The Turkish Journal of Pediatrics 2023; 65: 809-821
https://doi.org/10.24953/turkjped.2023.469

Original Article

The effect of granulocyte colony stimulating factor on genotoxicity in allogeneic peripheral blood stem cell transplantation donors: a prospective case-control study

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ABSTRACT

Background. Every year, thousands of donors are exposed to granulocyte-colony stimulating factor (G-CSF) for stem cell mobilization in hematopoietic stem cell transplantations (HSCT). Previous studies about the genotoxicity of G-CSF were inconclusive. In this study, the genotoxic effects of G-CSF in peripheral blood stem cell (PBSC) donors were evaluated prospectively by using three different validated and reliable methods for the first time in the literature to the best of our knowledge.

Methods. Donors of PBSC transplantation (n=36), who received G-CSF were evaluated for genotoxicity by micronucleus test (MNT), nuclear division index (NDI), and comet assay (CA). Genotoxic effects are expected to cause an increase in MNT and CA values and decrease in NDI. Blood samples were collected at three time-points (TP): before starting G-CSF (TP1), after G-CSF for five days (TP2), and one month after the last dose (TP3). Sixteen controls were included for baseline comparison of genotoxicity tests. CD34 cell counts and hemograms were also analyzed.

Results. MNT and CA parameters; comet and tail length, tail DNA%, and tail moment, showed no change in time whereas another CA parameter, Olive's tail moment (OTM) was increased significantly at TP3 compared to both baseline and TP2 ($p=0.002$ and $p=0.017$, respectively). Nuclear division index decreased significantly at TP2 ($p<0.001$), then increased above baseline at TP3 ($p=0.004$). Baseline comparison with controls showed higher MN frequency in donors without statistical significance ($p=0.059$). Whereas, CA results were significantly higher in controls. CD34 cell count showed moderate positive correlation with white blood cell count at TP2 (Pearson $R=0.495$, $p=0.004$).

Conclusions. Our results showed the genotoxic effect of G-CSF in healthy donors, in two of the three tests performed, short-term effect in NDI, and long-lasting effect in OTM. So, this study provides novel information for the debate about the genotoxicity of G-CSF and supports the need for further studies with a larger sample size and longer follow-up.

Key words: peripheral blood stem cell transplantation, granulocyte colony-stimulating factor, comet assay, micronucleus tests, genotoxicity tests, hematopoietic stem cell mobilization, hematopoietic stem cell donor, tissue donor.

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Received 25th June 2023, revised 8th July 2023, 26th July 2023, accepted 28th July 2023.

This study was presented as an oral presentation at the 46th Turkish National Hematology Congress (virtual) in 2020 and received an award in the field of Experimental Hematology and was published in abstract form in the proceedings of the congress.

Annually, > 90,000 hematopoietic stem cell transplantations (HSCT) are performed worldwide, including different indications for malignant and benign diseases.[1] Between two major methods of obtaining hematopoietic stem cells; peripheral blood stem cell (PBSC) collection by apheresis is the most common one (80% of all allogeneic HSCT) and generally preferred over bone marrow harvest, due to its advantages including faster engraftment, practicability for donor and medical staff, and lower risk of relapse for patients with high risk malignant disease.[2]

Granulocyte-colony stimulating factor (G-CSF) is the drug of choice for mobilizing stem cells. The short-term side effects of G-CSF are generally well-tolerated but the long-term effects remain unclear.[3] For many years, there have been some concerns that G-CSF may increase the risk of malignancy. Several preclinical and clinical studies performed to clarify this issue have been inconclusive regarding the increased genotoxicity associated with the use of G-CSF.[4] There are some anecdotal reports about acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) cases after PBSC donation.[5,6] But, in large series except one[7]; the incidence of cancer after PBSC and bone marrow transplantation was not different and did not detect an increase in the risk of MDS-AML.[8-10] The World Marrow Donor Association (WMDA) stated that there is not an increased risk of developing cancer after the use of G-CSF compared to donors not receiving G-CSF.[11] On the other hand, many centers continue to record the family history of leukemia and follow the donors for malignancies.[4]

Genotoxicity is defined as damage to genetic material by chemical, physical or biological agents. The relationship between genotoxicity and carcinogenicity has been clearly demonstrated. The detection of DNA damage in cells is fundamental for studying carcinogenesis. Therefore, genotoxicity studies are important in identifying carcinogens.[12] Today, different methods are used in genotoxicity studies. Micronucleus test (MNT), nuclear division index (NDI) and comet assay (CA) are the current reliable tests in 'International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use' classification but these methods have not been used in any previous study for evaluating the G-CSF genotoxicity.[13] An increase in MNT and CA values suggests there is a genotoxic effect, whereas NDI is expected to decrease with genotoxicity.[14,15]

In this prospective case-control study, our primary aim was to evaluate the possible genotoxic effects of G-CSF on healthy peripheral stem cell donors. For this purpose, donors were prospectively analyzed by MNT, NDI and CA at 3 time-points (TP), baseline (TP1), 5 days after G-CSF administration just before apheresis (TP2), and 1 month after stem cell collection (TP3). These tests were also performed on age- and sex-matched volunteers as a control group. Our secondary aim was to evaluate the effects of G-CSF on CD34 counts and hemograms.

Material and Methods

Participants and study design

Thirty-six HLA-matched related peripheral blood stem cell transplantation (PBSCT) donors and 16 age- and sex-matched healthy volunteers were included in the study. The data related to the donors and controls, including age, sex, body weight, smoking status and drugs being used, were recorded.

For mobilization of stem cells, the dose of G-CSF was 10 μg/kg/day subcutaneously for 5 days. Donors received two forms of G-CSF; filgrastim or lenograstim. On the 5th day, the G-CSF dose was applied at 6 a.m. and the apheresis was performed at 9 a.m.

Samples were collected for genotoxicity tests and hemograms at three time-points; TP1-3. Samples for genotoxicity tests were immediately transported to the laboratory and MNT and NDI were studied fresh and the samples for CA were stored frozen at -80°C.

The laboratory team were single-blinded during the genotoxicity studies. The CD34 counts after 5 days of G-CSF were measured as a routine procedure for transplantation at TP2. As a secondary outcome, for investigating the effect of G-CSF on hemogram and CD34 counts, results were recorded for the three TPs of the study.

To confirm the internal consistency and reliability, two samples one week apart were collected from 10/16 of controls.

The study, including sample collection and laboratory studies, were performed between May 2012- June 2013. The MNT and NDI were performed in the Pediatric Hematology Laboratory and the CA, in the Genetic Toxicology Laboratory of Forensic Sciences in Ankara University.

The study was approved by Ankara University Clinical Ethics Committee (Date: 28.05.2010, Number: 09-281-12). All samples were collected after written informed consent of donors and/or guardians before study entry.

Micronucleus test and nuclear division index

The micronucleus test was performed as defined in detail by Fenech et al.[14] In brief, for the preparation of cell cultures, fresh blood samples were added to the chromosome medium (Chromosome Medium B) and incubated at 37°C for 72 hours and cytochalazine-B was added to stop cytokinesis at 44 hours. At the end of previously defined procedures, the preparations were stained homogeneously in 5% Giemsa for the detection of micronucleus (MN) formations.

In these preparations, MN in 1000 binucleate cells were evaluated in total. During these examinations, 1000 binuclear cells were examined for each donor and those containing MN were noted. The following formula was used to calculate MN frequency and an increase in this value is interpreted as increased genotoxicity. "MN frequency=(1X 1MN)+(2X 2MN)+3X (3+4MN)/1000".[14]

Nuclear division index was calculated from the same preparations, after counting 500 cells and determining the number of cells with one, two, three and four nuclei and a decrease in NDI is interpreted as increased genotoxicity. The calculation was made according to the formula below. "NDI= [(1xN1)+(2xN2)+(3xN3)+(4xN4)] / n" (n: Total number of cells).[16]

Comet Assay

DNA damage was determined according to five CA parameters; comet length (CL), tail length (TL), tail DNA %, tail moment (TM), and Olive's tail moment (OTM). CA was conducted under alkaline Ph with some modifications from the original method as described earlier.[17-19] The levels of DNA damage were measured by the BAB Bs CA system and an increase in CA parameters is interpreted as increased genotoxicity. Approximately 15 regions of the preparation for each individual were scanned and 50 randomly selected lymphocytes were examined under an Olympus BX50 fluorescent microscope.

Pretest for genotoxicity tests: self-control

No significant difference was detected between two separate samples withdrawn at a one week interval from 10 volunteers in control group, confirming the internal consistency and reliability of genotoxicity tests ($p>0.05$).

Statistical analysis

All statistical analyses were performed using SPSS 21.0 (Statistical Package for Social Sciences, SPSS Inc., Chicago, USA). Since the test results obtained (MNT, NDI, CA) were repetitive measurements obtained from the same subjects, analysis of variance method was used for repeated measurements. Dependent groups T test was used to compare the results of the samples taken at different times from the control group. The results of the donors and controls were compared with the independent groups T-test. In the evaluation of the relationship between variables, correlation coefficients and

statistical significance were calculated with the Pearson test. The statistical significance limit was accepted as p<0.05 for all tests. The mean and standard deviation (SD) values were used as descriptives.

Results

Participants

The study group consisted of 36 donors (mean age±SD=32.3±14.5 years, min-max=8-66 years, M/F: 18/18) and 16 volunteers as the control group (mean age±SD=38±8.7 years, min-max=18-51 years, M/F: 8/8). Twenty-six of 36 donors (mean age±SD=32.7±14.6, min-max: 8-66 years, M/F: 13/13) completed all 3 sampling for genotoxicity tests, 10 donors who did not have three samples were excluded from the analysis of the genotoxicity tests. Eight of ten donors could not come for the third sampling due to geographic distance and the other two donors did not want to continue the study. Additionally, MNT could not be studied in two donors due to technical problems. As not all of the donors came for the originally planned first month control on time, the third samples were collected on average of the 42nd day (Median:36, IQR:10.8, min-max:30-148 days). The age and sex distributions of donors and controls were similar (p>0.05).

The drugs used for mobilization of stem cells were filgrastim (n=28) and lenograstim (n=8). CD34 cell count was available for 34 donors. Hemograms were evaluated in 26 donors who had three TP samples.

Micronucleus tests of donors

The MNT results were similar between the three TPs (TP1, TP2, TP3) (n= 24, p=0.819). (Fig. 1. A-C, Fig. 2, Table I).

Nuclear division index of donors

The nuclear division index of donors (n=24) was significantly decreased (p<0.001) at TP2 and significantly increased at TP3 compared to both TP1 and TP2 (p=0.004 and p<0.001, respectively) (Fig. 3 A-B, Fig. 4, Table I).

Comet assay of donors

Comet assay was studied with 5 parameters in 26 donors at three TPs. There was no significant difference between TPs for CL, TL, tail DNA%, TM. On the other hand, OTM at TP3 was

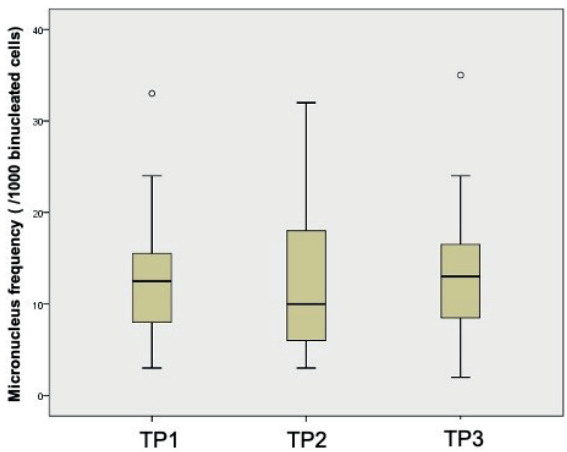

Fig. 2. Micronucleus frequency (/1000 binucleated cells) of donors at three time-points shown as a box-plot graph (TP1, TP2, TP3) (n= 24, p=0.819). TP: time-point.

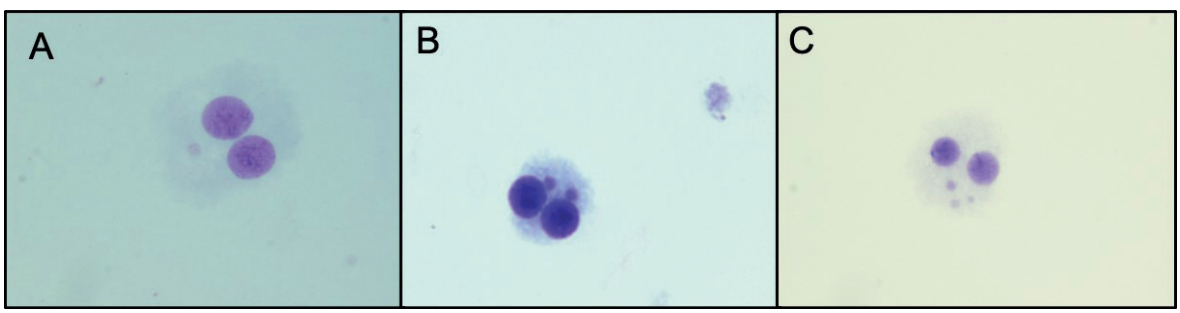

Fig. 1. Micronucleus tests (MNT). Micronuclei are seen as intracytoplasmic inclusions in two-nucleated cells, A. one micronucleus, B.two micronuclei, C. three micronuclei.

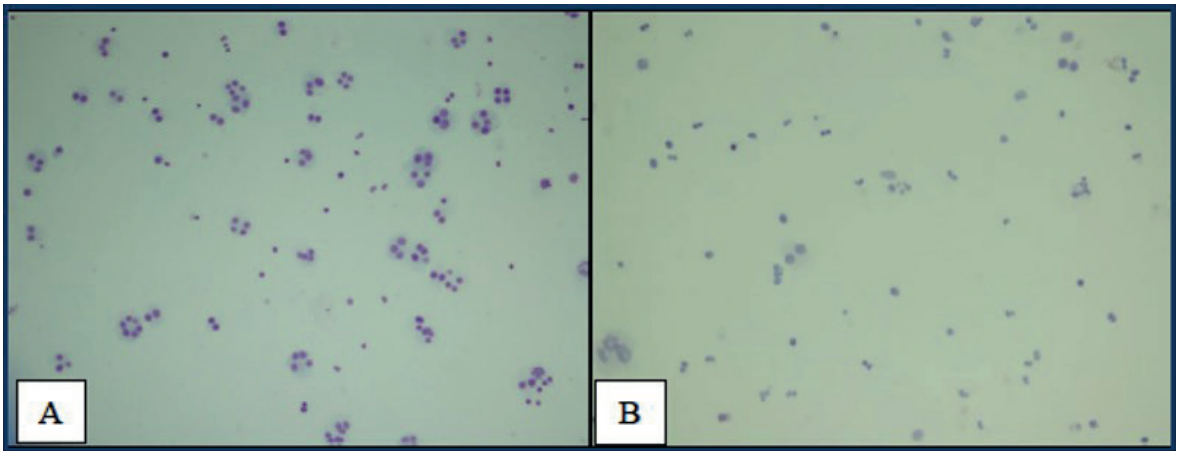

Fig. 3. Representative smear photographs used for calculating NDI of donors, A. before G-CSF, at TP1, normal induced dividing cells, B. after 5 days of G-CSF, at TP2, decreased nuclear division. G-CSF: granulocyte-colony stimulating factor, NDI: nuclear division index, TP: time-point.

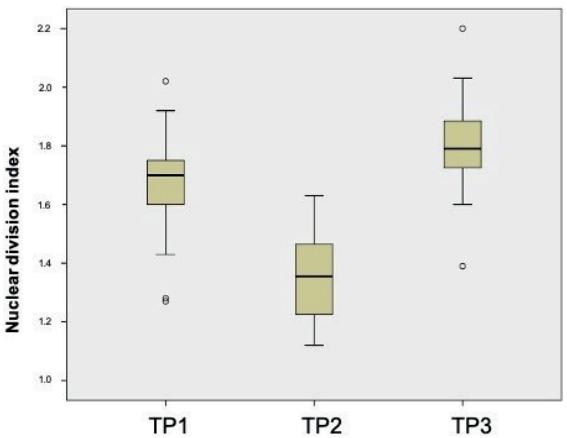

Fig. 4. Nuclear division index of donors at three time-points shown as a box-plot graph. (TP1 vs. TP2: p<0.001, TP2 vs. TP3: p<0.001, TP1 vs. TP3: p=0.004). TP: time-point.

significantly increased compared to both TP1 and TP2 (p=0.002 and p=0.017, respectively) (Fig. 5 A-E, Fig. 6, Table I).

Comparison of basal genotoxicity tests between donors and the control group

In terms of MNT, baseline results of donors (TP1) were higher compared to the control group (13.08±7.0 vs 9.19±4.82, respectively) without statistical significance (p=0.059) (Table II). For NDI, no statistically significant difference was found between groups (p=0.45) (Table II). In CA, results of controls, in terms of all parameters, were significantly higher than those of donors.

Factors affecting the CD34 count of donors

CD34 count of donors just before stem cell collection (TP2) was 86.6±46.0/µl (min-max: 16-246/µl). Age, body weight, gender and the form of G-CSF used (filgrastim or lenograstim) did not have a relationship with CD34 counts. Only the white blood cell (WBC) count at TP2 showed statistically significant and moderate correlation with CD34 count in the positive direction (Pearson R= 0.495, p=0.004) (Fig. 7, Table III).

Effects of G-CSF on hemogram parameters

Neutrophil, monocyte, basophil, eosinophil, and platelet counts showed significant increases at TP2 and decreased back to their baseline at TP3 (n=26). Lymphocyte counts, which had significantly increased at TP2, significantly decreased to even lower levels than the baseline count at TP3. There was no statistically significant change in hemoglobin levels (Table IV).

Table I. Genotoxicity test results of the control group at baseline and the donors at three TPs.

		TP	Donors*		Controls (n=16)	
			Mean±SD	Min-max	Mean±SD	Min-max
MNT (/1000 binucleated cells)		1	13.08±7.0	3.0-33.0	9.19±4.82	4.0-20.0
		2	12.31±6.94	3.0-32.0		
		3	13.3±7.75	2.0-35.0		
NDI		1	1.68±0.18	1.27-2.02	1.73±0.2	1.43-2.13
		2	1.35±0.16	1.12-1.70		
		3	1.81±0.16	1.39-2.20		
Comet	Comet length (μm)	1	26.43±4.37	20.19-30.39	31.82±3.27	28.4-39.56
Assay		2	26.60±4.51	19.30-35.85		
		3	27.63±5.96	11.09-36.40		
	Tail length (μm)	1	7.06±2.43	3.03-14.24	9.30±1.41	6.83-12.63
		2	6.73±1.60	3.86-10.20		
		3	7.51±2.22	2.65-12.56		
	Tail DNA%	1	72.20±10.9	48.1-88.82	78.65±4.98	67.82-84.72
		2	70.77±5.76	58.23-79.77		
		3	73.75±7.80	53.29-87.41		
	Tail moment	1	5.50±2.39	1.58-12.17	7.45±1.41	4.66-10.51
		2	5.03±1.42	2.47-8.08		
		3	5.90±2.13	1.91-11.16	24.95±1.07	19.85-32.91
	Olive's tail moment	1	19.99±3.80	12.47-26.33		
		2	20.39±5.33	10.18-30.88		
		3	23.21±4.12	13.73-35.85		

*: For MNT and NDI n=24, For Comet Assay n=26. MNT: micronucleus test, Min-max: minimum-maximum, NDI: nuclear division index, SD: standart deviation, TP: time-point

Table II. Comparison of the basal values of donors and the control group in terms of genotoxicity tests.

		Donors			Controls			p
		n	Mean±SD	Min-max	n	Mean±SD	Min-max	
MNT (/1000 binucleated cells)		24	13.08±7.0	3.0-33.0	16	9.19±4.82	4.0-20.0	0.059
NDI		24	1.68±0.18	1.27-2.02	16	1.73±0.2	1.43-2.13	0.45
Comet Assay	Comet length (μm)	26	26.43±4.37	20.19-30.39	16	31.82±3.27	28.4-39.56	<0.001
	Tail length (μm)	26	7.06±2.43	3.03-14.24	16	9.30±1.41	6.83-12.63	0.002
	Tail DNA%	26	72.20±10.9	48.1-88.82	16	78.65±4.98	67.82-84.72	0.013
	Tail moment	26	5.50±2.39	1.58-12.17	16	7.45±1.41	4.66-10.51	0.005
	Olive's tail moment	26	19.99±3.80	12.47-26.33	16	24.95±1.07	19.85-32.91	<0.001

MNT: micronucleus test, Min-max: minimum-maximum, NDI: nuclear division index, SD: standart deviation.

Table III. Comparison of CD34 counts by gender and G-CSF type.

	Sex			G-CSF		
	Female (n=17)	Male (n=17)	p	Filgrastim (n=27)	Lenograstim (n=7)	p
CD 34/ μl (mean±SD)	78.42±44.0	94.8±47.8	0.306	85.1±45.9	91.5±48.9	0.736

CD 34: CD 34 cell count, G-CSF: granulocyte-colony stimulating factor, SD: standart deviation.

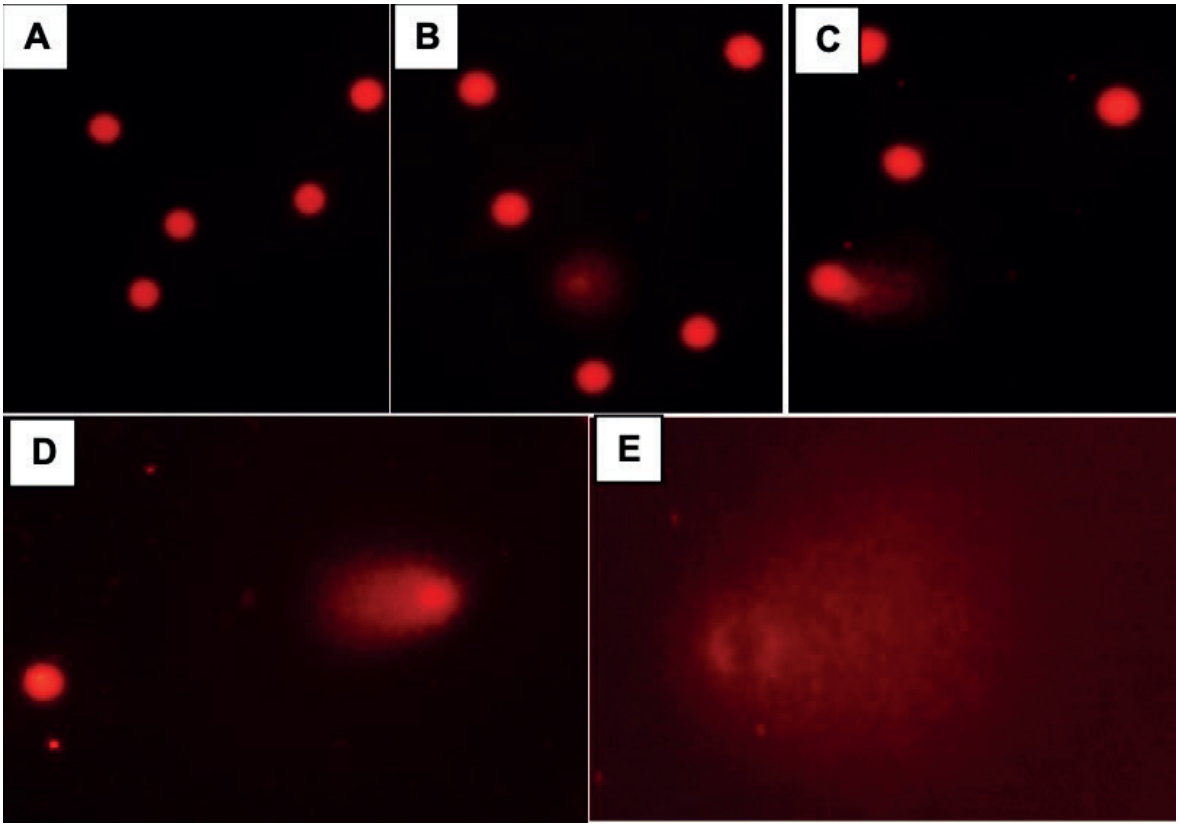

Fig. 5. Comet Assay, fluorescent microscobic images of comet assay showing increasing levels of DNA damage in lymphocytes in the order from A to E.

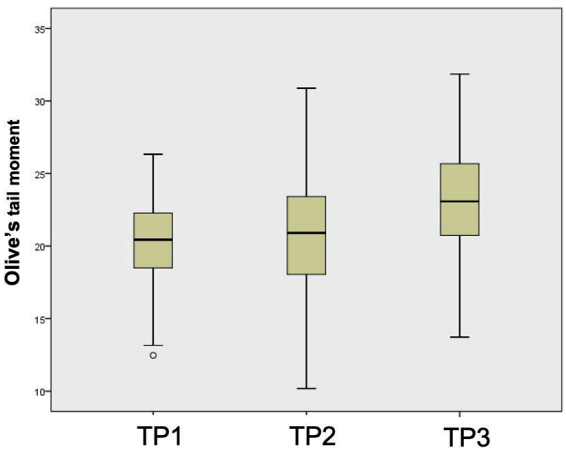

Fig. 6. Olive's tail moment of donors at three TPs shown as a box-plot graph. (TP1 vs. TP2: p=0.631, TP2 vs. TP3: p=0.017, TP1 vs. TP3: p=0.002). TP: time-point.

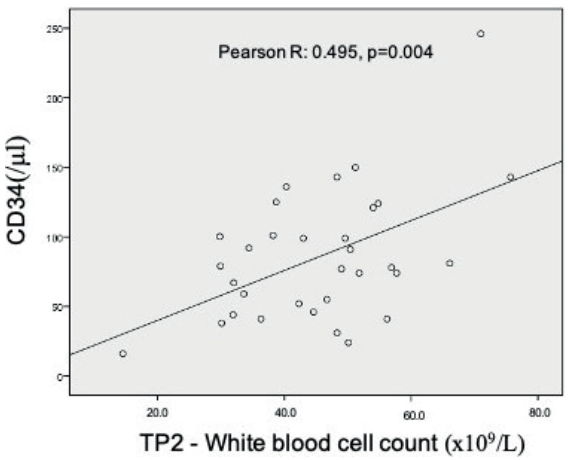

Fig. 7. Scattered dot graph showing the relationship between TP2 WBC count (x10^9/L) and CD34 count of donors (/µl). TP: time-point, WBC: white blood cell.

Table IV. Effect of Granulocyte-Colony Stimulating Factor on hemogram (n=26).

	TP1	TP2	TP3	P
WBC (x10^9/L)	5.340	46.807	45.80	*TP1 vs. TP2* <0.001
				TP1 vs. TP3 =0.043
				TP2 vs. TP3 <0.001
Neutrophil(x10^9/L)	4.396	33.024	4.013	*TP1 vs. TP2* <0.001
				TP1 vs. TP3 =0.380
				TP1 vs. TP3 <0.001
Lymphocyte (x10^9/L)	2.465	3.760	1.937	*TP1 vs. TP2* <0.001
				TP1 vs. TP3 =0.005
				TP2 vs. TP3 <0.001
Monocyte (x10^9/L)	0.440	9.197	0.451	*TP1 vs. TP2* =0.002
				TP1 vs. TP3 =0.781
				TP2 vs. TP3 =0.002
Eosinophil (x10^9/L)	0.155	0.464	0.126	*TP1 vs. TP2* <0.001
				TP1 vs. /TP3 =0.102
				TP2 vs. TP3 <0.001
Basophil (x10^9/L)	0.044	0.216	0.042	*TP1 vs. TP2* =0.047
				TP1/TP3 =0.908
				TP2/TP3 =0.056
Thrombocyte (x10^9/L)	251	221	249	*TP1/TP2* =0.007
				TP1/TP3 =0.746
				TP2/TP3 =0.008
Mean Platelet Volume (fL)	8.8	8.2	8.3	*TP1/TP2* =0.032
				TP1/TP3 =0.049
				TP2/TP3 =0.659
Hemoglobin (gr/dL)	14.1	13.9	13.8	0.414

TP: time-point, WBC: white blood cell

Discussion

In this study, the genotoxicity tests, namely MNT, NDI and CA, have been performed at 3 different TPs to explore whether G-CSF causes a genotoxic effect in healthy PBSCT donors for the first time in the literature. While NDI showed a short-term genotoxic effect at TP2 which normalized at TP3; OTM, one of the most sensitive components of CA for genotoxicity, revealed delayed genotoxic effect of G-CSF at TP3.[15,20,21] On the other hand, MNT did not detect any genotoxic effect.

The relationship between increased micronucleus formation due to known carcinogens, like gama radiation and ultraviolet light has been demonstrated.[22,23] Also, increased MN and elevated risk of cancer have been shown in clinical, prospective, and long-term follow-up studies.[24-26] Although MNT is a reliable method used in the detection of chromosomal damage, genome instability and cancer risk, it did not show a genotoxic effect in our study group.

Nuclear division index, which is a marker of cell proliferation, is expected to decrease with genotoxic effects. NDI was found to be lower in patients with lung cancer compared to healthy controls, and it was found to be lower in patients with colonic polyps or colon cancer compared to individuals with normal colonoscopy.[27,28] The NDI results of our donor group were

interpreted as G-CSF having an inhibitory effect on lymphocytes in the early period, but this effect was short-term. At TP3 this inhibitory effect disappeared and NDI increased above the baseline in a compensatory manner. Rutella et al.[29] showed that G-CSF inhibited the cell cycle progression in lymphocytes. Although our NDI results are in parallel with this finding, in our study this effect was reversible in about one month. The inhibiting effect on cell division may be an indicator of genotoxicity, but its disappearance at TP3 is in favor of reversibility.

The comet assay is a method that is frequently used in the evaluation of DNA damage and is used to investigate the possible genotoxic effects of newly defined drugs and chemicals.[15,30]As far as we know, this is the first study using CA to detect possible genotoxic effects of short-term G-CSF application in healthy individuals. The best parameters that predict genotoxicity in the CA are tail DNA% and Olive's tail moment.[15,20,21,31] In our study, CL, TL, tail DNA % and TM did not show a statistically significant change between the three TPs. On the other hand, OTM, which unites the TL and tail density as a single variable and therefore more sensitive in showing the genotoxic damage, was found to be increased at TP3 compared to TP1 and TP2. It is noteworthy that the genotoxic damage became obvious at TP3 in this assay, necessitating longer follow-up studies to determine the exact duration and the reversibility of this finding.

While no genotoxic effect was detected with MNT in our study, a statistically significant effect was demonstrated by OTM parameter of CA at TP3. On the contrary, a study comparing CA and MNT, found that the two methods had the same sensitivity in determining mutagenicity, while MNT was more powerful in determining low-level genotoxic damage potential, most likely because only the whole length parameters of CA was used in this study.[32] Our results showed that OTM, as one of the most sensitive parameter of CA, may be superior to MNT in detecting genotoxicity.

In the literature, laboratory studies investigating the possible genotoxic effect of G-CSF in healthy PBSCT donors with different methods provided various evidence which were inconclusive. Nagler et al.[33] detected asynchrony in the timing of allelic replication, changes in the capacity of DNA methylation, and aneuploidy that continued in the 6th month in lymphocytes. On the other hand, Schapira et al. found that DNA destabilization increased on the 5th day, returning to normal within 1-2 months.[34] According to Hirsch et al., G-CSF did not lead to any chromosomal instability and can be used safely.[35] More recent studies similarly found conflicting results such as; Baez et al.[36] showed that G-CSF treatment in healthy donors led to differential expression of a group of genes and microRNAs in CD34 cells, which was persistent after one year; whereas Leitner et al.[37] evaluated methylation in peripheral lymphocytes and did not find any significant change. As can be seen from these various studies, the methods of genotoxicity evaluation, target cells studied and time-points are not standard, long-term follow up data is lacking, leading to a confusion about the safety of G-CSF use in healthy PBSCT donors.

The comparison of basal genotoxicity tests between donors and controls showed that MN frequency was higher in donors at baseline. Although this was not statistically significant, it may support a possible increased genotoxicity in the donor group before G-CSF exposure. This may be due to the pre-procedural stress of the stem cell donation or cigarette smoking (there were no smokers in control group while 5 donors were smokers), which are possible genotoxic factors not present in control group.[38,39] On the other hand, unexpectedly, CA results of controls were significantly higher than the baseline values of donors. The samples of the donors and the controls arrived at the laboratory out of order and have been analyzed blindly by the investigator, excluding any possible technical problem responsible for these conflicting results. There may be other confounders such as air pollution, diet, alcohol

use, sedentary lifestyle and other genetic factors that are not easy to detect. In our study, as same individuals were evaluated longitudinally for possible genotoxic effects of G-CSF, we believe that the confounding effect of these factors has been minimized in the comparison of TPs in the donor group.

In our study, the the CD34 count showed a statistically significant, moderate and positive correlation with the WBC count at TP2 and there was no difference in CD34 mobilization effect of filgrastim and lenograstim consistent with the literature.[40,41] Additionally, the number of CD34 cells was not related to the donor's age, body weight or gender. However, there are controversial results in the literature.[42,43] It is thought that CD34 yield decreases, especially in females and with advanced age (>55 years).[44,45]

Following 5-day G-CSF, the WBC and lymphocyte counts increased significantly at TP2 compared to TP1, then returned to the normal range at TP3 but were significantly lower than baseline. Holig et al., in their study of 3928 unrelated donors, found that the WBC count was significantly lower than baseline in the 1st month after G-CSF administration, increased partially in the 4-year follow-up, but never reached baseline values. On the other hand it was found that the low lymphocyte count returned to normal one year later.[46] The reason for the prolonged slightly lower WBC counts relative to baseline is not clear but may be due to slow replacement of stem cells, "down-regulation" of G-CSF receptors, or other disturbances in cytokine linkages. In donors, leukocytosis secondary to possible pre-procedural stress may also be a reason for these values not returning to normal.[47] It was observed that the neutrophil, monocyte, basophil and eosinophil counts significantly increased at TP2 after G-CSF application, and decreased back to their previous values at TP3, consistent with the literature.[7,47]

In our study, there was a statistically significant decrease in platelet counts compared to baseline at TP2, coming back to normal at TP3. In large-scale studies, a decrease in platelet counts due to G-CSF use has been reported.[7] There may be two possible explanations; the partial suppression of platelet production secondary to the orientation of the stem cells to the myeloid series and hypersplenism secondary to the enlargement of the spleen due to G-CSF.[47]

There are some limitations to this study. First, fewer participants than planned at the beginning were recruited due to the difficulty of obtaining TP3 samples from individuals living in other cities. However, the sample size was found to be sufficient for this prospective study. Additionally, TP3 samples could not be collected homogenously on day 30 from all donors. The second limitation was not having a longer follow up of the donors, as there is no standard protocol for the follow-up of healthy donors in our country. Long-term follow-up of all donors and collection of follow-up data by the international study groups are recommended in order to understand the delayed effects of G-CSF. In France, these donors are covered by a 10-year health insurance and follow-up is planned for 10 years.[48] Last but not least, the conflicting results of CA in controls could not be clarified with additional experiments (/tests), as the sample collection part of the study was already completed.

In conclusion, in this study the genotoxic effects of G-CSF were detected in healthy PBSCT donors in two of the three tests perfomed (NDI and CA). Although NDI values decreased back to normal, OTM detected an actual persistent genotoxic effect at TP3. Future studies with larger donor groups with a longer follow-up are needed.

Acknowledgements

The donor group for the study was collected with the support of Ankara University Department of Pediatric Hematology and Oncology, Ankara University Department of Adult Hematology and Ankara University Department of Pediatric Immunology,

Hacettepe University Department of Pediatric Hematology and Oncology, Gülhane Military Academy Department of Pediatric Hematology. As the authors, we would like to express our gratitude to these centers. Special thanks to Assoc. Prof. Murat Akyıldız for his contribution in statistical analysis.

Ethical approval

Ankara University Clinical Ethics Committee (Date: 28.05.2010, Number: 09-281-12). All samples were collected after written informed consent of donors and/or guardians before study entry.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: HFÇ, Tİ; data collection: HFÇ, Tİ, HG, PT; analysis and interpretation of results: HFÇ, HG, ES, PT, ZK, Tİ, draft manuscript preparation: HFÇ, Tİ, ES, ZK. All authors reviewed the results and approved the final version of the manuscript.

Source of funding

This study was supported by Ankara University Scientific Study Fund with project number 12B3330022.

Conflict of interest

The authors declare that there is no conflict of interest.

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