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RESEARCH ARTICLE

Initial evaluation and follow-up of acute radiation syndrome in two patients from the Dakar accident

Jean Marc Bertho[1], Laurence Roy[1], Maâmar Souidi[1], Marc Benderitter[1], Eric Bey[2], Radjini Racine[1], Thierry Fagot[3], and Patrick Gourmelon[1]

[1]*Institut de Radioprotection et de Sûreté Nucléaire, DRPH/SRBE, Fontenay aux Roses, France,* [2]*Service de Chirurgie, Hôpital d'Instruction des Armées Percy, Clamart, France, and* [3]*Service d'Hématologie, Hôpital d'Instruction des Armées Percy, Clamart, France*

Abstract

The aim of this work was to evaluate and follow up the evolution of radiation damage in two victims of a radiation accident. Blood samples were used for cytogenetic evaluation of radiation dose and heterogeneity. The radiation dose estimates were 1 Gy and 2.3 Gy in the two most exposed patients. Plasma was used for the measurement of the Flt3 ligand as a marker of haematopoietic aplasia, citrulline for damage to the jejunal mucosal epithelium and oxysterols for damage to the liver, the central nervous system and the vascular compartment. The use of these biological indicators demonstrated the presence of a haematopoietic syndrome and suggested the presence of subclinical radiation-induced damage to the liver in one of the two patients. These results support the interest in using these biological indicators in order to evaluate radiation damage, especially in complex accidental situations.

Keywords: *Radiation accident; biological indicators; Flt3-ligand; citrulline; oxysterols*

Introduction

On June–July 2006, in the city of Dakar (Senegal), an iridium 192 source was lost from an industrial radiation device. The source stayed in a guide which was stored near a workplace for 2 months, before being discovered during a transfer of material. As a result, it was suspected that 63 people received an unwanted and uncontrolled irradiation dose (Clairand et al. 2008). However, as in most cases of accidental irradiation (Nenot 1998, Goans 2002), the radiological nature of the accident was recognized late, 2 months after the initial event, i.e. the detachment of the iridium source. Moreover, the determination of circumstances demonstrated that the irradiation was protracted, at a low dose rate, and with additional localized irradiation for at least two patients (Clairand et al. 2008). In such a case, the evaluation of radiation-induced damage to vital organs remains difficult. This in turn makes the choice of a therapeutic strategy difficult.

The evaluation of radiation damage is classically based upon the radiation dose received as determined by the frequency of chromosomal aberrations (IAEA 2001, Voisin et al. 2001). However, in a case of either protracted exposure or delayed cytogenetic analysis, the radiation dose may be underestimated, due to the progressive death of cells bearing dicentrics and rings. Although this can be at least partly overcome by the determination of translocations frequency (Gregoire et al. 2006), the information provided by the radiation dose received (understood as a physic measurement) does not take into account individual parameters such as the individual radiation sensitivity or the possible presence of underlying pathologies before the accident. Thus there is a need for an estimate of radiation damage to the main, life-threatening physiological systems.

Such an evaluation is classically based upon the occurrence and severity of symptoms from the initial syndrome, i.e. nausea, vomiting, headache, hypotension,

Address for Correspondence: J. M. Bertho, IRSN, DRPH/SRBE, BP n°17, F-92262 Fontenay aux Roses cedex, France. Tel: +33(1) 58 35 95 48. Fax: +33(1) 58 35 84 67. E-mail: jean-marc.bertho@irsn.fr

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hyperthermia and other described symptoms (Young 1987). Other parameters may be useful, such as the initial decrease in lymphocyte numbers (Silini & Gouskova 1991) or the modification in serum amylase (Barrett et al. 1982). However, these symptoms are either transitory or impossible to assess in a reliable way either in the case of protracted irradiation or several weeks after irradiation. Others have proposed a multiparametric approach, including numerous indicators for haematopoietic, gastrointestinal, neurological and cutaneous systems. This approach, termed METREPOL (Fliedner et al. 2001, 2007) was developed as an aid to define a therapeutic strategy adapted to each case. Nevertheless, this approach uses only blood cell counts for the evaluation of haematopoietic damage, and does not evaluate damage to other organs such as the liver and the vascular compartment.

We recently used new biological indicators in the follow-up of radiation damage after an accidental overexposure (Bertho et al. 2008), namely Flt3 ligand concentration for the haematopoietic system (Prat et al. 2006a), citrulline for the jejunum integrity (Crenn et al. 2003) and oxysterols for the lipid metabolism in the liver, the vascular compartment and the central nervous system (Souidi et al. 2004). This approach was applied to the two patients that received the highest radiation dose during the Dakar accident, namely patients (designated by their unique patient number) 06-20 and 06-23. In fact, the other potential victims did not show clinical symptoms of an irradiation and the biological dosimetric analysis confirmed that the other patients received a radiation dose below 0.4 Gy (Vaurijoux et al., 2009). The results demonstrated a haematopoietic syndrome under recovery and radiation-induced damage to the lipid metabolism in the patient who received the highest radiation dose, patient 60-20. These results support the interest in such a multiparametric approach, including cytogenetic analysis and recently validated bioindicators for the evaluation of radiation damage, especially in the case of complex overexposure.

Material and methods

Case presentation

On 3 June 2006, following the use of an industrial radiography device, an iridium source of an initial activity of 3.6 TBq detached from its support and stayed in the guide. This guide containing the source was stored for a few weeks near a workplace, until 31 July 2006. At that time, the material was sent to another site by express mail. When the radiography device was reused for the first time, the iridium source was found in the guide, suggesting the occurrence of a radiation accident (Clairand et al. 2008). Among the 63 people suspected to have received a significant radiation dose, four patients showed cutaneous symptoms. Due to the possible occurrence of a haematopoietic syndrome, two of them (patients 06-20 and 06-23) were admitted to the haematology department of Percy Hospital (Clamart, France) and were followed up for blood parameters with their informed consent, starting on 30 August 2006.

Cytogenetic dosimetry

Blood was drawn onto heparinized tubes (Vacutainer; BD Biosciences, Le pont de Claix, France) and was treated as described elsewhere (IAEA 2001, Roy et al. 2006). Total blood was diluted 1:10 in RPMI 1640 medium supplemented with penicillin, streptomycin, glutamine, fetal calf serum (FCS) (all from BD Biosciences) and 1% PHA-M. After 46 h of culture at 37°C and 5% CO_2, colchicine (BD Biosciences) was added in order to block proliferative cells during their first division, and cultures were stopped at 48 h. After a KCl shock (0.075 M; Sigma-Aldrich, Saint Quentin Fallavier, France), cells were then spread out onto glass slides, fixed and stained with Hoechst and Giemsa stains, as described elsewhere (IAEA 2001). Metaphases were then scored together with chromosomal aberrations. Fluorescence *in situ* hybridization (FISH) was also performed on some slides to detect translocations as previously described (Gregoire et al. 2006). Three chromosome pairs were labelled and both total and reciprocal translocations were scored. The radiation dose received by the patient was calculated according to reference curves established in the lab, using blood samples irradiated *in vitro* with a Cobalt-60 source at a dose rate of 0.5 Gy min^{-1} (Voisin et al. 2001).

Blood monitoring

Blood samples were drawn daily onto EDTA-coated tubes (Vacutainer, BD Biosciences). A complete blood cell count including reticulocytes was made onto an ADVIA 120 haematology counter (Siemens, Cergy Pontoise, France). Plasma was isolated by centrifugation 10 min at 800 *g* and frozen for later use. White blood cells (WBC) were then isolated by ammonium chloride lysis of red blood cells (RBC) and washed twice in phosphate-buffered saline solution (PBS) supplemented with 0.5% bovine serum albumin (BSA; Sigma-Aldrich). WBC were then counted and the viability assessed by trypan blue dye exclusion.

Phenotypic analysis of WBC

The following directly labelled antibodies were used: IgG1-FITC, -PE and Cy5, anti-CD2-Cy5, anti-CD8-FITC, anti-CD11b-FITC and -PE, anti-CD14-PC5, anti-CD16-FITC

(all from Beckman-Coulter, Marseille, France), anti-CD4-PE, anti-CD20-PerCP, anti-CD34-PE and anti-CD56-PE (all from BD Biosciences). WBC (1×10^5) were resuspended in 100 µl PBS 0.5% BSA, and the antibodies were added at predefined concentrations. After 20 min incubation at 4°C, the cells were washed twice in PBS 0.5% BSA, and 7-amino-actinomycin D was added at a final concentration of 1 µg ml^{-1} in order to exclude dead cells from the analysis. Cells were then analyzed on a FACScalibur (BD Biosciences), with the acquisition of at least 10 000 events per point.

Colony-forming assay

WBC were resuspended in minimum essential medium alpha at a cell concentration of 2×10^6 cells ml^{-1}, and 300 µl was mixed into 3 ml methylcellulose medium (Stem Cell Technologies, France) supplemented with the following recombinant human cytokines: 20 ng ml^{-1} interleukin-3, 50 ng ml^{-1} granulocyte-colony stimulating factor, 5 ng ml^{-1} granulocyte macrophage-colony stimulating factor, 50 ng ml^{-1} stem cell factor and 3 U ml^{-1} erythropoietin. Cultures were then incubated at 37°C and 5% CO_2, and colony-forming units–granulocyte-macrophages (CFU-GM) and burst-forming units–erythroids (BFU-E) were counted on day 12 of culture.

Flt3-ligand measurement

Flt3-ligand (FL) was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) system (R&D Systems, Abingdon, UK), according to the manufacturer's recommendations. The sensitivity of the assays was less than 7 pg ml^{-1}.

Citrulline measurement

Twice a week, a blood sample was taken in heparinized tubes. Plasma was isolated by centrifugation at 800 *g* for 10 min and frozen. Citrulline was measured by chromatographic methods, as described elsewhere (Lutgens et al. 2004).

Oxysterols analysis and biochemical parameter measurements

Creatinine, urea, alanine aminotransferase (ALT), aspartate transferase (AST), total cholesterol, high-density lipoprotein (HLD), low-density lipoproteins (LDL), apolipoproteins (ApoA1 and ApoB), triglycerides, creatine kinase (CK and CK-MB) were measured starting from plasma samples on a Konelab 20 biochemical automatic device using specific kits (Thermo Fischer Scientific, Illkirch, France) and following the manufacturer's recommendations.

Oxysterols were analyzed as described elsewhere (Gueguen et al. 2006) with a minor modification. Briefly, an alkaline hydrolysis was carried out on a 0.5 ml EDTA plasma sample. Oxysterols were extracted by hexane and were then treated by cholesterol oxidase. Samples were then analyzed by high-performance liquid chromatography at 240 nm, by comparison with plasma samples containing known quantities of each of the analyzed oxysterols, namely 24*S*-hydroxycholesterol (24S-OH-Chol), 27-hydroxycholesterol (27-OH-Chol) and 7α-hydroxycholesterol (7α-OH-Chol).

Statistical analysis

Unless otherwise indicated, the data are presented as individual measurements. The mean ± SD was used for reference values when indicated.

Results

Biological dosimetry

At admission, a cytogenetic analysis was made in order to determine the radiation dose received by the two patients (06-20 and 06-23). Results (Table 1) indicated a mean radiation dose to the whole body of 2.6 Gy (95% confidence interval (CI) 2.3–2.9) for patient 06-20 and 1 Gy (95% CI 0.8–1.1) for patient 06-23 (Table 1). Moreover, as the two patients showed localized radiation burns, an estimation of radiation heterogeneity was made based upon the Qdr method (Sasaki & Miyata 1968). This indicated that 84% of the blood of patient 06-20 received a mean radiation dose of 3.4 Gy (95% CI 2.8–3.9), and that 56% of the blood of patient 06-23 received a mean radiation dose of 1.6 Gy (95% CI 1.4–2.6).

However, as the irradiation took place over several weeks, the dose could be underestimated due to the disappearance of cells bearing complex chromosomal aberrations such as dicentrics and centric rings (Gregoire et al. 2006). Thus, an analysis of translocations (total and reciprocal) by FISH was made on the cells of patient 06-20. Based upon the reference curve from

Table 1. Results of biological dosimetry performed onto blood lymphocytes from patients 06-20 and 06-23.

Patient number	Type of aberration	Number of cells	Number of aberrations	Radiation dose (Gy)	95% confidence interval
06-20	Dicentrics and rings	246	115	2.6	2.3–2.9
	Reciprocal translocations	698	47	3.2	2.9–3.5
	Total translocations	698	115	3.1	2.7–3. 8
06-23	Dicentrics and rings	494	42	1.0	0.8–1.1

the lab (Voisin et al. 2001), a dose estimate of 3.15 Gy (95% CI 2.9–3.5) was obtained for total translocations and a dose of 3.06 Gy (95% CI 2.68–3.78) for reciprocal translocations (Table 1).

Monitoring of blood cell counts

At the onset of hospitalization, patient 06-20 showed a pan-cytopenia with 0.5×10^9 WBC l^{-1} blood, 0.14×10^9 neutrophils l^{-1} blood and 0.33×10^9 lymphocytes l^{-1} blood (Figure 1A). Moreover, the patient showed a severe thrombopenia with 6×10^9 platelets (Plt) l^{-1} blood and a mild anaemia with 3.2×10^{12} RBC l^{-1} blood (Figure 2A). Overall, this was indicative of a severe pan-cytopenia with high risks of haemorrhages and opportunistic infections.

The blood cell count of patient 06-23 did not show such signs of pan-cytopenia (Figure 1B). In fact, although the number of WBC was slightly below the normal range (2.9×10^9 WBC l^{-1} blood compared with $3.1 - 10.3 \times 10^9$ WBC l^{-1}) (Cheng et al. 2004) and the lymphocyte number was decreased to 0.33×10^9 l^{-1} blood, patient 06-23 did not show thrombopenia, anaemia or neutropenia.

The two patients were monitored for blood cell count over 25 days. During this period, the blood cell counts of patient 06-23 did not vary significantly (Figure 1B), indicating that this patient either did not develop a pan-cytopenia or recovered from it before admission. By contrast, patient 06-20 was rapidly treated with platelet transfusions and cytokine injections, (T. Fagot, personal communication), in order to avoid bleeding and haemorrhages and to sustain a rapid recovery. The cytokine treatment was the same as that previously used during the treatment of another radiation accident victim (Fagot et al. 2006). In fact, as soon as 24 h after the beginning of the treatment, patient 06-20 showed an increase in WBC, neutrophils and lymphocytes (Figure 1A). The progressive recovery of platelet numbers appeared later, from day 15 of monitoring (Figure 1C). Also, the increase in reticulocyte percentage started on day 10, peaking on day 22 of follow-up (Figure 1D). Overall, after a period of hyperplasia due to cytokine treatment, patient 06-20 showed a return to a normal blood cell count by day 25 of the follow-up.

Radiation damage to the haematopoietic system

Damage to the bone marrow was evaluated in these two patients by plasma Flt3 ligand concentration, number of circulating progenitors (CFU-GM and BFU-E) and absolute number of circulating $CD34^+$ cells. At the onset of admission, patient 06-23 had a plasma Flt3 ligand concentration of 107 pg ml^{-1}, slightly above the normal

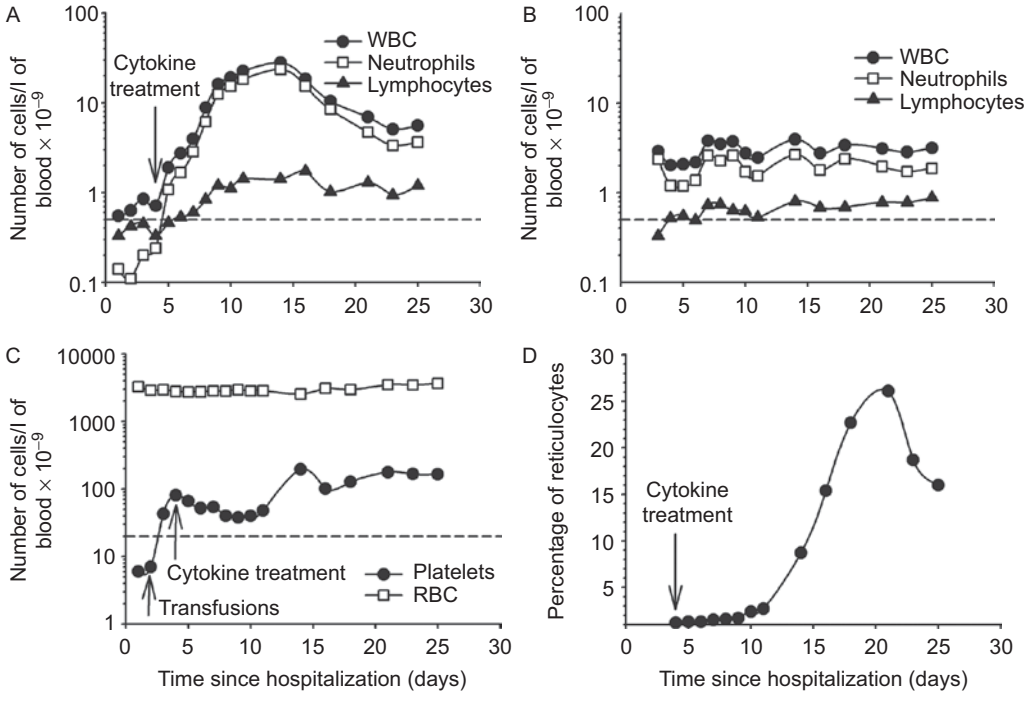

Figure 1. Follow-up of white blood cells (WBC; closed circles), neutrophils (open squares) and lymphocytes (closed triangles) (A) in patient 06-20 and (B) in patient 06-23. Horizontal dotted lines indicate the upper limit for severe neutropenia. The beginning of cytokine treatment for patient 06-20 is indicated by an arrow. (C) Follow-up of platelets (closed circles) and red blood cells (RBC; open squares) in patient 06-20. The horizontal dotted line indicates the upper limit for a severe thrombopenia. (D) Percentage of reticulocytes according to the time in the blood of patient 06-20.

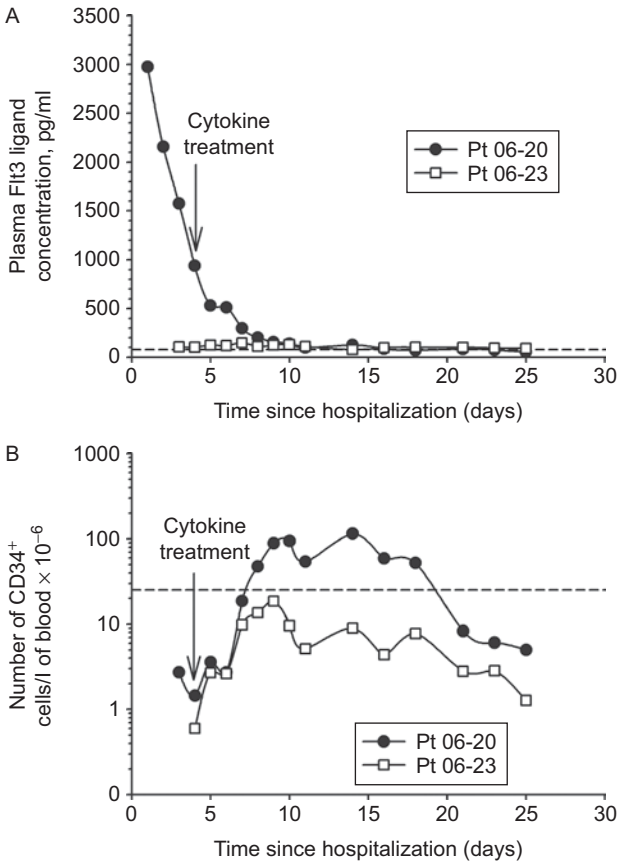

Figure 2. Evaluation of bone marrow damage by the mean of (A) plasma Flt3 ligand concentration follow-up and (B) absolute number of circulating CD34⁺ cells follow-up in patients 06-20 (closed circles) and 06-23 (open squares). The arrow indicates the beginning of cytokine treatment for patient 06-20. Horizontal dotted lines indicate mean normal values for these two parameters.

Flt3 ligand concentration for an African population (78.8 ± 27.7 pg ml^{-1}, as defined in 33 healthy donors) (J. M. Bertho, personal communication). Progenitor frequency in the blood was lower than the normal range (3.6×10^4 l^{-1} of blood compared with $1.4 \times 10^5 \pm 0.9 \times 10^5$ for CFU-GM and 4.0×10^4 l^{-1} of blood compared with $2.4 \times 10^5 \pm 1.0 \times 10^5$ for BFU-E), and the number of circulating CD34⁺ cells was below normal values (0.6×10^6 CD34⁺ cells l^{-1} blood compared with $25.1 \times 10^6 \pm 13.5 \times 10^6$ CD34⁺ cells l^{-1} in a control population; see Figure 2). Flt3 ligand concentration did not change significantly during the follow-up period, and both progenitor frequency and CD34⁺ cells increased up to the normal range. Overall, these results indicated that no significant radiation-induced bone marrow damage could be demonstrated in patient 06-23 during the hospitalization period or alternatively that the patient fully recovered before admission. As a result, patient 06-23 did not receive any specific treatment for a haematopoietic syndrome.

In contrast, patient 06-20 had a plasma Flt3 ligand concentration of 2975 pg ml^{-1} (almost 40-fold above the normal range value in the African population), in the range of a severe bone marrow aplasia, and the number of circulating CD34⁺ cells was below the normal range (2.7×10^6 CD34⁺ cells l^{-1}; see Figure 2). The number of circulating CFU-GM and BFU-E were also decreased compared with normal values (4.3×10^4 l^{-1} of blood compared with $1.4 \times 10^5 \pm 0.9 \times 10^5$ for CFU-GM and 1.2×10^4 l^{-1} of blood compared with $2.4 \times 10^5 \pm 1.0 \times 10^5$ for BFU-E). The Flt3 ligand concentration decreased rapidly, before the beginning of the cytokine treatment, strongly suggesting that patient 06-20 was in the bone marrow recovery phase at the onset of admission. This was confirmed by the rapid increase in both WBC numbers, circulating progenitors and circulating CD34⁺ cells, especially after the beginning of cytokine injection.

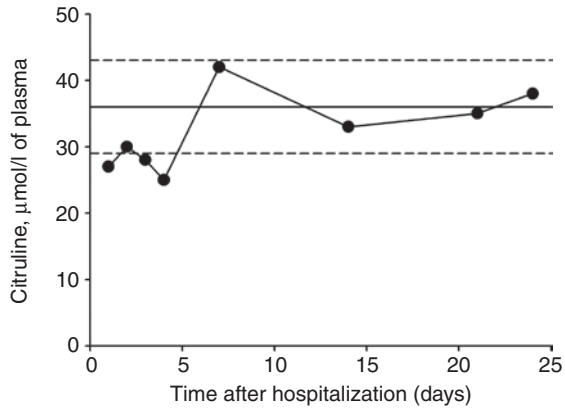

Figure 3. Citrulline plasma concentration as a bioindicator of enterocyte mass. Horizontal lines indicate the mean ± 1 SD of citrulline concentration in a control group of males aged between 20 and 40 years.

Radiation damage to the gastrointestinal system

Neither of the two patients described nausea and vomiting within hours following irradiation. Moreover, neither of these patients showed classical signs of a gastrointestinal syndrome such as haemorrhagic diarrhoea and loss of water and electrolytes. However, as these patients were hospitalized 1 month after irradiation and as the irradiation has been demonstrated to be heterogeneous, the occurrence of a discrete gastrointestinal syndrome was not excluded. Mucosal denudation of the intestine was then evaluated with plasma citrulline concentration in patient 06-20, as previously described (Crenn et al. 2003, Lutgens et al. 2004).

Citrulline concentration was between 29 and 43 µmol l^{-1} of plasma for both patients (Figure 3). These concentrations were within the normal range of citrulline concentration for a male in the 20–40-years-old age bracket (36 ± 7 µmol l^{-1} plasma as previously defined (Lutgens et al. 2004)), except during the first 3 days of follow-up. This demonstrated the absence of significant mucosal damage to the small bowel.

Use of oxysterols as bioindicators of radiation damage and follow-up of liver metabolism

Three oxysterols were followed in the plasma: 27-OH-Chol which is mainly produced by the lungs and the vascular compartment (Harik-Khan & Holmes 1990, Rajappa & Sharma 2005), 24S-OH-Chol which is produced by the central nervous system (Lutjohann et al. 2000) and 7α-OH-Chol, which is exclusively produced by the liver (Oda et al. 1990). These oxysterols may be used as a damage bioindicator, particularly for liver metabolism (Oda et al. 1990, Souidi et al. 2004).

27-OH-Chol consistently decreased in the plasma of the two patients compared with the normal range (defined as 4.1–10.1 μg ml^{-1} in previous studies; Figure 4A) (Hari-Khan et al. 1990, Bertho et al. 2008). This suggested a perturbation of lipid metabolism in the cardiovascular system (Rajappa & Sharma 2005). However, both creatinine kinase (CK) and CK-MB concentration were within normal values (data not shown) and no sign of a cardiovascular pathology was observed. 24S-OH-Chol concentration was also consistently below 133 ± 24 ng ml^{-1}, the normal range values (Lutjohann et al. 2000) in the two patients, with the exception of one time point (Figure 4B). However, no specific sign of a cerebrovascular disease was observed and 24S-OH-Chol concentration returned to normal values by the end of the monitoring period. In patient 06-20, 7α-OH-Chol concentration was increased 7-fold on day 5 of follow-up compared with the normal range (244 ± 42 ng ml^{-1} in the normal physiological situation; Figure 5A). At that time AST and ALT, two liver enzymes classically used to assess liver integrity showed a normal blood concentration (Figure 5C). Thereafter, while 7α-OH-Chol concentration returned to normal values, an increase in both AST and ALT indicated a perturbation of liver metabolism. By contrast, in patient 06-23, no significant modification in 7α-OH-Chol, AST or ALT was detected.

Overall, these results indicated that ionizing radiations induced a perturbation in the lipid metabolism of both the central nervous system and the vascular compartment, although no clinical consequences were observed in these two patients. Moreover, this also suggested that a perturbation of 7α-OH-Chol metabolism is indicative, and might be predictive of radiation-induced liver damage.

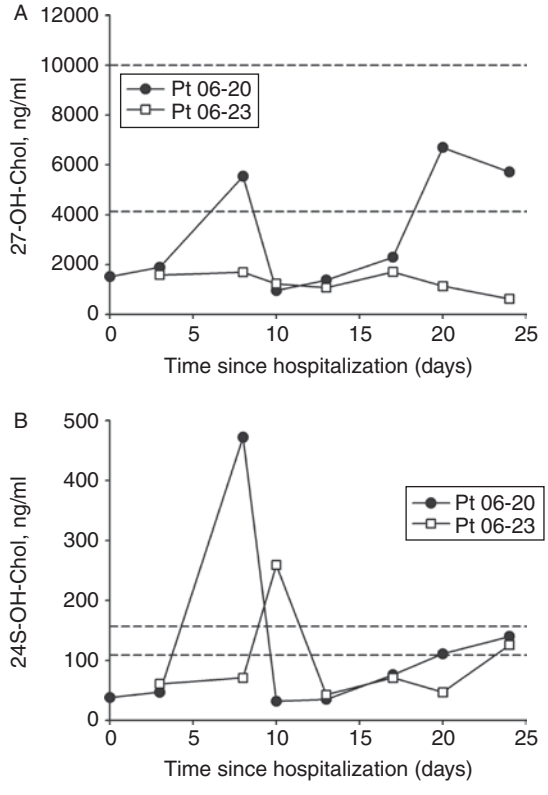

Figure 4. Evolution of (A) 27-OH-Cholesterol and (B) 24*S*-OH-Cholesterol in patients 06-20 (closed circles) and 06-23 (open squares). Horizontal dotted lines indicate the normal range for each of these two oxysterols.

Discussion

Each radiation accident appears as unique, according to the circumstances, the delay between irradiation and detection of the accident, the radiation dose, dose rate and quality, and the fractionation of irradiation. Thus, health consequences are specific for each radiation accident victim. For instance, for a Belgium victim of accidental irradiation (Bertho et al. 2008) the radiation dose was elevated (4.5 Gy) with a high dose rate, raising mainly a haematopoietic syndrome. By contrast, in the present case, the recognition of the accident was delayed by 2 months, the irradiation was protracted with a low dose rate and mainly raising radiological burns for the two most severely irradiated patients. Thus, although these two radiation accidents appeared similar in the biological follow-up, they were managed in different ways, and in fact, each radiation accident must be considered as an orphan pathology.

In the present case of accidental overexposure, the global radiation doses were in the mild range (1.2 Gy and 2.3 Gy), as determined by cytogenetic dosimetry. However, the irradiation was complex, with a combination of protracted low dose rate irradiation with localized high dose rate irradiation. This was confirmed by the use of the Qdr method (Sasaki & Miyata 1968) applied to the frequency of dicentrics in these two patients, which showed that only a fraction of blood cells was irradiated to higher doses. Thus, given that in such complex situations with a long-lasting period between irradiation and cytogenetic analysis, the frequency of dicentrics may underestimate the radiation dose (Gregoire et al. 2006).

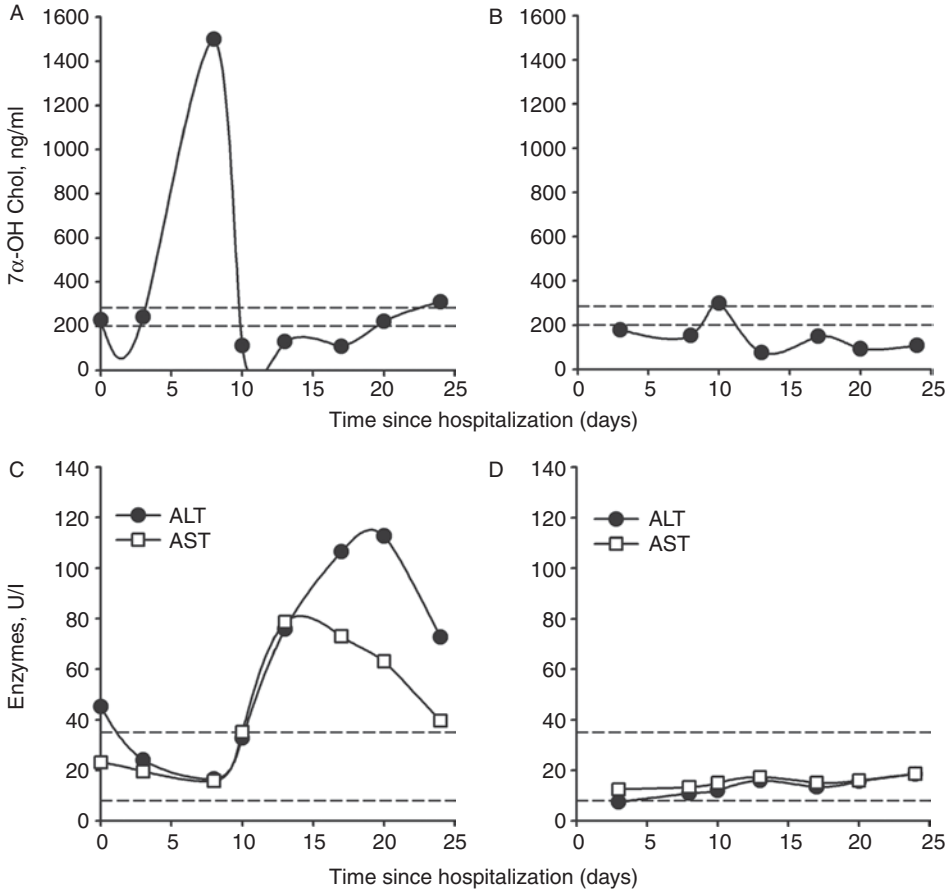

Figure 5. Variations in 7α-OH-cholesterol (A, B) and in plasma AST and ALT (C, D) in patient 06-20 (A, C) and in patient 06-23 (B, D). Horizontal dotted lines indicate the normal range for each of these parameters.

Therefore an analysis by translocation frequency was made for patient 06-20. Results indicated a good agreement between dicentric frequency and translocation frequency, with a mean radiation dose of 3.4 Gy (95% CI 2.8–3.9) for dicentrics and 3.15 Gy (95% CI 2.9–3.5) for total translocations. This suggests that it might be useful to evaluate the radiation dose by several methods in cases of delayed analysis or protracted overexposure (Wojcik et al. 2004).

Although the radiation doses received by the two patients were not life-threatening, an evaluation of radiation damage to the haematopoietic system was made, based on the use of Flt3 ligand concentration as a biological indicator of marrow function (Huchet et al. 2003, Prat et al. 2006a). Patient 06-23 showed a normal blood formula at the onset of hospitalization, suggesting the absence of a haematopoietic syndrome. This was confirmed by both circulating CFU-GM and BFU-E, circulating $CD34^+$ cells and Flt3 ligand concentration which were within the normal ranges for humans (Huchet et al. 2003). By contrast, at the onset of hospitalization, blood cell counts of patient 06-20 were suggestive of a severe haematopoietic syndrome, with both neutropenia, lymphopenia and severe thrombopenia. This was confirmed by an elevated Flt3 ligand concentration, indicative of severe radiation-induced bone marrow damage compared with previous studies in humans (Huchet et al., 2003, Prat et al., 2006b). However, the presence of circulating $CD34^+$ progenitors, circulating CFU-GM and BFU-E and the rapid decrease of blood Flt3 ligand concentration in the following days strongly suggested that patient 06-20 was in a haematopoietic syndrome recovery phase, compared with previous results obtained in haematopoietic stem cell transplanted patients (Prat et al. 2006b). In fact, as soon as cytokine treatment was started, patient 06-20 rapidly recovered a normal blood cell count. Overall, these results indicated that Flt3 ligand concentration in the blood allowed the prediction of haematological recovery in patient 06-23, supporting the interest in using Flt3 ligand concentration in the management of radiation-accident victims (Bertho et al. 2008).

A recent analysis of past radiation accidents (Fliedner et al. 2005) suggested that the acute radiation syndrome must be considered as a multiple organ dysfunction syndrome (MODS) rather than the addition of specific diseases from one physiological system. According to this view, radiation doses as low as those observed here may induce some modifications in the physiology

of specific organs, even in the absence of any clinical symptoms. We thus evaluated radiation damage to other organs using specific biological indicators. Although the two patients did not show signs of a gastrointestinal syndrome, the integrity of the jejunum mucosal epithelium was tested in patient 60-20 by measurement of citrulline concentration, as previously described (Crenn et al. 2003, Lutgens et al. 2004). Citrulline concentration was in the normal range for 20–40-year-old males as previously defined (Lutgens et al. 2004), indicating that there was no significant radiation damage to the mucosal epithelium. Although no clinical sign of a specific pathology was observed, we used plasma oxysterols as bioindicators of lipid metabolism in several organs. Results indicated that there was a perturbation of lipid metabolism mainly in the most severely irradiated victim, and mainly in association with liver physiology. Interestingly, the increase in plasma concentration of 7α-OH-Chol, which is specifically produced by the liver (Oda et al. 1990, Kuroki et al. 1995), preceded an increase of ALT and AST, two classical markers of liver pathology. The occurrence of a radiation-induced damage to the liver was in accordance with the physical dosimetry reconstruction of radiation doses received by patient 06-20 (Clairand et al. 2008). In contrast, 7α-OH-Chol remained below the normal range in patient 06-23 and the two liver enzyme values remained within the normal range. In a previous radiation accident (Bertho et al. 2008), an increase in plasma 7α-OH-Chol also preceded a perturbation of lipid metabolism, and a decrease in 27-OH-Chol preceded an increase in CK-MB above normal values, suggesting a cardiovascular perturbation. Such association between variations in plasma oxysterol concentration and either liver (Oda et al. 1990, Kuroki et al. 1995), cardiovascular (Harik-Khan & Holmes 1990) or central nervous system pathology (Lutjohann et al. 2000) have already been demonstrated in humans. Moreover, a severe liver disease was also observed during the course of a high-dose radiation-induced MODS (Uozaki et al. 2005). However, in the two present cases, perturbations in plasma oxysterols were limited and no specific pathology appeared. This suggested that a variation in oxysterol concentration in patients suffering from an acute radiation syndrome should be considered as a sign of a potential pathology to a specific physiological system, but not necessarily requiring specific treatment in the present case. Of course, this conclusion should be adapted to each individual case. Therefore, although needing some experimental confirmation in either animal models or in clinical studies, the present work confirms previous results (Fliedner et al. 2005, Konchalovsky et al. 2005, Bertho et al. 2008) suggesting that accidental irradiation induces a mixed pathology involving not only the haematopoietic system, the digestive tract and the skin, but also other organs and systems such as the liver and the cardiovascular system, even in the range of sublethal radiation doses.

Overall, this work also supports the interest in using these new biological indicators for the evaluation of radiation damage, in association with the evaluation of radiation dose and heterogeneity by classical cytogenetic analysis. Such a multiparametric approach, included in a more general evaluation score such as the METREPOL score (Fliedner et al. 2001) is essential for the evaluation of radiation damage and the choice of a therapeutic strategy adapted to each case of accidental irradiation.

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