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Considerations for radiotherapy in Bloom Syndrome: A case series[☆]

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ABSTRACT

Bloom Syndrome (BS) is a genetic DNA repair disorder, caused by mutations in the *BLM* gene. The clinical phenotype includes growth retardation, immunodeficiency and a strong predisposition to different types of malignancies. Treatment of malignancies in BS patients with radiotherapy or chemotherapy is believed to be associated with increased toxicity, but clinical and laboratory data are lacking. We collected clinical data of two Dutch BS patients with solid tumors. Both were treated with radiotherapy before the diagnosis BS was made and tolerated this treatment well. In addition, we collected fibroblasts from BS patients to perform *in vitro* clonogenic survival assays to determine radiosensitivity. BS fibroblasts showed less radiosensitivity than the severely radiosensitive Artemis fibroblasts. Moreover, studies of double strand break kinetics by counting 53BP1 foci after irradiation showed similar patterns compared to healthy controls. In combination, the clinical cases and laboratory experiments are valuable information in the discussion whether radiotherapy is absolutely contraindicated in BS, which is the Case in other DNA repair syndromes like Ataxia Telangiectasia and Artemis.

1. Introduction

Bloom Syndrome (BS) was first described by David Bloom in 3 children with severe growth deficiency and a telangiectatic erythematous rash on the face (Bloom, 1954). The clinical spectrum currently includes a number of additional features such as type 2 diabetes, immunodeficiency, infertility, and most importantly, predisposition to early onset cancer in multiple different organ systems (Flanagan and Cunniff, 1993). BS is an autosomal recessive disorder caused by mutations in the *BLM* gene, which encodes a RecQ helicase that plays a role in DNA replication, recombination and repair (de Renty and Ellis, 2017; Ellis et al., 1995). Unlike other RecQ helicase disorders such as Werner Syndrome and Rothmund-Thomson Syndrome, which predispose

patients primarily to sarcomas, individuals with BS are at risk for a wide array of tumor types in multiple organ systems (Lindor et al., 2000; Oshima et al., 1993). The Bloom registry in New York collected clinical data on BS patients from all over the world, including 145 BS patients with a malignancy. In these patients a total of 226 malignancies were described and the majority (149 or 66%) were carcinomas (Flanagan and Cunniff, 1993). The most common solid tumor in BS is colorectal carcinoma, which occurs at a median age of 35 (range 16–49) years (Cunniff et al., 2017). BS is often mentioned among the other DNA repair syndromes Ataxia Telangiectasia (A-T), Fanconi Anemia and Nijmegen Breakage Syndrome, which are all characterized by early onset cancer and an immunodeficiency (Taylor et al., 2019). Besides a similar predisposition to malignancies, these other DNA repair syndromes are all

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extremely susceptible to the toxicity of several routinely recommended cancer treatments. Therefore, for each of these syndromes specific recommendations exist for the use of chemo- and radiotherapy (Mehta et al., 2017; Pastorczak et al., 2016; Schoenaker et al., 2016). However, for BS there is no such protocol (Cunniff et al., 2018). According to a recent published article of several patient societies, a BS adapted approach is of great importance for the BS community (Campbell et al., 2018). Even though, the treatment of malignancies in BS has not been studied systematically, most treating physicians have used reduced or omitted (weight-based) doses of chemotherapy and radiotherapy to avoid extreme toxicity. Here we present two clinical cases of BS with solid tumors to address the therapeutic dilemmas involved and show *in vitro* data on radiosensitivity in cultured skin fibroblasts of BS. The aim of this study is to share our clinical experiences of treating patients with BS and a solid tumor, and to present the results of studies on radiosensitivity in cultured skin fibroblasts of BS patients.

2. Methods

2.1. Clinical data

The Radboud University Medical Centre (in Nijmegen, The Netherlands) is a national referral center for patients with BS and A-T and offers systematic follow up with regular screening for malignancies. We reviewed clinical data of two patients with a solid malignancy and requested clinical data from other centres who follow and treat BS patients (BLM01 and BLM02). We obtained detailed clinical information on two BS patients with a solid tumor, concerning diagnosis, treatment and outcome. This study was approved by the ethical committee of the Radboud University Nijmegen Medical Center (METC number 2019–5809).

2.2. Clonogenic survival assay

To study clonogenic survival after irradiation, primary fibroblasts from BS patients were cultured in DMEM (Gibco), supplemented with 10% FCS and penicillin (100U/ml) and streptomycin (100 µg/ml). After trypsinisation cells were plated in two different concentrations: 1000–2000 cells per dish and 10,000–20,000 cells per dish. The more sparsely plated fibroblasts were used for irradiation at doses 0.1 and 2 Gy (200 kV, 5.5 mA), whereas the more densely plated cells were irradiated at 4 and 6 Gy. Afterwards, all irradiated fibroblasts were cultured at 37°. After 14 days, the cells were fixed and coloured with a solution of ethanol absolute and methylene blue. Survival assessment was performed by counting all cell colonies (defined as more than 20 cells) in the dish (van der Burg et al., 2009). As controls, we included healthy donor derived non-radiosensitive fibroblasts and Artemis-deficient patient derived radiosensitive fibroblasts. All tests were performed in duplicate and scored by two independent observers.

2.3. 53BP1 foci

In addition, 53BP1 foci were counted to determine the kinetics of DNA double strand breaks (DSBs). Fibroblasts of four BS patients, one radiosensitive A-T patient and healthy non-radiosensitive fibroblasts were seeded in petridishes and grown confluent. The cells were starved for 48 h in DMEM with 1% FCS. Cells were irradiated with 1 Gy and fixated with 2% formaldehyde at different time points after irradiation: 0 h, 1 h, 2 h, 4 h, 24 h and 72 h. Cells were incubated for 90 min with the primary antibody 53BP1 and subsequently with a secondary antibody (Goat anti-rabbit Alexa Fluor 488). Afterwards, at each time point the number of cell foci was counted by fluorescence microscopy by two independent observers.

3. Results

3.1. Case series

BLM01 and BLM02, described in detail below, were irradiated for their tumors because at that time the diagnosis BS was not made yet. The treating clinicians who exposed their patients to radiation observed that both patients tolerated the radiotherapy well and had relative mild side-effects (Table 1).

3.1.1. BLM01

Patient 1 had a small stature, pulmonary bronchiectasis, and pulmonary bleeding requiring embolisation. Her family history listed two sisters deceased from gastric cancer at 28 years and ovarian cancer at 34 years respectively, and several other family members with cancer at a young age. Prior to the diagnosis of BS, she had already had several malignant tumors that were treated according to standard protocols. At 40 years of age, she suffered from her first malignancy, a T1N0 gingival carcinoma, that was primarily treated with photodynamic therapy. The recurrent tumor was treated with resection and post-operative radiotherapy on the right neck (46 Gy in 2 Gy fractions, with a sequential boost with 56 Gy in 2 Gy fractions). At 41 years she had a Dukes-C sigmoid carcinoma, treated with resection and adjuvant chemotherapy (oxaliplatin, leucovorin and 5-FU, with dose reductions up to 50% during treatment because of neutropenia with fever and stomatitis). At 49 years a triple negative breast cancer T1N0M0 was diagnosed, that was treated with local resection and radiotherapy of the breast (46.2 Gy in 21 fractions to the breast, with an integrated boost with 55.86 Gy to the tumor area). In the years thereafter, several benign bowel polyps and a dysplastic gastric polyp were removed. At 48 years she was diagnosed with BS based on bi-allelic mutations in the *BLM* gene detected by whole exome sequencing. At 52 years she presented with a T2aN0M0 non-small cell lung cancer, irresectable due to severe pulmonary bronchiectasis. Based on the clinical observation that prior standard radiotherapy regimens were tolerated well despite the underlying diagnosis of BS (no signs of severe late damage like pigmentation or telangiectasia in irradiated skin areas, no severe fibrosis, no severe oral complications), she was treated with standard dose stereotactic radiotherapy aiming to achieve maximum local control (56 Gy in 3 fractions). This treatment was also tolerated well, without unusual regional radiotherapy effects and with an adequate response on CT-scan at 6 months. After that her condition slowly deteriorated further due to progression of bronchiectasis until her death at the age of 54, but no other tumors or complications occurred.

3.1.2. BLM02

A 30-year-old male presented with a tubulovillous adenoma and an adenocarcinoma in the sigmoid (stage T4N1). The patient was treated according to a regular protocol that included surgery, chemotherapy (capecitabine) and radiotherapy (25 × 2 Gy). After that, the clinical diagnosis BS was made by a clinical geneticist based on his slightly dysmorphic appearance, and subsequently genetically confirmed. He developed erythema of the skin due to radiotherapy toxicity. He has been cancer free for six years now without any signs of significant late toxicity of the radiotherapy.

3.2. Sensitivity towards ionizing radiation

To determine the degree of radiosensitivity of normal tissues for ionizing radiation, a clonogenic survival assay was performed (van der Burg et al., 2006). Available fibroblast cell lines of four patients with BS were compared to healthy control fibroblasts and Artemis-deficient fibroblasts, the latter being known for its severe radiosensitivity (Fig. 1). BS fibroblasts showed no (n = 1) to mild (n = 3) increased radiosensitivity; whereas the Artemis-deficient fibroblasts showed severe radiosensitivity.

Table 1
Overview of *BLM* mutations, solid tumors and treatment in the cases.

Case	sex	mutations	malignancy	age at diagnosis	surgery	chemotherapy	radiotherapy	remission	side-effect	
BLM01	F	c.3855C>G p. (Tyr1285*)	Gingival carcinoma (T1N0M0)	40	resection and photodynamic therapy resection		yes 28 × 2 Gy	yes		
			Dukes-C sigmoid carcinoma	41		yes			neutropenia, fever, stomatitis	
			Breast cancer (T1N0M0)	49		local resection			yes 21 × 2.66 Gy	yes
			Non-small cell lung cancer	52		not possible, due to bronchiectasias			yes 3 × 18 Gy	6 months free
BLM02	M	98 kb del 15q26.1 c.3558+1G>T	Adenocarcinoma sigmoid (T4N1M0)	30		yes (capecitabine)	yes 25 × 2 Gy	multiple yrs in remission	Skin erythema	

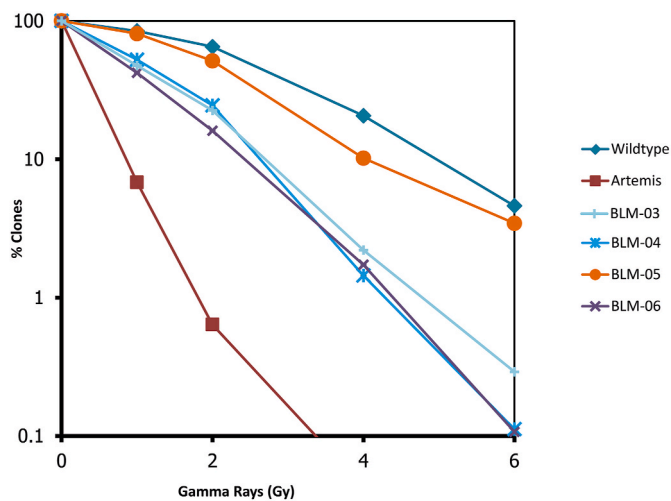


Fig. 1. Clonogenic survival assay of a non-radiosensitive control fibroblast cell line (WT), a radiosensitive Artemis-deficient fibroblast cell line and fibroblast cell lines of four patients with BS (BLM 3,4,5 and 6). The percentage of clones were determined two weeks after irradiation with increasing doses of irradiation.

3.3. DSB repair kinetics

To determine the DSB repair kinetics, the number of 53BP1 foci was counted 1, 2, 24 and 72 h after irradiation in fibroblasts of BS patients, in healthy control fibroblasts and in radiosensitive fibroblasts from an Artemis-deficient and A-T patient. The four BS fibroblast cell lines displayed the same DSB repair kinetics as the control, which is characterized by a strong increase in the number of 53BP1 until 1 h after irradiation and a decrease to zero after 72 h, indicating complete repair of DSBs (Fig. 2, for primary data see supplemental 1). In contrast, in the radiosensitive fibroblast lines (Artemis-deficient and A-T), where the initial repair kinetics seems normal, some unrepaired DSBs remain present after 48 h. The results of the Artemis-deficient and A-T cell lines are consistent with previously published data (Noordzij et al., 2003). In summary, BS fibroblasts show a similar pattern as the non-radiosensitive healthy control.

4. Discussion

BS is a very rare disease with only scarce information on treatment of solid tumors, although their occurrence is the major cause of death in this syndrome. This study describes two BS patients with malignancies, the effects of radiotherapy, and the degree of radiosensitivity tested by *in vitro* experiments.

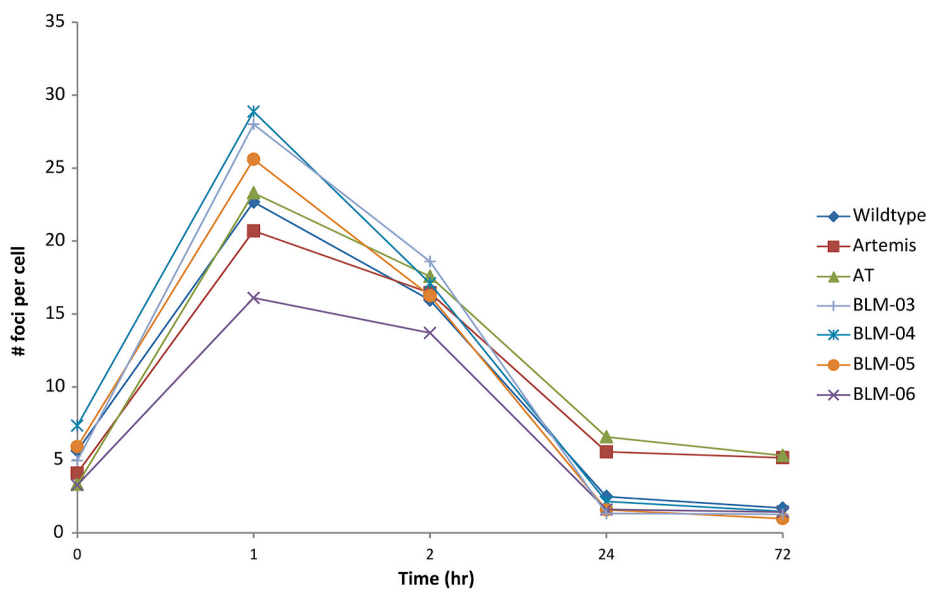


Fig. 2. DSB repair kinetics. The number of 53BP1 foci per cell was determined in a non-radiosensitive control fibroblast cell line, a radiosensitive Artemis-deficient fibroblast cell line and fibroblast cell lines of four patients with BS (BLM 3,4,5 and 6). Per fibroblast line, a total of 40 cells were counted by two independent observers.

First of all, these cases demonstrate the importance of considering cancer predisposition syndromes like BS in young patients presenting with (multiple) solid tumors. Especially, in the context of a complex medical history, such as growth retardation or a neurological disorder. The diagnosis is essential for considering syndrome adapted treatment. For Ataxia Telangiectasia, Fanconi Anemia and Nijmegen Breakage Syndrome reduction of recommended dosage was advised in recent publications (Dembowska-Baginska et al., 2009; Schoenaker et al., 2016). For BS little is known about optimal dosages of both chemo- and radiotherapy. In the absence of consensus guidelines, most Case reports consider dose-reduction for safety reasons. In the international literature, radiotherapy in patients with BS and solid carcinomas is only rarely described (Table 2). (Bouman et al., 2018; Cairney et al., 1987; Goudge et al., 2007; Jain et al., 2001; Kataoka et al., 1989; Ma et al., 2001; Martinez et al., 2016; Mizumoto et al., 2013; Moreira et al., 2013; Thomas et al., 2008) BLM01 and BLM02, who were not yet diagnosed with BS at the time of cancer diagnosis, show that radiation was tolerated well in these two cases. In this context, it is of particular relevance that in BLM01 the radiotherapy was applied by hypofractionation, i.e. fewer fractions with a higher dose per fraction. For the breast cancer treatment of this patient 21 fractions of 2.66 Gy were used, whereas with conventional fractionation the dose per fraction is 1.8–2.2 Gy. For the bronchus carcinoma stereotactic treatment was applied with an even higher doses of 18 Gy. With these high doses much more instant DNA damage is caused compared to conventional fractionation. Despite this, no unusual toxicity was noted in this patient. Although these cases tolerated this therapy well, a definitive conclusion cannot be drawn based on these observations.

To further explore the effects of radiotherapy in BS we performed *in vitro* irradiation on fibroblasts of 4 BS patients. In this study, BS fibroblasts show less radiosensitivity than radiosensitive cells of a patient with Artemis deficiency using two different assays, w the clonogenic survival assay (CSA) and 53BP1 foci. In previous articles, some authors tested a small number of BS cell lines and have shown a mild radiosensitivity in these cells. Two BS cell lines were irradiated with 0, 1, 2, 3 and 4 Gy and measured at a single time point 72 h later. These cell lines were compared to very radiosensitive A-T cells and non-radiosensitive healthy controls and classified as mildly radiosensitive (Beamish et al., 2002). Joubert et al. tested some DNA repair disorders, including BS and A-T, for a survival fraction at 2 Gy. BS was classified as intermediate

radiosensitive, in contrast to A-T (high radiosensitivity) (Joubert et al., 2008).

Some clinical Case reports mention, in contrast to our cases and laboratory data, toxicity from radiation. Kataoka et al. reported an esophageal stricture caused by radiation in a 38 year old male with BS who had a squamous cell carcinoma of the bronchus and was treated with a total dose of 50.4Gy (Kataoka et al., 1989). Two months after radiotherapy the patient suffered from dysphagia and radiography showed progressive narrowing of the esophageal lumen. Eventually transabdominal tubal feeding was required. Of note, however, esophageal stricture is reported in 3% of all patients after radiation of the head and neck area in the general population and thus might be unrelated to the underlying BS. Especially dosages above 45 Gy are identified as a risk factor for esophageal strictures (Ahlberg et al., 2010). A second BS patient was treated with proton beam therapy with 59.4Gy in 33 fractions in 71 days and had grade II mucositis which is also expected for patients without BS (Mizumoto et al., 2013). Both examples of toxicity are well known complications of radiotherapy in any patient. Secondary tumors, as a consequence of the administered radiotherapy, have not been described. Altogether, our two cases and *in vitro* data challenge the current paradigm that radiotherapy is absolutely contraindicated in BS patients. Future clinical and laboratory studies in more BS patients will help form better guidelines on both safe and effective treatment of cancer in BS patients.

Besides radiotherapy as therapeutic treatment, solid tumors are often treated with chemotherapy, especially when the cancer is more advanced (Burbach et al., 2016). increased toxicity after chemotherapy in BS has been described in malignancies in some of the Case reports for other malignancies, mostly leukemias and lymphoma (Adams et al., 2013; Emir et al., 2013). Wilm's tumors are the only reported solid tumors in BS patients that have been treated with chemotherapy. (Cairney et al., 1987; Jain et al., 2001). Both clinical data and *in vitro* studies on chemotherapy in BS patients are lacking. The effect of chemotherapy on BS cells has been studied for camptothecin, cisplatin, and 5 fluorouracil for the development of biomarkers for chemotherapeutic response and these biomarkers showed an increased reaction (Kohzaki et al., 2007; Mao et al., 2010). Laboratory studies suggest cautiousness for the use of cisplatin in combination with radiotherapy. Cisplatin has been used in some cases in the available literature (Table 2). Currently, evidence-based recommendations for the exact dosage of chemotherapy

Table 2
Overview of BS patients with solid tumors treated with radiotherapy in the available literature.

Publication	sex	malignancy	age at diagnosis	age at death	surgical resection	Chemotherapy	radiotherapy	remission	side-effects	survival
Ma2001	F	Squamous ca oropharynx T2N2cM0	33	died several months after completion therapy	no	cisplatin, 5-FU	yes 60 Gy	no	erythema skin and mucositis (treatment well tolerated)	5 months (progression disease)
Bouman et al. Eur J Med Genet	F	Nasopharyngeal carcinoma	36	died		cisplatin	yes, 70 Gy	no	acute renals insufficiency, pancytopenia. Mucositis and colitis after start cisplatin	A few weeks
Mizumoto2013	F	Poorly differentiated squamous cell carcinoma oropharyngeal T2N2bM0	32	died 9 months after start treatment	no		proton beam therapy, 59,4 GyE in 33 fractions		mucositis grade II, decreased dietary intake, grade II dermatitis	9 months (deterioration after lung metastasis)
Kataoka1989	M	Squamous cell carcinoma left lung	38	died, 18 months after treatment			yes 50.4 Gy in a split-course schedule	?	oesophageal stricture	18 months
Cairney J. pediat. 1987	F	Wilms tumor stage III	8 yrs	survived	yes	vincristine, actinomycineD; doxorubicin, cyclophosphamide	yes 10 Gy	yes		

in BS do not exist. The chemotherapy dosage will be determined by clinical condition, the general protocol for non-BS patients and the preferences of patients.

Severe limitations of this study are the small number of patients and the small amount of patient material. Therefore, clinicians should interpret these conclusions with caution when deciding on the application of radiotherapy in BS patients. Ultimately, the clinical condition of a BS patient is most important in this decision process. The strength of this study is that it presents cases of a very rare syndrome of which two were treated with radiotherapy and it has supportive laboratory tests to confirm clinical observations.

In conclusion, we show that two BS patients tolerated radiotherapy in normal dosages relatively well, and that the fibroblasts of 4 BS patients showed mild to no radiosensitivity at all. This suggests that further studies are needed to optimize evidence based treatment protocols for solid cancers in BS, in particular when using radiotherapy.

Author statement

MS, IP, CW and MB designed the experiments. MS, ST and IP performed experiments. MS, CW, MB and JK analyzed the results and wrote the manuscript. CD, WV and MD wrote Case reports. CD, SH, WV, MD and MW reviewed and edited the manuscript. CW, MB and JK supervised the whole process.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejmg.2021.104293>.

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