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Chromosomal breakage tests in the differential diagnosis of Fanconi anemia and aplastic anemia

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Abstract

Background: FA patients are hypersensitive to preconditioning of bone marrow transplantation.

Objective: Assessment of the power of mitomycin C (MMC) test to assign FA patients.

Methods: We analysed 195 patients with hematological disorders using spontaneous and two types of chromosomal breakage tests (MMC and bleomycin). In case of presumed Ataxia telangiectasia (AT), patients' blood was irradiated in vitro to determine the radiosensitivity of the patients.

Results: Seven patients were diagnosed as having FA. The number of spontaneous chromosomal aberrations was significantly higher in FA patients than in aplastic anemia (AA) patients including chromatid breaks, exchanges, total aberrations, aberrant cells. MMC-induced ≥10 break/cell was 83.9 ± 11.4% in FA patients and 1.94 ± 0.41% in AA patients ($p < .0001$). The difference in bleomycin-induced breaks/cell was also significant: 2.01 ± 0.25 (FA) versus 1.30 ± 0.10 (AA) ($p = .019$). Seven patients showed increased radiation sensitivity. Both dicentric + ring, and total aberrations were significantly higher at 3 and 6 Gy compared to controls.

Conclusions: MMC and Bleomycin tests together proved to be more informative than MMC test alone for the diagnostic classification of AA patients, while in vitro irradiation tests could help detect radiosensitive—as such, individuals with AT.

KEYWORDS
aplastic anemia, bleomycin test, chromosomal breakage test, Fanconi anemia, in vitro irradiation, mitomycin test

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Novelty statement

What is the new aspect of your work?

We performed both mitomycin C, bleomycin and spontaneous chromosome aberration tests on our cohort of hematological patients before stem cell transplantation.

What is the central finding of your work?

MMC-test provides a useful method to differentiate between FA and other AA patients.

What is (or could be) the specific clinical relevance of your work?

This simple tool can help to reduce the need of FANC genetic testing, and accelerate accurate diagnosis.

1 | INTRODUCTION

Fanconi anemia is a rare autosomal recessive disorder, which is phenotypically and genetically heterogenous.[1] The consequential bone marrow failure usually starts with thrombocytopenia and typically progresses to aplastic anemia (AA). Transplantation with hematopoietic stem cells is currently the only curative treatment to correct the bone marrow failure and also reduce the risk of leukemia.[2] Patients with FA are hypersensitive to cyclophosphamide, busulfan, ionizing radiation and other genotoxic agents. FA patients are also prone to suffer from the damaging inflammatory consequences of graft versus host disease. Therefore, FA patients need special treatment and the diagnosis should be established to distinguish FA from other inherited hematological diseases.[3] Efforts have also focused on reducing the dose of toxicity of preparative regimens required for transplant and using alternative conditioning treatments.[4]

Approximately one-third of FA patients have no overt physical/somatic abnormalities.[5] On the other hand, patients having FA-like symptoms may not be suffering from FA.[6] Several genetic conditions exist in which bone marrow failure is an overlapping feature: Diamond–Blackfan anemia, Shwachman–Diamond syndrome or Nijmegen breakage syndrome, for example—whose differential diagnosis may require a multitude of genetic and nongenetic tests. Currently, pathogenic variants in 23 genes have been reported to cause FA.[7] However, absence of detected biallelic mutations in the FA genes cannot be understood as exclusion of the diagnosis of FA, and variants of unknown significance need further functional testing to decide on their pathogenicity. Therefore, other confirmatory tests are of paramount importance. Increased frequency of chromosome breakage was first described in FA patients by Schroeder et al.[8] After that, Schuler et al. showed that supplementing the culture media with DNA cross-linking agents increased the measured frequency of DNA breaks in the samples of FA patients.[9] It was suggested that mitomycin C (MMC) or diepoxybutane (DEB) sensitivity may reliably distinguish between FA and non-FA cases.[1,10] These chromosomal breakage tests can be considered equally valuable but much cheaper first-tier assays than DNA-based molecular testing of FA genes. After a positive breakage test result has been obtained, screening for mutations in the known FA genes is warranted. In a study, approximately 80% of chromosome breakage tests from patients referred to FA diagnostic testing with confirmed bone marrow failure had a negative result. However, chromosome tests are not 100% specific, overlapping bone marrow failure syndromes may score positive as well.[11] Furthermore, lymphocyte mosaicism was estimated to occur in 10%–30% among the FA patients.

There is no consensual cutoff value for chromosomal breakage percentage in peripheral blood lymphocytes to differentiate FA patients with mosaicism from FA heterozygotes or from healthy individuals without FA. Castella et al. proposed a new chromosome fragility index (CFI) in order to distinguish the patients with FA mosaicism from germ-line FA. The CFI considers the aberrant cell number and breaks per multiaberrant cells.[12]

In our study patients with hematological disorders were tested for chromosome aberrations prior to bone marrow transplantation to exclude FA. MMC-induced chromosomal breakage test, bleomycin test (to obtain information on postreplication repair in the case of AA) and spontaneous chromosomal aberration test were performed. We also applied the above mentioned CFI value to characterize chromosome fragility. Elevated alpha-fetoprotein level of some patients raised the suspicion of Ataxia telangiectasia (AT). Blood samples of these patients were irradiated in vitro with 3 and 6 Gy to determine their radiosensitivity.

2 | PATIENTS AND METHODS

2.1 | Patients and samples

We retrospectively analysed the data of 195 patients with hematological disorders (among them 56 patients with AA) who underwent chromosomal breakage tests in our department during the period of 2007–2021. Anamnestic data, symptoms, blood count results and history of medication of the patients with clinical suspicion of FA were obtained from their clinicians. Healthy volunteers ([$n = 20$] hospital workers) were used as controls for the chromosome breakage studies. This study was performed according to the Declaration of Helsinki and was approved by the National Ethical Committe (permit number 190 80-2/2016/EKU).

2.2 | Lymphocyte cultures

Heparinised blood samples were collected and spontaneous, MMC-induced and bleomycin-induced chromosome preparations were applied. Blood (0.8 mL) was cultured in RPMI-16740 cell culture medium complemented with bovine serum albumin (15%) and penicillin/streptomycin (100 U/mL/100 μg/mL, Gibco, Thermo Fisher Scientific Inc., Waltham, MA) and phytohaemagglutinin M (2% v/v, Gibco, Waltham, MA) for induction of cell division. Incubation was 48 h at 37°C, in order to perform chromosome analysis in the first cell division. Further proliferation was stopped with colcemid (0.1 μg/mL, Gibco) at the 46th h. Lymphocytes were then suspended in hypotonic solution (0.075 M KCl) for 15 min at 37°C and fixed with ice-cold methanol-acetic acid 3:1 mixture. Following several washes in fixative, concentrated cell suspension was dropped onto glass slides, stained with Giemsa solution (3%, RAL Diagnostics).

2.3 | Mitomycin test

Whole blood cultures from the patients and healthy controls were prepared like in standard cytogenetic analysis and 50 ng/mL MMC (mitomycin-C Kyowa) was added and the culture was incubated for 72 h on 37°C. Proliferation was inhibited with 0.1 μg/mL colcemid (Gibco) at the 67th hour. From each patient 100 metaphases were analyzed with light microscopes (1500× magnification, Olympus CH 2). Only round and clear metaphase cells were counted. The following aberrations were evaluated: chromatid gap (an interruption in the staining of a chromatid 1–2 times the width of the chromatid); chromatid break (the interruption is more than two times the width or the broken piece of chromatid appears dislocated); triradial chromosome (an interchange figure presumably having resulted from the misrepair of two breaks in two distinct chromosomes); quadriradial chromosome (an interchange figure resulting from the misrepair of two broken chromatids in different chromosomes). Triradial and quadriradial chromosomes were counted as two breaks.

Aberrations were recorded as total breaks per cells, percentage of aberrant cells, and percentage of cells of ≥10 b/c. Cultures from healthy controls show 1%–5% of 5–10 b/c (break/cell).[6]

In our practice, patients with >60% of cells with 10 or more breaks are diagnosed as having FA and after genetic confirmation of FA they are treated with alternative bone marrow transplantation techniques. In the case of more than 10% of cells having ≥10 b/c or more than 10% aberrant cells present caution in the application of genotoxic agents in the treatment of patients is warranted.

2.4 | Study of spontaneous chromosomal aberrations

We assessed frequencies of chromatid (chromatid break, exchange) and chromosome type aberrations (fragments, translocations, dicentric, and ring chromosome aberrations). The value of terminal and interstitial deletions were summed up as excess fragments. Numerical aberrations were calculated from the same metaphases by counting the centromeres. Cells with 44, 45 or 47 chromosomes were considered aneuploid. The chromosome analysis was performed in accordance with the ICPEMC[13] requirements. The samples were coded and the prepared slides (CAs) were evaluated blindly by two well-trained scorers.

2.5 | Bleomycin as a radiomimetic test

For performing BLM test, we used the protocol published by Hsu et al.[14] The lymphocytes were cultured in the same conditions as in spontaneous chromosomal aberration test, but incubated for 72 h (for second division). Bleomycin was added (30 μg/mL) to the preparations at the 67th hour to introduce DNA breaks. Addition of colcemid, treatment with hypotonic solution, fixation and purification was performed as described above. One hundred metaphases per patient were counted. Lesions wider than a chromatid width were considered as chromatid breaks. Chromatid exchanges were calculated as two breaks. If more than 12 breaks/cell were counted, we described the cell as having 12 breaks. The mean number of chromatid breaks per cell (b/c) was used as an indicator of repair disability. Based on the chromosome results, all individuals (patients and healthy as well) were divided into three groups: Hypersensitivity to BLM: >1.25 b/c, Intermediately sensitive: 1.0–1.24 b/c; Not sensitive (hyposensitive) <1.0 b/c.[15–17]

2.6 | In vitro irradiation with 3 and 6 Gy

In case of suspected non-FA radiosensitivity, two milliliters heparinised blood was placed in water filled plastic phantom and irradiated with 3 and 6 Gray at 6 MV and 1400 Monitor Unit/min on a Varian True Beam linear accelerator as suggested by Borgmann et al.[18] Then, the lymphocytes were cultured by standard cytogenetic techniques in order to compare the chromosomal aberrations between patients and healthy subjects.

2.7 | Statistics

The frequencies of CAs do not follow normal distribution as it was concluded by earlier investigations.[19] Therefore, non-parametric Mann–Whitney *U* test was used to compare individual groups. Value of $p < .05$ was considered as the limit of significance. Graph Pad Prism 8 (San Diego, CA) and OriginPro 8.5 software packages were used for the calculations and presentation of the data.

3 | RESULTS

We have investigated a large group of patients ($N = 195$) with hematological disorders by using MMC-chromosomal breakage, bleomycin

mutagenity test and spontaneous chromosome fragility tests. The hematological disorders of the patients are shown in Table 1. The majority of patients were referred to us for testing because of AA (37.1%) bi—tricytopenia or pancytopenia (19%), or myelodysplastic syndrome 12.3%. In total, 148 of the 195 cases had only hematological symptoms, 18 patients (9.2%) had both hematological features and congenital malformations. Most common somatic abnormalities were: intellectual disability, hearing impairment, ophthalmological abnormalities, chest deformity, microcephaly, radius hypophasia, cleft lip and palate, skeletal abnormalities. The proportion of males and females was the same (1:1). Two-thirds of the patients were under 19 years of age.

3.1 | Chromosome fragility

We performed MMC-induced chromosome fragility assay in all tested patients with cytopenias and in 20 healthy volunteers. The most common chromosomal abnormalities were chromatid breaks followed by exchanges. Acentric fragments, dicentric and ring chromosomes were rarely seen. Chromosome fragility is usually reported as breaks/cell and aberrant cell %. Summary of the results obtained on MMC-induced fragility is provided in Table 2. Seven patients were diagnosed with FA (CFI:537-1000) based on sensitivity to MMC (50 ng/mL). Sequencing data of only one patient is available to us. This patient was homozygous for *FANCF* c.613dup,p.(Ile205Asnfs*61).

The percentage of aberrant cells and the number of breaks per cell were both low in AA patients and control subjects. As a group, patients with FA could be clearly distinguished from AA patients within the analysis of MMC cultures by the percentage of cells with 10 or more breaks (83.9 ± 11.4 vs. 1.94 ± 0.41, $p < .0001$) (Table 2). The number of breaks/cell was more than five times higher in the FA population when compared to the AA population (9.4 ± 0.6 vs. 1.62 ± 0.13, $p < .0001$), while the percent of aberrant cells was increased nearly two fold in the AA groups (98.2 ± 1.19 vs. 49.5 ± 5.12, $p < .0001$) (Figure 1.). AA patients showed significant difference from healthy controls regarding break/cell value ($p = .0041$) and aberrant cell percent ($p = .0024$). The AA patient group's elevated values can have genetic or non-genetic causes.

We compared breaks/multiaberrant cell values of the two groups and found significant differences ($p < .0001$ FA vs. AA, $p = .024$ control vs. AA, respectively). We combined percentage (%) of aberrant cells and breaks/multiaberrant cell in the same graph, as Castella et al.[12] suggested (Figure 2), while proposed CFI was calculated in the following way CFI = % aberrant cells × (breaks/multiaberrant cell) value that confirmed the previously demonstrated differences ($p < .0001$ FA vs. AA, $p = .032$ control vs. AA, respectively) (Figure 3) also in this parameter. Seven patients were diagnosed with FA: CFI values: 537–1000.

TABLE 1 Demographic data and some characteristics of the patients.

Patients	*N*
Age	
0.5–19 years	140 (71.8%)
20–63 years	55 (28.2%)
Gender	
Female	97 (50%)
Male	98 (50%)
Clinical diagnosis/reason for referral	
MDS (Myelodysplastic syndromes)	30
CML (Chronic myeloid leukemia), AML (Acute myeloid leukemia)	6
Bloom syndrome	3
Ataxia telangiectasia	10
Aplastic anemia	56
Bicytopenia, tricytopenia[a]	13
Pancytopenia[a]	24
Thrombocytopenia	7
Granulocytopenia	3
Congenital malformations[b]	14
Fanconi anemia?	6
Head and neck tumor	2
Immunodeficiency, highAFP	2
Others[c]	19

[a]Do not meet the diagnostic criteria for AA.
[b]Congenital malformation which do not meet AA diagnostic criteria.
[c]Others: 1–1 cases: Blackfan Diamond anemia, Sideroblastic anemia, Haemolytic anemia, von Willenbrand disease, Ewing sarcoma and pancytopenia, etc.

3.2 | Spontaneous chromosome fragility

Spontaneous chromosome fragility was evaluated in all patients by using exactly the same conditions. The results obtained are described in Table 2. The number of spontaneous chromosome aberrations was significantly higher in the FA patients than in the AA patients including gaps ($p = .0003$), exchanges ($p < .0001$), chromatid breaks ($p = .0002$), total aberrations ($p < .0001$), aberrant cells ($p < .0001$). Overall higher spontaneous chromosome fragility was observed in FA patients, that was 8.0-fold in chromatid breaks, 7-fold in total aberrations and 6.4-fold in aberrant cells, when compared with AA patients. On the other hand, chromatid breaks ($p = .0141$), total aberrations ($p = .0013$) and aberrant cells ($p = .011$) were significantly higher in the AA group than in healthy subjects, resulting from underlying cellular and genetic mechanisms that act in favour of DNA damage and/or impaired repair.[2]

3.3 | Bleomycin mutagenity test

Bleomycin test evinces chromatid breaks induced by in vitro treatment of peripheral lymphocytes with the antitumor (radiomimetic) drug bleomycin, which has been utilized as an indicator of individual

TABLE 2 Numerical and structural aberrations in Fanconi anemia, AA patients and in healthy control subjects and results of mitomycin (MMC) and bleomycin tests.

Code/ gender/age	Spontaneous chromosome aberrations± SE/100 cells								MMC ≥10 breaks/ cell, %	Bleomycin Breaks/ cell
	Aneuploid	Gap	Chromatid break	Exchange	Chromosome fragment	Dicentric + ring	Total aberrations	% Aberrant cells		
SH 47, M, 31	1.5	9	12.5	7	0	0	26.5	22	69	2.68
SH 733, M,7	1	3.5	27.5	4.5	0	0	32	23.5	100	1.26
SH 977, M, 8	3	5	13.5	1.5	2	0.5	19	17	100	1.74
DH 277, M 4	1	2	15	0	0	0	15	14	100	3.16
DH 332, F 7	0	13	26	0	0	1	27	22	98	1.63
SH 721, M 8	8	6.5	1	0	0	0	1	1	20.5	1.68
DH 535, M, 0,5	2	5	10	0	0	0	10	9	100	1.92
FA $N = 7$	2.14 ±0.81	5.50[a] ±1.59	16.14[b] ±3.58	1.86[c] ±1.06	0.21 ±0.21	0.07 ±0.07	18.5[d] ±4.21	15.36[e] ±3.22	83.9[f] ±11.4	2.01[g] ±0.25
AA patients $N = 56$	1.59 ±0.20	2.06[a] ±0.25	2.05[b,h] ±0.31	0.21[c] ±0.08	0.26 ±0.13	0.07 ±0.04	2.61[d,i] ±0.41	2.40[e,j] ±0.35	1.94[f] ±0.41	1.30[g] ±0.10
Controls $N = 20$	2.06 ±0.39	1.53 ±0.37	0.71[h] ±0.19	0.06 ±0.06	0.12 ±0.12	0.06 ±0.06	0.82[i] ±0.25	0.82[j] ±0.25	0.68 ±0.32	1.08 ±0.21

Note: Significant differences: FA patients vs. AA patients: [a]($p = .0003$), [b]($p < .0001$), [c]($p = .0002$), [d]($p < .0001$),[e]($p < .0001$), [f]($p < .0001$), [g]($p = .0198$) AA patients vs. controls: [h]($p = .0141$), [i]($p = .013$).

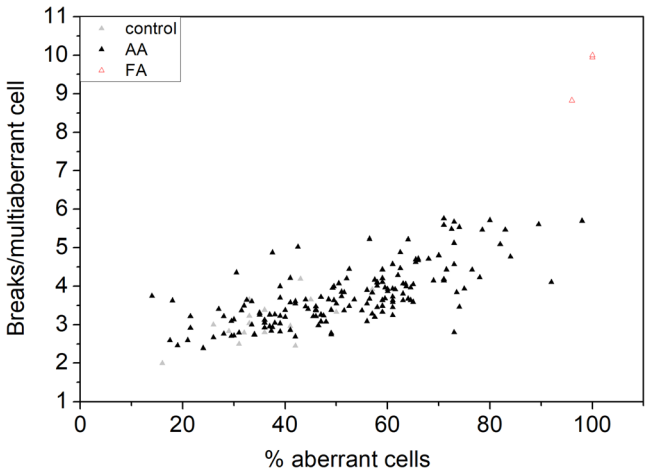

FIGURE 1 Mitomycin-induced chromosome fragility in Fanconi anemia, AA and control groups expressed by breaks/ multiaberrant cell.

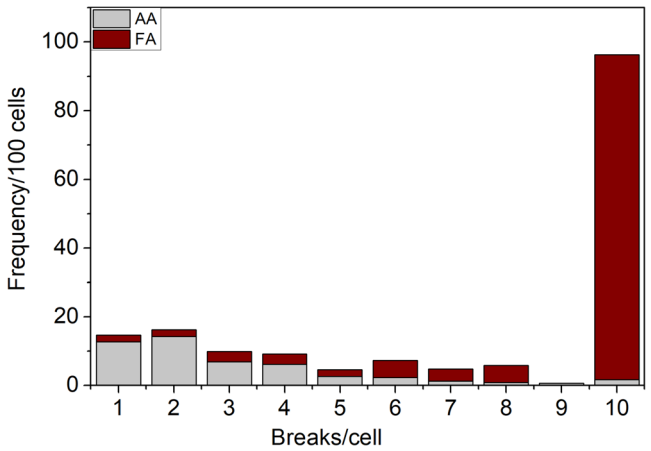

FIGURE 2 Comparison of chromosome aberrations (breaks/cell) in Fanconi anemia ($n = 7$) and AA ($n = 56$) patients with mitomycin (MMC) test.

DNA repair capacity. Depending on the level of chromatid breaks induced by the bleomycin treatment, individuals can be classified as nonsensitive, sensitive or hypersensitive. The individual b/c values ranged between 0.17 and 5.36 in 181 patients (14 out of 195 patients had no BMC test data because of blood shortage), while the most sensitive patient had conjunctival telangiectasia and high alpha fetoprotein (AFP) level.

In total, 80 patients were considered nonsensitive (0–1.0 b/c), 23 patients were sensitive (1.00–1.24 b/c), and 71 patients were hypersensitive (≥1.25 b/c). All of the Fanconi anemia patients were hypersensitive (2.01 ± 0.28). Only seven bleomycin tests were available in control individuals, but the average value was 1.08 ± 0.19.

3.4 | Results of in vitro irradiation

Among patients there were some individuals suspected to suffer in AT. As AT patients are usually sensitive to ionizing radiation, the blood of AT-suspect patients was irradiated in vitro with 3 and 6 Gy in a linear accelerator (1400 MU/min, 6MV FFF). Four of six patients had elevated frequency of dicentric + ring ($p = .025$, $p = .0107$, respectively) and total aberrations ($p = .0004$, $p = .001$, respectively) both at 3 Gy and 6 Gray compared with controls (Table 3). These patients were not sensitive to mitomycin, but they were sensitive to bleomycin (except one patient). Among them two patients were genetically confirmed to have ataxia teleangiectasia (*ATM* c7189 C > T; ac 2376 G > T, and ac7630-2A > c; ac8986-1G > C).

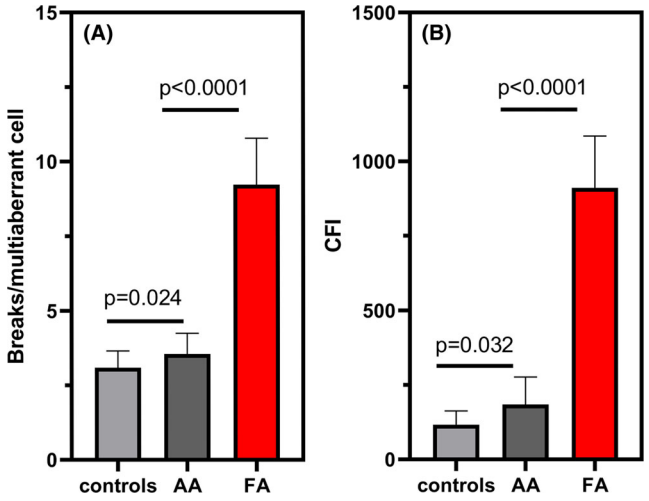

FIGURE 3 Mitomycin-induced chromosome fragility (average ± SEM) in Fanconi anemia, ($n = 7$) AA ($n = 56$) and control groups ($n = 20$) expressed by breaks/multiaberrant cell and calculation of CFI [(% aberrant cells)x(Breaks/multiaberrant cell)]. Statistical significance is shown.

Another patient had a bone marrow transplantation because of FA at the age of 6 years and he developed head and neck tumor at the age of 35, when we tested him. He showed only 2 cells with 10 or more breaks in MMC test, CFI 218, 3/100 cells total aberration in spontaneous chromosome aberration test and only 0.88 breaks/cells in bleomycin test. Therefore, he was assumed to be cured from FA, but he was moderately sensitive to irradiation (Table 3). Given this increased radiosensitivity his tumor was irradiated using proton therapy.

One CVID (Common variable immunodeficiency) patient who developed retroperitoneal lymphadenomegaly and splenomegaly was also radiosensitive.

Correlation between the spontaneous and induced chromosome aberrations.

Spearman correlation was used to find correlation between spontaneous and induced chromosome aberrations. There were weak correlations between mitomycin-induced and spontaneous aberrant cell % ($p = .020$), as well as mitomycin-induced and total aberrations ($p = .015$). The bleomycin-induced break/cell value correlated with the spontaneous aberrant cell percent ($p < .0001$), with total aberrations ($p < .0001$) and with chromatid breaks ($p < .0001$), as well.

TABLE 3 Chromosome aberrations induced by in vitro irradiation of blood lymphocytes, mitomycin and bleomycin tests of five suspected Ataxia-telangiectasia, one head and neck tumor and one CVID patient.

	3 Gy		6 Gy				
Code	Dic + ring/ 100 cells	Total ab./ 100 cells	Dic + ring/ 100 cells	Total ab./ 100 cells	MMC ≥10 b/c, %	Bleomycin b/c	Diagnosis
DH 152	48	162	112	448	2.5	2.66	Presumed AT? Confirmatory: *ATM* gene test c7189 C > T; ac2376 G > T
DH 475	84	117	228	304	2	2.25	Presumed AT: conjunctival teleangiectasias without neurological symptoms
DH 498	31	66	219	312	0	1.14	Presumed AT? Confirmatory:non AT CF
DH 513	57	86	284	339	1	1.24	Pancytopenia
DH 514	67	153	140	327	2	1.88	Presumed AT? Confirmatory: *ATM* genetic test: ac 7630-2A > C; ac 8986-1G > C
DH 431	83	141	208	269	2	0.88	FA diagnosed at 6 years—bone marrow transplant, Solid tumor of the head and neck at 35 years (carcinoma planocellulare)-proton therapy
DH 512	123	147	259	325	1	1.14	CVID (common variable immunodeficiency), retroperitoneal lymphadenomegaly and splenomegaly
Ave-rage $N = 7$	70.4 ± 11.3[a]	124.6 ± 13.8 [b]	207.1 ± 23.3[c]	332.0 ± 21.1 [d]	1.5 ± 0.3	1.60 ± 1.0	
Control $N = 5$	36.5 ± 5.24 [a]	75 ± 5.03 [b]	148 ± 6 [c]	287 ± 17[d]	–	–	–

Note: Significant difference: [a]$p = .025$; [b]$p = .011$; [c] $p = .0004$; [d]$p = .001$.

4 | DISCUSSION

Fanconi anemia results in increased risk of hematological disorders developing usually by 40 years of age in 90% of the patients.[20] Furthermore, the patients are more prone to solid tumors of which the cumulative incidence is 28% by the same age. The largest likelihood is related to head and neck cancer, which is 500–700 times more frequent than in the healthy population.[20] Of note, once developed, the treatment of these tumors are difficult, because the affected patients are chemo- and radiosensitive. Stem cell transplantation for the hematological disorders is also dangerous, as FA patients are extremely sensitive to DNA cross-linking agents. Therefore, reduced intensity conditioning must be used (e.c. cyclosphamide).[21]

Unfortunately, patients with other diseases, such as inherited bone marrow failure syndromes are reported to have similar clinical symptoms and similar blood test results. The fact that the lymphocytes of FA patients show increased spontaneous chromosome fragility can help in the differential diagnosis of FA. However, as Dokal and Luzatto suggested, in the case of increased chromosome fragility results, these patients might be misdiagnosed as having FA.[11] FA mosaic patients are even more difficult to identify due to coexisting normal and abnormal cell lines.[22]

In the early 2000s MMC and DEB-induced breakage tests became the gold standard in differentiation of FA and other aplastic anemia syndromes.[23] Furthermore, Castella et al.[12] proposed a new CFI by using DEB-induced cultures, which can better differentiate between FA patients and healthy individuals. They found a correlation between the number of congenital malformations and frequency of chromosome breaks due to DEB or MMC induction. Fargo et al., however, found partial overlap between the CFI of mosaic and non-mosaic FA patients. Unfortunately, they could study only four FA mosaic patients. According to their results, MMC caused more aberrant cells, breaks per cell and breaks per aberrant cells than DEB, which was due to the higher frequency of radials after MMC induction.[2]

We have investigated a large group ($n = 56$) of AA patients using MMC-chromosome breakage test in order to distinguish FA cases from other AAs. Patients were categorized according to cytogenetic criteria and in a single patient, sequencing results. A total of seven patients were identified as having FA, their age ranged between 0.5–31 years. In one case, limb anomaly (supernumerary finger) was found. Allogenic hematopoietic transplantation was the curative therapy for five patients. One patient is under close follow-up and is well. Transplantation-related mortality occurred in one patient. Another patient died of pneumonia before transplantation. In our study the proportion of males and females among aplastic patients studied was equal, but there was a predominance of males in the FA patient group. Similarly, a total of 124 patients were diagnosed as having Fanconi anemia between 2014 and 2020 in Pakistan and male predominance (1.9:1) was seen.[24]

Spontaneous chromosome aberrations showed 7, 6.4, and 7.9-fold rise in total aberrations, aberrant cell frequency and chromatid breaks, respectively, when FA patients were compared to AA patients ($p < .0001$; $p < .0001$ and $p < .0001$, respectively). The MMC-induced aberrant cell frequencies with equal to or more than 10 breaks of these patients were significantly higher ($p < .0001$, 43.2-fold change) than the values of other AA patients. In our FA patient cohort, the ratio of 10 or more breaks/cell was 21%–100% (Table 2.). In the study of Talmoudi et al., 22.22% (38 patients) were diagnosed as affected with FA and 77.17% (132 patients) as unaffected according to their sensitivity to MMC.[25] By contrast, in our work using the same concentration of MMC (50 ng/mL) only 3.6% of the patients were confirmed to be FA positive and 96.4% were not.

We also calculated CFI (Figure 3) for each studied group, but found less increase (4.9-fold difference between FA and AA patients compared, $p < .0001$) than that observed by other authors—regarding the aberrant cell number with 10 or more breaks induced by MMC (43.2-fold, see above) between the FA and AA group.[12] There was no overlap between the CFI of the AA (94–429) and FA patients (557–1000).

Bleomycin test measures sensitivity to a double strand break inducing mutagenic agent. The sensitivity to this agent probably has a hereditary basis and is suggested to be in connection with reaction to mutagenic assults of individuals at acellular level.[26] Cloos et al, suggested, that high b/c is due to impaired cell cycle checkpoints which do not allow enough time for DNA repair.[27] We performed bleomycin test on 181 patients' blood samples and found three distinct populations: hypersensitive (≥1.25b/c), sensitive (1.24–1.0 b/c) and non-sensitive patients (<1.0 b/c). We found 1.30 ± 0.10 b/cell value for AA anemia patients (and 1.08 ± 0.21 for healthy subjects) which is higher but comparable with the results of Cloos et al, which was 0.79 ± 0.32 b/c.[27] Bleomycin sensitivity was characteristic for FA patients (1.26–3.16 break/cell), but it was not exclusive. The other AA patients, showed sensitivity in a very wide range: 0.17–5.36 b/c.

We also included in our study population potentially radiosensitive non-FA patients, for example the ones with suspected ataxia telangiectasia. AT is an inherited DNA repair defect and its symptoms affect multiple organs increasing tumor risk.[28] Similarly to FA, radiotherapy should be restricted in these patients with great caution and reduced doses. The dose of any radiomimetic drugs used in the therapy of AT patients should also be modified to minimize cytotoxicity. Higher aberration frequencies were found in these five patiens as a result of 3 and 6 Gy in vitro irradiation. In the case of AT patients, increased response presenting with chromosome breakages were confirmed in the bleomycin fragility test, but it was not detected by MMC, which phenomenon can help in the differential diagnosis of this disease.

5 | CONCLUSIONS

Our data illustrate that MMC-test provides a useful method to differentiate between FA and other AA patients. This simple tool can help to reduce the need of FANC genetic testing, and accelerate accurate diagnosis and the early recognition of these disorders, also allowing for appropriate therapy planning. We have also proved that the parallel use of MMC, bleomycin tests and in vitro irradiation that induce chromosome breakages can be included in the investigation of AT patients.

AUTHOR CONTRIBUTIONS

Gyöngyi Farkas wrote the manuscript. Gyöngyi Farkas, Gábor Székely, Veronika Goda, Krisztián M. Kállay, Katalin Szakszon, Gábor Benyó, Dániel Erdélyi, Zoltán Liptai, Katalin Csordás, Gabriella Kertész, István Szegedi, and Gergely Kriván performed the measurements. Zsuzsa S. Kocsis performed the analytic calculations. Zoltán Takácsi-Nagy, Csaba Polgár, and Zsolt Jurányi discussed the results and commented on the manuscript. All authors discussed the results and contributed to the final manuscript.

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CONFLICT OF INTEREST STATEMENT

No potential conflict of interest was reported by the authors.

DATA AVAILABILITY STATEMENT

The authors would not like to share the data due to the regulation of GDPR.

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