® Postepy Hig Med Dosw (online), 2018; 72: 846-852
e-ISSN 1732-2693

www.phmd.pl
Original Article

Received: 16.11.2017
Accepted: 18.04.2018
Published: 01.10.2018

The assessment of radiosensitivity in patients with ataxia-telangiectasia syndrome and in carriers of the mutated *ATM* gene using lymphoblastoid cell lines*

Ocena radiowrażliwości u pacjentów z zespołem ataksja-teleangiektazja oraz u nosicieli zmutowanego genu *ATM* z użyciem limfoblastycznych linii komórkowych

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Barbara Pietrucha[1] A D E F, Hanna Gregorek[2] C D E F, Edyta Heropolitańska-Pliszka[1] D E F, Bożena Cukrowska[3] A B E, Ewa Konopka[3] B E, Ewa Bernatowska[1] D E

[1]Department of Immunology, The Children's Memorial Health Institute, Warsaw, Poland
[2]Department of Microbilogy and Clinical Immunology, The Children's Memorial Health Institute, Warsaw, Poland
[3]Department of Pathology, The Children's Memorial Health Institute, Warsaw, Poland

Summary

Introduction: Hypersensitivity to ionising radiation is most often observed in the course of primary immunodeficiency diseases, which are associated with dysfunctional DNA repair, especially with the repair of double-strand breaks. Due to phenotypic similarities between primary immunodeficiency diseases, radiosensitivity testing can prove useful in early differential diagnosis, when attempting to identify patients with increased toxic reactivity to radio- and chemotherapy, and can have an impact on the process of their preparation for stem cell transplantation.

Aim: The aim of the study was to assess the radiosensitivity *in vitro* of patients with ataxia-telangiectasia (A-T) syndrome, and their parents, carriers of one copy of the mutated *ATM* gene.

Material/Methods: Lymphoblastoid cell lines (LCLs) from 15 A-T patients (remaining under the care of the Immunology Clinic and Immunology Outpatient Clinic of the Children's Memorial Health Institute) and 11 mothers and 11 fathers of A-T patients, were used for radiosensitivity assessment. A standard colony survival assay (CSA) was applied in the tests.

Results: A markedly decreased survival fraction (SF) of LCLs after *in vitro* exposure to X-rays was observed in all A-T patients when compared to control cells. A clear diversification of radiosensitivity to ionising radiation was observed among obligate heterozygotes. SF for heterozygotes was between 1% and 53%, i.e. varied from the values in healthy individuals to the extreme values observed in A-T patients.

Conclusion: The assessment of cell radiosensitivity in A-T patients using CSA may be a useful additional test for confirming a clinically suspected disease. In heterozygous carriers, it can be an indicator of increased risk of carcinogenesis, and in both A-T patients and their parents can be helpful in making decisions with regard to radio- and/or chemotherapy.

Keywords: **ataxia-telangiectasia • radiosensitivity • lymphoblastoid cell lines • *ATM* gene heterozygotes**

*The work was conducted under statutory task No. S223/2013 and internal grant No. S137/2014 – the Children's Memorial Health Institute in Warsaw.

GICID 01.3001.0012.5856
DOI: 10.5604/01.3001.0012.5856
Word count: 4111
Tables: 3
Figures: –
References: 40

Author's address: Barbara Pietrucha MD, Department of Microbiology and Clinical Immunology, The Children's Memorial Health Institute, Al. Dzieci Polskich 20, 04-730 Warsaw, Poland; e-mail: B.Pietrucha@IPCZD.PL

INTRODUCTION

Hypersensitivity to ionising radiation is most often seen in autosomal recessive hereditary disorders that are associated with dysfunctional DNA repair, especially with the repair of DNA double-strand breaks (DNA DSBs) [10, 12, 25, 28, 33]. DNA repair disorders share certain common phenotypic traits, including hypersensitivity to X-radiation, increased predisposition to developing neoplasms, immunodeficiency, neurological abnormalities, and DNA DSB repair dysfunction. Therefore, due to the common phenotype, it was proposed that these disease entities should be gathered under one group, called XCIND (X-irradiation sensitivity, cancer susceptibility, immunodeficiency, neurological abnormality and double-strand DNA breakage) [11, 24]. The XCIND syndromes which have been described to date are presented in Table 1. Among these, the most often diagnosed is ataxia-telangiectasia (A-T) syndrome, caused by mutations in the *ATM* (ataxia telangiectasia mutated) gene.

Radiosensitivity tests evaluate cell viability after exposure to ionising radiation. B-lymphoblastoid cell lines (LCLs) transformed by Epstein-Barr virus (EBV) to obtain immortalised cell lines are used in this analysis. LCLs, in addition to being useful in radiosensitivity testing, have a broad range of applications in laboratory diagnosis. These include being a source of genetic material - the RNA obtained from LCLs can be used to detect splice site mutations in the diagnosis of certain diseases; they can also be used to extract nuclear proteins, and characterise them using the western-blot method. Furthermore, LCLs can be frozen and stored for many years and sent to other specialised centres.

Colony survival assay (CSA) is one of the first and most common radiosensitivity tests. It facilitates the assessment of cell viability after X-irradiation. This test, which had initially been used to confirm early diagnosis of A-T syndrome, later played a critical role in the studies that led to the identification of the *ATM* gene. The normal values for CSA were determined using LCLs obtained from A-T patients, from heterozygous carriers of the mutated *ATM* gene, and from phenotypically normal individuals. The cell survival fraction (SF) after the application of 1Gy radiation, expressed as a percentage, was <21% for A-T cells and >36% for cells collected from healthy people. The "grey zone" or "non-diagnostic" intermediate values were between 21% and 36%. This zone could include patients with DNA damage response disorders, as well as healthy carriers of one copy of the mutated gene [18, 36].

It is worth noting that the *in vitro* determination of cell radiosensitivity does not always correlate with clinical radiosensitivity. Clinical hypersensitivity to X-rays and/or chemotherapy employed in neoplasm treatment has been documented for at least four disease entities

Table 1. XCIND syndromes

Name of syndrome	Gene	Year described
Ataxia-telangiectasia syndrome	ATM	1926
Fanconi anaemia	FANC	1927
X-linked agammaglobulinemia	BTK	1952
ADA-SCID	ADA	1979
Nijmegen breakage syndrome	NBS1	1981
ATLD	MRE11A	1999
Ligase IV syndrome	LIG4	1999
SCID Artemis	ARTEMIS	2001
SCID Cernunnos	XLF	2006
RIDDLE syndrome	RNF168	2009
RAD50 deficiency	RAD50	2009
SCID	DNA-PKCS	2009

SCID – severe combined immunodeficiency, ATLD – ataxia-telangiectasia-like disease, RIDDLE – radiosensitivity, immunodeficiency, dysmorphic features, learning difficulties

associated with deficient DNA repair, i.e. A-T, Nijmegen breakage syndrome (NBS), Fanconi anaemia and LIG4 syndrome [11, 14, 28, 32].

Ataxia-telangiectasia (OMIM#208900) is an autosomal recessive multisystem genetic disease caused by mutations in the *ATM* gene located on chromosome 11q22.3 [1, 29]. The ATM protein kinase is a product of this gene. It plays a central role in DNA damage response (DDR), cell cycle regulation, apoptosis, and telomere and mitochondrial maintenance. DNA-damaging agents activate the ATM protein, which starts the repair process through the phosphorylation of hundreds of effector particles participating in the DDR process [20].

The cardinal clinical sign of A-T is progressive cerebellar ataxia, most often observed between the 12th and 14th month of life, when the child is starting to walk, and is evident in most patients by the 6th year of life. Patients usually become wheelchair dependent by puberty. The other main sign of the syndrome is telangiectasias (dilated blood vessels), which appear slightly later (around the 4th to the 6th year of life). Intellectual development is normal in most patients; however, with age a slight decrease in mental ability and difficulties in learning are observed in approx. 30% of patients [27].

Other characteristics of A-T include humoral and cell-mediated immunity deficiencies of a varying degree, which underlie recurrent respiratory infections (chronic bronchitis and chronic sinusitis are pathognomonic of this syndrome) [21]. Increased susceptibility to the development of neoplasms, mainly of the lymphoid origin, is also observed. These neoplasms are the second leading cause of death, next to chronic pulmonary lesions. Also, carriers of the mutated *ATM* gene display increased susceptibility to carcinogenesis. This applies mainly to women, in whom the risk of developing breast cancer is 5 times greater than in the healthy population [34, 37, 39]. Laboratory tests show elevated and slowly increasing serum alpha-fetoprotein (AFP) levels after two years of age in approximately 95% of patients, as well as IgA deficiency observed in approx. 70%, and often lymphopenia [3]. The most significant characteristic of cell disorders in A-T is the clinically and *in vitro* confirmed hypersensitivity to ionising radiation, and cytostatic drugs belonging to the group of radiomimetic agents (e.g. bleomycin).

Early suspicion of the A-T syndrome is usually based on the presence of characteristic clinical signs, and must be confirmed by genetic tests. It should be noted, however, that in younger patients, and those with milder neurological signs manifesting later in life [7, 40], early diagnosis is often difficult, which, in turn, delays genetic testing and necessary genetic counselling. Early A-T diagnosis requires highly specialised tests, such as cell radiosensitivity evaluation, ATM protein expression and function assessment using the western-blot method, and *ATM* gene sequencing for mutation detection. These tests are usually time-consuming and require experienced laboratory staff. Nevertheless, they can provide valuable diagnostic assistance, especially useful in the case of young couples planning to have children, and in prenatal diagnosis.

The aim of this study was to assess the radiosensitivity of LCLs isolated from the whole blood of A-T patients and clinically symptomless heterozygous carriers of the mutated *ATM* gene, using CSA.

MATERIALS AND METHODS

The study involved 15 A-T patients aged 2.5-22 yrs (median: 8 yrs), including 11 boys and 4 girls, who were under the care of the Immunology Clinic and Immunology Outpatient Clinic of the Children's Memorial Health Institute, diagnosed on the basis of definitive (13 pts) or probable (2 pts) criteria set for A-T by ESID [https://esid.org/Education/Diagnostic-Criteria-PID].

Furthermore, the study also included 22 parents of A-T patients, obligate carriers of the mutated *ATM* gene aged 25 – 52 yrs (median: 38 yrs), i.e. 11 mothers and 11 fathers.

From each individual, 5-8 ml of venous peripheral blood was collected into EDTA tubes to perform a CSA radiosensitivity test. The correct course of the test was controlled using LCL collected from a healthy donor.

The study was approved by the Bioethics Committee of the Children's Memorial Health Institute (approval No. 91/KBE/2013 and 161/KBE/2014), and informed consent was obtained from all parents and/or patients.

LYMPHOBLASTOID CELL LINES, LCLS

In the first stage of the study, to get a medium containing proliferated EBV (i.e. modified EBV culture medium), the virus was grown for 7 days on a B95-8 cell line (obtained courtesy of Prof. Ricard Gatti, UCLA, USA) in a complete RPMI medium (Sigma-Aldrich) with the addition of 10% fetal bovine serum (FBS; Sigma-Aldrich) and 1% antibiotic solution (1% Antibiotic-Antimycotic Solution; Sigma-Aldrich) at 37°C and 5% CO_2. To remove the cell line used for EBV growth, the culture was centrifuged (5 min. 300xG) and the supernatant was passed through a 0.22 µm pore filter. To obtain a modified EBV culture medium this supernatant was supplemented with a complete RPMI medium at a 3:7 ratio.

The lymphocytes of A-T patients and A-T heterozygotes (parents) were isolated from 5-8 ml of peripheral blood. After mixing the blood with PBS (Biomed, Lublin) at a 1:2 ratio, the suspension was spread on Ficoll-Hypaque Plus (GE Healthcare Life Sciences) and subjected to density gradient centrifugation (20 min. 800xG). The isolated peripheral blood lymphocytes were EBV-transformed using a modified EBV culture medium, in 25 cm^3 culture flask, at 37°C and 5% CO_2.Half of the modified EBV cul-

ture medium was replaced every 10 days until intensive growth of lymphoblastoid cells was observed (numerous, large cell aggregates and an increase in cell count clearly visible microscopically). Then, the LCL cultures were maintained in a complete RPMI medium.

RADIOSENSITIVITY TESTING USING CSA

Evaluation of radiosensitivity was based on the method and criteria published by Sun X et al. [36].

Lymphoblastoid cells were placed in two 96-well culture plates (NUNC) in concentration 200 cells per well in 100 µl of a complete RPMI medium. One of the plates was subjected to ionising radiation at 1 Gy, and the other constituted the control sample. LCL cultures in both plates were maintained at 37°C and 5% CO_2 for 8-13 days depending on the degree of colony (cell aggregate) growth. Next, the cells in both plates were stained by adding into each well 100 µl of 0.1% MTT (Thiazolyl Blue Tetrazolium Bromide, Sigma-Aldrich) dissolved in PBS (Biomed, Lublin). After 1 hour of incubation every well was assessed microscopically for the presence of living cells, i.e. dark blue cells. The presence of colonies containing more than 32 living cells was assumed as a positive result for the well. Colony-forming efficiency (CFE) was calculated on the basis of the obtained results using the following formulae:

$$CFE_{ir} = \frac{-\ln \frac{neg_{ir}}{96}}{W}$$

$$CFEc = \frac{-\ln \frac{neg_{c}}{96}}{W}$$

CFEir – colony-forming efficiency irradiated plate
CFEc – colony-forming efficiency control plate
negir – number of negative wells in the irradiated plate
negc – number of negative wells in the control plate
W – number of cells seeded per well

Next, the survival fraction (SF) was calculated using the following formula:

$$SF = \frac{CFE_{ir}}{CFE_{c}} \times 100\%$$

The test was deemed to have been correctly conducted if the percentage of positive wells in the control plate exceeded 60%. If there were fewer positive wells, the radiosensitivity test was repeated with 300 cells per well introduced into both plates.

SF <21% indicated cell radiosensitivity, >36% no radiosensitivity, whereas results between 21-36% had no diagnostic value.

RESULTS

The characteristics of the studied patients are presented in Table 2.

The CSA radiosensitivity test was conducted on 15 LCLs from A-T patients and 22 LCLs obtained from the carriers of the mutated *ATM* gene, using a uniform dose of 1 Gy. The control LCL was obtained from a healthy donor.

In all A-T patients, SF after *in vitro* exposure to X-rays was between 1% and 19% (median: 3%) when compared to the control cells (SF >36%), which confirms significantly increased radiosensitivity in this syndrome. In 10 out of 15 patients (66.7%), the SF was <5%, which was only about one-seventh of the lower threshold of the percentage of living normal cells. Values between 5% and 10% were observed in 20% of the studied individuals, and between 10% and 21 in 13.3% (Table 3).

Among the LCLs from obligate heterozygotes, a significant diversity was observed in cell viability after *in vitro* exposure to ionising radiation, which varied from the values characteristic of healthy individuals to the extreme values recorded in the A-T patients. SF ranged

Table 2. The clinical characteristics of the 15 studied A-T patients

Studied parameters		A-T patients	
		No.= 15	(%)
Age in years	Median (range)	8 (2.5 – 14.1)	
Gender	Boys	11	73
	Girls	4	27
Comorbidities	Diabetes	1	7
	Hypothyroidism	1	7
	Skin granuloma	1	7
	Albinism	1	7
	Kidney stone disease	1	7
	Leukopenia	2	13
	Aplastic anaemia	1	7
Tumours	T-cell ALL	2	13

Table 3. The results of CSA in A-T patients and ATM gene carriers

The study group (size)	Survival fraction of LCLs after irradiation in %				
	< 5%	5% - 10%	11% - 20%	21% - 36%	> 36%
A-T patients (no. =15)	10/15 (66.7%)	3/15 (20%)	2/15 (13.3%)	0/15 (0%)	0/15 (0%)
Carriers of *ATM* gene (no.=22)	8/22 (36.4%)	6/22 (27.3%)	4/22 (18.1%)	3/22 (13.6%)	1/22 (4.6%)

from 1% to 53% (median: 7.5%). In 18 out of 22 (81.8%) tested parents, SF was within the positive range, and was lower than 21% of living cells. In 36.4% (8/22) parents SF was below 5%, in 13.6% SF values were in the "non-diagnostic" range, and, only in the case of one mother, no hypersensitivity to ionising radiation was determined (SF: 53%) (Table 3).

One carrier of the mutated *ATM* gene, whose SF of LCLs was 2%, developed breast cancer. The performed radiosensitivity test had a practical aspect in relation to this patient, as due to cell radiosensitivity diagnosed *in vitro*, radiotherapy was abandoned as a treatment method.

DISCUSSION

Radiosensitivity, as one of the main characteristics of A-T, was first documented in 1967 in a 10-year-old A-T patient, who experienced a severe topical response to radiation in the face and neck area, after being exposed to 30 Gy radiation intended to treat lymphosarcoma. As a result of complications, the patient died 8 months later [13]. In the subsequent years, radiosensitivity was confirmed *in vitro* by Higurashi and Conen [17], who observed a higher level of chromosome aberrations in lymphocytes of patients with A-T, Bloom syndrome and Fanconi syndrome, after exposure to a 1 Gy dose of radiation. Taylor et al. [38] extended these observations using fibroblasts collected from the skin of A-T patients. In the 1980s, Coquerelle et al. [4, 5] demonstrated a slower course of DNA DSB repair caused by ionising radiation in skin fibroblasts of patients with A-T and Fanconi syndrome, when compared with healthy controls.

In 1994, Huo Y. et al. [18] published a modified test for evaluating hypersensitivity to ionising radiation (CSA). In 2002, Sun X et al. [36] published radiosensitivity evaluation criteria for CSA, and, at present, the test is regarded as the most reliable "gold standard" for assessing cell viability following X-irradiation.

Since 2007, the Pathology Department of the Children's Memorial Health Institute has successfully derived LCLs from patients with primary immunodeficiencies, and since 2014 the Institute has conducted CSAs to measure cell sensitivity to ionising radiation, based on the criteria prepared in detail by Sun et al. [36].

In our tests evaluating cell viability after X-irradiation using CSA, significant radiosensitivity was demonstrated in all LCLs obtained from A-T patients. Extremely low cell viability (SF < 5%) was observed in 66.7% of the studied individuals, and SF did not exceed 21% in any of them, which was the threshold assumed for "positive" results. These results are in line with the majority of earlier findings, indicating increased radiosensitivity in patients with genetically conditioned dysfunctional DNA repair, especially the repair of DNA double-strand breaks [22, 25]. In light of the increased sensitivity of A-T patients to ionising radiation (X or γ-rays), which has a toxic effect on them in most cases, exposure to X-rays should be limited to medically necessitated diagnostic purposes. Radiotherapy, employed in cancer treatment and for other reasons, is generally harmful to A-T patients. It should be used in rare cases, using reduced doses [6, 16, 23]. Furthermore, it was demonstrated in *in vitro* experiments that the mechanism of repair of DNA damage caused by other genotoxic factors, e.g. UV, is deficient in A-T cells [15, 30]. Despite this, patients do not display increased susceptibility to skin cancer and there are no contraindications to sunbathing.

Suspicion of A-T syndrome is usually based on the presence of characteristic clinical symptoms early in life. However, in recent years more and more findings have been appearing which indicate that in younger patients and those with milder neurological symptoms manifesting later in life [7, 40], early diagnosis is often difficult, which delays genetic testing. On the other hand, there are situations in which A-T is suspected, but access to genetic testing is limited, and obtaining a result takes time. In both cases, the *in vitro* testing of radiosensitivity in conjunction with other laboratory tests, such as increased alpha-fetoprotein levels, immunodeficiencies, or the lack or a low level of ATM protein, can be a very useful test supplementing A-T diagnosis in the period of waiting for the results of genetic tests [36].

In the literature there are not many findings concerning the evaluation of radiosensitivity in heterozygous carriers of the mutated *ATM* gene using CSA. In most cases, these were population studies or were conducted on skin fibroblasts, characterised by poor growth in culture media [36]. Most papers have demonstrated that cells collected from heterozygous carriers of the mutated *ATM* gene presented variable but "intermediate" sensitivity to ionising radiation, i.e. their cells were more sensitive than the cells of healthy individuals, but less sensitive than the cells of A-T patients [19, 31]. In our own studies, a considerable diversity was observed in the SF of LCLs obtained from obligate carriers of the mutated *ATM* gene (patients' parents), which was

between 1% and 53%, with, unexpectedly, as many as 36.4% of results falling within SF < 5%. The actual degree of interrelation between *in vitro* radiosensitivity and clinical radiosensitivity to therapeutic radiation doses is unknown, as persons participating in such tests usually avoid any contact with ionising radiation. On the other hand, a number of studies indicated that heterozygous carriers of the *ATM* gene are more predisposed, when compared to the healthy population, to not only breast cancer development, but also pancreatic, stomach, bladder or ovarian cancer, or chronic lymphocytic leukaemia [37]. A series of studies involving women with breast cancer, and patients suffering from unexpectedly severe responses to radiation during or after cancer treatment, displayed the presence of a mutated *ATM* gene in a significant percentage of patients [2, 9, 35]. Ostendorf et al. [26] described a case of a 30-year-old woman with toxic side effects during acute lymphocytic leukaemia (ALL) treatment and a severe response to radiation during conditioning before stem cell transplantation. Tests demonstrated the presence of the mutated *ATM* gene, which had been causing the side effects of radiotherapy. In the studied material by us, 2 patients with A-T developed ALL, and 1 of the carrier mothers, whose SF of LCL in radiosensitivity test was at 2%, developed breast cancer. In all those cases, assessment of radiosensitivity could have its practical aspect, because it allows clinicians to implement suitable medical intervention not involving conventional radiotherapy.

CONCLUSIONS

A greater sensitivity to ionising radiation was observed in all A-T patients, as well as in 81.8% of the carriers of the mutated *ATM* gene.

CSA is a useful laboratory tool for early A-T diagnosis, especially during the period of waiting for the results of genetic testing, conducted to confirm the diagnosis. In asymptomatic carriers, it can be a valuable indicator of increased risk of carcinogenesis, or, in the case of already-diagnosed cancer and confirmed increased radiosensitivity, an indication to avoid radiotherapy or modifying chemotherapy.

REFERENCES

[1] Boder E., Sedgwick R.P.: Ataxia-telangiectasia; a familial syndrome of progressive cerebellar ataxia, oculocutaneous telangiectasia and frequent pulmonary infection. Pediatrics, 1958; 21: 526-554

[2] Byrd P.J., Srinivasan V., Last J.I., Smith A., Biggs P., Carney E.F., Exley A., Abson C., Stewart G.S., Izatt L., Taylor A.M.: Severe reaction to radiotherapy for breast cancer as the presenting feature of ataxia telangiectasia. Br. J. Cancer, 2012; 106: 262-268

[3] Chun H.H., Gatti R.A.: Ataxia-telangiectasia, an evolving phenotype. DNA Repair, 2004; 3: 1187-1196

[4] Coquerelle T.M., Weibezahn K.F.: Rejoining of DNA double-strand breaks in human fibroblasts and its impairment in one ataxia telangiectasia and two Fanconi strains. J. Supramol. Struct. Cell Biochem., 1981; 17: 369-376

[5] Coquerelle T.M., Weibezahn K.F., Lücke-Huhle C.: Rejoining of double strand breaks in normal human and ataxia-telangiectasia fibroblasts after exposure to ^{60}Co γ-rays, ^{241}Am α-particles or bleomycin. Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med., 1987; 51: 209-218

[6] DeWire M.D., Beltran C., Boop F.A., Helton K.J., Ellison D.W., McKinnon P.J., Gajjar A., Pai Panandiker A.S.: Radiation therapy and adjuvant chemotherapy in a patient with a malignant glioneuronal tumor and underlying ataxia telangiectasia: A case report and review of the literature. J. Clin. Oncol., 2013; 31: e12-e14

[7] Dörk T., Bendix-Waltes R., Wegner R.D., Stumm M.: Slow progression of ataxia-telangiectasia with double missense and in frame splice mutations. Am. J. Med. Genet. A, 2004; 126A: 272-277

[8] ESID Diagnostic Criteria PID. https://esid.org/Education/Diagnostic-Criteria-PID (25.04.2017)

[9] Fang Z., Kozlov S., McKay M.J., Woods R., Birrell G., Sprung C.N., Murrell D.F., Wangoo K., Teng L., Kearsley J.H., Lavin M.F., Graham P.H., Clarke R.A.: Low levels of ATM in breast cancer patients with clinical radiosensitivity. Genome Integr., 2010; 1: 9

[10] Gatti R.A.: The inherited basis of human radiosensitivity. Acta Oncol., 2001; 40: 702-711

[11] Gatti R.A., Boder E., Good R.A.: Immunodeficiency, radiosensitivity, and the XCIND syndrome. Immunol. Res., 2007; 38: 87-101

[12] Gennery A.R.: Primary immunodeficiency syndromes associated with defective DNA double-strand break repair. Br. Med. Bull., 2006; 77-78: 71-85

[13] Gotoff S.P., Amirmokri E., Liebner E.J.: Ataxia telangiectasia. Neoplasia, untoward response to x-irradiation, and tuberous sclerosis. Am. J. Dis. Child., 1967; 114: 617-625

[14] Gutiérrez-Enríquez S., Fernet M., Dörk T., Bremer M., Lauge A., Stoppa-Lyonnet D., Moullan N., Angèle S., Hall J.: Functional consequences of *ATM* sequence variants for chromosomal radiosensitivity. Genes Chromosomes Cancer, 2004; 40: 109-119

[15] Hannan M.A., Hellani A., Al-Khodairy F.M., Kunhi M., Siddiqui Y., Al-Yussef N., Pangue-Cruz N., Siewertsen M., Al-Ahdal M.N., Aboussekhra A.: Deficiency in the repair of UV-induced DNA damage in human skin fibroblasts compromised for the *ATM* gene. Carcinogenesis, 2002; 23: 1617-1624

[16] Hart R.M., Kimler B.F., Evans R.G., Park C.H.: Radiotherapeutic management of medulloblastoma in a pediatric patient with ataxia telangiectasia. Int. J. Radiat. Oncol. Biol. Phys., 1987; 13: 1237-1240

[17] Higurashi M., Conen P.E.: *In vitro* chromosomal radiosensitivity in "chromosomal breakage syndromes". Cancer, 1973; 32: 380-383

[18] Huo Y.K., Wang Z., Hong J.H., Chessa L., McBride W.H., Perlman S.L., Gatti R.A.: Radiosensitivity of ataxia-telangiectasia, X-linked agammaglobulinemia, and related syndromes using a modified colony survival assay. Cancer Res., 1994; 54: 2544-2547

[19] Kiuru A., Kämäräinen M., Heinävaara S., Pylkäs K., Chapman K., Koivistoinen A., Parviainen T., Winqvist R., Kadhim M., Launonen V., Lindholm C.: Assessment of targeted and non-targeted responses in cells deficient in *ATM* function following exposure to low and high dose X-rays. PLoS One, 2014; 9: e93211

[20] Lavin M.F.: Ataxia-telangiectasia: from a rare disorder to a paradigm for cell signalling and cancer. Nat. Rev. Mol. Cell Biol., 2008; 9: 759-769

[21] Lefton-Greif M.A., Crawford T.O., Winkelstein J.A., Loughlin G.M., Koerner C.B., Zahurak M., Lederman H.M.: Oropharyngeal dysphagia and aspiration in patients with ataxia-telangiectasia. J. Pediatr., 2000; 136: 225-231

[22] Matsuoka S., Ballif B.A., Smogorzewska A., McDonald E.R. 3rd, Hurov K.E., Luo J., Bakalarski C.E., Zhao Z., Solimini N., Lerenthal Y., Shiloh Y., Gygi S.P., Elledge S.J.: ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. Science, 2007; 316: 1160-1166

[23] Meyer A., John E., Dörk T., Sohn C., Karstens J.H., Bremer M.: Breast cancer in female carriers of ATM gene alterations: outcome of adjuvant radiotherapy. Radiother. Oncol., 2004; 72: 319-323

[24] Mizutani S., Takagi M.: XCIND as a genetic disease of X-irradiation hypersensitivity and cancer susceptibility. Int. J. Hematol., 2013; 97: 37-42

[25] Nahas S.A., Gatti R.A.: DNA double strand break repair defects, primary immunodeficiency disorders, and 'radiosensitivity'. Curr. Opin. Allergy Clin. Immunol., 2009; 9: 510-516

[26] Ostendorf B.N., Terwey T.H., Hemmati P.G., Böhmer D., Pleyer U., Arnold R.: Severe radiotoxicity in an allogeneic transplant recipient with a heterozygous ATM mutation. Eur. J. Haematol., 2015; 95: 90-92

[27] Perlman S.L., Boder Deceased E., Sedgewick R.P., Gatti R.A.: Ataxia-telangiectasia. Handb. Clin. Neurol., 2012; 103: 307-332

[28] Pollard J.M., Gatti R.A.: Clinical radiation sensitivity with DNA repair disorders: An overview. Int. J. Radiat. Oncol. Biol. Phys., 2009; 74: 1323-1331

[29] Savitsky K., Bar-Shira A., Gilad S., Rotman G., Ziv Y., Vanagaite L., Tagle D.A., Smith S., Uziel T., Sfez S., Ashkenazi M., Pecker I., Frydman M., Harnik R., Patanjali S.R., et al.: A single ataxia telangiectasia gene with a product similar to PI-3 kinase. Science, 1995; 268: 1749-1753

[30] Shiloh Y.: ATM: Expanding roles as a chief guardian of genome stability. Exp. Cell Res., 2014; 329: 154-161

[31] Shiloh Y., Parshad R., Frydman M., Sanford K.K., Portnoi S., Ziv Y., Jones G.M.: G2 chromosomal radiosensitivity in families with ataxia-telangiectasia. Hum. Genet., 1989; 84: 15-18

[32] Slack J., Albert M.H., Balashov D., Belohradsky B.H., Bertaina A., Bleesing J., Booth C., Buechner J., Buckley R.H., Ouachée-Chardin M., Deripapa E., Drabko K., Eapen M., Feuchtinger T., Finocchi A., et al.: Outcome of hematopoietic cell transplantation for DNA double-strand break repair disorders. J. Allergy Clin. Immunol., 2018; 141: 322-328.e10

[33] Slatter M.A., Gennery A.R.: Primary immunodeficiencies associated with DNA-repair disorders. Expert Rev. Mol. Med., 2010; 12: e9

[34] Stankovic T., Kidd A.M., Sutcliffe A., McGuire G.M., Robinson P., Weber P., Bedenham T., Bradwell A.R., Easton D.F., Lennox G.G., Haites N., Byrd P.J., Taylor A.M.: ATM mutations and phenotypes in ataxia-telangiectasia families in the British Isles: Expression of mutant ATM and the risk of leukemia, lymphoma, and breast cancer. Am. J. Hum. Genet., 1998; 62: 334-345

[35] Su Y., Swift M.: Outcomes of adjuvant radiation therapy for breast cancer in women with ataxia-telangiectasia mutations. JAMA, 2001; 286: 2233-2234

[36] Sun X., Becker-Catania S.G., Chun H.H., Hwang M.J., Huo Y., Wang Z., Mitui M., Sanal O., Chessa L., Crandall B., Gatti R.A.: Early diagnosis of ataxia-telangiectasia using radiosensitivity testing. J. Pediatr., 2002; 140: 724-731

[37] Swift M., Morrell D., Massey R.B., Chase C.L.: Incidence of cancer in 161 families affected by ataxia-telangiectasia. N. Engl. J. Med., 1991; 325: 1831-1836

[38] Taylor A.M., Harnden D.G., Arlett C.F., Harcourt S.A., Lehmann A.R., Stevens S., Bridges B.A.: Ataxia telangiectasia: a human mutation with abnormal radiation sensitivity. Nature, 1975; 258: 427-429

[39] Thompson D., Duedal S., Kirner J., McGuffog L., Last J., Reiman A., Byrd P., Taylor M., Easton D.F.: Cancer risks and mortality in heterozygous. ATM mutation carriers. J. Natl. Cancer Inst., 2005; 97: 813-822

[40] Worth P.F., Srinivasan V., Smith A., Last J.I., Wootton L.L., Biggs P.M., Davies N.P., Carney E.F., Byrd P.J., Taylor A.M.: Very mild presentation in adult with classical cellular phenotype of ataxia telangiectasia. Mov. Disord., 2013; 28: 524-528

The authors have no potential conflicts of interest to declare.