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G-CSF administration favours SDF-1 release and activation of neutrophils and monocytes in recipients of autologous peripheral blood progenitor cells

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ABSTRACT

G-CSF is a growth factor widely used to mobilise CD34+ progenitor cells for clinical applications. The present study aimed to assess expression of G-CSF receptor (CSF3R) on neutrophils and monocytes, as well as SDF-1 and G-CSF serum levels in relation to efficacy of G-CSF-induced mobilisation for autologous transplantation. For this purpose, 105 patients with haematological disorders and 46 healthy controls were investigated.

Before mobilisation patients were characterised with significantly higher percentage of CSF3R expressing neutrophils ($p < 0.001$) and monocytes ($p = 0.002$), than controls. G-CSF administration resulted in a decrease of CSF3R+ neutrophils ($p < 0.001$) and monocytes ($p < 0.001$), while presence of G-CSF receptor on neutrophils tended to negatively affect mobilisation yield ($p = 0.075$).

G-CSF concentration increased during mobilisation ($p < 0.001$). On the 5th day of mobilisation a positive correlation was observed between G-CSF and SDF-1 serum levels ($p < 0.001$) and the number of CD34+ cells released from bone marrow seemed to be related to both G-CSF ($p = 0.036$) and SDF-1 levels ($p = 0.084$).

As compared to Hodgkin's lymphoma patients, those with multiple myeloma had lower basal percentage of CSF3R+ neutrophils ($p = 0.014$) while Non-Hodgkin's lymphoma cases exhibited higher G-CSF ($p = 0.026$) and SDF-1 ($p = 0.006$) concentration on mobilisation day 5. Hodgkin's lymphoma patients were also characterised with worse mobilisation efficacy than multiple myeloma ($p = 0.022$) and Non-Hodgkin's lymphoma ($p = 0.013$) patients.

These results suggest that both SDF-1 and G-CSF play a role in HSC release into peripheral blood and show that G-CSF administration affects expression of CSF3R on monocytes and neutrophils, implying potential role of these cell subpopulations in mobilisation process.

1. Introduction

Mobilised peripheral blood (PB) is the predominant source of haematopoietic stem cells (HSC), also referred to as CD34+ cells, for autologous and allogeneic transplantation (HSCT). The main indications for this procedure are lymphoproliferative disorders and leukaemias, although it is also used in a variety of other diseases like solid tumours, bone marrow failure syndromes, haemoglobinopathies, immune deficiencies, inherited metabolic disorders or autoimmune disorders [23]. Currently, over 68 thousand HSCTs using PB are performed each year worldwide [43].

Granulocyte colony-stimulating factor (G-CSF), also known as colony stimulating factor 3 (CSF3), is the main growth factor that controls both proliferation and differentiation of myeloid progenitor cells into neutrophils [63]. It was one of the first described cytokines and was introduced to clinical practice for depleting neutropenia and infections in cancer patients [8,22]. This approach has not changed, as described in the 2010 EORTEC guidelines [1]. G-CSF is administered to patients with high risk of severe neutropenia upon receiving chemotherapy and also to allow performing intensive chemotherapy. However, this cytokine needs to be dosed carefully because prolonged G-CSF treatment may lead to myelodysplastic syndromes (MDS) and acute myeloid leukaemia (AML) development [52].

Currently, recombinant G-CSF, sold under various names, e.g.

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lenograstim, filgrastim, pegfilgrastim and others [17,49,1], is widely utilised as a mobilising factor during HSC collection for transplantation and for other clinical purposes. It is the most common mobilisation agent that is administered either alone or in conjunction with chemotherapy [5].

G-CSF is the primary ligand for colony-stimulating factor 3 receptor (CSF3R), also referred to as G-CSFR or CD114 antigen. It is encoded by the *CSF3R* gene, mapped to chromosome 1p32-35. This gene contains 17 exons that code for an 812-813 amino acid-long transmembrane protein [20,62,4]. The receptor for G-CSF is a member of Type-I cytokine/haematopoietin receptor family characterised with four highly conserved cysteine residues and a tryptophan–serine repeat (WSXWS) located in the cytokine receptor homology (CRH) region of the extracellular domain [62,64].

Clinical HSC mobilisation has been successfully performed for the last thirty years [18]. This process is similar to natural HSC migration in response to inflammatory factors or tissue damage [16]. Haematopoietic progenitor cells are retained in bone marrow through interactions with adhesion molecules. Among those, stromal cell-derived factor 1 (SDF-1; also known as CXC motif chemokine ligand 12, CXCL12) is regarded as the key mediator [70,38,41], as HSCs express its receptor: chemokine CXC motif receptor 4 (CXCR4). It is believed that upon G-CSF binding to CSF3R, bone marrow leukocytes and endothelial cells are stimulated to release proteolytic enzymes. Those, in turn, degrade molecules anchoring progenitor cells in haematopoietic niches [2,28]. Disruption of the CXCR4/SDF-1 chemotactic interaction during G-CSF-induced mobilisation facilitates HSC release from bone marrow into peripheral circulation [33].

Neutrophils, which are greatly affected by G-CSF signalling, are believed to play the leading role in HSC mobilisation. However, it is important to note that G-CSF receptor is expressed on many different cell types, including platelets, monocytes/macrophages, endothelial cells and other cell lines [9,25,58,3,35,40,47]. Activity of different blood cell subpopulations was shown to be modulated during G-CSF-induced clinical mobilisation [10]. Results obtained from studying murine models also suggested that CSF3R signals in monocytes could be sufficient to mediate haematopoietic progenitor cell mobilisation [13] and that defective activation of granulocytes and monocytes could be responsible for poor mobilisation efficacy [71]. This prompted us to analyse involvement of both neutrophils and monocytes in mobilisation in humans.

Our previous studies documented the effect of *SDF-1* and *CSF3R* polymorphisms on peripheral blood stem cell enrichment in autologous patients and allogeneic healthy donors [6,7].

In the present study, we aimed to find out whether expression of G-CSF receptor on neutrophils and monocytes could be associated with effectiveness of G-CSF-induced mobilisation in recipients of autologous peripheral blood progenitor cells. For this purpose, CSF3R expression was analysed at two time points: on day 0 (before G-CSF administration) and on day 5 of clinical mobilisation. In addition, serum levels of G-CSF and SDF-1 were also measured and compared with mobilization efficacy and expression of CSF3R.

2. Materials and methods

2.1. Patients and controls

One hundred and five recipients (F/M = 57/48, aged 20–70, mean 55; median 59 years) of autologous transplantation of peripheral blood progenitor cells (PBPCT) were investigated. There were 58 (55.2%) patients with multiple myeloma (MM), 29 (27.6%) with non-Hodgkin's lymphoma (NHL), 13 (12.4%) with Hodgkin's lymphoma (HL) and 5 (4.8%) with acute myeloid leukaemia (AML). Brief patients' characteristics are given in Table 1.

The study group fulfilled the following criteria: age ≥ 18 years, diagnosed blood cancer, complete remission status and qualification for the HSCT procedure following high-dose chemotherapy. Patients were transplanted at the Clinic of Haematology, Blood Neoplasms and Bone Marrow Transplantation of the Wroclaw Medical University. CD34+ cells numbering 6×10^6 or more per kg of body weight (cells/kg b. w.) were considered an optimal quantity for transplantation [57,39].

Table 1
Patients' characteristics and differences between mobilisation efficacy in regard to the type of blood cancer. Various blood cancers were compared in regard to listed parameters. Analysis was performed with *T* Test for two independent samples with the usage of the real statistics resource pack for Microsoft excel 2013; ns – not statistically significant ($p > 0.05$). Significant differences were observed while comparing total number of separated CD34+ cells/kg b. w. in NHL vs. HL ($p = 0.030$) (A) as well as total number of separated CD34+ cells/μl in MM vs. HL ($p = 0.022$) (B) and in NHL vs. HL ($p = 0.013$) (C).

Haematological malignancy	MM (n = 58)	NHL (n = 29)	HL (n = 13)	AML (n = 5)
Age [years]	37–70	24–66	20–60	46–61
Mean	57	54	39	54
Sex (F/M)	30/28	14/15	7/6	3/2
Number of performed separations	1–5	1–5	1–4	1–5
Mean	1.8	1.3	1.4	1.5
Number of CD34+ cells/kg b. w. in the first leukapheresis product	0–33	0–14	0–8	0–7
Mean	6.4	4.5	3.7	3.4
Number of CD34+ cells/μl in the first leukapheresis product	0–1547	0–676	0–76	0–66
Mean	126.3	100.7	40.9	35.3
Total number of separated CD34+ cells/kg b. w.	0–50	0–14	0–8	3–7
Mean	7.9	5.3[A]	3.9[A]	4.6
Total number of separated CD34+ cells/μl	0–2825	0–1245	0–503	204–482
Mean	555.1[B]	430.6[C]	280.5[B,C]	334.7
Non-responders	8 (14% MM)	4 (14% NHL)	1 (8% HL)	0 (0% AML)

For 30 (28.6%) patients, the number of haematopoietic progenitor cells collected during the first separation was optimal for transplantation (exceeded 6×10^6 CD34+ cells/kg b. w.).

In regard to all performed leukaphereses (up to five), there was a group of 13 (12.4%) patients, for whom the level of CD34+ cells in the peripheral circulation never rose in response to clinical mobilisation and so collection of HSCs was not possible. Consequently, this group was described as non-responders (those who did not respond to G-CSF treatment). Among them, eight were diagnosed with MM, four with NHL and one with HL (Table 1).

Additionally, a group of 45 (F/M = 15/30) healthy blood donors from the Regional Centre of Transfusion Medicine and Blood Bank in Wroclaw, aged between 19 and 64 (mean 36; median 35), served as controls.

All the patients and controls provided written informed consent. The study was approved by the Wroclaw Medical University Ethics Committee and all the procedures were in accordance with the ethical standards of the Helsinki Declaration, as revised in 2013.

2.2. Surface expression of G-CSF receptor

The analysis of CSF3R surface expression on neutrophils and monocytes via flow cytometry was performed in all 45 healthy controls and in 37 patients (16, 15 and 6 persons with NHL, MM and HL, respectively) on day 0 and day 5 of G-CSF administration.

The presence of the G-CSF receptor was assessed with G-CSFR/CD114 Fluorescein-labelled antibody (monoclonal mouse anti-human IgG1, Clone #38660; R&D Systems Inc., Minneapolis, Minnesota, USA). Neutrophils were identified as CD16-positive granulocytes using CD16

PE-Cy5-canjugated antibody (monoclonal mouse anti-human IgG1, Clone 3G8; Becton Dickinson Biosciences, San Jose, California, USA), while for monocytes, CD14 PerCP-tagged antibody (monoclonal mouse anti-human IgG2b, Clone MΦP9; Becton Dickinson Biosciences, San Jose, California, USA) was utilised. For every antibody, appropriate isotype control was purchased: fluorescein-mouse IgG1 isotype control (Clone 11711), PE-Cy5-mouse IgG1 isotype control (Clone MOPC-21) and Per-CP-mouse IgG2b isotype control (Clone 133303), respectively, all from R&D Systems Inc. (Minneapolis, Minnesota, USA). As PE-Cy5 fluorescent dye has emission maximum close to that of Per-CP, analysis of G-CSFR+/CD16+ granulocytes and G-CSFR+/CD14+ cells was performed separately.

For each sample, 40 µl of the EDTA-preserved peripheral blood (2×10^5 cells) was incubated either with optimized amount of a specific antibody or isotype control or without antibody (for background fluorescence measurement). The BD FACS Lysing Solution (Becton Dickinson Biosciences, San Jose, California, USA) reagent was used for lysis of erythrocytes. After two washing steps with PBS buffer with 1% fetal bovine serum (EURx, Gdańsk, Poland), samples were suspended in Flow Fix, a 1% formaldehyde Fixation buffer (Polysciences, Inc., Warrington, Pennsylvania, USA) and analysed on FACS Canto II instrument with FACS Diva 8.0 software (Becton Dickinson Biosciences, San Jose, California, USA).

2.3. G-CSF and SDF-1 serum levels

Serum samples were collected from 105 patients before and on day 5 of G-CSF administration and from 35 healthy blood donors and stored at −20 °C. The samples were screened for the simultaneous detection of G-CSF and SDF-1 proteins with the use of a customized Magnetic Luminex Screening Assay (R&D Systems Inc., Minneapolis, Minnesota, USA). Analyses were performed according to the manufacturer's manual. The serum samples collected on day 5 of G-CSF administration were diluted 4 times with the Calibrator Diluent (provided with the assay), while remaining samples of patients before mobilisation and those of healthy controls were not diluted. For analysis purposes, Luminex 200 instrument (Luminex Corp., Austin, Texas, USA) was used.

2.4. Statistical analysis

The results were related to clinical data. Relations between efficacy of CD34+ cell mobilisation (expressed as the average number of CD34+ progenitor cells in the leukapheresis product) and different patient groups were calculated with *T* Test for two independent samples (results are shown in Table 1). The *U* Mann-Whitney test for two independent samples was introduced for assessing possible differences in surface expression of CSF3R on neutrophils and monocytes, and G-CSF and SDF-1 concentration in serum with respect to type of disease (Supplementary Tables 1 and 2). The Spearman's Rho correlation test was used for G-CSF, SDF-1 and CSF3R protein expression analysis at different time points. All calculations were performed with the real statistics resource pack for Microsoft Excel 2013 or using Social Science Statistics website (http://www.socscistatistics.com/tests/Default.aspx). Differences between samples were considered significant at $p < 0.05$. Probability (p) values between 0.05 and 0.10 were considered as indicative of a trend.

3. Results

3.1. Mobilisation yield in regard to blood cancer type (in patients with various blood cancers)

The efficacy of mobilisation procedure was expressed by (I) number of leukaphereses performed to obtain the required number of CD34+ cells for transplantation, (II) number of CD34+ cells collected during the first leukapheresis (either per kg of body weight or per µl of peripheral blood) and (III) total number of separated CD34+ cells (per kg of b. w. or µl of blood).

No correlation was observed between patients' age and mobilisation efficacy measured as number of CD34+ cells separated either per kg of body weight or per µl of blood. Patients' sex also did not seem to affect haematopoietic stem cells release.

There were no differences between mean numbers of CD34+ cell separations performed in patients with various blood neoplasms. Average number of performed leukaphereses equalled 1.5, 1.4, 1.3 and 1.8 for AML, HL, NHL and for MM patients, respectively (as shown in Table 1).

No statistically significant correlation between different types of blood cancer and number of CD34+ cells separated per kg of body weight or per µl of blood was found for the first leukapheresis (Table 1).

However, when comparing all mobilisation attempts between different types of blood cancer, NHL and MM patients were characterised with better mobilisation efficacy in comparison to individuals with HL. The total number of separated haematopoietic stem cells was higher for NHL patients as compared to HL cases ($p = 0.030$ for the total number of CD34+ cells/kg b. w., and $p = 0.013$ for the total number of CD34+ cells/µl) (Fig. 1). Similarly, patients with MM released more CD34+ cells in response to G-CSF than HL patients ($p = 0.022$ for the total number of CD34+ cells per µl of blood). Also, when total number of CD34+ cells/kg b. w. was considered, MM patients seemed to mobilise HSCs more effectively than HL patients ($p = 0.092$).

3.2. G-CSF serum levels and the association with mobilisation efficiency

G-CSF serum levels were assessed in patients before and after mobilisation (day 0 and day 5), and in healthy controls. As expected, G-

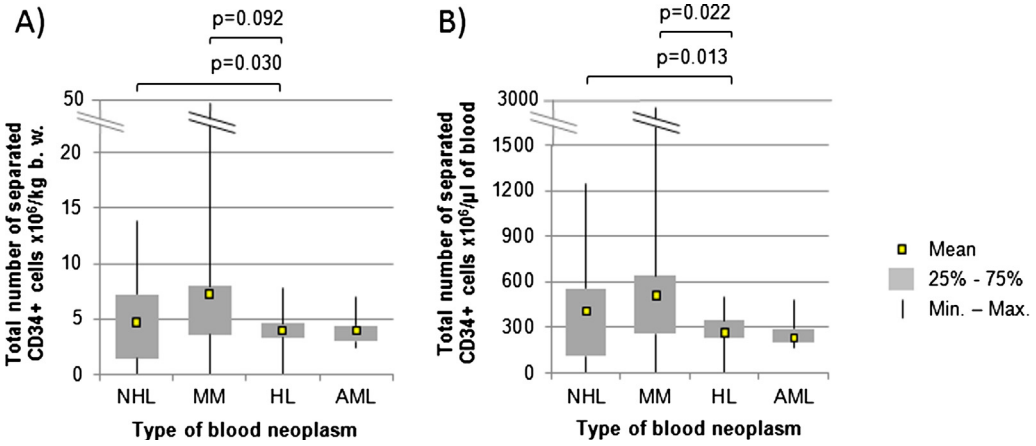


Fig. 1. Comparison of the total number of CD34+ cells separated in different blood cancers. Mobilisation efficacy was measured as the number of cells separated per kg of body weight (A) or per µl of blood (B). Analyses were performed using the *T* Test for two independent samples of the real statistics resource pack for Microsoft Excel 2013. Probability values were considered statistically significant for $p < 0.05$, while values between 0.05 and 0.10 were indicative of a trend.

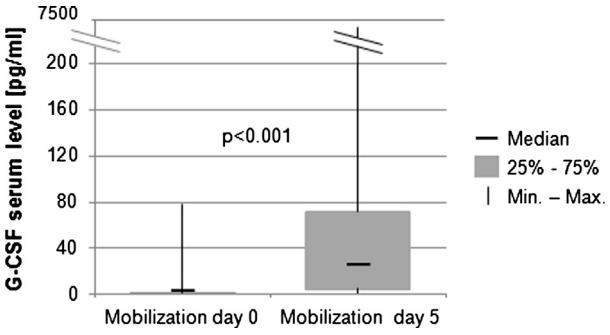


Fig. 2. An increase in G-CSF serum levels in patients during mobilisation. Statistical analysis was performed with the *U* Mann-Whitney test for two independent samples of the real statistics resource pack for Microsoft Excel 2013. Probability values were considered statistically significant for $p < 0.05$.

CSF levels significantly increased in patients during G-CSF administration ($p < 0.001$; Fig. 2). This association was seen in all the patients irrespective of the type of blood cancer (Figs. 3 and 4). However, no relations were found between mean G-CSF serum levels and patients' age or sex (Supplementary Table 1).

A slightly higher basal G-CSF level was observed in patients when compared to healthy controls ($p = 0.072$; Fig. 3A). When each group of patients was analysed separately, some significant relations emerged. Before mobilisation, controls were characterised with lower G-CSF serum levels as compared to NHL ($p = 0.015$) and HL patients ($p = 0.049$) (Fig. 3B).

Of note, before mobilisation no significant differences were seen in G-CSF levels when patients with distinct haematological malignancies were compared (Supplementary Table 1). However, on the 5th day of G-CSF administration some distinctions were observed between different types of blood cancer. At this time point patients with NHL were characterised with the highest G-CSF level compared to other groups (Fig. 4). The greatest difference was observed between NHL and AML ($p = 0.009$), followed by distinction between NHL and HL ($p = 0.026$). Some differences, although not statistically significant, were observed in G-CSF levels between NHL and MM ($p = 0.087$) as well as between MM and AML ($p = 0.070$).

The initial G-CSF levels (on day 0) before mobilisation were not associated with the mobilisation yield expressed as the number of CD34+ cells separated either per kg of body weight, or per µl of blood (Supplementary Table 1).

However, G-CSF level on the 5th day of mobilisation corresponded with procedure effectiveness, as higher serum G-CSF titre was related to increased number of HSCs separated per µl of blood ($p = 0.036$; Fig. 5).

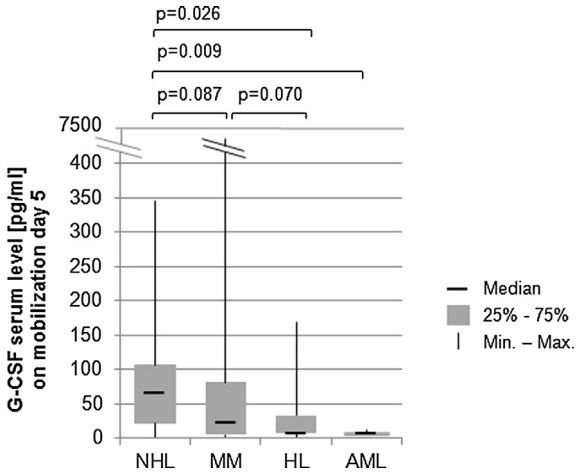


Fig. 4. Comparison of G-CSF serum levels on mobilisation day 5 in patients with different blood cancers. Calculations were performed with the *U* Mann-Whitney test for two independent samples of the real statistics resource pack for Microsoft Excel 2013. Probability values were considered statistically significant for $p < 0.05$, while values between 0.05 and 0.10 were indicative of a trend.

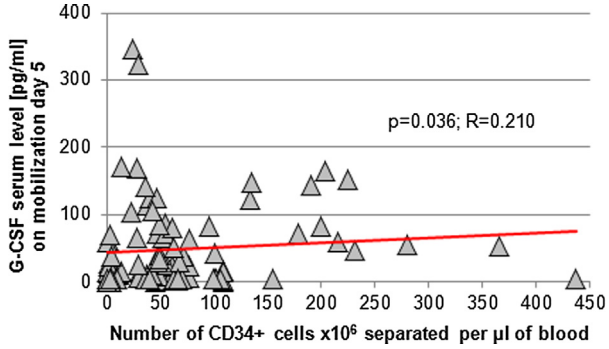


Fig. 5. Correlation between mobilisation efficacy (measured as number of CD34+ cells separated per µl of blood) and G-CSF serum concentration on mobilisation day 5. Spearman's Rho correlation test of the Social Science Statistics website (http://www.socscistatistics.com/tests/Default.aspx) was used for calculations. R stands for Spearman's Rho correlation coefficient. Probability values were considered statistically significant for $p < 0.05$.

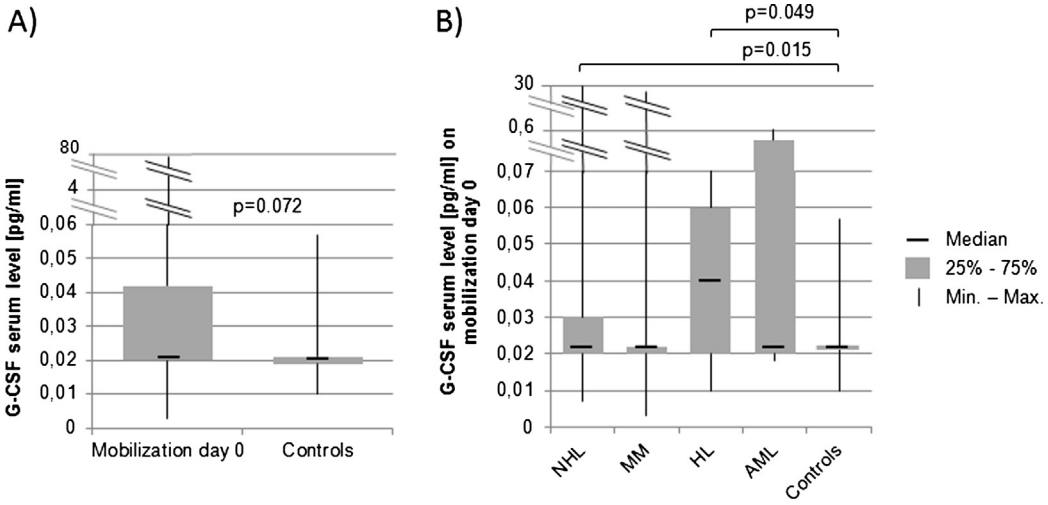
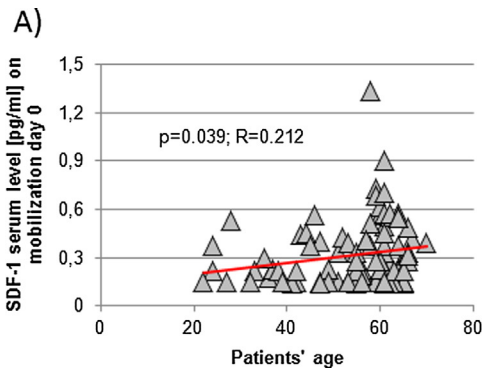


Fig. 3. Basal G-CSF serum levels in patients and healthy controls. G-CSF levels did not significantly differ between healthy controls and the whole patient group (A). When patients were separated according to respective blood cancers (B), individuals with NHL and HL were found to have significantly higher basal G-CSF serum levels compared to controls. Calculations were performed with the *U* Mann-Whitney test for two independent samples of the real statistics resource pack for Microsoft Excel 2013. Probability values were considered statistically significant for $p < 0.05$, while values between 0.05 and 0.10 were indicative of a trend.



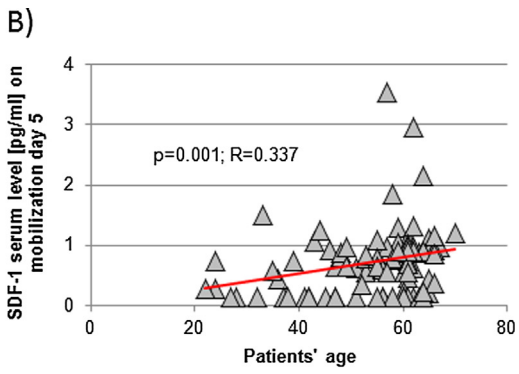


Fig. 6. Correlation between age of an individual and SDF-1 serum level. SDF-1 levels were compared in patients before (A) and on the 5th day (B) of G-CSF administration. Spearman's Rho correlation test of the Social Science Statistics website (http://www.socscistatistics.com/tests/Default.aspx) was used for calculations. R stands for Spearman's Rho correlation coefficient. Probability values were considered statistically significant for $p < 0.05$.

A tendency was also observed in regard to the number of HSCs separated per kg of body weight ($p = 0.085$).

3.3. Relationships between SDF-1 and G-CSF levels and the effect on mobilisation yield

Similarly to the G-CSF concentrations, SDF-1 serum levels were also assessed in patients at two time points - before and after mobilisation (day 0 and day 5) – and in healthy controls. Irrespective of the time of analysis, the levels were relatively low and did not exceed 4 pg/ml. They did not differ depending on sex (Supplementary Table 1), however, they were found to be correlated with patients age. Older individuals were found to have higher SDF-1 serum levels than younger patients both before ($p = 0.039$) and on the 5th day of mobilisation ($p = 0.001$) (Fig. 6).

No statistically significant differences were observed while comparing SDF-1 serum levels in patients before G-CSF administration and healthy controls (Supplementary Table 1). It is worth noting that similarly to G-CSF level, SDF-1 serum concentration significantly rose in response to mobilisation (Fig. 7). SDF-1 concentrations before and on the 5th day of mobilisation were significantly different ($p < 0.001$), although not correlated (Supplementary Table 1).

Some interesting results were obtained while comparing SDF-1 levels in various blood cancers. Before mobilisation no statistically significant differences were observed (Fig. 8A), though MM patients had slightly higher SDF-1 levels than individuals with NHL ($p = 0.076$). SDF-1 concentration increased in response to mobilisation regardless of blood cancer type. On the 5th day of mobilisation, HL patients were clearly characterised with lower SDF-1 serum concentrations when compared with NHL ($p = 0.006$) and MM ($p = 0.025$) patients (Fig. 8B). Difference between individuals with HL and AML were also observed, however not statistically significant ($p = 0.067$).

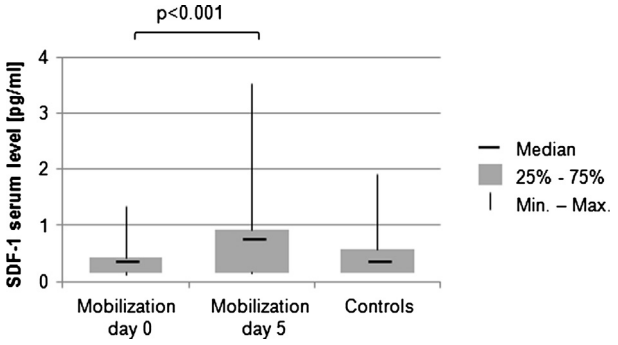


Fig. 7. Differences in SDF-1 concentration between patients on mobilisation day 0 and 5 and healthy controls. Calculations were performed with the *U* Mann-Whitney test for two independent samples of the real statistics resource pack for Microsoft Excel 2013. Probability values were considered statistically significant for $p < 0.05$.

There was no relationship between G-CSF and SDF-1 serum levels before mobilisation (Fig. 9A). However, SDF-1 levels detected on the 5th day of mobilisation procedure positively correlated with G-CSF serum levels at this time point ($p < 0.001$) (Fig. 9B).

No statistically significant correlations were observed between mobilisation efficacy (measured as number of CD34+ cells separated per kg b. w. or per µl of blood) and SDF-1 concentrations either before or on the 5th day of G-CSF treatment (Supplementary Table 1). Only a trend between number of HSC released per µl of blood and SDF-1 concentration on mobilisation day 5 was found ($p = 0.084$) (Fig. 10).

3.4. Changes in the CSF3R expression on neutrophils and monocytes

When considering autologous HSCT patients, mean number of both neutrophils and monocytes rose on day 5 of mobilisation in comparison to levels before G-CSF administration ($p = 0.033$ and $p = 0.004$; for neutrophils and monocytes, respectively) (Fig. 11).

Interestingly, number of neutrophils and monocytes did not correlate with percentage of CSF3R-expressing cells of those two subpopulations, neither on day 0 nor day 5 of mobilisation (Supplementary Table 2). G-CSF receptor surface expression was also not related to patient's age or sex.

However, some differences were observed between expression of the receptor for G-CSF on neutrophils and monocytes when patients and healthy controls were compared. For both cell subpopulations, percentage of cells expressing CSF3R on their surface before mobilisation was higher in autologous HSCT recipients than in healthy controls ($p < 0.001$ for neutrophils and $p = 0.002$ for monocytes) (Fig. 12). During mobilisation, mean percentage of CSF3R-positive cells in patients dropped for both cell subpopulations to levels comparable with those observed in the control group, and was significantly lower than before G-CSF administration ($p < 0.001$ for both neutrophils and monocytes).

Although the aforementioned decrease in cells expressing G-CSF receptor was evident in both subpopulations, CSF3R expression on neutrophils was not found to be correlated with its expression on monocytes, either before or after G-CSF treatment (Supplementary Table 2).

In the most cases, percentage of neutrophils and monocytes possessing G-CSF receptor did not vary between different groups of patients. The only significant finding in this respect was higher percentage of CSF3R+ neutrophils before mobilisation in HL patients compared to the MM group ($p = 0.014$) (Fig. 13). When all other blood cancers were considered, no differences were seen in regard to numbers of cells possessing G-CSF receptor either before or on the 5th day of mobilisation (Supplementary Table 2).

Interestingly, a trend was found between percentage of neutrophils expressing G-CSF receptor on their surface and mobilisation yield (Fig. 14). CSF3R expression on neutrophils before G-CSF administration tended to be inversely correlated with the number of CD34+ cells separated per kg of body weight ($p = 0.064$). Also, a similar negative

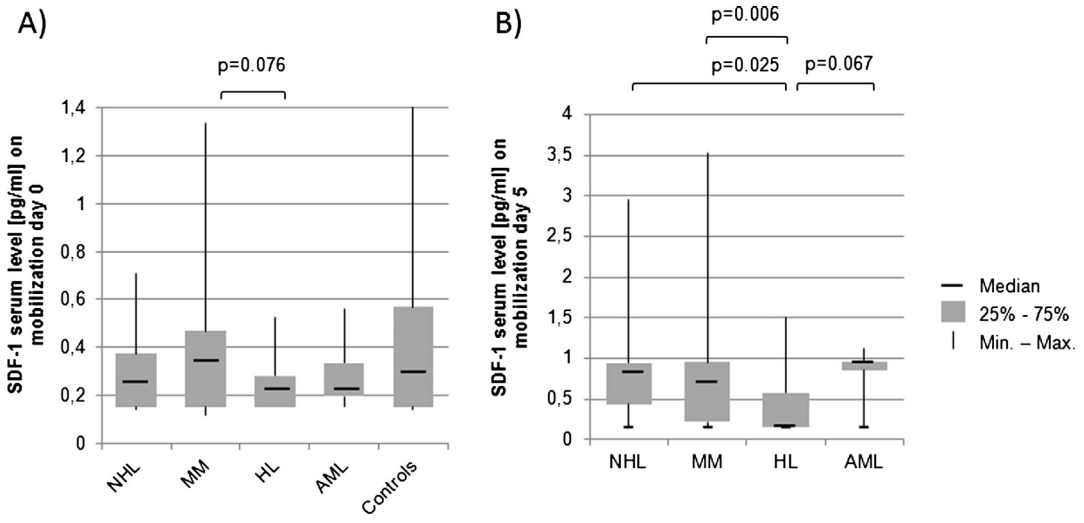


Fig. 8. Differences in SDF-1 concentration during mobilisation between various haematological malignancies. SDF-1 levels were compared in different blood neoplasms before (A) and on the 5th day (B) of G-CSF administration. Calculations were performed with the *U* Mann-Whitney test for two independent samples of the real statistics resource pack for Microsoft Excel 2013. Probability values were considered statistically significant for $p < 0.05$, while values between 0.05 and 0.10 were indicative of a trend.

trend was observed on the 5th day of mobilisation in regard to number of HSC separated per kg b. w. ($p = 0.075$) and per µl of blood ($p = 0.082$). However, no such relationship was observed for the monocytes subpopulation, either on mobilisation day 0 or day 5 (Supplementary Table 2).

Additionally, when comparing G-CSF and SDF-1 serum levels with surface expression of CSF3R on neutrophils and monocytes, some interesting relationships were detected. A negative correlation was found between G-CSF level and percentage of neutrophils ($p = 0.048$) and monocytes ($p = 0.001$) expressing CSF3R protein on their surface before mobilisation. Similar, though not statistically significant relationships were observed for serum SDF-1 levels and percentage of CSF3R expressing cells ($p = 0.099$ and $p = 0.082$; for neutrophils and monocytes, respectively). On the 5th day of G-CSF administration no such relationships for G-CSF and SDF-1 levels in regard to CSF3R expression either on neutrophils or monocytes were detected (Supplementary Table 2).

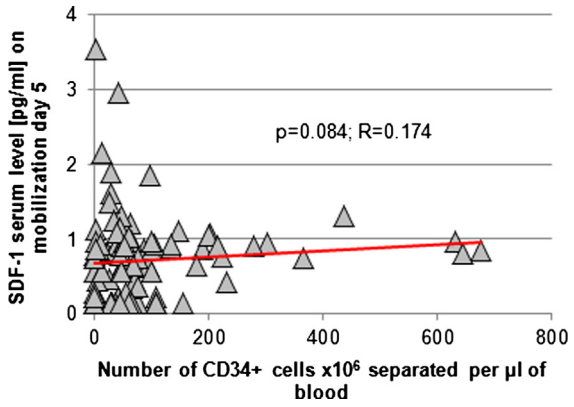


Fig. 10. Relation between SDF-1 concentration on the 5th day of G-CSF administration and mobilisation efficacy. Spearman's Rho correlation test of the Social Science Statistics website (http://www.socscistatistics.com/tests/Default.aspx) was used for calculations. R stands for Spearman's Rho correlation coefficient. Probability values were considered statistically significant for $p < 0.05$, while values between 0.05 and 0.10 were indicative of a trend.

4. Discussion

Although granulocyte colony-stimulating factor was introduced to clinical practice many years ago and is widely used as a mobilising factor for haematopoietic stem cells, molecular mechanisms and pathways in which it is engaged remain unclear.

In the present study, the detected G-CSF concentrations in the serum of healthy volunteers are relatively low, which supports previously reported observations [68,19]. Its basal levels are similar to patients with haematological malignancies and they increase after G-CSF administration regardless of blood cancer type. A variety of non-

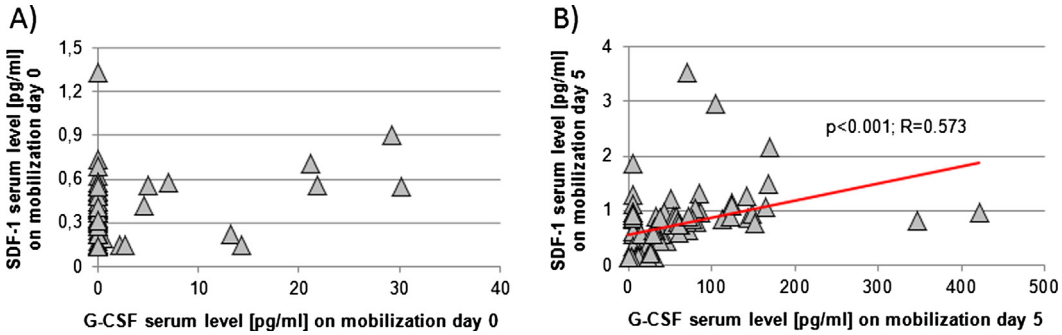


Fig. 9. Relations between SDF-1 and G-CSF serum levels. Analysis was performed before (A) and on the 5th day of mobilisation (B). Spearman's Rho correlation test of the Social Science Statistics website (http://www.socscistatistics.com/tests/Default.aspx) was used for calculations. R stands for Spearman's Rho correlation coefficient. Probability values were considered statistically significant for $p < 0.05$.

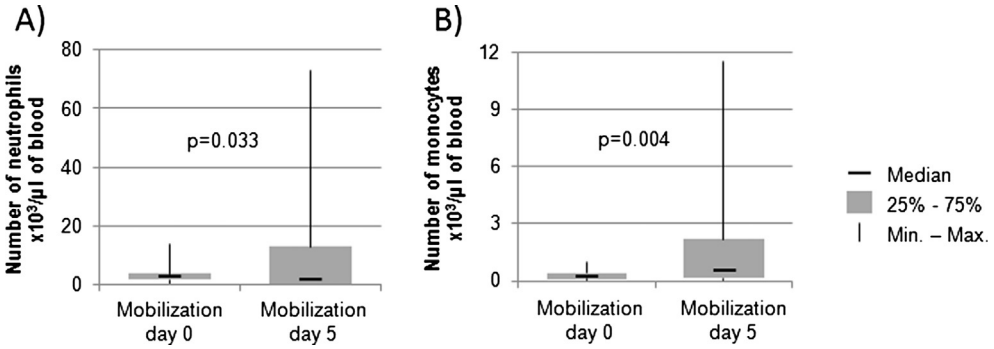


Fig. 11. An increase in (A) neutrophil and (B) monocyte counts in patients in response to G-CSF administration. Calculations were performed with the *U* Mann-Whitney test for two independent samples of the real statistics resource pack for Microsoft Excel 2013. Probability values were considered statistically significant for $p < 0.05$.

haematopoietic tumours – such as hepatomas [21], sarcomas [54], glioblastomas [66] and carcinomas [55] – were found to release G-CSF, presumably to promote tumour cell growth, migration and angiogenesis. For haematological malignancies, some incidences of an increase in basal G-CSF serum levels were reported in idiopathic aplastic anaemia, Fanconi's anaemia, myelodysplastic syndrome, acute leukaemia without any blast cells in the blood, chronic myeloid leukaemia, chronic lymphoid leukaemia, cyclic neutropenia and malignant lymphoma [68]. Results of the present study additionally imply that G-CSF initial levels are elevated in NHL and HL patients.

Neutrophils are believed to play the leading role in G-CSF-induced signalling and thus in clinical mobilisation [31,46,24,12]. It is known that their number rises in response to clinical mobilisation, as shown in the current study and by other groups [61,11].

Also, interestingly, a reverse correlation between blood neutrophil count and serum G-CSF level upon G-CSF treatment was reported [68,60,61]. Our current findings reveal a similar correlation between basal percentage of CSF3R+ neutrophils and G-CSF levels before mobilisation. *In vitro* studies suggest that this may occur upon G-CSF clearance by internalization via its receptors [42], as well as upregulation of receptor mRNA in response to presence of the ligand [69,59,65,61].

However, one should remember that CSF3R is present on many cell types [9,25,58,3,35,40,47] and clinical administration of G-CSF affects many different blood cell types [10]. Though neutrophils remain the most probable target cells for G-CSF [48,14,46], monocytes seem to be the other most promising cell population to assist with HSC release into peripheral blood. Interestingly, monocytes not only react to G-CSF treatment with increased cytokine secretion [67,53], but can also release G-CSF in response to a variety of stimuli [44]. Experiments performed in murine models [13] showed promising results supporting the

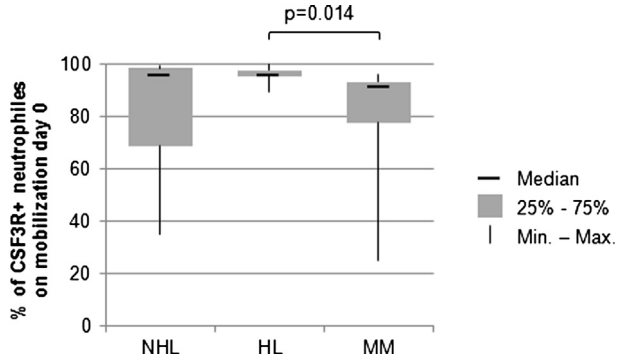


Fig. 13. Basal percentage of CSF3R+ neutrophils in distinct blood cancers. Calculations were performed with the *U* Mann-Whitney test for two independent samples of the real statistics resource pack for Microsoft Excel 2013. Probability values were considered statistically significant for $p < 0.05$.

hypothesis that monocyte activity does indeed affect HSC mobilisation. In mice with monocyte-restricted expression of G-CSF receptor, clinical release of haematopoietic progenitors into peripheral circulation was at least as high as that observed in control mice. As to date, effect of monocytes on mobilisation in humans has not been investigated. In the current study we did observe a significant increase in both neutrophil and monocyte count in autologous HSCT patients upon G-CSF treatment. Importantly, the number of the above mentioned cells did not affect efficacy of HSC mobilisation. Also, in contradiction to an earlier report on lung and ovarian cancer patients [61], instead of an increase, we observed a decrease in percentage of neutrophils and monocytes with surface expression of CSF3R on the 5th day of mobilisation. Nonetheless, G-CSF serum levels before treatment were lower in

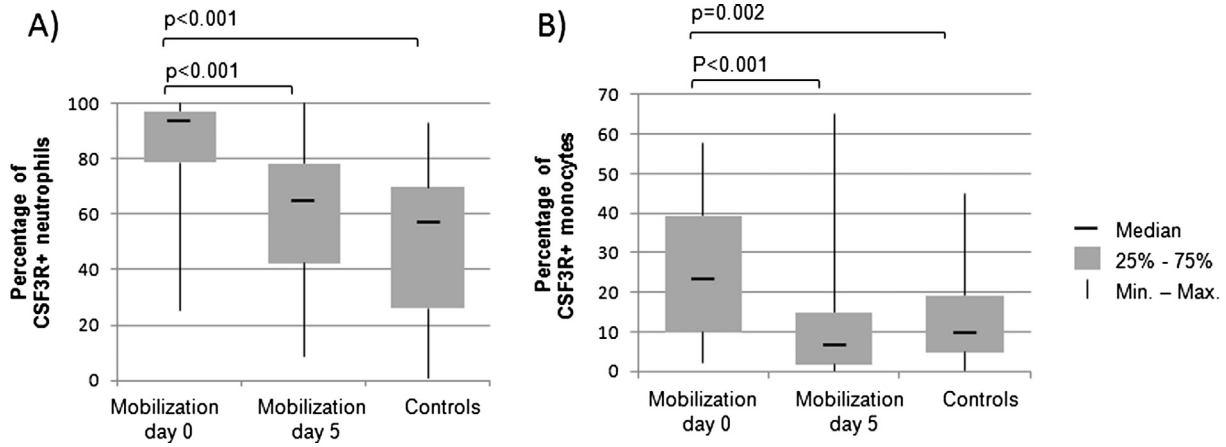


Fig. 12. Comparison of CSF3R surface expression on neutrophils (A) and monocytes (B) among healthy individuals and patients undergoing HSC mobilisation. Calculations were performed with the *U* Mann-Whitney test for two independent samples of the real statistics resource pack for Microsoft Excel 2013. Probability values were considered statistically significant for $p < 0.05$.

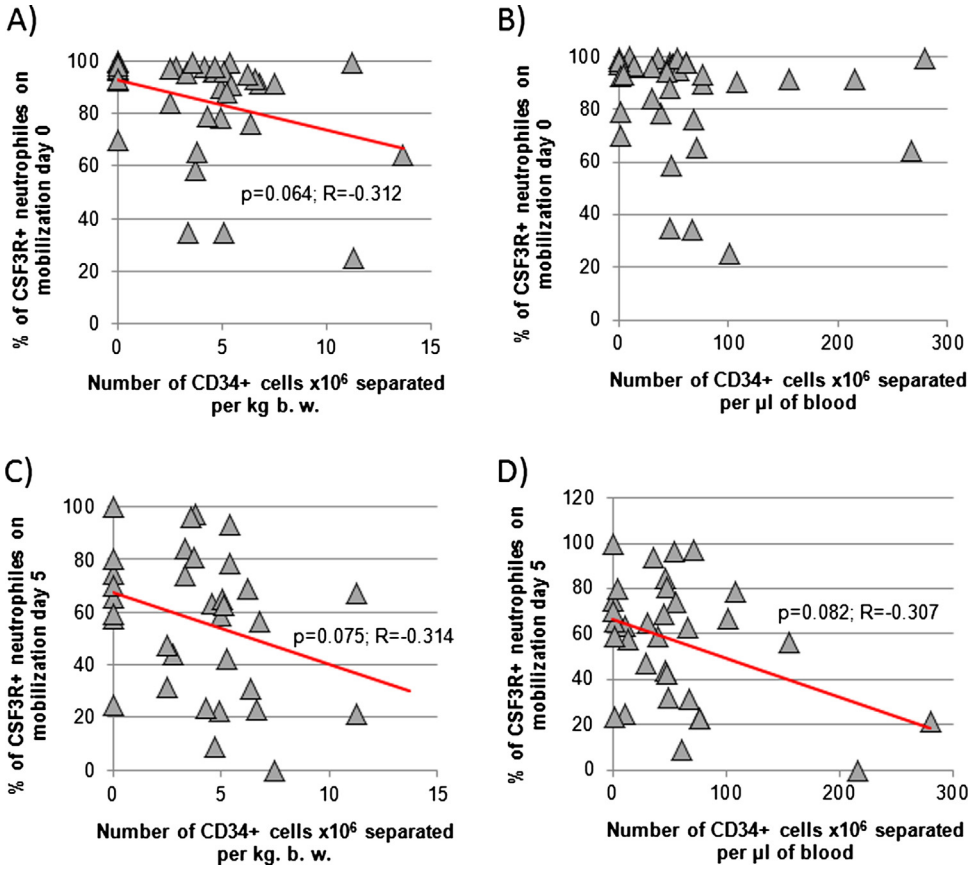


Fig. 14. Relations between HSC mobilisation effectiveness and percentage of neutrophils expressing G-CSF receptor on their surface. Expression of CSF3R on neutrophils was assessed before (A and B) and on the 5th day of G-CSF administration (C and D). Mobilisation yield was measured as number of CD34+ cells separated per kg b. w. (A and C) or per µl of blood (B and D). Spearman's Rho correlation test of the Social Science Statistics website (http://www.socscistatistics.com/tests/Default.aspx) was used for calculations. R stands for Spearman's Rho correlation coefficient. Probability values were considered statistically significant for $p < 0.05$, while values between 0.05 and 0.10 were indicative of a trend.

patients expressing more surface CSF3R, supporting the aforementioned theory of G-CSF clearance by intracellular processing through receptors [61].

These findings further support the hypothesis of both neutrophil and monocyte activation upon G-CSF administration and so their putative role in HSC mobilisation.

As for CD34+ release efficacy, the lower mobilisation potential of HL patients in comparison with NHL and MM patients, affirmed in the current study, corresponds to the results of our previous research [7]. It is worth noting that, current results show that HL patients were characterised with lower G-CSF levels on mobilisation day 5 compared to NHL patients as well as with higher basal percentage of CSF3R+ neutrophils in comparison with MM patients. However, low mobilisation potential in HL can also be linked to intensity of previous treatment, which is used to induce remission. In Hodgkin's lymphoma, refractory/resistant disease serves as an indication for autologous HSCT. First line therapy for HL usually contains aggressive chemotherapy, not only with ABVD regimen (adriamycin, bleomycin, vinblastine and dacarbazine), but also with escalated BEACOPP (bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine, prednisone) in more advanced stages of HL at diagnosis. Mobilisation chemotherapies do not differ between non-Hodgkin's and Hodgkin's lymphoma, so the impact of intensity of first line therapy could be an important, negative factor in further efficacy of stem cell collection. On the other hand, multiple myeloma patients are mostly transplanted early during first line treatment, when they achieve at least partial response to initial therapy.

Another presumed factor affecting G-CSF-induced mobilisation is SDF-1. It is known as a potent chemoattractant for HSCs, which express its receptor – CXCR4 [30,74]. It is believed that a local SDF-1 depletion in the bone marrow, following G-CSF application, causes release of blood progenitor stem cells into peripheral circulation [32,33]. For this reason, the CXCR4/SDF-1 axis is the best-studied target for HSC mobilisation [2,28]. A hypothesis was established that SDF-1 gradient, forming between the bone marrow and peripheral blood during mobilisation, is the key factor in the release of progenitor cells [56,26,50]. However, several reports contradict this hypothesis [36,45,15,29]. That is why SDF-1 role in cell trafficking during the state of emergency needs further clarification.

Our results show comparable SDF-1 concentrations in the peripheral blood of patients (regardless of blood cancer type) before G-CSF treatment and in healthy controls. Other researchers found both concentration of this chemokine and expression of its receptor, CXCR4, to be elevated in individuals with haematologic malignancies [72,73,27]. We observed an increase in SDF-1 level in peripheral blood upon G-CSF administration, which was not reported by others [37,50,51,34]. Regarding the bone marrow, SDF-1 mRNA and protein level was also reported to decrease in response to G-CSF administration [48,33,12].

The current study shows that both G-CSF and SDF-1 concentrations increase in the peripheral blood on the 5th day of mobilisation. Furthermore, their levels tend to be related to the number of HSCs released into peripheral circulation. This was predictable considering a very strong positive correlation between G-CSF and SDF-1 serum concentrations upon mobilisation, described herein. That is why one cannot neglect the possibility that relation between SDF-1 concentration and HSC release may be in fact dependent on G-CSF levels alone.

In conclusion, although clinical HSC mobilisation has been successfully performed for the last thirty years [18], molecular mechanisms behind this process are still to be uncovered. Our current study shows that, apart from neutrophils, monocyte population is also affected by G-CSF administration and, therefore, may be involved in progenitor cells mobilisation. Also, the role of SDF-1 in this process remains controversial and requires further investigation.

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Declarations of interest

None.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2018.12.011.

References

[1] M.S. Aapro, J. Bohlius, D.A. Cameron, L. Dal Lago, J.P. Donnelly, N. Kearney, G.H. Lyman, R. Pettengell, V.C. Tjan-Heijnen, J. Walewski, D.C. Weber, C. Zielinski, European Organisation for Research and Treatment of Cancer. 2010 update of EORTC guidelines for the use of granulocyte-colony stimulating factor to reduce the incidence of chemotherapy-induced febrile neutropenia in adult patients with lymphoproliferative disorders and solid tumours, Eur. J. Cancer 47 (2011) 8–32.

[2] A. Aiuti, I.J. Webb, C. Bleul, T. Springer, J.C. Gutierrez-Ramos, The chemokine SDF-1 is a chemoattractant for human CD34+ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34+ progenitors to peripheral blood, J. Exp. Med. 185 (1997) 111–120.

[3] B.R. Avalos, Molecular analysis of the granulocyte colony-stimulating factor receptor, Blood 88 (1996) 761–777.

[4] B.R. Avalos, The granulocyte colony-stimulating factor receptor and its role in disorders of granulopoiesis, Leukemia Lymphoma 28 (1998) 265–273.

[5] L.J. Bendall, K.F. Bradstock, G-CSF: from granulopoietic stimulant to bone marrow stem cell mobilizing agent, Cytokine Growth Factor Rev. 25 (2014) 355–367.

[6] K. Bogunia-Kubik, A. Gieryng, D. Dlubek, A. Lange, The CXCL12-3'A allele is associated with a higher mobilization yield of CD34 progenitors to the peripheral blood of healthy donors for allogeneic transplantation, Bone Marrow Transplant. 44 (2009) 273–278.

[7] K. Bogunia-Kubik, A. Gieryng, K. Gębura, A. Lange, Genetic variant of the G-CSF receptor gene is associated with lower mobilization potential and slower recovery of granulocytes after transplantation of autologous peripheral blood progenitor cells, Cytokine 60 (2012) 463–467.

[8] M.H. Bronchud, J.H. Scarffe, N. Thatcher, D. Crowther, L.M. Souza, N.K. Alton, N.G. Testa, T.M. Dexter, Phase I/II study of recombinant human granulocyte colony-stimulating factor in patients receiving intensive chemotherapy for small cell lung cancer, Br. J. Cancer 56 (1987) 809–813.

[9] F. Bussolino, J.M. Wang, P. Defilippi, F. Turrini, F. Sanavio, C.J. Edgell, M. Aglietta, P. Arese, A. Mantovani, Granulocyte- and granulocyte-macrophage-colony stimulating factors induce human endothelial cells to migrate and proliferate, Nature 337 (1989) 471–473.

[10] B.J. Capoccia, R.M. Shepherd, D.C. Link, G-CSF and AMD3100 mobilize monocytes into the blood that stimulate angiogenesis in vivo through a paracrine mechanism, Blood 108 (2006) 2438–2445.

[11] G. Carlsson, A. Ahlin, G. Dahllof, G. Elinder, J.I. Henter, J. Palmblad, Efficacy and safety of two different rG-CSF preparations in the treatment of patients with severe congenital neutropenia, Br. J. Haematol. 126 (2004) 127–132.

[12] M.J. Christopher, F. Liu, M.J. Hilton, F. Long, D.C. Link, Suppression of CXCL12 production by bone marrow osteoblasts is a common and critical pathway for cytokine-induced mobilization, Blood 114 (2009) 1331–1339.

[13] M.J. Christopher, M. Rao, F. Liu, J.R. Woloszynek, D.C. Link, Expression of the G-CSF receptor in monocytic cells is sufficient to mediate hematopoietic progenitor mobilization by G-CSF in mice, J. Exp. Med. 208 (2011) 251–260.

[14] K.W. Christopherson II, S. Cooper, H.E. Broxmeyer, Cell surface peptidase CD26/DPPIV mediates G-CSF mobilization of mouse progenitor cells, Blood 101 (2003) 4680–4686.

[15] K.W. Christopherson II, G. Hangoc, C.R. Mantel, H.E. Broxmeyer, Modulation of hematopoietic stem cell homing and engraftment by CD26, Science 305 (2004) 1000–1003.

[16] M.H. Cottler-Fox, T. Lapidot, I. Petit, O. Kollet, J.F. DiPersio, D. Link, S. Devine, Stem cell mobilization, Hematol. Am. Soc. Hematol. Educ. Program 2003 (2003) 419–437.

[17] D. Dale, Current management of chemotherapy-induced neutropenia: the role of colony-stimulating factors, Semin. Oncol. 30 (2003) 3–9.

[18] S.A. Ezzone, History of hematopoietic stem cell transplantation, Semin. Oncol. Nurs. 25 (2009) 95–99.

[19] N. Kröger, S. Sonnenberg, L. Cortes-Dericks, P. Freiberger, H. Mollnau, A.R. Zander, Kinetics of G-CSF and CD34+ cell mobilization after once or twice daily stimulation with rHu granulocyte-stimulating factor (lenograstim) in healthy volunteers: an intraindividual crossover study, Transfusion 44 (2004) 104–110.

[20] R. Fukunaga, E. Ishizaka-Ikeda, C.X. Pan, Y. Seto, S. Nagata, Functional domains of the granulocyte colony stimulating factor receptor, EMBO J. 10 (1991) 2855–2865.

[21] J.L. Gabrilove, K. Welte, L. Lu, H. Castro-Malaspina, M.A. Moore, Constitutive production of leukemia differentiation, colony-stimulating, erythroid burst-promoting, and pluripoietic factors by a human hepatoma cell line: characterization of the leukemia differentiation factor, Blood 66 (1985) 407–415.

[22] J.L. Gabrilove, A. Jakubowski, H. Scher, C. Sternberg, G. Wong, J. Grous, A. Yagoda, K. Fain, M.A. Moore, B. Clarkson, H.F. Oettgen, K. Alton, K. Welte, L. Souza, Effect of granulocyte colony-stimulating factor on neutropenia and associated morbidity due to chemotherapy for transitional-cell carcinoma of the urothelium, New England J. Med. 318 (1988) 1414–1422.

[23] A. Gratwohl, H. Baldomero, M. Aljurf, M.C. Pasquini, L.F. Bouzas, A. Yoshimi, J. Szer, J. Lipton, A. Schwendener, M. Gratwohl, K. Frauendorfer, D. Niederwieser, M. Horowitz, Y. Kodera, Worldwide network of blood and marrow transplantation: hematopoietic stem cell transplantation: a global perspective, JAMA 303 (2010) 1617–1624.

[24] A.D. Gregory, L.A. Hogue, T.W. Ferkol, D.C. Link, Regulation of systemic and local neutrophil responses by G-CSF during pulmonary Pseudomonas aeruginosa infection, Blood 109 (2007) 3235–3243.

[25] Y. Hanazono, T. Hosoi, T. Kuwaki, S. Matsuki, K. Miyazono, K. Miyagawa, F. Takaku, Structural analysis of the receptors for granulocyte colony-stimulating factor on neutrophils, Exp. Hematol. 18 (1990) 1097–1103.

[26] P. Hanel, P. Andréani, M.H. Graler, Erythrocytes store and release sphingosine 1-phosphate in blood, FASEB J. 21 (2007) 1202–1209.

[27] B.K. Khandany, G. Hassanshahi, H. Khorramdelazad, Z. Balali, A. Shamsizadeh, M.K. Arababadi, H. Ostadebrahimi, A. Fatehi, M. Rezazadeh, Z. Ahmadi, M.N. Karimabad, Evaluation of circulating concentrations of CXCL1 (Gro-α), CXCL10 (IP-10) and CXCL12 (SDF-1) in ALL patients prior and post bone marrow transplantation, Pathol. Res. Pract. 208 (2012) 615–619.

[28] C.H. Kim, H.E. Broxmeyer, In vitro behavior of hematopoietic progenitor cells under the influence of chemoattractants: stromal cell-derived factor-1, steel factor, and the bone marrow environment, Blood 91 (1998) 100–110.

[29] C.H. Kim, W. Wu, M. Wysoczynski, A. Abdel-Latif, M. Sunkara, A. Morris, M. Kucia, J. Ratajczak, M.Z. Ratajczak, Conditioning for hematopoietic transplantation activates the complement cascade and induces a proteolytic environment in bone marrow: a novel role for bioactive lipids and soluble C5b–C9 as homing factors, Leukemia 26 (2012) 106–116.

[30] T. Lapidot, A. Dar, O. Kollet, How do stem cells find their way home? Blood 106 (2005) 1901–1910.

[31] J.P. Lévesque, Y. Takamatsu, S.K. Nilsson, D.N. Haylock, P.J. Simmons, Vascular cell adhesion molecule-1 (CD106) is cleaved by neutrophil proteases in the bone marrow following hematopoietic progenitor cell mobilization by granulocyte colony-stimulating factor, Blood 98 (2001) 1289–1297.

[32] J.P. Lévesque, J. Hendy, Y. Takamatsu, B. Williams, I.G. Winkler, P.J. Simmons, Mobilization by either cyclophosphamide or granulocyte colony-stimulating factor transforms the bone marrow into a highly proteolytic environment, Exp. Hematol. 30 (2002) 440–449.

[33] J.P. Lévesque, J. Hendy, Y. Takamatsu, P.J. Simmons, L.J. Bendall, Disruption of the CXCR4/CXCL12 chemotactic interaction during hematopoietic stem cell mobilization induced by GCSF or cyclophosphamide, J. Clin. Investig. 111 (2003) 187–196.

[34] S. Li, Q. Zhai, D. Zou, H. Meng, Z. Xie, C. Li, Y. Wang, J. Qi, T. Cheng, L. Qiu, A pivotal role of bone remodeling in granulocyte colony stimulating factor induced hematopoietic stem/progenitor cells mobilization, J. Cell. Physiol. 228 (2013) 1002–1009.

[35] F. Liu, J. Poursine-Laurent, D.C. Link, Expression of the G-CSF receptor on hematopoietic progenitor cells is not required for their mobilization by G-CSF, Blood 95 (2000) 3025–3031.

[36] Q. Ma, D. Jones, T.A. Springer, The chemokine receptor CXCR4 is required for the retention of B lineage and granulocytic precursors within the bone marrow microenvironment, Immunity 10 (1999) 463–471.

[37] S. Massberg, P. Schaerli, I. Knezevic-Maramica, M. Köllnberger, N. Tubo, E.A. Moseman, I.V. Huff, T. Junt, A.J. Wagers, I.B. Mazo, U.H. von Andrian, Immunosurveillance by hematopoietic progenitor cells trafficking through blood, lymph, and peripheral tissues, Cell 131 (2007) 994–1008 (Kröger, N., Sonnenberg, S., Cortes-Dericks, L.).

[38] S. Mendez-Ferrer, D.T. Scadden, A. Sanchez-Aguilera, Bone marrow stem cells: current and emerging concepts, Ann. NY Acad. Sci. 1335 (2015) 32–44.

[39] M. Montgomery, M. Cottler-Fox, Mobilization and collection of autologous hematopoietic progenitor/stem cells, Clin. Adv. Hematol. Oncol. 5 (2007) 127–136.

[40] K. Morikawa, S. Morikawa, M. Nakamura, T. Miyawaki, Characterization of granulocyte colony-stimulating factor receptor expressed on human lymphocytes, Br. J. Haematol. 118 (2002) 296–304.

[41] S.J. Morrison, D.T. Scadden, The bone marrow niche for haematopoietic stem cells, Nature 505 (2014) 327–334.

[42] N.A. Nicola, L. Peterson, D.J. Hilton, D. Metcalf, Cellular processing of murine colony-stimulating factor (multi-CSF, GM-CSF, GCSF) receptors by normal hemopoietic cells and cell lines, Growth Factor 1 (1988) 41–49.

[43] D. Niederwieser, H. Baldomero, J. Szer, M. Gratwohl, M. Aljurf, Y. Atsuta, L.F. Bouzas, D. Confer, H. Greinix, M. Horowitz, M. Iida, J. Lipton, M. Mohty,

N. Novitzky, J. Nunez, J. Passweg, M.C. Pasquini, Y. Kodera, J. Apperley, A. Seber, A. Gratwohl, Hematopoietic stem cell transplantation activity worldwide in 2012 and a SWOT analysis of the Worldwide Network for Blood and Marrow Transplantation Group including the global survey, Bone Marrow Transplant. 51 (2016) 778–785.
[44] S. Nioche, A. Tazi, D. Lecossier, A.J. Hance, Production of granulocyte colony-stimulating factor (G-CSF) by human cells: T lymphocyte-dependent and T lymphocyte-independent release of G-CSF by blood monocytes, Eur. J. Immunol. 18 (1988) 1021–1026.
[45] N. Onai, Y.Y. Zhang, H. Yoneyama, T. Kitamura, S. Ishikawa, K. Matsushima, Impairment of lymphopoiesis and myelopoiesis in mice reconstituted with bone marrow-hematopoietic progenitor cells expressing SDF-1-intrakine, Blood 96 (2000) 2074–2080.
[46] L.M. Pelus, H. Bian, A.G. King, S. Fukuda, Neutrophil-derived MMP-9 mediates synergistic mobilization of hematopoietic stem and progenitor cells by the combination of G-CSF and the chemokines GRObeta/CXCL2 and GRObetaT/CXCL2delta4, Blood 103 (2004) 110–119.
[47] A.R. Pennington, Effects of G-CSF on Monocytes and Neurons: in vitro and in vivo studies in a Mouse Model of Alzheimer's Disease. Graduate Theses and Dissertations, University of South Florida Scholar Commons, 2012.
[48] I. Petit, M. Szyper-Kravitz, A. Nagler, M. Lahav, A. Peled, L. Habler, T. Ponomaryov, R.S. Taichman, F. Arenzana-Seisdedos, N. Fujii, J. Sandbank, D. Zipori, T. Lapidot, G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4, Nat. Immunol. 3 (2002) 687–694.
[49] I. Pusic, J.F. DiPersio, The use of growth factors in hematopoietic stem cell transplantation, Curr. Pharm. Des. 14 (2008) 1950–1961.
[50] M.Z. Ratajczak, H. Lee, M. Wysoczynski, W. Wan, W. Marlicz, M.J. Laughlin, M. Kucia, A. Janowska-Wieczorek, J. Ratajczak, Novel insight into stem cell mobilization-plasma sphingosine-1-phosphate is a major chemoattractant that directs the egress of hematopoietic stem progenitor cells from the bone marrow and its level in peripheral blood increases during mobilization due to activation of complement cascade/membrane attack complex, Leukemia 24 (2010) 976–985.
[51] M.Z. Ratajczak, C.H. Kim, W. Wojakowski, A. Janowska-Wieczorek, M. Kucia, J. Ratajczak, Innate immunity as orchestrator of stem cel mobilization, Leukemia 24 (2010) 1667–1675.
[52] P.S. Rosenberg, C. Zeidler, A.A. Bolyard, B.P. Alter, M.A. Bonilla, L.A. Boxer, Y. Dror, S. Kinsey, D.C. Link, P.E. Newburger, A. Shimamura, K. Welte, D.C. Dale, Stable long-term risk of leukaemia in patients with severe congenital neutropenia maintained on G-CSF therapy, Br. J. Haematol. 150 (2010) 196–199.
[53] M. Saito, N. Kiyokawa, T. Taguchi, K. Suzuki, T. Sekino, K. Mimori, T. Suzuki, H. Nakajima, Y.U. Katagiri, J. Fujimura, H. Fujita, K. Ishimoto, Y. Yamashiro, J. Fujimoto, Granulocyte colony-stimulating factor directly affects human monocytes and modulates cytokine secretion, Exp. Hematol. 30 (2002) 1115–1123.
[54] N. Sakai, M. Kubota, M. Shikita, M. Yokota, K. Ando, Intraclonal diversity of fibrosarcoma cells for the production of macrophage colony-stimulating factor and granulocyte colony-stimulating factor, J. Cell. Physiol. 133 (1987) 400–404.
[55] K. Sato, K. Terada, T. Sugiyama, T. Sugiyama, H. Masuda, H. Kakinuma, T. Kato, Granulocyte colony-stimulating factor produced by bladder carcinoma of a patient with leukemoid reaction did not affect proliferation of the tumor cells, J. Urol. 151 (1994) 1687–1690.
[56] G. Seitz, A.M. Boehmle, L. Kanz, R. Möhle, The role of sphingosine 1-phosphate receptors in the trafficking of hematopoietic progenitor cells, Ann. NY Acad. Sci. 1044 (2005) 84–89.
[57] T.C. Shea, J.F. DiPersio, Mobilization of autologous peripheral blood hematopoietic cells for cellular therapy, in: F.R. Applebaum, S.J. Forman, R.S. Negrin, K.G. Blume (Eds.), Thomas' Hematopoietic Cell Transplantation, Wiley-Blackwell, Hoboken, NJ, 2009, pp. 590–604.
[58] K. Shimoda, S. Okamura, N. Harada, S. Kondo, T. Okamura, Y. Niho, Identification of a functional receptor for granulocyte colony-stimulating factor on platelets, J. Clin. Investig. 91 (1993) 1310–1313.
[59] R.A. Steinman, D.J. Tweardy, Granulocyte colony-stimulating factor receptor mRNA upregulation is an immediate early marker of myeloid differentiation and exhibits dysfunctional regulation in leukemic cells, Blood 83 (1994) 119–127.
[60] H. Takatani, H. Soda, M. Fukuda, M. Watanabe, A. Kinoshita, T. Nakamura, M. Oka, Levels of recombinant human granulocyte colonystimulating factor in serum are inversely correlated with circulating neutrophil counts, Antimicrob. Agents Chemother. 40 (1996) 988–991.
[61] K. Terashi, M. Oka, S. Ohdo, T. Furukubo, C. Ikeda, M. Fukuda, H. Soda, S. Higuchi, S. Kohno, Close association between clearance of recombinant human granulocyte colony-stimulating factor (G-CSF) and G-CSF receptor on neutrophils in cancer patients, Antimicrob. Agents Chemother. 43 (1999) 21–24.
[62] I.P. Touw, G.M. van de Geijn, Granulocyte colony stimulating factor and its receptor in normal myeloid cell development, leukemia and related blood cell disorders, Front. Biosci. 12 (2007) 800–815.
[63] I.P. Touw, K. Palande, R. Beekman, Granulocyte colony-stimulating factor receptor signaling: implications for G-CSF responses and leukemic progression in severe congenital neutropenia, Hematol. Oncol. Clin. North Am. 27 (2013) 61–73.
[64] K. Tsuji, Y. Ebihara, Expression of G-CSF receptor on myeloid progenitors, Leukemia Lymphoma 42 (2001) 1351–1357.
[65] T. Tsuruta, K. Tani, M. Shimane, K. Ozawa, S. Takahashi, D. Tsuchimoto, K. Takahashi, S. Nagata, N. Sato, S. Asano, Effects of myeloid cell growth factors on alkaline phosphatase, myeloperoxidase, defensin and granulocyte colony-stimulating factor receptor mRNA expression in haemopoietic cells of normal individuals and myeloid disorders, Br. J. Haematol. 92 (1996) 9–22.
[66] D.J. Tweardy, L.A. Cannizzaro, A.P. Palumbo, S. Shane, K. Huebner, P. Vantuinen, D.H. Ledbetter, J.B. Finan, P.C. Nowell, G. Rovera, Molecular cloning and characterization of a cDNA for human granulocyte colony-stimulating factor (G-CSF) from a glioblastoma multiforme cell line and localization of the G-CSF gene to chromosome band 17q21, Oncogene Res. 1 (1987) 209–220.
[67] M.L. Varney, K. Ino, A.G. Ageitos, D.G. Heimann, J.E. Talmadge, R.K. Singh, Expression of interleukin-10 in isolated CD8+ T cells and monocytes from growth factor-mobilized peripheral blood stem cell products: a mechanism of immune dysfunction, J. Interferon Cytokine Res. 19 (1999) 351–360.
[68] K. Watari, S. Asano, N. Shirafuji, H. Kodo, K. Ozawa, F. Takaku, S. Kamachi, Serum granulocyte colony-stimulating factor levels in healthy volunteers and patients with various disorders as estimated by enzyme immunoassay, Blood 73 (1989) 117–122.
[69] A. Wilson, P. Corthesy, P. Reichenbach, H.R. MacDonald, M. Nabholz, Interleukin (IL)-1 and IL-2 receptor alpha and beta expression in immature thymocytes, Eur. J. Immunol. 24 (1994) 1729–1735.
[70] A. Wilson, A. Trumpp, Bone-marrow haematopoietic-stem-cell niches, Nat. Rev. Immunol. 6 (2006) 93–106.
[71] M. Wysoczynski, M. Adamiak, M. Suszynska, A. Abdel-Latif, J. Ratajczak, M.Z. Ratajczak, Poor mobilization in T-cell-deficient nude mice is explained by defective activation of granulocytes and monocytes, Cell Transplant. 26 (2017) 83–93.
[72] A.C. Zannettino, A.N. Farrugia, A. Kortesidis, J. Manavis, L.B. To, S.K. Martin, P. Diamond, H. Tamamura, T. Lapidot, N. Fujii, S. Gronthos, Elevated serum levels of stromal-derived factor-1alpha are associated with increased osteoclast activity and osteolytic bone disease in multiple myeloma patients, Cancer Res. 65 (2005) 1700–1709.
[73] D.F. Zeng, P.Y. Kong, X.H. Chen, X.G. Peng, L. Wei, C. Chang, L. Liu, H. Liu, Q.Y. Wang, Expression of stromal cell derived factor-1(SDF-1) and its receptor CXCR4 in hematologic malignancies, Zhongguo Shi Yan Xue Ye Xue Za Zhi 13 (2005) 274–277.
[74] B. Zhou, Z.C. Han, M.C. Poon, W. Pu, Mesenchymal stem/stromal cells (MSC) transfected with stromal derived factor 1 (SDF-1) for therapeutic neovascularization: enhancement of cell recruitment and entrapment, Med. Hypotheses 68 (2007) 1268–1271.