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Mobilization of hematopoietic stem cells in patients undergoing HSCT as a treatment of early diabetes type 1

Mobilizacja macierzystych komórek krwiotwórczych u chorych z niedawnorozpoznaną cukrzycą typu 1 przed przeszczepieniem autologicznych komórek krwiotwórczych

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ABSTRACT

Background: The immunoablation with autologous hematopoietic stem cell transplantation is a new experimental treatment of early diabetes type 1. The treatment is based on destruction of immune system with cytotoxic drugs which leads to halt of immune reaction directed against beta cells of pancreas. During that treatment young patients with diabetes type 1 who are otherwise healthy undergo mobilization with cyclophosphamide (CY) and G-CSF. They are naïve to cytotoxic drugs and mobilization is their first contact with chemotherapy. We analyzed the efficiency of mobilization with cyclophosphamide and G-CSF in this population. **Methods:** We analyzed the medical records of 25 patients with diabetes who underwent mobilization with cyclophosphamide and G-CSF. **Results:** The median white blood cell count on the first day of apheresis was $14.6 \times 10^3/\mu L$ (range 1.5–33.3) in CY + G-CSF mobilized patients. Median absolute CD 34+ cell count in peripheral blood on the first apheresis day was 0.095 $127 \times 10^3/\mu L$ (range 0.026–0.477). The median total number of collected CD34+ cells during one or two (if needed) aphereses was 466×10^6 (range 204–816) or 7.24×10^6 CD34+ cells per kg of patient body weight (range 3.03–13.1). There were no poor mobilizers who were unable to collect sufficient

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cell numbers. **Conclusion:** The mobilization of hematopoietic stem cells with CY + G-CSF in patients with early diabetes type 1 is efficient and the underlying diabetes does not impair the efficiency of hematopoietic stem cell collection.

Introduction

Since 2008 the experimental autologous hematopoietic stem cell transplantation (AHSCT) program in early type 1 diabetes mellitus (T1DM) was conducted at our institution [1]. The transplantation can only be performed if there are a sufficient number of autologous hematopoietic stem cells mobilized prior to transplantation. The standard protocol for mobilization in patients with autoimmune diseases is cyclophosphamide (CY) (2–4 g/m^2) with granulocyte colony stimulating factor (G-CSF) [1–3]. This population of patients with T1DM is naïve to cytotoxic drugs, and mobilization is their first contact with chemotherapeutics (such as CY). T1DM has been diagnosed only a few weeks prior to the procedure, thus reducing the likelihood of influence of this disease on the stem cell compartment in the bone marrow. Therefore, patients with T1DM as they cannot be described as healthy, they are, so far, the healthiest population of patients that have been exposed to upfront mobilization with CY + G-CSF. There is a published report of actual patient data treated with HSCT on mobilization in early T1DM which shows very good efficacy in mobilization [3]. On the other hand, diabetic patients were shown to have impaired mobilization of CD34+ hematopoietic stem cells [4]. As the diabetes is chronic disease some of the changes observed might be connected with prolonged influence of impaired glucose metabolism on bone marrow niche. Mobilization efficiency in other autoimmune diseases varies with disease – the patients with multiple sclerosis having the highest CD34+ cell counts and patients with systemic sclerosis the lowest [2]. The reasons for differences are not clear – it can be speculated that the observed differences could be caused by the disease itself or the other drugs used prior to the use of cyclophosphamide in systemic sclerosis or in multiple sclerosis [5].

The aim of this retrospective study was to analyze the results of mobilization with CY + G-CSF in patients with early diabetes type 1 and no other hematologic disease.

Methods

The medical records of 25 patients with early T1DM who were mobilized at our institution were reviewed. T1DM patients were enrolled into the study 4–8 weeks after diagnosis.

Patients with early T1DM were mobilized as previously described [1]. They received CY (2 g/m^2) and subsequently G-CSF 10 μg/kg per day from day +1 after CY until the day of collection (usually between day +7 and +9) of over 3.0 × 10^6 CD34+ cells/kg of body weight. The CD34+ cells were collected through the double lumen central venous catheter when the CD34+ count reached over 15 cells/μL. The collection of HSC was performed on Cobe Spectra cell separator (Caridian BCT, Lakewood, CO, USA) according to the centers' standard operating procedures. Evaluation of CD34+ cells was performed according to ISHAGE guidelines. The study was approved by the local bioethics committee.

Results

The group of T1DM consisted of 25 patients of median age of 23 (18–35). There were 16 males and 9 females. The patients were generally healthy except for the new onset diabetes. Patients 5 and 9 had mild anemia due to the iron deficiency, patients 8 and 14 autoimmune thyroid disorder in euthyreosis. No other serious conditions that could influence stem cell mobilization were reported. One apheresis was sufficient for collection of hematopoietic stem cells for transplantation in 13 out of 25 diabetic patients (52%). Two aphereses were sufficient for collection of hematopoietic stem cells in the rest of patients (12 patients – 48%). The collection data are shown in Table I. White blood cell count

Table I – The clinical data of the patients with early diabetes type 1 mobilized with Cy + G-CSF

	Median (range)
Age (years)	23 (18–35)
Sex distribution	9 females, 16 males
Weight (kg)	70 (48–91)
Pre first leukapheresis	
White blood cell count on 1st apheresis day (×10^3/μL)	14.6 (1.5–33.3)
CD 34+ cells in peripheral blood (percent)	0.70% (0.21–4.20%)
CD 34+ absolute cell count in peripheral blood (×10^3/μL)	0. 095 (0.026–0.477)
First leukapheresis (n = 25)	
Total nucleated cell count in leukapheresis (×10^8)	103 (56–202)
CD 34+ cell in leukapheresis (percent)	1.425 (0.76–3.45%)
Total CD34+ cell leukapheresis (×10^6)	300 (150–814)
Second leukapheresis (n = 12)	
Total nucleated cell count in leukapheresis (×10^8)	114 (61–174)
CD 34+ cell in leukapheresis (percent)	1.15% (0.83–2.19%)
Total CD34+ cell leukapheresis (×10^6)	300 (159–601)
Total of first and second leukaphereses	
Total CD34+ cell leukaphereses (×10^6)	466 (204–816)
Total CD34+ cell × 10^6/kg of patient weight	7.24 (3.03–13.1)

(WBC) on the collection day ranged from 1.5 G/L to 33.3 G/L (median $14.6 \times 10^3/\mu L$) in T1DM patients. CD34+ cells accounted for 0.21–4.2% WBC in T1DM (median 0.70%). The absolute numbers of circulating CD34+ cells varied between 26 and 477 per microliter in T1DM patients (median 127 cells/μL). In terms of collection efficacy, the median yield of CD34+ cells during the first leukapheresis was 300×10^6 (range 150–814 $\times 10^6$). When the total collection was analyzed (day 1 plus day 2) the median yield of CD34+ cells was 466×10^6 (range 204–816 $\times 10^6$). The median mobilized number of CD34+ cells per kilogram of body weight was 7.24×10^6 (range 3.03–13). There were no poor mobilizers who were unable to collect sufficient cell numbers

The mobilization was well tolerated with no serious adverse effects that have been reported. Most of the patients reported mild nausea which was well controlled with ondansetron – as the symptoms were mild the detailed data on nausea have not been recorded. No infections during the mobilization were reported.

Discussion

In conclusion, the CY+ G-CSF mobilization is the standard protocol used in hematologic and non-hematologic patients undergoing autologous hematopoietic stem cell transplantation. Our results show that CY+ G-CSF used for HSC mobilization in chemotherapy naïve individuals works well. The rationale to CY in mobilization is reduction in the risk of flare (in case of autoimmune diseases) and antineoplastic activity in hematologic patients. Elimination of CY from such protocols has disadvantage of losing the antineoplastic or anti-autoimmunity effect of mobilization – however, the data supporting this view come from limited number of cases [2]. Here we show that the addition of CY does not influence negatively the cell yield of mobilization in diabetic patients as all of the diabetic patients reached the desired level of over 3×10^6 CD34+ cells per kg of weight.

The obvious limitation of the study is relatively small group of patients – however, it is the biggest described group of patients undergoing this treatment and comparable with group described by De Santis et al. [3]. We also cannot be sure to what extent diabetes itself influences mobilization. Our institution has the experience with collection of hematopoietic stem cells from healthy unrelated donors (over 500 collections). When the results of mobilization among the patients with diabetes mobilized with Cy + G-CSF are compared with results of mobilization with G-CSF among group unrelated donors adjusted for sex, age and body weight the stem cell yield is higher among patients with diabetes (data not shown). Our results show that CY + G-CSF combination in this population is very efficient and if diabetes has any negative influence on mobilization addition of CY clearly overcomes it. We did not have the control group of patients with early diabetes with sole use of G-CSF for mobilization to reduce the risk of flare of autoimmune disorder [2].

Concluding, mobilization protocol with the CY and G-CSF in patients with early diabetes type 1 is safe and results in sufficient CD34+ cells yield with no risk of poor mobilization.

Authors' contributions/Wkład autorów

ES – study design, data collection, statistical analysis, data interpretation, manuscript preparation, literature search. AM, WWJ, EF – study design. KH, MłK, MK, EU, TT, AM, KJ – data collection.

Conflict of interest/Konflikt interesu

None declared.

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None declared.

Ethics/Etyka

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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