the lowest dose (taking ~5 min); exposure was stopped for 1 min and two tubes were removed. This procedure was repeated five times to cover the whole dose range. Dose was estimated using an ionizing chamber 30001-1402 UNIDOS-1001-0576. The dose measurement error did not exceed 5%.

2.3. *Lymphocyte culture, slide preparation*

About 5 ml venous blood was collected into heparinized vacutainers before whole-body irradiation of patients and repeated either 1 or 18–68 h after each exposure (table 1). Duplicate 0.5 ml vols blood were placed into plastic flasks with 4.5 ml RPMI-1640 medium supplemented with heat-inactivated (56°C for 30 min) 15% foetal bovine serum, 2.5% PHA (Murex), antibiotics, 9.3 μg ml^{-1} 5-bromodeoxyuridine (5-BrdU). Lymphocytes were incubated at 37°C for 48–54 h, the last 3 h in the presence of colcemide at a final concentration of 0.1 μg ml^{-1}. After incubation, the cells were collected and exposed to hypotonic solution (KCl 0.075 M) for 14 min, followed by three changes of methanol:glacial acetic acid (3:1). Chromosome preparation followed a standard air-drying procedure. For both conventional and FISH staining, FPG technique was applied (Perry and Wolff 1974, Kulka *et al.* 1995, respectively), and chromosome aberrations were analysed in cells that had undergone only one cell division.

FISH was carried out using different cocktails of whole-chromosome specific DNA probes: for patients B10 and B13, chromosome 1 (biotinylated, detected with Texas-Red), chromosome 4 (FITC labelled), chromosome 8 (biotinylated and FITC labelled) and pancentromeric probe (FITC-labelled); for patient B16, chromosomes 1, 4 and 8 (FITC labelled) and pancentromeric probe (biotinylated, detected with Texas–Red); for patients B17 and B18, chromosome 1 (biotinylated and FITC labelled), chromosome 4 (biotinylated, detected with Texas-Red), chromosome 8 (FITC labelled) and pancentromeric probe (FITC labelled). All probes were directly labelled, purchased from Cambio (UK) and used in accordance with the recommended protocol. DAPI-Vectashield mixture was used for counter-staining. For patient B17, parallel slides were prepared with the cocktail of whole-chromosome specific DNA probes for chromosomes 1, 4 and 12. In this case plasmid DNA of chromosome-specific Hind III PBS of human chromosomes 1, 4, 12 was biotinylated as described (Pinkel *et al.* 1986, Natarajan *et al.* 1992, Schmid *et al.* 1992). Pancentromeric probe was produced from whole-genome human DNA, using degenerative primers for α-satellite DNA (Weier *et al.* 1991). Post-hybridization washes and staining of bound DNA probes with FITC-avidin (chromosome-specific probes) and AMCA-conjugated antibodies (pancentromeric probe) were performed as described (Bauchinger *et al.* 1993). Counter-staining of the remaining chromosomes was performed with propidium iodide in an antifade solution (Vectashield®).

Table 1. Intervals between irradiation of patients and starting the lymphocyte culture (h).

Dose, cGy	B10	B13	B16	B17	B18
11.5	1	18	18	68	18
23	18	1	1	1	1
34.5	1	18	18	18	43
46	1	68	1	1	1
57.5	1	1	18*	–	68

*Last fraction was given after a delay of 3 days.

2.4. *Aberration scoring*

Conventional scoring was performed using light microscopes: Axioplan microscope (Carl Zeiss, Germany) and BIMAM (LOMO, Russia) with ×100 oil objective and a 10× ocular. Scoring of aberrations by FISH was carried out using fluorescent microscopes: Axioplan or Axioscop (Carl Zeiss, Germany) equipped with ×100 oil objectives in combination with the triple band pass filter for simultaneous observation of green (FITC), red (Texas-Red) and yellow (FITC+ Texas-Red) fluorescence. Only metaphases of the first division were examined, on coded slides. The number of cells scored per dose per donor varied between 500 and 1000 metaphases in conventional analysis and between 900 and 4000 metaphases using the FISH-assay, depending on the dose of irradiation.

Translocations were classified in accordance with classical (not PAINT) nomenclature as reciprocal (two-way) and terminal (one-way). Insertions as well as occasionally complex aberrations (classified as apparently simple) were included in the total number of exchanges. Genomic frequency of translocations and dicentrics was calculated by dividing number of observed aberrations on cell equivalent. Cell equivalents were calculated using Lucas formula:

$$N = 2.05 \times f_p \times (1 - f_p),$$

where N is the number of cells scored and f_p is the fraction of genome painted (Morton 1991) by FISH (Lucas *et al.* 1992). For patient B17, the data obtained with different chromosome cocktails were combined after correction for target size. All types of chromosome aberrations were recorded; data are presented for dicentrics and translocations.

2.5. *Statistics*

Data were processed using the Poisson Iteratively Reweighted Least Squares (PIRLS) computer program. The Student's *t*-test was used to estimate the significance of differences.

3. Results

Table 1 gives the schedule of blood sampling after radiation exposure of the patients. In the majority of cases, blood culture was started within 1–18 h of irradiation.

Translocations and dicentrics were detected by FISH and dicentrics were also detected by Giemsa staining; the data are presented in tables 2–4. The individual yields of translocations and dicentrics fitted well the linear dose–response model ($p < 0.05$). Table 5 shows the resulting values of coefficients C and α for equation

$$Y = C + \alpha D,$$

where Y is the frequency of exchange aberrations (per 100 cells) and D is the dose (cGy). The total dose–response coefficients also presented in table 5 were calculated on the basis of the number of events and number of cells scored for each patient and each dose (tables 2–4). The dicentric yield (both FISH and Giemsa) increased significantly faster with dose in lymphocytes irradiated *in vitro*, compared to those irradiated *in vivo*. No difference between *in vivo* and *in vitro* dose–response curves was observed for translocations. The frequency of translocations after *in vivo* irradiation of lymphocytes was higher than the frequency of dicentrics. Dose–response curves and the corresponding straight-line fits (table 5) are shown in figures 1–5.

4. Discussion

A number of previous studies have examined the *in vitro* dose–effect relationship for asymmetrical chromosomal exchanges (Luchnik and Sevan'kaev 1976, Lloyd *et al.* 1988) and recently for symmetrical ones (Bauchinger *et al.* 1993, Straume and Lucas 1993, Tucker *et al.* 1994a, Vorobtsova *et al.* 1997, Savage *et al.* 2000). On the basis of animal experiments (Brewen and Gengozian 1971, Preston *et al.* 1972, Clemenger and Scott 1973), the assumption has been made that lymphocytes exposed *in vivo* respond to irradiation in the same manner as *in vitro*. There are few quantitative data on this issue in case of human whole-body irradiation (Buckton *et al.* 1971, Schmid and Bauchinger 1974, Leonard *et al.* 1995). All these studies compare *in vivo* and *in vitro* dose–responses for unstable chromosome exchanges, detected by the conventional Giemsa-staining method. Good agreement between the yields of dicentrics in lymphocytes exposed *in vivo* and *in vitro* was observed in these studies. As to the stable chromosome aberrations (i.e. translocations) detected by chromosome painting, the *in vivo* dose–response curve has not so far been studied, although for the purpose of biological dosimetry in cases of post- and chronic-radiation exposure such studies are extremely important. As suggested and pointed out by others, in contrast to dicentrics, translocations are not eliminated from the blood with time (Lucas *et al.* 1992, Natarajan *et al.* 1998, Darroudi and Natarajan 2000).

The present study presents a comparison between dose–response curves for stable and unstable exchanges in lymphocytes irradiated *in vivo* and *in vitro* at the dose range of 0–57.5 cGy. Table 2 presents

Table 2. Genomic frequency of translocations in lymphocytes irradiated *in vivo* and *in vitro* (per 100 cells).

Code of patient		Dose 0			Dose 1			Dose 2			Dose 3			Dose 4			Dose 5		
		Number			Number			Number			Number			Number			Number		
		C. eq.	Tr	F_G	C. eq.	Tr	F_G	C. eq.	Tr	F_G	C. eq.	Tr	F_G	C. eq.	Tr	F_G	C. eq.	Tr	F_G
B10	*vivo*	384	4	1.04	780	21	2.69	384	18	4.69	924	38	4.11	402	21	5.23	409	25	6.12
	vitro				376	8	2.13	419	12	2.86	350	13	3.72	220	9	4.09	409	21	5.13
B13	*vivo*	450	8	1.78	309	12	3.88	597	31	5.19	836	39	4.67	289	16	5.53	–	–	–
	vitro				300	10	3.33	–	–	–	–	–	–	–	–	–	161	9	5.60
B16	*vivo*	599	15	2.50	575	16	2.78	266	13	4.89	565	27	4.78	145	6	4.14	584	34	5.83
	vitro				1346	39	2.90	1460	50	3.43	1294	51	3.94	1133	42	3.71	351	14	3.99
B17	*vivo*	990	12	1.21	1423	36	2.53	744	20	2.69	1325	60	4.53	1655	80	4.83	–	–	–
	vitro				893	23	2.58	769	15	1.95	1430	60	4.20	1131	47	4.16	1045	50	4.78
B18	*vivo*	1614	20	1.24	669	10	1.49	1637	32	1.96	745	22	2.95	729	29	3.98	1243	38	3.06
	vitro				1161	21	1.81	1260	22	1.75	928	20	2.15	698	21	3.01	785	32	4.08

Dose 1–5: *in vivo*. 11.5; 23; 34.5; 46; 57.5 cGy; *in vitro*. 8, 16, 24, 32, 40 cGy (B10, B13, B16, B17) and 10, 20, 30, 40, 50 cGy (B18).
C. eq., cell equivalent.
Tr, translocations.
F_G, genomic frequency.

Table 3. Genomic frequency of dicentrics (by FISH) in lymphocytes irradiated *in vivo* and *in vitro* (per 100 cells).

Code of patient		Dose 0			Dose 1			Dose 2			Dose 3			Dose 4			Dose 5		
		Number			Number			Number			Number			Number			Number		
		C. eq.	Dc	F_G	C. eq.	Dc	F_G	C. eq.	Dc	F_G	C. eq.	Dc	F_G	C. eq.	Dc	F_G	C. eq.	Dc	F_G
B10	*vivo*	384	1	0.26	780	4	0.51	384	6	1.56	924	11	1.19	402	8	1.99	409	10	2.45
	vitro				376	4	1.06	419	4	0.95	350	4	1.14	220	5	2.27	409	15	3.67
B13	*vivo*	450	1	0.22	309	1	0.32	597	5	0.84	836	12	1.44	289	5	1.73	–	–	–
	vitro				300	0	0.00	–	–	–	–	–	–	–	–	–	161	5	3.11
B16	*vivo*	599	4	0.67	575	3	0.52	266	2	0.75	565	5	0.89	145	4	2.76	584	8	1.37
	vitro				1346	15	1.11	1460	15	1.03	1294	15	1.16	1133	17	1.50	351	10	2.85
B17	*vivo*	990	3	0.30	1423	9	0.63	744	6	0.81	1325	14	1.06	1655	32	1.93	–	–	–
	vitro				893	3	0.34	769	4	0.52	1430	18	1.26	1131	26	2.30	1045	30	2.87
B18	*vivo*	1614	1	0.06	669	2	0.30	1637	12	0.73	745	4	0.54	729	10	1.37	1243	13	1.05
	vitro				1161	3	0.26	1260	12	0.95	928	18	1.94	698	10	1.43	785	28	3.57

Dose 1–5: *in vivo*. 11.5; 23; 34.5; 46; 57.5 cGy; *in vitro*. 8, 16, 24, 32, 40 cGy (B10, B13, B16, B17) and 10, 20, 30, 40, 50 cGy (B18).
C. eq., cell equivalent.
Dc, dicentrics.
F_G, genomic frequency.

the background level of translocations in these five patients; interindividual variation in the frequency of spontaneously occurring translocations is evident. The age factor may contribute to this effect as has been shown that for healthy control populations (Tucker *et al.* 1994b, Tucker and Moore 1996, Vorobtsova *et al.* 2000). We find no significant correlation between age and background frequency of translocations; this could be due to patient B16, whose background level of translocations were much higher than values reported for control donors of the same age (Tucker *et al.* 1994b, Ramsey *et al.* 1995, Sorokine-Durm *et al.* 2000, Vorobtsova *et al.* 2000). The reason for the high level of translocations in B16 is unclear.

The spontaneous frequency of dicentrics (tables 3 and 4) for all patients is higher than the range seen by others in normal donors (0.5–1.5 dicentrics per 1000 cells; Lloyd *et al.* 1988). This could be due to genomic instability as shown in various studies (Hsu *et al.* 1985, Cloos *et al.* 1994).

Inconsistent results have been reported about the ratio of translocations and dicentrics measured by FISH after *in vitro* exposure of lymphocytes (Natarajan *et al.* 1992, Bauchinger *et al.* 1993, Straume and Lucas 1993, Kanda and Hayata 1996, Fomina *et al.* 2000). The present data (table 5) provide evidence for a higher frequency of translocations compared to the frequency of dicentrics, after *in vivo*

Table 4. Frequency of dicentrics by Giemsa staining in lymphocytes irradiated *in vivo* and *in vitro* (per 100 cells).

Code of patient		Dose 0			Dose 1			Dose 2			Dose 3			Dose 4			Dose 5		
		Number			Number			Number			Number			Number			Number		
		Cells	Dc	*F*	Cells	Dc	*F*	Cells	Dc	*F*	Cells	Dc	*F*	Cells	Dc	*F*	Cells	Dc	*F*
B10	*vivo*	912	1	0.11	953	12	1.26	1154	14	1.21	1000	17	1.70	727	15	2.06	914	32	3.50
	vitro				779	4	0.51	536	6	1.12	662	10	1.51	663	14	2.11	844	27	3.32
B13	*vivo*	1279	6	0.47	1038	11	1.06	886	11	1.24	1138	17	1.49	568	11	1.94	728	15	2.06
	vitro				736	5	0.68	582	6	1.03	316	5	1.58	705	14	1.99	592	20	3.38
B16	*vivo*	970	5	0.52	565	5	0.88	998	9	0.90	1001	15	1.50	771	12	1.62	642	14	2.18
	vitro				1081	9	0.83	500	4	0.80	501	8	1.60	994	22	2.21	307	9	2.93
B17	*vivo*	1000	3	0.30	1000	7	0.70	1000	12	1.20	1000	19	1.90	700	25	3.57	–	–	–
	vitro				1007	9	0.89	916	11	1.20	1001	18	1.80	501	10	2.00	703	17	2.42
B18	*vivo*	1000	3	0.30	500	3	0.60	500	4	0.80	710	11	1.55	800	10	1.25	1643	28	1.70
	vitro				544	2	0.37	750	7	0.93	919	21	2.29	713	18	2.52	1535	37	2.41

Dose 1–5: *in vivo*. 11.5; 23; 34.5; 46; 57.5 cGy; *in vitro*. 8, 16, 24, 32, 40 cGy (B10, B13, B16, B17) and 10, 20, 30, 40, 50 cGy (B18).
Dc, dicentrics.
F, frequency of dicentrics.

Table 5. Dose–response coefficients for five patients.

Code of patient	*In vitro*		*In vivo*	
	$(C \pm SE)*10^{-2}$	$(\alpha \pm SE)*10^{-2}$	$(C \pm SE)*10^{-2}$	$(\alpha \pm SE)*10^{-2}$
Translocations				
B10	1.15±0.46	10.18±2.59	1.47±0.47	8.58±1.79[c]
B13	1.98±0.59	10.11±5.04	2.27±0.62	8.35±2.56
B16	2.65±0.43	4.07±2.10	2.24±0.52	5.97±1.84[c]
B17	1.36±0.31	9.25±1.54	1.34±0.30	8.10±1.28[d]
B18	1.18±0.23	4.55±1.09	1.20±0.24	4.05±0.88
Pooled data	1.67±0.16	6.55±0.81	1.63±0.17	6.41±0.64[d]
Dicentrics by FISH				
B10	0.25±0.23	6.65±1.66	0.22±0.20	3.56±0.91
B13	0.14±0.17	5.29±2.73	0.18±0.19	3.29±1.02
B16	0.70±0.24	2.84±1.23	0.53±0.24	1.59±0.89
B17	0.19±0.13	5.49±0.84	0.27±0.14	3.05±0.67[a]
B18	0.05±0.05	5.24±0.67	0.07±0.06	2.11±0.40[b]
Pooled data	0.21±0.07	5.24±0.46	0.24±0.07	2.61±0.32[b]
Dicentrics by Giemsa				
B10	0.09±0.10	6.76±0.99	0.18±0.13	5.13±0.71
B13	0.40±0.16	5.67±1.21	0.54±0.18	2.89±0.75
B16	0.45±0.19	5.14±1.26	0.50±0.20	2.60±0.76
B17	0.34±0.16	5.56±1.12	0.24±0.14	5.42±0.94
B18	0.25±0.15	4.83±0.73	0.31±0.16	2.48±0.56[a]
Pooled data	0.32±0.07	5.40±0.45	0.39±0.08	3.48±0.33[b]

Differences between *in vivo* and *in vitro* are significant as indicated: [a]$p<0.05$; [b]$p<0.01$.
Differences between translocations and dicentrics (by FISH) are significant as indicated: [c]$p<0.05$; [d]$p<0.01$.

(but not *in vitro*) irradiation of lymphocytes in the dose range studied.

Schmid *et al.* (1995) reported that the yield of radiation-induced dicentrics in *in vitro* human lymphocytes estimated by conventional analysis is significantly higher than when measured by FISH, at doses >100 cGy. In the doses-range studied here (up to 57.5 cGy), we have found no significant difference between the two techniques.

The results of regression analysis of data presented in table 5 and figures 1–3 show that in the dose range 0–57.5 cGy the dose–responses for translocations and dicentrics both *in vivo* and *in vitro* are linear. That is true both for individual patients and for the

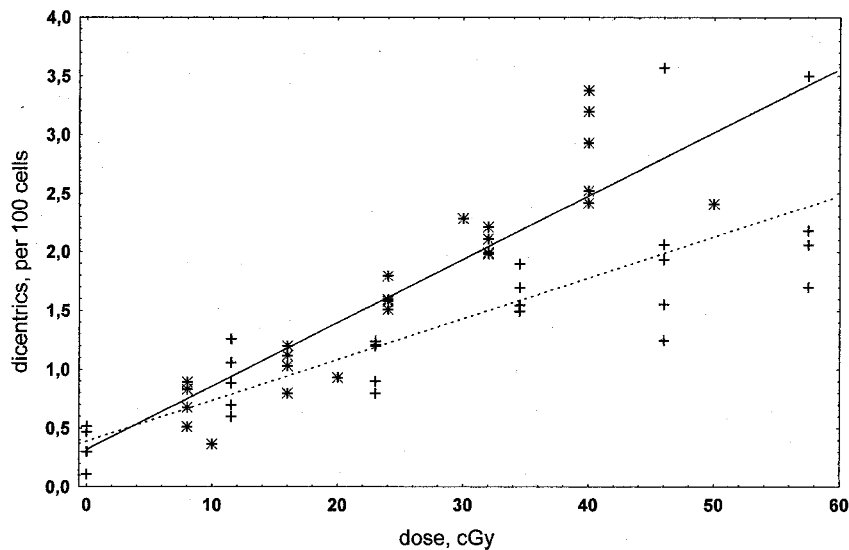

Figure 1. Dose–response for dicentrics detected by Giemsa in lymphocytes, irradiated *in vivo* and *in vitro*. ∗, *in vitro*; +, *in vivo*.

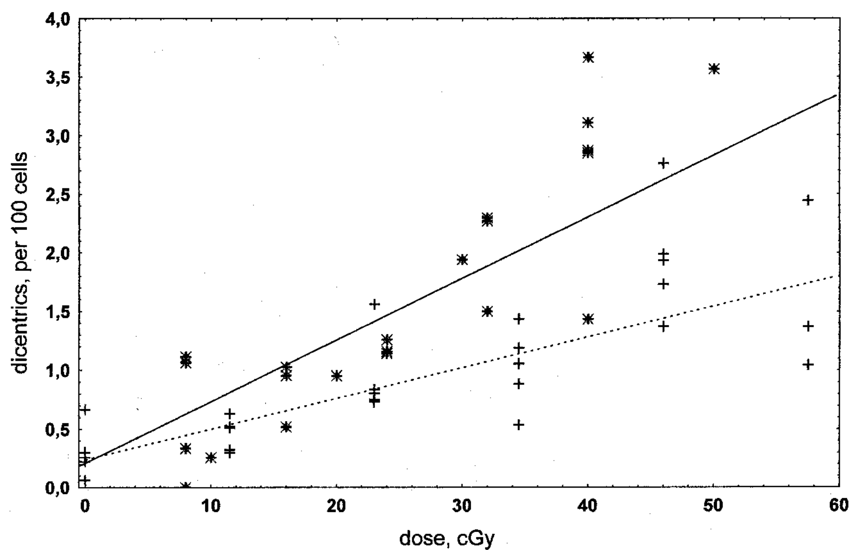

Figure 2. Dose–response for dicentrics detected by FISH in lymphocytes, irradiated *in vivo* and *in vitro*. ∗, *in vitro*; +, *in vivo*.

pooled data. A linear response has been obtained in earlier studies using the conventional Giemsa-staining technique for dicentrics in a similar dose range (Buckton *et al.* 1971, Kucerova *et al.* 1972, Schmid and Bauchinger 1974). However, it has been emphasized that for the application of these curves in biological dosimetry, a large number of cells should be analysed (Kucerova *et al.* 1972, Littlefield and Lushbaugh 1990). In our five patients we scored ~20 000 (genome equivalent) and 25 000 (cells), respectively in the FISH and FPG assays.

When a comparison is made of the α-coefficients (table 5) for dicentrics (both FISH and Giemsa), the dose–response curve *in vitro* was significantly higher than *in vivo*. A non-significant trend for a more pronounced reaction in lymphocytes irradiated *in vitro* than *in vivo* has been described (Leonard *et al.* 1995). In the present study, this presumably could be due to the schedule of whole-body irradiation of patients (see Section 2.2) and more effective repair of DNA damage in lymphocytes irradiated *in vivo* (protracted irradiation) than *in vitro* (acute irradiation). The difference in temperature during exposure of blood *in vivo* and *in vitro* also could play a role. However, for translocations, no significant difference was found between *in vitro* and *in vivo* irradiation. This could be considered as evidence for a difference in misrepair/repair processes leading to formation of stable and unstable exchanges as has been suggested earlier (Darroudi *et al.* 1998).

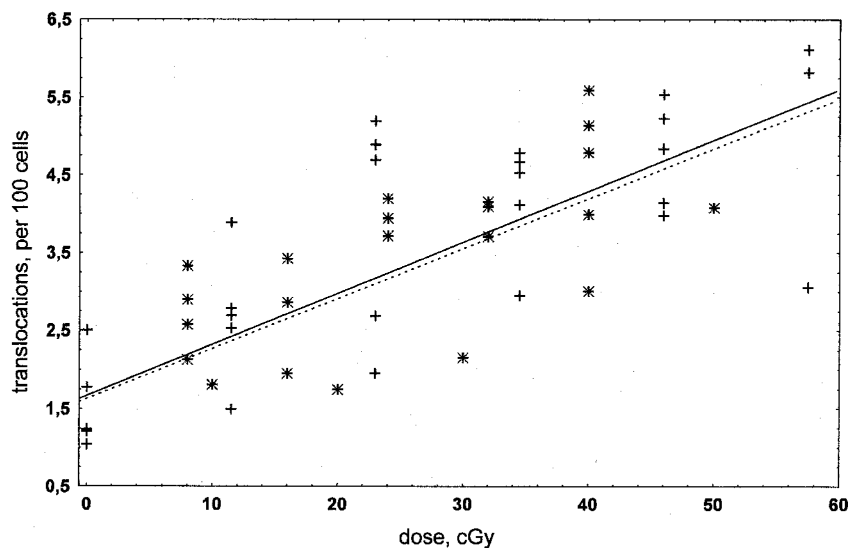

Figure 3. Dose–response for translocations in lymphocytes, irradiated *in vivo* and *in vitro*. $*$, *in vitro*, solid line; $+$, *in vivo*, dotted line.

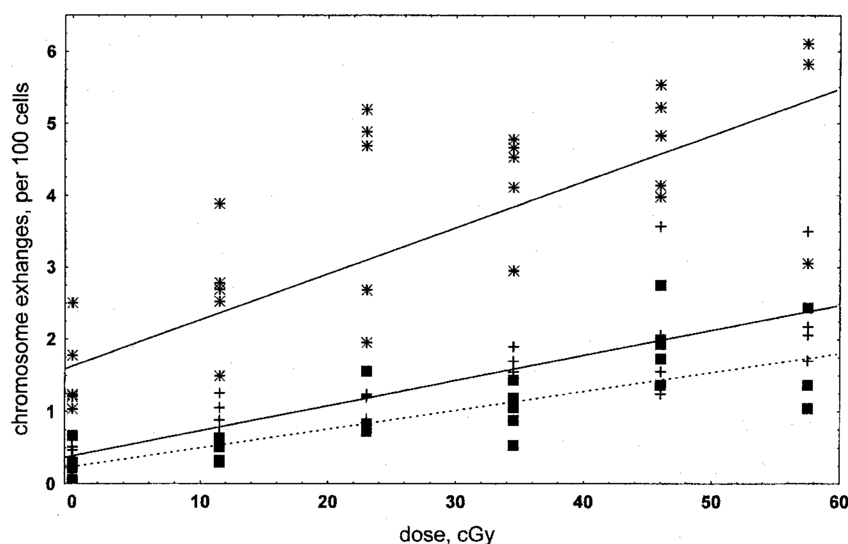

Figure 4. Dose–response for translocations and dicentrics (FISH and Giemsa) in lymphocytes, irradiated *in vivo*. $*$, Translocations, solid line; $+$, dicentrics Giemsa, solid line; ■, dicentrics FISH, dotted line.

We recognize that *in vivo* dose–response curves obtained on cancer patients should be applied with great care to the dose estimation of accidentally exposed people. Nevertheless, the data obtained in this study seem to be significant for biodosimetry based on the scoring of dicentrics. Since for dose reconstruction the individual frequency of dicentrics is usually referred to the *in vitro* calibration dose–response curve, the actual absorbed radiation dose in case of protracted exposure is likely to be underestimated. As our data show, for better estimation of radiation dose the individual frequency of dicentrics should be referred to the *in vivo* calibration dose–response curve. In contrast, translocations seem to be independent of the irradiation regimen and seem to be a better end-point both for early and retrospective biodosimetry using the *in vitro* dose–response calibration curve. Further studies of several additional patients exposed to higher doses of irradiation are under way.

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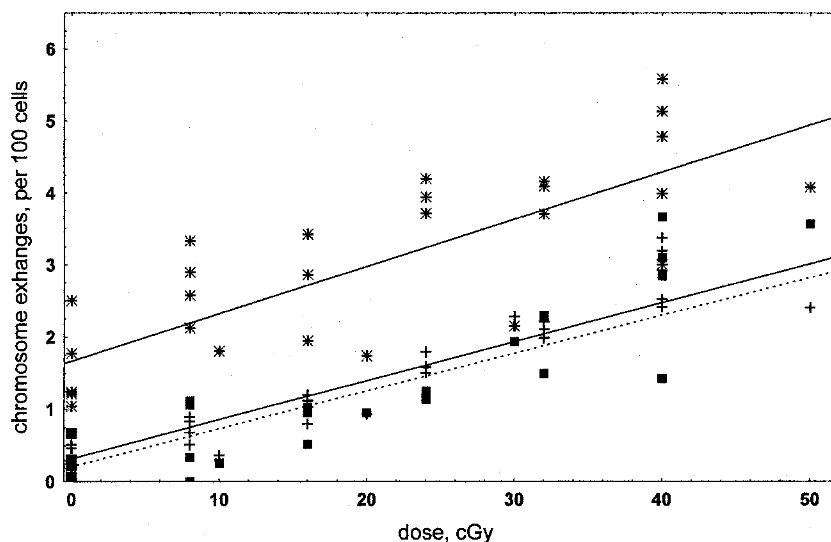

Figure 5. Dose–response for translocations and dicentrics (FISH and Giemsa) in lymphocytes, irradiated *in vitro*. *, Translocations, solid line; +, dicentrics Giemsa, solid line; ■, dicentrics FISH, dotted line.

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