Review article

Oxidative stress, mitochondrial abnormalities and antioxidant defense in Ataxia-telangiectasia, Bloom syndrome and Nijmegen breakage syndrome

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Mateusz Maciejczyk[a,*], Bozena Mikoluc[b], Barbara Pietrucha[c], Edyta Heropolitanska - Pliszka[c], Malgorzata Pac[c], Radosław Motkowski[b], Halina Car[a]

[a] *Department of Experimental Pharmacology, Medical University of Bialystok, Szpitalna 37 Str., 15-295 Bialystok, Poland*
[b] *Department of Pediatrics, Rheumatology, Immunology and Metabolic Bone Diseases, Medical University of Bialystok, Waszyngtona 17 Str., 15-274 Bialystok, Poland*
[c] *Department of Immunology, The Children's Memorial Health Institute, Av. Dzieci Polskich 20, 04-730 Warsaw, Poland*

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ABSTRACT

Rare pleiotropic genetic disorders, Ataxia-telangiectasia (A-T), Bloom syndrome (BS) and Nijmegen breakage syndrome (NBS) are characterised by immunodeficiency, extreme radiosensitivity, higher cancer susceptibility, premature aging, neurodegeneration and insulin resistance. Some of these functional abnormalities can be explained by aberrant DNA damage response and chromosomal instability. It has been suggested that one possible common denominator of these conditions could be chronic oxidative stress caused by endogenous ROS overproduction and impairment of mitochondrial homeostasis. Recent studies indicate new, alternative sources of oxidative stress in A-T, BS and NBS cells, including NADPH oxidase 4 (NOX4), oxidised low-density lipoprotein (ox-LDL) or Poly (ADP-ribose) polymerases (PARP). Mitochondrial abnormalities such as changes in the ultrastructure and function of mitochondria, excess mROS production as well as mitochondrial damage have also been reported in A-T, BS and NBS cells. A-T, BS and NBS cells are inextricably linked to high levels of reactive oxygen species (ROS), and thereby, chronic oxidative stress may be a major phenotypic hallmark in these diseases. Due to the presence of mitochondrial disturbances, A-T, BS and NBS may be considered mitochondrial diseases. Excess activity of antioxidant enzymes and an insufficient amount of low molecular weight antioxidants indicate new pharmacological strategies for patients suffering from the aforementioned diseases. However, at the current stage of research we are unable to ascertain if antioxidants and free radical scavengers can improve the condition or prolong the survival time of A-T, BS and NBS patients. Therefore, it is necessary to conduct experimental studies in a human model.

1. Introduction

Ataxia-telangiectasia (A-T; OMIM #208900), Bloom syndrome (BS; OMIM #21090) and Nijmegen breakage syndrome (NBS; OMIM #251260) are rare pleiotropic genetic disorders with the prevalence of 1 per 40,000–100,000 [1–3]. They belong to a group of chromosomal breakage syndromes (CBS) and are characterised by an increased rate of chromosomal rearrangements and genomic instability, which

may arise either spontaneously or as a consequence of exposition to various DNA-damaging factors such as ionising radiation (IR), external radiomimetic chemicals and/or ultraviolet (UV) light [3–8]. Indeed, cells obtained from A-T, BS and NBS patients show signs of DNA injury, cytoskeletal and chromatin reorganisation, defective or excessive apoptotic cell death and aberrant cell cycle checkpoint function [3,5,9–11]. Although the type of DNA injury is different in each syndrome (A-T results from biallelic mutations in the *Ataxia Telangiectasia Mutated* gene *(ATM)*; BS is caused by the *BLM* gene; NBS is caused by the *NBN* gene), all of them are inherited in an autosomal recessive pattern and exhibit a few similar clinical features including growth retardation, premature ageing, progressive cerebral degeneration, variable immunodeficiency, characteristic telangiectasias and higher cancer susceptibility [3,6,11]. Some of these functional abnormalities can be explained by the aberrant DNA damage response and/or genomic instability [12]. Reports suggest that A-T, BS and NBS multifaceted phenotypes are inextricably linked to high levels of reactive oxygen species (ROS) and thereby constant activation of cellular stress response pathways [9,13], and also impairment of antioxidant defenses [1,2,13]. In view of the fact that supplementation with ROS scavengers may alleviate and/or delay some of the symptoms of these diseases [12,14–16], it has been proposed that endogenous ROS overproduction may play an important role in the molecular basis of A-T, BS and NBS, especially in the context of mitochondrial anomalies [2,13].

Considerable attention has been paid in the literature to the involvement of ROS-mediated chromosomal abnormalities in DNA repair and maintenance [13]. Several studies have also indicated the role of mitochondrial alterations in a number of oxidative stress-related genetic disorders, e.g. Cockayne Syndrome (CS), Down Syndrome (DS), Fanconi anaemia (FA), Hutchinson-Gilford syndrome (HGS), Werner syndrome (WS), Xeroderma pigmentosum (XP) and in the aforementioned A-T, BS and NBS [13,17]. All of these disorders are also classified as premature ageing (progeroid) syndromes and are characterised by genomic instability, degeneration of the central nervous system, dysfunction of cutaneous appendices, heightened risk of malignancies as well as increased susceptibility to infection and immunological senescence [3]. It has been suggested that one possible common denominator of these conditions could be constant oxidative stress (Fig. 1) caused by endogenous ROS overproduction and impairment of mitochondrial homeostasis. Although the first paper on this topic was published in 1983 [18], knowledge of the role of oxygen metabolism in the pathophysiology and clinical manifestations of A-T, BS and NBS as well as their multiple complications is still limited. It is clear that a better understanding of pro-oxidant and antioxidant homeostasis may provide relevant information about the essence of these diseases and may possibly represent a new therapeutic strategy for patients. Therefore, the purpose of this mini-review is to examine the latest findings concerning the relationship between oxidative stress, mitochondrial dysfunctions and antioxidant defense in patients with Ataxia-telangiectasia, Bloom syndrome and Nijmegen breakage syndrome.

2. Ataxia-telangiectasia, ATM protein kinase and oxidative stress

A-T is a recessively inherited disorder caused by null mutations in the *Ataxia Telangiectasia Mutated* (*ATM*; 607585) gene. *ATM* encodes a high molecular weight (~350 kDa) multifunctional serine/threonine nuclear kinase [5,19] which possesses a C-terminal protein kinase domain and belongs to the large PIKK (phosphoinositide 3-kinase related kinase) family [20–22]. This group also includes ATR (ATM and Rad3-related), DNA-PKcs (DNA-dependent protein kinase catalytic subunit), mTOR (mammalian target of rapamycin), and SMG-1 (suppressor with morphological effect on genitalia family member) protein [19,20]. Three of these (ATM, ATR, DNA-PKcs) are considered

sensors of DNA injury and play an essential role in maintaining the integrity of the human genome [20]. ATM, a central mediator in the DNA damage response (DDR) pathway, is responsible for signalling and repair of ionising radiation (IR)-induced double strand breaks (DSBs) in DNA, the most cytotoxic and lethal form of DNA injury [4]. The ATM protein kinase participates therefore in multiple signal-transduction pathways and cellular networks, including control of gene expression, replication, recombination, apoptosis, and cellular senescence which occurs through the phosphorylation of numerous intermediary proteins, e.g. p53, Chk2, Brca1, and Nbs1 [4,10,15,19,20,22]. A fraction of ATM is also localised in mitochondria [12] and ATM plays a crucial role in the anti-oxidative response to enhanced ROS levels and accumulated oxidative DNA damage [23–26]. ATM can therefore be regarded as an important part of the cellular redox regulatory system [21,22,27].

Cells obtained from A-T patients are characterised by extreme sensitivity to DSBs-inducing agents including exposure to ionising radiation (IR) and various radiomimetic chemicals (e.g. bleomycin and neocarzinostatin). Importantly, in patients with A-T either the DNA repair of DSBs is defective or detection of their presence in the cell is impaired [4,21]. Furthermore, loss or inactivation of the ATM protein kinase is associated with an inadequate response to the p53 protein or c-Abl tyrosine kinase [4,21,22,27] and contributes to the development of T-cell malignancies [12]. Therefore, extreme radiosensitivity, one of the main features of A-T cells, may reflect an innate susceptibility to increased oxidative stress caused by the accumulation of oxidative-damaged DNA and defects in DNA repair mechanisms [9,27]. Cellular DNA may also be affected by normal metabolic byproducts, among which ROS appear to play a major role in all living organisms [28].

The rate of ROS production depends on the cell's changing physiopathological conditions and is determined by the balance of many endogenous and exogenous pro-oxidant agents [29]. In order to prevent oxidative damage, the cell's redox equilibrium is maintained by remarkably efficient enzymatic and non-enzymatic antioxidants such as catalase (CAT), superoxide dismutases (SOD1 and SOD2), glutathione peroxidases (GPx), vitamin A, C and E or ubiquinone (Q_{10}) [28,30–32].

Relatively little research has been conducted on the role of oxidative stress in the cellular and clinical manifestations of A-T disease. In addition, the majority of studies have been performed only on cell lines or animal models (*Atm*-deficient (*Atm$^{-/-}$*, *Atm$^{y/y}$* and *Atm*-ΔSRI) mice). Early studies examined the response of A-T cells to oxidative stress induced by hydrogen peroxide (H_2O_2) and the response of inflammatory cells (neutrophils) activated with 12-O-tetradecanoyl-phorbol-13-acetate (TPA) or the ROS-generating (H_2O_2 and superoxide radical ($O_2^{•-}$) enzymatic xanthine/xanthine oxidase (X/XO) system. Yi et al. [33] and Ward et al. [34] demonstrated that A-T fibroblasts were more vulnerable to oxidative injury than normal cells. The authors speculated that chromosomal damage in A-T patients may be caused by endogenous sources of ROS (e.g. hydrogen peroxide and superoxide anion) and such sources may play an important role in spontaneous tumors [33]. An alternative hypothesis may be a defect in glutathione metabolism [18], decreased catalase (CAT) activity [35], defective DNA damage repair by ROS and oxygen free radical attack [18,33] as well as exogenous sources of ROS and their oxygen metabolites [33]. It is well known that ROS possess a large oxidising capacity and in adverse conditions may lead to permanent changes in the structure and function of both purine or pyrimidine bases as well as the deoxyribose backbone of DNA [28,31,36]. Ward et al. [34] claim that the genotype of A-T cells affects their response to DNA damaging sources through inflammatory processes, which can also be regarded as a major source of oxidative stress.

Neurodegeneration and cancer susceptibility are the most debilitating manifestations of A-T disease and a major cause of death in A-T patients [37]. Kamsler et al. [38] and Barlow et al. [39] demonstrated ROS overproduction in brain tissue of *Atm*-deficient mice. The authors showed increased activity of thioredoxin in Atm (-/-) cerebella, changes
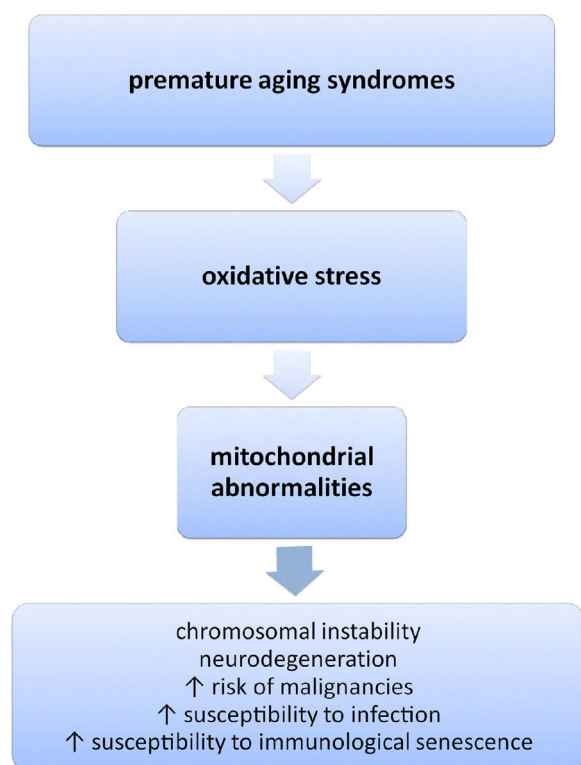
Fig. 1. Potential involvement of oxidative stress and mitochondrial abnormalities in the pathogenesis of premature ageing (progeroid) syndromes. Many data indicate that premature ageing (progeroid) syndromes: Cockayne Syndrome (CS), Down Syndrome (DS), Fanconi anaemia (FA), Hutchinson-Gilford syndrome (HGS), Werner syndrome (WS), Xeroderma pigmentosum (XP), Ataxia-telangiectasia (A-T), Bloom syndrome (BS) and Nijmegen breakage syndrome (NBS) may be considered as oxidative stress-related genetic disorders. It is believed that many pathologies such as chromosomal instability, neurodegeneration, dysfunction of cutaneous appendages, heightened risk of malignancies as well as increased susceptibility to infection and immunological senescence may result from chronic oxidative stress and oxidative damage caused by endogenous ROS overproduction, especially in the context of mitochondrial anomalies.

in the levels of thiol-containing compounds in the cerebella and cerebra of Atm (+/+) and (-/-) mice including one of the most important brain antioxidant – reduced glutathione (GSH) and its precursor – cysteine. They also demonstrated that alternations in the activity of enzymatic antioxidants (↓CAT and ↑Mn-SOD) may be the result of a response to superoxide anion overproduction ($O_2^{\cdot-}$) and may lead to increased levels of endogenous hydrogen peroxide (H_2O_2). Additionally, it can be assumed that an increase in GSH content, as well as higher thioredoxin and Mn-SOD activity, may be a major compensatory mechanism associated with elevated oxidative stress in the brain of *ATM*-deficient mice. In addition, significantly increased activity of the antioxidant enzyme heme oxygenase (HO), combined with enhanced levels of nitrotyrosine, a biomarker of oxidative nitric oxide (NO) damage, indicate cellular redox disturbances in the cerebellum of Atm (-/-) mice [39]. One can therefore assume that the severity of oxidative stress and oxidative damage is different in different tissues of the *ATM-*

deficient brain and oxidative stress may play an important role in A-T neurodegeneration, particularly cerebellar degeneration, which is linked to ATM deficiency.

It is well known that A-T cells are particularly sensitive to ROS-generating agents (e.g. IR, hydrogen peroxide (H_2O_2), nitric oxide (NO) as well as *t*-butyl hydroperoxide [7,40,41]) resulting in oxidative stress and oxidative damage to important biomacromolecules and cell structures [42]. In contrast to exogenous factors, the well-established endogenous ROS-generating sources include the metabolism of cytochrome P450, microsomes and peroxisomes as well as the activities of xanthine oxidase (XO), NADPH oxidase, lipoxygenases (LOX) and peroxidases (Px) [28,31]. However, one of the main sources of ROS is the mitochondrial electron transport chain (mtETC) and therefore, mitochondria are the most vulnerable to oxidative injury [28,31,32]. Recently, a growing body of research has indicated a link between mitochondrial alternations and higher oxidative stress in A-T cells (Table 1) [13,17,43]. ATM inactivation is known to be linked with oxidative stress in A-T cells, but the precise mechanism of these changes is not fully understood. Moreover, there is no conclusive evidence that ATM kinase is activated by mitochondrial dysfunction through the increased mitochondrial reactive oxygen species (mROS) production. Ambrose et al. [6] explored the hypothesis that mitochondrial dysfunction may directly contribute to an elevated oxidative stress level in A-T cells. In the study, the authors visualised the mitochondria from A-T lymphoblastoid cell lines (LCLs), determined their membrane potential ($\Delta\psi_m$) and mitochondrial respiratory function as well as the expression of mtDNA repair and various ROS detoxifying genes (e.g. *mitochondrial topoisomerase I (TOP1mt), mitochondrial peroxiredoxin 3 (Prx 3, thioredoxin peroxidase-2), polymerase gamma (POLG) and SOD-2*). The study demonstrated abnormal structural organisation of mitochondria in A-T cells, a reduction of their $\Delta\psi_m$ and total respiratory activity and increased expression of mitochondrial-targeted *TOP1mt, Prx 3, POLG and SOD2* genes [6]. However, the number of mitochondria in A-T cells did not differ from the control group. In addition, the mitochondrial-specific antioxidant supplementation with α-lipolic acid (ALA) led to an increase in the respiration rates of A-T cells as compared to wild-type (WT) cells [6]. Overall, the presented data provide evidence that ATM may directly or indirectly modulate mitochondrial homeostasis and is associated with continuous oxidative stress in A-T patients.

Kalifa et al. [44] demonstrated that double strand breaks (DSBs) in DNA activated ATM kinase in the absence of mitochondrial alternations in A549 human lung adenocarcinoma cells. The authors believe that mitochondrial DNA (mtDNA) damage may promote their repair and lead to reduced mROS production and mitochondrial dysfunctions [44]. In contrast, Kobayashi et al. [26] reported that elevated oxidative stress levels, expressed as excessive concentrations of endogenous ROS from mitochondrial defects, reduced ATM activation and impaired homologous recombination (HR) repair in AOA3 (ataxia with oculomotor apraxia type 3) lymphoblastoid cells. Resseguie et al. [45] showed that prolonged exposure to hyperoxia in A549 cells activated ATM kinase, irrespective of mitochondrial alternations and the accumulation of mROS. They also demonstrated that ATM may regulate enhanced ROS production during hyperoxia regardless of elevated

Table 1
Recent advances on the role of oxidative stress in Ataxia-telangiectasia (A-T).

| Cells	Endpoints	Refs.
Lymphoblastoid cell lines from A-T patients	aberrant structural organisation of mitochondria, ↓ $\Delta\psi_m$, ↓ total respiratory activity, ↑ TOP1mt, ↑ Prx 3, ↑ POLG, ↑ SOD2	[6]
Thymocytes and fibroblasts from ATM-null mice	aberrant structural organisation of mitochondria, ↑ mROS, ↑ respiratory capacity, ↓ mitophagy	[12]
Fibroblasts from A-T patients	↓ heme oxygenase−1 (HO−1), ↑ intracellular ROS, ↑ oxidative stress-induced apoptosis	[46]
Fibroblasts from primary A-T fibroblast cell line	ox-LDL-induced ↑ ROS, ↑ DSBs, ↑ chromosome breaks, ↑ micronuclei, ↓ cell viability	[47]
Fibroblasts from A-T patients	↑ NADPH oxidase 4 (NOX4), ↑ oxidative DNA damage	[48]
Fibroblasts from ATM-defective cell line	↑ mROS, ↑ mitochondrial mass, ↓ $\Delta\psi_m$, altered mitophagy	[66]
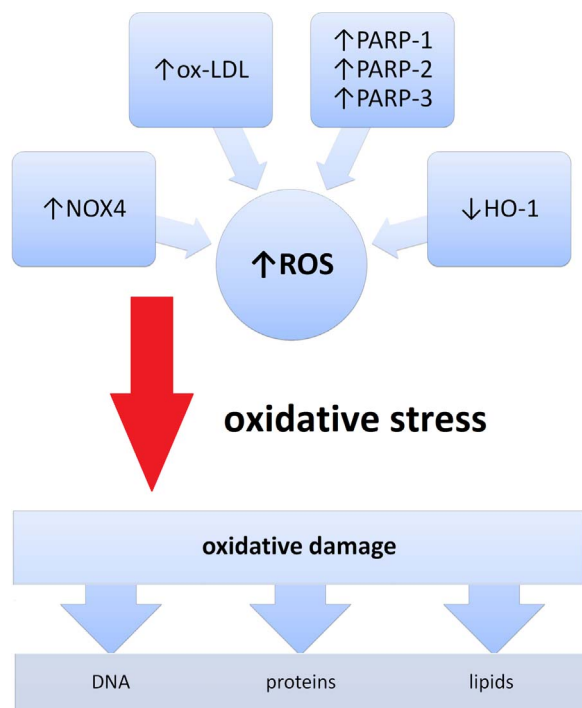
Fig. 2. New sources of endogenous ROS overproduction in Ataxia-telangiectasia (A-T), Bloom syndrome (BS) and Nijmegen breakage syndrome (NBS) and their role in oxidative cellular damage. Recent studies indicate new sources of endogenous ROS overproduction in A-T, BS and NBS cells including NADPH oxidase 4 (NOX4), oxidised low-density lipoprotein (ox-LDL) or Poly (ADP-ribose) polymerases (PARP-1, PARP-2, PARP-3), which may contribute to oxidative damage to major cellular components such as nucleic acids (DNA mutations), proteins (protein oxidation) and lipids (lipid peroxidation).

mROS levels. Yasmine et al. [12] also emphasised that ATM played a major role in both the cellular response to DNA-DSBs as well as mitochondrial homeostasis. Therefore, A-T may be considered a mitochondrial disease, similarly to other progressive neurodegenerative disorders [12,32]. It can be assumed that the observed decrease in ATP levels and a decline in the activity of complex I of mtETC may be associated with increased mROS production in A-T cells. Additionally, the authors demonstrated a significant increase in the number of altered mitochondria in A-T thymocytes with a simultaneous increase in their mass and elevated mtDNA content. The authors suggested that it may be due to abnormal mitochondrial autophagy (mitophagy), but presumably not to mitochondrial biogenesis [12]. These data support the hypothesis that *ATM* loss is indeed associated with intrinsic mitochondrial defects in thymocytes and T-cells, including altered structural organisation of mitochondria, high levels of mROS production, elevated respiratory capacity and decreased mitophagy as well as contributes to the cancer-prone phenotype in A-T cells [12]. Previous studies have also demonstrated elevated ROS levels in $ATM^{-/-}$ cells, but their source has not been determined yet (Table 1).

In one of the recent works, Hoon Yu et al. [46] reported that ATM protein kinase induced biliverdin-producing enzyme heme oxygenase-1 (HO-1) in cells exposed to hydrogen peroxide (H_2O_2). They also showed that a loss of HO-1 induction in A-T cells resulted in an increase in DNA fragmentation and cell death. Additionally, transfection of ATM induced HO-1 expression through the activation of protein kinase C (PKC-δ) and nuclear factor-κB (NF-κB). It also reduced elevated intracellular ROS levels and oxidative stress-induced apoptosis in A-T cells. This may indicate why A-T patients are particularly sensitive to oxidative stress and ROS-mediated oxidative DNA damage [46]. HO-1 protects cells against higher oxidative stress and may therefore be considered a major antioxidant target in A-T patients. On the other hand, Semlitsch et al. [47] demonstrated that a specific

oxidation product, ox-LDL (oxidised low-density lipoprotein), induced the phosphorylation of ATM and downregulated p21 expression in normal fibroblasts and endothelial cells. They also showed that ox-LDL was responsible for chromosomal instability, increased ROS production, and reduced cell viability in A-T cells. They concluded that ox-LDL played an important role in oxidative stress-mediated DNA injury in A-T cells. Supplementation with exogenous antioxidant and NF-κB inhibitor PDTC (ammonium pyrrolidinedithiocarbamate) resulted in a decrease in ROS levels in ATM-deficient cells [47].

Interesting data concerning alternative sources of ROS in A-T were published by Weyemi et al. [48], who showed that NADPH oxidase 4 (NOX4) may be a key sensor of oxidative stress in A-T cells. In the study, the authors evaluated a relationship between ATM deficiency, oxidative DNA damage, neurodegeneration and cancer susceptibility in A-T disease. They demonstrated that NOX4, which constitutively produces ROS in a gamut of cell types and tissues as well as plays a crucial role in oxidative DNA damage and the subsequent senescence [49], was highly up-regulated in A-T patients, similarly to normal cells with a lack of ATM protein kinase [48]. Additionally, NOX4 correlated with higher oxidative damage and apoptosis. The study demonstrated that a 10-fold increase in the concentration of 8-hydroxy-2-deoxyguanosine (8-OHdG) in A-T fibroblasts, as compared to normal cells, while silencing the expression of NOX4 reduced the level of oxidative DNA lesions by approximately 60%. The authors also showed that administration of fulvene-5, a specific inhibitor of NOX4 and NOX2 (NADPH oxidase 2), significantly reduced ROS levels, DNA breaks and oxidative DNA damage in A-T primary cells. Furthermore, inactivation of NOX4 was found to be responsible for a decrease in cancer incidence (lymphoma) in ATM-deficient mouse in comparison with the control group. As a major member of the NOX/DUOX family of NADPH oxidases, NOX4 may therefore be considered a crucial mediator in A-T multifaceted phenotype, together with chronic oxidative stress from other potential cellular sources. Hence, it can be assumed that NOX4 may prove a therapeutic strategy in A-T disease, particularly in progressive cerebellar degeneration (Fig. 2) [48].

3. Antioxidant defense and oxidative damage in Ataxia-telangiectasia

To date, there have been few studies evaluating the oxidant/antioxidant status in A-T, BS and NBS patients (Tables 2 and 3). In one of the first reports on the subject, Reichenbach et al. [50] observed a decrease in the levels of total antioxidant capacity (TEAC; ↓77% of normal capacity) and major plasma lipophilic antioxidants: vitamin A (retinol) and vitamin E (α-tocopherol) in A-T patients. In contrast, the levels of reduced coenzyme Q10 (ubiquinol, an important endogenous fat-soluble antioxidant) were not significantly reduced. Although there was no correlation between age, activated T-cells and the analysed oxidative stress parameters, it can be assumed that A-T patients display redox equilibrium dysfunction as well as possess a reduced ability to counteract oxidative damage by ROS and other free radicals. It has been suggested that the efficiency of non-enzymatic antioxidants (retinol and α-tocopherol) may be decreased by excessive production of ROS in A-T cells [50]. A few years later Reichenbach et al. [42] demonstrated that reduced antioxidant capacity in A-T patients has consequences in the cellular redox homeostasis and affects a clinical manifestations of A-T phenotype. The authors showed significantly higher levels of total lipid peroxides and 8-OHdG in A-T patients indicating that oxidative stress indeed damages lipids and DNA in A-T cells. The authors also suggested that neurodegeneration in A-T patients may be caused by lipoperoxidation to the membrane lipids, as well as oxidative damage to the cellular proteins [42]. The elevated levels of oxidant-modified lipids (F2-isoprostanes) and proteins (3-nitrotyrosine) in the brain of *ATM*-deficient mice have previously shown Kamsler et al. [38] and Barlow et al. [39].

Da Silva et al. [51] studied the relationship between nutritional

Table 2
Antioxidant/oxidant parameters in patients with Ataxia-telangiectasia (A-T).

Antioxidant/oxidant parameter	Parameter changes	Biological material	Method	Number of patients (*n*)	Refs.
Total oxidant/antioxidant capacity					
Total antioxidant capacity (TEAC)	↓	plasma	colorimetric	10	[50]
Lipophilic antioxidants					
Retinol	↓	plasma	HPLC	10	[50]
	≈		HPLC	14	[51]
β-carotene	≈	plasma	HPLC	14	[51]
α-tocopherol	↓	plasma	HPLC	10	[50]
	≈	plasma	HPLC	9	[53]
γ-tocopherol	≈	plasma	HPLC	9	[53]
Ubiquinol (Q10)	≈	plasma	HPLC	10	[53]
Other non-enzymatic antioxidants					
Total glutathione	≈	plasma	HPLC	8	[52]
Oxidised glutathione (GSSG)	↓	whole blood	HPLC	8	[52]
Reduced glutathione (GSH)	≈	whole blood	HPLC	8	[52]
GSSG:GSH ratio	≈	whole blood	HPLC	8	[52]
	≈		HPLC	8	[53]
Glyoxal (Glx)	≈	plasma	HPLC	8	[52]
	≈		HPLC	8	[53]
Methylglyoxal (MGlx)	↓	plasma	HPLC	8	[52]
	≈		HPLC	8	[53]
Uric acid (UA)	≈	plasma	HPLC	8	[53]
Ascorbic acid (AA)	≈	plasma	HPLC	8	[53]
Enzymatic antioxidants					
Superoxide dismutase (SOD)	↑	erythrocytes	colorimetric	6	[54]
	≈		colorimetric	20	[55]
	≈	lymphocytes	colorimetric	15	[54]
Catalase (CAT)	↑	erythrocytes	colorimetric	9	[54]
	≈		colorimetric	20	[55]
	≈	lymphocytes	colorimetric	14	[54]
Glutathione peroxidase (GSH-Px)	↑	erythrocytes	colorimetric	5	[54]
	≈	lymphocytes	colorimetric	5	[54]
Glutathione reductase (GSSG-R)	≈	erythrocytes	colorimetric	5	[54]
	≈	lymphocytes	colorimetric	5	[54]
Glutathione S-transferase (GST)	≈	erythrocytes	colorimetric	5	[54]
	≈	lymphocytes	colorimetric	2	[54]
Glucose–6 phosphate dehydrogenase (G6PD)	≈	erythrocytes	colorimetric	12	[54]
	≈	lymphocytes	colorimetric	14	[54]
Oxidative modification products					
8-hydroxy–2-deoxyguanosine (8-OHdG)	≈	leukocytes	LC	8	[52]
	≈		LC	9	[53]
	↑	PBMC	HPLC-MS	6	[42]
	≈	urine	LC	9	[53]
Malondialdehyde (MDA)	≈	plasma	colorimetric	14	[51]
	≈		colorimetric	20	[55]
Total lipid peroxides	↑	plasma	colorimetric	33	[42]
Antioxidant metals					
Zinc (Zn)	≈	serum	AAS	14	[51]
	↓		ICP-MS	16	[1]
	≈	erythrocytes	AAS	14	[51]
Copper (Cu)	↑	serum	ICP-MS	16	[1]

Abbreviations: ↑ - statistically significant increase *vs* control, ↓ - statistically significant decrease *vs* control, ≈- changes not statistically significant *vs* control, AAS - Atomic Absorption Spectroscopy, HPLC - High-Performance Liquid Chromatography, ICP-MS - Inductively Coupled Plasma - Mass Spectrometry, LC - Liquid Chromatography, PBMC - peripheral blood mononuclear cells.

Table 3
Antioxidant/oxidant parameters in patients with Bloom syndrome (BS).

Antioxidant/oxidant parameter	Parameter changes	Biological material	Method	number of patients (*n*)	Refs.
Lipophilic antioxidants					
α-tocopherol	≈	plasma	HPLC	4	[62]
	≈		HPLC	4	[53]
γ-tocopherol	≈	plasma	HPLC	4	[62]
	≈		HPLC	4	[53]
Other non-enzymatic antioxidants					
Oxidised glutathione (GSSG)	↓	whole blood	HPLC	4	[62]
GSSG: GSH ratio	↓	whole blood	HPLC	4	[62]
	↓	whole blood	HPLC	4	[53]
Glyoxal (Glx)	≈	plasma	HPLC	4	[62]
	≈		HPLC	4	[53]
Methylglyoxal (MGlx)	≈	plasma	HPLC	4	[62]
	≈		HPLC	4	[53]
Uric acid (UA)	↑	plasma	HPLC	4	[53]
	↑		HPLC	4	[62]
Ascorbic acid (AA)	≈	plasma	HPLC	4	[62]
	≈		HPLC	4	[53]
Oxidative modifications products					
8-hydroxy–2-deoxyguanosine (8-OHdG)	↑	leukocytes	LC	4	[62]
	↑		LC	4	[53]
	≈	urine	LC	4	[62]
	↑		LC	4	[53]

Abbreviations: ↑ - statistically significant increase *vs* control, ↓ - statistically significant decrease *vs* control, ≈- changes not statistically significant *vs* control, HPLC - High-Performance Liquid Chromatography, LC - Liquid Chromatography.

parameters and oxidative status in patients with A-T. They also evaluated the concentration of retinol which proved to be similar to the control group. The concentrations of beta-carotene (precursor of retinol) and malondialdehyde (MDA; lipid peroxidation marker) were also comparable to the control group. On the other hand, vitamin A concentrations showed a significant positive correlation with the immunoglobulin IgA level and a negative correlation with the MDA serum level. Research conducted by Degan et al. [52] may, in turn, indicate an adaptive response to increased oxidative stress levels in A-T cells, as evidenced by decreased concentrations of blood oxidised glutathione (GSSG) and plasma methylglyoxal (MGlx). The levels of leukocyte and urinary 8-OHdG, plasma glyoxal (Glx) and other non-enzymatic antioxidants (i.e. uric acid (UA) and ascorbic acid (AA)) were similar to those in the control group [52,53]. An assessment of the enzymatic antioxidant profile was conducted by Aksoy et al. [54] who found higher activity of catalase (CAT) and superoxide dismutase (SOD) in erythrocytes, in contrast to lymphocytes. The levels of other oxidative stress markers (glucose-6 phosphate dehydrogenase (G6PD), glutathione reductase (GSSG-R), glutathione peroxidase (GSH-Px) and glutathione-S-transferase (GST)) were similar to those found in the control group. Ludwig et al. [55], however, reported no statistically significant changes in the antioxidant profile (CAT, SOD) or lipid oxidation markers (MDA) in patients with A-T.

The results of a recent study indicate that disturbances in key transition metal ions and their related antioxidant enzymes may be associated with ATM inactivation and may play a major role in the A-T

multi-faceted phenotype. Squadrone et al. [1] evaluated blood concentration levels of copper (Cu), iron (Fe), selenium (Se) and zinc (Zn) and the expression and activity of ROS-scavenging enzymes (CAT, GPx, SOD1 and SOD2) in the lymphoblastoid cell lines (LCLs) from A-T patients. The concentrations of important antioxidant metals such as Fe and Se were similar in the control and experimental groups. However, abnormal levels of Cu and Zn in A-T patients (↑Cu, ↓Zn concentrations) may be responsible for the observed decrease in the gene expression (↓30%) and activity (↓40%) of Cu/Zn dependent superoxide dismutase 1 (SOD1), and may, consequently promote further oxidative damage. It is believed that Cu and Zn participate in the oxidative stress response, e.g. elevated levels of copper (component of the cytochrome c oxidase) may account for an increase in ROS production by the Fenton and Haber-Weiss reactions [1,28]. Squadrone et al. [1] speculated that Cu/Zn alternations may therefore be considered potential biomarkers as well as a novel therapeutic strategy for A-T patients. The expression of other antioxidant enzymes (GPx and SOD2), with the exception of catalase (CAT), remained unchanged. The observed decrease in SOD2 activity (↓30%) may be a consequence of changes in feedback between SOD1 and SOD2 activities [1]. Changes in oxidant/antioxidant parameters in A-T patients are presented in Table 2.

4. Oxidative stress in Bloom syndrome and Nijmegen breakage syndrome

Chromosome instability disorders, such as Bloom syndrome and Nijmegen breakage syndrome (NBS), share a number of common cellular features which are linked to defective DNA repair mechanisms and cycle abnormalities [3,56,57]. The very rare BS (Bloom-Torre-Machacek syndrome) is caused by a single gene, *BLM* (604610), located on 15q26.1, whereas NBS results from hypomorphic mutation in the *NBN* gene (c.657_661del5) [56,58]. *BLM* encodes DNA helicase RecQ protein-like-3 which plays an important role in maintaining the stability of DNA through the replication processes [3,58]. Nibrin, a protein encoded by the *NBN* gene, plays a similar role in the cellular response to DNA injury and is regarded as a major component of the MRE11/RAD50/NBN (MRN) double strand breaks (DSBs) repair complex [59,60]. BS and NBS are characterised by microcephaly, growth failure, increased cancer incidence and immunodeficiency [56,60]. With regard to radiation sensitivity, BS and NBS cells show pleiotropic characteristics including chromosome rearrangements, abnormal S-phase cycle checkpoint as well as decreased homologous recombination [56,61]. It has been suggested that BS and NBS cells, similarly to A-T cells, exist in a state of permanent oxidative stress (Table 4). Nonetheless, few studies evaluating the oxidant/antioxidant status in BS and NBS patients have been conducted to date.

Zatterale et al. [62] did not notice any statistically significant differences in the levels of selected plasma non-enzymatic antioxidants: glyoxal (Glx), methylglyoxal (MGlx), ascorbic acid (AA), α- and γ-tocopherol. However the concentrations of uric acid (UA), one of the most important blood antioxidants, as well as leukocyte levels of oxidative DNA damage marker −8-OHdG were significantly elevated in BS patients as compared to healthy controls and BS heterozygotes. On the other hand, the GSSG/GSH ratio was significantly lower in BS

Table 4
Recent advances on the role of oxidative stress in Nijmegen breakage syndrome (NBS).

Cells	Endpoints	Refs.
Fibroblasts from *Nbn* null mutant mice	↑ intracellular ROS, ↑ peroxiredoxin 6, ↑ SOD–2, ↓ NAD+	[65]
Fibroblasts from *Nbn* null mutant mice	↑ ROS, ↑ Poly(ADP-ribose) polymerase, ↓ NAD+	[59]
Fibroblasts from NBS-defective cell line	↑ mROS, ↑ mitochondrial mass, ↓ Δψ$_m$, altered mitophagy	[66]

patients, which may suggest an adaptive response to oxidative stress and increased ROS levels in BS cells, as in A-T patients. By contrast, Lloret et al. [53], showed a significant increase in both leukocyte and urinary concentrations of 8-OHdG as well as a decrease in the GSSG/ GSH ratio. The authors did not report any statistically significant changes in Glx and MGlx concentrations in BS patients. Additionally, studies conducted on fibroblast cell lines (e.g. GM3403 B-lymphoblastoid BS cell line) confirmed the role of cellular redox alternations in the BS phenotype pointing to elevated levels of SOD activity and enhanced production of ROS in BS cells [63]. Table 3 summarises the antioxidant/oxidant parameters and products of oxidative modifications in patients with BS.

Research conducted by Krüger et al. [64] and Melchers et al. [65] demonstrated that a higher cancer incidence in NBS resulted from increased oxidative stress due to DSBs and inadequate DNA damage response mechanisms. The authors revealed radiation-induced alterations in proteins involved in oxidative stress (e.g. peroxiredoxin 6 and SOD-2), augmented intracellular ROS levels and decreased concentrations of oxidised nicotinamide adenine dinucleotide (NAD+) in NBS cells [64]. Furthermore, Krenzlin et al. [59] showed that *Nbn* deficiency was inextricably linked with elevated oxidative stress following DNA damage in NBS patients. The authors speculated that the depletion of NAD+ due to the hyperactivation of Poly(ADP-ribose) polymerases (PARP-1, PARP-2, PARP-3), which play a critical role in the detection of homologous recombination and DNA DSBs, may be responsible for increased ROS levels and oxidative stress-mediated cellular damage in NBS cells [59]. Moreover, they also suggested that a high prevalence of cancer in *Nbn* null mutant mice and NBS patients may be associated with a primary deficiency in DSBs detection in DDR pathways as well as secondary ROS-mediated DNA injury [59]. Additionally, Shimura et al. [66] demonstrated that long-term, low-dose ionising radiation (FR) induced mitochondrial damage, mitochondrial biogenesis and oxidative stress in the AT5BIVA (ATM-deficient) and GM7166 (NBS1-deficient) radiosensitive cell lines. The authors revealed higher mitochondrial mass, reduced $\Delta\psi_m$, altered mitophagy, mROS accumulation and abnormal mitochondria as well as the induction of apoptosis in irradiated A-T and NBS cells. Administration of the non-enzymatic thiol-containing antioxidant N-acetyl-L-cysteine (NAC) protects A-T and NBS cells from FR-induced mitochondrial damage and, therefore, NAC might be considered a useful strategy against radiation toxicity in these conditions [66].

5. Future prospects of antioxidant strategies in Ataxia-telangiectasia, Bloom syndrome and Nijmegen breakage syndrome

The results of many studies not only confirm the important role of oxidative stress in the A-T, BS and NBS phenotypes, but may also indicate new therapeutic strategies in these syndromes, as in other oxidative stress-related genetic disorders characterised by a higher cancer prevalence and neurological degeneration [13,17,22,32,67]. It has been demonstrated that the elevated production of ROS in A-T, BS and NBS patients leads to an excess activity of antioxidant enzymes (e.g. CAT, GSH-Px and SOD) with the simultaneous low concentration of low molecular weight antioxidants (LWMA), such as α-tocopherol, GSH, retinol and UA [1,50–52,55,62,66]. These alternations suggest an adaptive physiological response to elevated oxidative stress and accumulated oxidative damage in these diseases. It is believed that the induction of antioxidant defense systems (AOS) is one of the most important mechanisms for limiting the formation of ROS-induced oxidative injury as well as regulating the biological activity of ROS in various pathological states and human diseases [28]. Continuous oxidative stress, however, may lead to the depletion of enzymatic and non-enzymatic antioxidants and therefore, supplementation with exogenous antioxidants may prove very beneficial.

Several studies have evaluated the influence of antioxidant treatment (e.g. tempol, NAC, L-carnitine, iron, EUK-189 and CTMIO) on oxidative stress, oxidative damage, neurodegeneration and cancer chemoprevention in ATM- and NBS-deficient cell lines and/or animals models [15,67]. Schubert et al. [68] showed that long-term dietary supplementation of synthetic SOD analog Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl) reduced ROS levels, restored normal $\Delta\psi_m$, decreased oxidative damage to proteins (↓carbonyl derivatives) and reduced the proliferation of A-T thymocytes and splenocytes. Tempol has also been found to extend the lifespan of *Atm*-deficient mice and can therefore be considered a potential cancer therapeutic agent, similarly to low molecular weight N-acetyl-L-cysteine (NAC). The antioxidant effects of chronic (2-year-study) oral supplementation of NAC in $Atm^{-/-}$ mice were investigated by Reliene et al. [16] who found a significantly reduced incidence (76.5% *vs* 37.5%) and multiplicity (4.6 *vs* 2.8) of lymphoma as well as an increased lifespan (↑18 weeks) of AT-mutated mice following NAC treatment. Similar results were obtained in a study conducted by Ito et al. [69]. Berni et al. [70] who investigated the impact of L-carnitine (β-hydroxy-γ-trimethyl-ammonium butyric acid) on cytogenetic DNA damage induced by the pro-oxidant agent *tert*-butyl-hydroperoxide (t-butyl-OOH) in A-T patients with different *ATM* mutations. The authors demonstrated that L-carnitine significantly decreased all types of chromosomal abnormalities and proved more effective than mannitol, a non-enzymatic scavenger of highly reactive hydroxyl radical (·OH). The authors also found that the kinetics of DNA repair in cells varied depending on the type of ATM deficiency [70]. Furthermore, it has been suggested that α-lipolic acid, EUK-189, CTMIO and dexamethasone can alleviate the symptoms of IR hypersensitivity, neurodegeneration and cancer susceptibility in A-T cells [15,67]. Finally, McDonald et al. [71] demonstrated that a high Fe status may determine constant oxidative stress and oxidative damage in A-T cells and therefore, antioxidant supplementation with Fe is not indicated in A-T patients.

It is still to be established whether antioxidants and free radical scavengers may improve the clinical condition and prolong the survival time of A-T, BS and NBS patients. In clinical practice, NAC, tempol, α-lipolic acid, L-carnitine and other antioxidants can be used. However, no research concerning antioxidant treatment with major lipophilic LWMA such as α-tocopherol, retinol or ubiquinol (Q10) has been conducted to date. It appears that antioxidant supplementation may provide an additional therapeutic strategy for these groups of patients. Further experimental studies in a human model are required to explore the issue.

6. Conclusions

Chronic oxidative stress may be considered a major phenotypic hallmark of A-T, BS and NBS diseases. It has been demonstrated that A-T, BS and NBS cells are more sensitive to oxidative stress than normal cells, being inextricably linked with endogenous ROS overproduction and the associated oxidative damage to major cellular biomacromolecules including nucleic acids (DNA mutations), proteins (protein oxidation) and lipids (lipid peroxidation) [42,53,62]. Oxidative stress leads to a permanent structural and functional changes of DNA, proteins and lipids, however the most damaging process appear to be a oxidative DNA damage, because these changes may directly contribute to the cellular senescence as well as carcinogenesis. Mitochondrial dysfunctions: changes in the structure and function of mitochondria, excess mROS production and oxidative stress-induced mitochondrial damage have also been reported in A-T, BS and NBS patients and/or corresponding animal models [6,12,17,22,45,66]. Furthermore, recent studies have indicated alternative, endogenous sources of oxidative stress in A-T, BS and NBS cells including NADPH oxidase 4 (NOX4), oxidised low-density lipoprotein (ox-LDL) or Poly(ADP-ribose) polymerases (PARP-1, PARP-2, PARP-3) (Fig. 2) [47,48,59,65] which may provide new pharmacological strategies for patients.
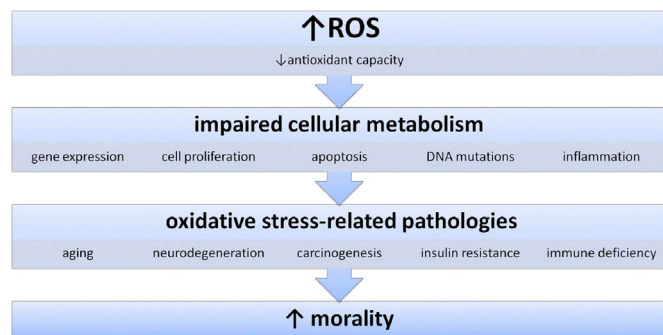
Fig. 3. The consequences of ROS overproduction in patients with Ataxia-telangiectasia (A-T), Bloom syndrome (BS) and Nijmegen breakage syndrome (NBS). Not all pathologies in A-T, BS and NBS patients must be explained by chromosomal rearrangements and genomic instability. It is very likely that premature aging, growth retardation, insulin resistance, endocrine abnormalities and immunodeficiency may be a result of chronic oxidative stress and accumulated oxidative injury. A-T, BS and NBS are inextricably linked to increased production of ROS, reduced antioxidant capacity and the associated oxidative damage. Disturbances in oxidant and antioxidant status together with oxidative cellular injury may contribute to clinical progression as well as increased mortality in patients with A-T, BS and NBS.

Some data indicate that a number of A-T, BS and NBS-related pathologies (i.e. premature aging, growth retardation, insulin resistance, endocrine abnormalities and immunodeficiency) may result from elevated oxidative stress and accumulated oxidative damage, but presumably not from chromosomal rearrangements and genomic instability. It is likely that oxidative stress, ROS-induced oxidative injury as well as mitochondrial alternations may modulate cellular metabolism including gene expression, signal transduction pathways, cell differentiation and apoptotic cell death, and thus contribute to the clinical progression in A-T, BS and NBS patients. Under abnormal mechanisms of the antioxidant defense, formation and accumulation of oxidised products of the cellular DNA, proteins and lipids may be directly responsible for the premature aging, neurodegeneration, carcinogenesis as well as chronic inflammation (Fig. 3). Quantitative and qualitative changes in non-enzymatic antioxidants suggest, in turn, that the supplementation of exogenous LWMA may be a means of compensating for the attenuated ROS-scavenging systems in these groups of patients.

It should be recalled that a proper index of oxidative stress is the presence of oxidative modification products in the cell, and thus, oxidative stress is inevitably associated with A-T, BS and NBS. It appears that differences in the assessed oxidative stress and oxidative damage parameters may arise from a very small number of subjects in the studies conducted to date and do not necessarily reflect the real oxidant/antioxidant status in these patients (Tables 2 and 3). Therefore, there is a need for further research in this area, especially research concerning the examination of total oxidant/antioxidant capacity markers, individual enzymatic and non-enzymatic antioxidants as well as cellular oxidative modification products. Only the clear understanding of the oxidant/antioxidant equilibrium and mechanisms leading to oxidative stress and oxidative damage in A-T, BS and NBS cells will allow development of new, additional therapeutic proposals for patients suffering from these diseases.

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