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Poor stem cell harvest may not always be related to poor mobilization: lessons gained from a mobilization study in beta-thalassemia major patients

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Abstract

BACKGROUND—Hematopoietic stem cell mobilization and leukapheresis in adult beta-thalassemia patients have been recently optimized in the context of clinical trials for obtaining hematopoietic stem cells for thalassemia gene therapy. In some patients, however, CD34⁺ cell yield was poor despite successful mobilization and the modification of apheresis settings was mandatory for harvest rescue.

STUDY DESIGN AND METHODS—The data of twenty adult β -thalassemia patients who were enrolled in a clinical trial for optimizing mobilization strategies for stem cell gene therapy were analyzed. The aim of this *post-hoc* analysis was to assess how certain hematological and/or clinical parameters may correlate with low collection efficiency in the presence of adequate numbers of circulating stem cells, after pharmacological mobilization and standard leukapheresis procedures.

RESULTS—Four patients among 19 optimally mobilized with Plerixafor or remobilized with G-CSF+Plerixafor patients demonstrated disproportionally poor CD34⁺ cell harvest, with respect to their circulating CD34⁺ cells after mobilization. All four patients who had undergone splenectomy presented at baseline and before first apheresis with lymphocytosis resulting in a lymphocyte/neutrophil ratio well above 1 and marked reticulocytosis as compared to patients with optimal mobilization/CD34⁺ cell harvest. Such unexpected expansion of specific cell populations disrupted the normal cell layer separation and necessitated modification of the apheresis settings in order to rescue the harvests.

CONCLUSIONS—By close examination of certain hematological and/or clinical parameters prior to leukapheresis, patients who, in spite of adequate mobilization are at risk for poor CD34⁺

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cell harvests, may be identified and harvest failure can be prevented by adjusting of the apheresis settings.

Keywords

CD34+ cell harvest; gene therapy; mobilization; thalassemia

INTRODUCTION

The collection of mobilized peripheral blood stem cells has been a standard approach for obtaining hematopoietic stem cell grafts in the majority of autologous and allogeneic transplants.¹ It is well-established that mobilized blood provides several-fold higher numbers of hematopoietic stem cells (HSCs) over bone marrow harvest,² and that peripheral blood CD34⁺ cell counts can reliably predict the harvest yields.³⁻⁵ Stem cell gene therapy applications also pursue the use of peripheral blood because of the high numbers of autologous genetically-modified cells needed for re-infusion into the patients. Although 2×10^6 CD34⁺ cells/kg have been considered as the minimum threshold for engraftment in autologous transplantation,^{6,7} in specific stem cell gene therapy protocols, as for β -thalassemia, significantly higher numbers are targeted for an optimal outcome.⁸⁻¹⁰

In order to define the optimal mobilization approach for adult β -thalassemia patients, before the implementation of gene therapy, we conducted two mobilization trials investigating four different mobilization modes: G-CSF alone, Hydroxyurea+G-CSF, Plerixafor alone, G-CSF +Plerixafor. In these studies we identified the combination of G-CSF+Plerixafor as the optimal mobilization approach and the G-CSF+Plerixafor-mobilized cells as the optimal graft source for gene therapy of β -thalassemia.¹¹⁻¹³

While peripheral blood CD34⁺ cell enumeration still remains the most reliable predictive factor of apheresis yield,¹³ there are rare cases of unexpectedly poor CD34⁺ cell harvests despite successful mobilization.^{14, 15} Here, we present four cases among 20 Plerixafor-mobilized or G-CSF+plerixafor remobilized thalassemic patients with optimal mobilization but inadequate stem cell harvests due to low collection efficiency. In these patients, disproportionate low yields of CD34⁺ cells with regard to the numbers of circulating CD34⁺ cells, were obtained under standard leukapheresis settings or would have been obtained should modification of the leukapheresis settings had not taken place.

Limited literature on this matter suggests that red cell abnormalities could represent a factor responsible for this discordance by interfering with the separation of cell layers during leukapheresis.^{16, 17} We here, analyzed the factors that, within a homogeneous patient population with overt red cell abnormalities, as the homozygous β -thalassemia patients, may account for failures to collect the circulating CD34⁺ cells by standard apheresis variables. Those patients with thalassemia who are at risk for harvest failure may be identified by certain hematological and/or clinical parameters and the failure to collect CD34⁺ cell numbers proportional to the circulating HSCs can be prevented by adjustment of the apheresis variables.

MATERIALS AND METHODS

Stem cell mobilization

Adult β -thalassemia patients were enrolled in a Plerixafor-based mobilization clinical trial (EudraCT number 2009-014136-37, NCT 01206075). The trial was approved by the Greek Independent Ethics Committee at the George Papanicolaou Hospital, the Institutional Review Board of the University of Washington, and the Greek National Organization for Medicines. Informed consent was obtained by all enrolled patients according to the Helsinki Declaration. Patients were mobilized with Plerixafor (provided by Sanofi-Genzyme, gratis) with a total CD34⁺ yield target of 6×10^6 cells/kg, collected in up to two leukaphereses sessions. Plerixafor was administered subcutaneously at 0.24 mg/kg/day in up to two consecutive evenings, followed by leukapheresis 10 hours after each dose. Subjects who failed to provide a total of 6×10^6 CD34⁺ cells/kg by Plerixafor alone per two aphereses, or those who had failed to mobilize effectively with G-CSF-alone in a previous mobilization trial, were offered the opportunity to be remobilized with the combination of G-CSF +Plerixafor, as previously described.¹¹

Harvest

Leukapheresis was performed with a Cobe Spectra apheresis system (Terumo BCT Corporate) using a 3x total blood volume. In the majority of patients the mononuclear cell collection layer was set according to the manufacturer's instructions. However, in some patients this interface was reset towards the red blood cells' layer and a higher hematocrit in the colorgram scale, in an effort to rescue the CD34⁺ harvest, as explained in the Results section. Collection efficiency (CE) of the stem cell harvest was calculated using the following formula : Collection efficiency = Total number of harvested CD34⁺ cells / [peripheral blood CD34⁺ cells (μ l) $\times$ apheresis blood volume (L)]^{14,16}

CD34⁺ enumeration and blood cell counts

Hematopoietic progenitor cells (CD34⁺) in both peripheral blood samples and harvest samples were enumerated by flow cytometry(XL-MCL, *Beckman Coulter, Brea, CA*) using Stem-Kit™ Reagents (Beckman Coulter) and a single-platform ISHAGE protocol, as previously described.¹⁸ Complete blood cell counts, automated differentials and reticulocyte counts were performed on a hematology analyzer (Sysmex XE 5000, TOA Medical Electronics Kobe, Japan)

Statistics

A descriptive analysis of all continuous variables was performed, including mean and standard deviation. Data are expressed as mean $\pm$ SD values. Means of continuous variables were compared using the Student's t-test.

RESULTS

Poor harvests in optimally mobilizing thalassemic patients may be anticipated and prevented by adjustment of apheresis variables

We previously reported the results of two mobilization trials in patients with β -thalassemia, conducted in order to optimize the mobilization strategy in this specific population for gene therapy purposes.^{11, 12} We here focus on four patients enrolled in the second trial who were mobilized with Plerixafor, and in whom, despite the high numbers of circulating CD34⁺ cells before leukapheresis, adjustments of the apheresis variables were needed to rescue the CD34⁺ cell harvest.

In this trial, 20 β -thalassemia major patients were enrolled and mobilized with Plerixafor or G-CSF+Plerixafor following previous mobilization failure. Overall, 23 mobilization rounds and 41 apheresis sessions were performed. We here refer to optimally mobilizing or re-mobilizing patients (CD34⁺cells>20/microL) after primary mobilization or re-mobilization, respectively (n=19), excluding from the analysis one patient who was not apheresed¹¹ and three primary mobilization failures.

Patient 10 (P10), a splenectomized patient mobilized with Plerixafor, experienced poor CD34⁺ cell collection by 2 aphereses (1.7×10^6 CD34⁺ cells/kg in total) in the presence of high numbers of circulating CD34⁺ cells (66 and 59 CD34⁺ cells/ μ L before apheresis 1 and 2, respectively) (**Table 1**). Repeated CD34⁺ cell enumeration, both in the blood sample and the apheresis product, confirmed the initial measurements. The poor harvest was attributed at that time, to a possible, albeit unconfirmed, technical failure.¹¹

Patient 14 (P14), a second splenectomized patient mobilized with Plerixafor, yielded, again unexpectedly, very low CD34⁺ cells by the first leukapheresis (0.75×10^6 CD34⁺ cells/kg) despite apparently adequate numbers of blood CD34⁺ cells (36 CD34⁺ cells/ μ L) (**Table 1**). This time, it was rather obvious that in some patients with thalassemia, the formation of separated layers by centrifugation during apheresis is altered and the mononuclear cell layer moves to a different depth. Thus, we decided to start the next day apheresis in this patient using standard settings, obtain an intermediate graft sample for CD34⁺ cell count, and then modify the apheresis variables to collect from a deeper layer (where usually the red cells are found) and measure again CD34⁺ cells in a second intermediate sample. These measurements at a given processed blood volume (5 L and 8.8 L) when extrapolated to the targeted blood volume for processing (15 L), predicted that by collecting at the standard depth or a deeper layer we would harvest at the end of apheresis 0.6×10^6 /kg or 4.6×10^6 /kg CD34⁺ cells/kg, respectively. Indeed, after processing 5 L of blood with standard settings, we completed the harvest with the modified instrument settings to collect from a deeper layer, an intervention that rescued both the second and a similarly performed third leukapheresis, yielding 2.73 and 4.33×10^6 CD34⁺ cells/kg, in the presence of 61 and 65 circulating CD34⁺ cells/ μ L, respectively (**Table 1**).

At that time, we went back to the patients' data in an effort to potentially identify a common haematological profile for P10 and P14 that could distinguish them from the rest of the mobilized thalassemic patients in whom the well-defined correlation between the circulating

CD34⁺ cell numbers and the apheresis output, was confirmed. An initial observation was that a common feature of these two patients, not shared by the rest of previously mobilized thalassemic patients, was a predominance of lymphocytes over neutrophils in the differential counts, both at baseline and before the first apheresis (**Table 2**).

P19, a third splenectomized patient mobilized with Plerixafor, presented with higher percentage and absolute number of lymphocytes over neutrophils, both at baseline and before the first apheresis. The circulating CD34⁺ cells were 76 and 37 per μL , before the first and second apheresis, respectively. We decided to harvest CD34⁺ cells upfront from a deeper layer and not follow the standard settings. Both collections were successful, yielding in total 6.51×10^6 CD34⁺ cells/kg (**Table 1**).

In P20, a fourth splenectomized patient mobilized with Plerixafor, because of borderline relative lymphocytosis before apheresis (**Table 2**), we decided to proceed with the standard instrument variables in the first apheresis, and if needed, modify the settings of the next day apheresis. Even this borderline redistribution of blood leukocytes resulted in a significantly disproportional yield of CD34⁺ cells with respect to circulating CD34⁺ cells (1.44×10^6 CD34⁺ cells/kg and 80 CD34⁺ cells/ μL , respectively). Following modification to collect from a deeper layer during the second and third leukapheresis sessions, the harvest was rescued, ultimately yielding 9.14×10^6 /kg CD34⁺ cells in total (**Table 1**).

Overall, in three of the four presented cases (P10, P14 and P20 - excluding P19 whose collection layer was pre-emptively modified from the first leukapheresis) the stem cell product from the first apheresis obtained under standard collection settings, was disproportionally low with respect to the circulating CD34⁺ cells/ μL (**Table 1**). Collectively, the mean yield of the first leukapheresis in these patients was only $1.02 \pm 0.38 \times 10^6$ CD34⁺ cells/kg in spite of 60.27 ± 22.48 on average circulating CD34⁺ cells/ μL (**Table 3**). In contrast, the first apheresis mean counterpart values in the rest patients who also mobilized well (CD34+cells>20/ μL) either during primary mobilization or re-mobilization (n=15, excluding one patient in whom we did not proceed to harvest¹¹ and three primary mobilization failures), were $5.11 \pm 3.14 \times 10^6$ CD34⁺ cells/kg (p=0.04) and 71.9 ± 54.4 CD34⁺ cells/ μL (p=ns), respectively (**Table 3**). Indeed, there was a 3-fold difference in yield in terms of CE between patients with optimal mobilization and harvest and patients who achieved optimal mobilization but experienced harvest failure (0.36 ± 0.11 vs 0.1 ± 0.01 , respectively, p=0.001) (**Table 3**).

Not unexpectedly, by collecting CD34⁺ cells at a deeper layer (towards the RBC layer), the hemoglobin (Hb) content of the leukaphereses products of these patients was significantly higher than the average Hb concentration in the leukaphereses products obtained from patients in whom leukaphereses were performed under standard operating settings (4.45 ± 1.40 vs 1.12 ± 0.75 g/dL, p<0.00001) (**Table 3**).

Predictive factors of harvest failure in good mobilizers with red cell abnormalities

In order to define factors which may negatively affect the apheresis output when mobilization *per se* is successful, we comparatively tested clinical, hematological and mobilization characteristics of the patients described above with their counterparts among

the adequately mobilizing patients, in whom the HSC harvest yield was well-correlated with the circulating CD34⁺ cells.

No differences were encountered with regard to age, weight, or ferritin levels at baseline, and with regard to platelets, hemoglobin levels or blood CD34⁺ cells both at baseline and before the first apheresis (**Table 3**). At that time points however, all 4 optimally mobilizing patients who either failed or were predicted to fail first harvest, presented predominant relative or/and absolute lymphocytosis (p 0.0001 and p 0.01, respectively) as well as marked reticulocytosis (p 0.0001) (**Table 2 and Table 3**). Importantly, the predominance of lymphocytes over neutrophils represented by lymphocyte to neutrophil count ratio (LNR) above 1, arose as a highly predictive factor for low CE with the standard apheresis settings, clearly discriminating good mobilizers with low CE from good mobilizers with predictable CE (p 0.000004) (**Table 2 and Table 3**). It is also worth mentioning that reticulocytosis only in combination with an LNR>1, adversely influenced the HSC harvest (see P2, P3 vs splenectomized patients with low CE, table 2).

In addition, all 4 subjects who failed the 1st harvest had undergone splenectomy and splenectomy correlated with lymphocytosis and reticulocytosis over the intact spleen, both at baseline (p 0.01 and 0.03, respectively) and before the first apheresis (p 0.05 and 0.09, respectively) (**Table 4**). Although splenectomy was associated with a higher LNR overall (**Table 4**), only those splenectomised patients with a baseline and 1st apheresis LNR>1 in association with reticulocytosis ultimately failed the harvest process (p 0.0007) (**Table 2 and Table 5**).

DISCUSSION

Factors like age, gender, ethnicity, baseline WBC, differential count, have been recognized as having an impact on successful stem cell mobilization by pharmacological agents, both in normal donors and in patients with hematologic malignancies.²¹ In addition, several studies have shown that the absolute number of circulating mobilized CD34⁺ cells strongly correlates with the CD34⁺ cell yield of the apheresis product.³⁻⁵ Nevertheless, the failure of hematopoietic stem cell collection to obtain yields proportional to circulating CD34⁺ cells as well as the identification of factors exclusively affecting the collection efficiency remains largely unexplored.

In the context of gene therapy for β -thalassemia, large numbers of genetically modified HSCs are required for amelioration or correction of the thalassemic phenotype. Mobilized peripheral blood is the preferable source of CD34⁺ cells because it provides significantly higher numbers than direct bone marrow harvest, under a less invasive procedure. In a recent clinical trial from our group,²⁰ mobilization with G-CSF was shown to be both safe and effective in non-splenectomized patients with thalassemia major. However in splenectomized patients, hyperleukocytosis emerged as a G-CSF-dose limiting factor, ultimately leading to suboptimal or poor CD34⁺ mobilization. In light of these results, in a subsequent trial, we explored the safety and efficacy of Plerixafor, a recently available mobilizing agent, used either as a single agent or in combination with G-CSF after primary mobilization failure, in 20 adults with thalassemia major.¹² Here, we performed a *post-hoc* analysis, focusing on the

fact that the CD34⁺ cell apheresis yield in some Plerixafor-mobilized patients was, or would have been, disproportionately poor to their otherwise, successful mobilization.

Among the 19 analyzed patients who all mobilized optimally either during primary or secondary mobilization, the stem cell content of the first leukapheresis product in three of them (P10, P14, P20) was disproportionately low with respect to their circulating CD34⁺ cells while in another subject (P19), harvest was rescued by pre-emptive device setting modifications. When poor harvest in the absence of mobilization failure was initially encountered (P10), an unconfirmed technical failure was considered possible. Upon re-encountering poor harvest while adequate circulating CD34⁺ cells were present in patient P14, we suspected that poor yields in such cases might be related to the apheresis variables. We hypothesized that in some patients the mononuclear cell layer containing the CD34⁺ cells may move towards a different depth and consequently, by using standard settings the apheresis product is deprived of the precious CD34⁺ cells. If however, apheresis at risk of failure could be timely recognized, harvests might be rescued by modifying the settings of the apheresis device. Indeed, by adjusting the depth of the collection layer towards the RBC layer, the ultimate yields of leukaphereses in P14, P19, and P20 met the expectations based on the pre-apheresis CD34⁺ counts.

Previously, in the scarce literature addressing the issue of poor stem cell harvest in spite of adequate mobilization, Wang and coworkers identified that the collection efficiency in healthy donors with low mean cell volume (MCV) was significantly lower than that of normal-MCV donors despite similar mobilization outcomes¹⁴. In our study, including a homogeneous patient population with corrected MCV due to regular transfusions, no significant MCV differences were seen between those who failed or succeeded harvest, as expected.

We show here that, within a thalassemic patient population who mobilized CD34⁺ cells efficiently, lymphocytosis associated with reticulocytosis negatively affected stem cell collection by standard apheresis settings. In particular, the combination of an expansion of lymphocytes over neutrophils represented by an LNR above 1 and reticulocytosis, emerged as a highly predictive factor for harvest failure under standard collection device settings. Because all four patients who experienced harvest failure presented with both LNRs >1.0 and reticulocytosis, we could not dissociate the impact of an LNR >1.0 and reticulocytosis in inducing low CE. However, reticulocytosis alone, if not accompanied by LNR>1, did not seem to adversely impact the HSC harvest. Apparently, the expanded layers of lymphocytes over neutrophils and of reticulocytes result in a rearrangement of the separation layers during harvest; and, consequently, the collection interface lies deeper than the expected level in subjects with normal differential blood counts.

By adjusting the mononuclear cell collection layer towards the RBC layer, the hemoglobin (Hb) content of the leukaphereses products was, as expected, significantly higher compared to that of the leukaphereses products obtained under standard apheresis settings. Although autologous grafts with higher hematocrits are not reported to intervene with engraftment, this parameter should be taken into account in collections containing high numbers of damaged and ruptured RBCs if subsequent CD34 cell enrichment is needed (as in

thalassemia gene therapy), because the hemolyzed plasma and the numerous dead cells may affect the final purity and recovery of CD34+ cells.

Interestingly, another common characteristic of all 4 patients in whom adjustments were needed to rescue the CD34+ cell yield, apart from lymphocytosis and reticulocytosis, was splenectomy. Post-splenectomy lymphocytosis, reticulocytosis and a decrease in red-cell deformability have been well-evidenced in the literature.^{16,17,22-24} In our thalassemic splenectomized patients, all these factors may have accounted for the altered cell separation during leukapheresis. In particular, splenectomy by increasing the probability of lymphocytosis, potentially indirectly increased the chance for a LNR>1. Reticulocytosis - albeit linked to the disease background and the interindividual level of anemia or hemolysis-, might have been also exacerbated by splenectomy. These post-splenectomy-induced hematological alterations interpret why all subjects experiencing low CE were among the splenectomized ones. We identified only one relevant published report of rescuing a harvest by adjusting the apheresis settings to collect in the RBC layer, in a splenectomized patient with β -thalassemia intermedia who had multiple myeloma, underwent G-CSF+Plerixafor mobilization for autologous transplantation, and failed the initial stem cell harvest¹⁵. The authors of that case report hypothesized that the red blood cell abnormalities and the wide distribution of RBC sizes because of the thalassemic background, were responsible for altering cell separation during apheresis.

Although there are not yet data in the literature to support that the conclusions from our observations could also apply to populations beyond thalassemic patients, caution should be taken before the initiation of leukapheresis in specific hematopoietic stem cell donors with splenectomy and lymphocytosis, with LNRs >1.0 or/and reticulocytosis, to avoid possible harvest failure when mobilization is optimal. Nevertheless, further studies are needed to solidify the upfront apheresis settings modifications or interim CD34+ graft cell counts in other than thalassemic, donor populations who exhibit the aforementioned findings. In conclusion, we present here, within a specific patient population with thalassemia, patients who had poor stem cell yields that were not associated with suboptimal mobilizations; and we highlight the need, in certain cases, for customized stem cell harvests on the basis of specific individual features.

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Table 1

Individual patient characteristics and aphereses parameters in subjects with 1st harvest failure (P10, P14, P20) or upfront rescue (P19)

Patientno	P10	P14	P19	P20
Age (years)	42	30	22	40
Weight (kg)	85	73	55	74
Sex	M	M	M	M
Splenectomy	Y	Y	Y	Y
Chelation	XJ	Fer	Fer+Desf	Fer+Desf
Mobilization	Plerixafor	Plerixafor	Plerixafor	Plerixafor
1st apheresis with standard device settings				
CD34 ⁺ cells in PB (/μL)	66	36	76	80
CD34 ⁺ cell yield (×10 ⁶ /kg)	0.8	0.75	3 [*]	1.44
2nd apheresis with modified device settings				
CD34 ⁺ cells in PB (/μL)	59	61	37	56
CD34 ⁺ cell yield (×10 ⁶ /kg)	0.9	2.73	3.51	3.2
3rd apheresis with modified device settings				
CD34 ⁺ cells in PB (/μL)	N/A	65	N/A	42
CD34 ⁺ cell yield (×10 ⁶ /kg)	N/A	4.33	N/A	4.5
Total CD34⁺ cell yield (×10⁶/kg)	1.7	7.81	6.51	9.14

Abbreviations: WBC: white blood cells; Neut: neutrophils; Lymph: lymphocytes; PB: peripheral blood; M: male; Y: Yes; XJ: Exjade; Fer: Ferriprox; Desf: Desferal; N/A: not applicable

* value refers to upfront device settings adjustments

Table 2

Hematological parameters of optimally mobilized patients, at baseline and before 1st apheresis

BASELINE										BEFORE 1st APHERESIS					
WBC	% neut	% lymph	neut (K/uL)	lymph (K/uL)	lympho/neutro ratio	Ret %	WBC	% neut	% lymph	neut (K/uL)	lymph (K/uL)	lympho/neutro ratio	Ret %		
NON-SPLENECTOMIZED															
P1	6,5	68	27	4,4	1,8	0,9	18,3	65	26	11,9	4,7	0,39	1,8		
P7	6,1	66	25	4	1,6	0,46	16,6	64	21	10,6	3,5	0,33	n/a		
P8	7,5	66	29	5	2,2	n/a	25,6	71	22	18	5,7	0,32	n/a		
P9	4,9	50	43	2,5	2,1	0,84	21,6	60	32	13,1	6,9	0,53	0,23		
P12	6,7	57	36	3,8	2,4	0,63	26,4	61	29	16,2	7,6	0,47	0,26		
P13b	8,0	68	26	5,44	2,08	0,38	59,5	70	10	41,60	5,95	0,14	0,13		
P16	5,7	63	29	3,59	1,6	0,45	22,9	70	25	15,9	5,8	0,36	n/a		
SPLENECTOMIZED															
P2	16,5	46	41	7,5	6,8	0,91	1,3	61,4	61	30	37,6	18,1	0,48	n/a	
P3	10,6	57	39	6,1	4,2	0,69	3,75	33	55	30	18,2	9,9	0,54	2,39	
P4	10,5	49	48	5,1	5	0,98	0,2	87,8	85	12	84,6	10,5	0,12	1,2	
P5	10,3	55	35	5,7	3,6	0,63	n/a	34	55	31	18,6	10,4	0,56	n/a	
P6	7,3	57	39	4,5	2,7	0,60	47,2	67	22	42,1	6,6	0,16	n/a		
P11b	8,3	43	41	3,57	3,4	0,95	0,8	60,2	n/a	n/a	n/a	n/a	1,46		
P17b	8,1	46	34	3,73	2,8	0,75	0,24	98,4	71	18	70,2	17,3	0,25	0,28	
P18	11	51	34	5,61	3,8	0,68	43,3	48	32	20,9	13,9	0,67	n/a		
SPLENECTOMIZED WITH LOW COLLECTION EFFICIENCY															
P10	8,5	27	48	2,3	4,1	1,78	2,62	41,9	n/a	n/a	n/a	n/a	3,21		
P14	5,9	36	62	2,1	3,7	1,76	4,1	15,2	29	70	4,4	10,6	2,41	3,91	
P19	15	40	43	5,9	6,4	1,08	1,4	48,8	31	56	15,1	27,2	1,80	n/a	
P20	21,1	26	65	5,5	13,65	2,48	4,8	37,8	40	49	15,1	18,5	1,23	5,66	

WBC: white blood cells; Neut: neutrophils; Lymph: lymphocytes; ret: reticulocytes; "b" next to the patient number refers to remobilization with G-CSF+plerixafor in patients who failed primary mobilization; n/a : values not available; P15 who was mobilized but not apheresed and all primary mobilization failures (n=3) have not been included in the analysis

Table 3

Cumulative data on hematological and mobilization/apheresis parameters

Baseline													
	Age (years)	Weight (kg)	Ferritin (mg/dL)	Hb (g/dL)	MCV	PLT (×10 ³ /μL)	% neu	neu (×103/μl)	% lymph	lymph (×10 ³ /μL)	lymph/neu ratio	RET (%)	CD34+/μL
Optimal mobilization and harvest (n=15)	31.8 ± 6.68	65.50 ± 7.42	728 ± 342	11.2 ± 1	81.24 ± 3.8	432.8 ± 191.1	55.97 ± 9.63	4.6 ± 1.15	34.5 ± 7.13	3 ± 1.4	0.67 ± 0.21	0.7 ± 0.9	3.4 ± 1.6
1st harvest failure or rescue following optimal mobilization (n=4)	33.5 ±9.25	71.75 ± 12.42	604 ± 444	11.23 ± 1.1	81.83 ± 4.1	544.5 ± 32.58	32.15 ± 6.95	3.95 ± 2.03	54.45 ± 1.36	7.15 ± 5.63	1.78 ±0.57	3.2 ± 1.5	4.5 ± 1
<i>p-value</i>	0.6	0.3	0.54	0.88	0.79	0.36	0.0001	0.37	0.0001	0.003	0.000001	0.0002	0.19

Before 1st apheresis													
	Hb (g/dl)	MCV	PLT (×10 ³ /μL)	% neu	neu (K/ul)	% lymph	Lymph (×10 ³ /μL)	lymph/neu ratio	RET (%)	CD34+ /μL (PB)	CD34+X10^6 /kg	Collection Efficiency	Hb content of LPs (g/dL)
Optimal mobilization and harvest (n=15)	10.9 ± 0.9	81.4 ± 3.6	396.6 ± 189.2	63.4 ± 9.72	29 ± 22.37	25.26 ± 7.90	9 ± 4.3	0.46 ± 0.28	0.8 ± 0.8	71.9 ± 54.4	5.11 ± 3.14	0.36±0.11	1.12 ± 0.75
1st harvest failure or rescue following optimal mobilization (n=4)	11.4 ± 0.67	82.55 ± 3.5	501.8 ± 99.7	33.33 ± 5.86	11.53 ± 6.18	58.33 ± 10.69	18.8 ± 8.3	1.81 ±0.59	4.3 ± 1.3	60.27 ± 22.48	*1.02 ± 0.38	*0.1±0.01	**4.45 ± 1.40
<i>p-value</i>	n/s	0.57	n/s	0.0001	0.21	0.00001	0.01	0.000004	0.0001	0.82	0.04	0.001	0.0000000005

Abbreviations: Hb: hemoglobin; PLT: platelets; Lymph: lymphocytes; RET: reticulocytes; PB:peripheral blood; LP: leukapheresis product. Data are expressed as mean ± SD; p-values are derived by the unpaired Student's t-test.One patient who was mobilized but not apheresed and all primary mobilization failures have not been included in the analysis;

* value refers only to patients in whom 1st apheresis was performed using standard settings (n=3)

** value refers to aphereses performed with the modified device settings

Table 4

Correlation of splenectomy with hematological parameters

		Baseline				First apheresis			
		% Lymph	Lymph ($\times 10^3/\mu\text{L}$)	lymph/neu ratio	RET (%)	% Lymph	Lymph ($\times 10^3/\mu\text{L}$)	lymph/neu ratio	RET (%)
Patients without splenectomy	n=7	29.6 $\pm$ 6.5	1.9 $\pm$ 0.4	0.5 $\pm$ 0.16	0.4 $\pm$ 0.3	24.1 $\pm$ 6.3	5.7 $\pm$ 1.2	0.37 $\pm$ 0.11	0.5 $\pm$ 0.7
Patients with splenectomy	n=12	43.4 $\pm$ 9.5	4.8 $\pm$ 2.8	1.08 $\pm$ 0.55	1.7 $\pm$ 1.6	36.2 $\pm$ 16.6	14 $\pm$ 5.5	0.85 $\pm$ 0.67	2.1 $\pm$ 1.8
	<i>p-value</i>	0.001	0.01	0.009	0.03	0.05	0.0003	0.06	0.09

Abbreviations: Lymph: lymphocytes; RET: reticulocytes. Data are expressed as mean $\pm$ SD. p-values are derived by the unpaired Student's t-test.

Table 5

Predictive factors within the splenectomized patient population

		Baseline				1st apheresis			
		% lymph	lymph ($\times 10^3/\mu\text{L}$)	lymph/neu ratio	RET (%)	% lymph	lymph ($\times 10^3/\mu\text{L}$)	lymph/neu ratio	RET (%)
Patients with splenectomy and harvest failure	n=4	54.45 $\pm$ 10.7	6.96 $\pm$ 4.61	1.78 $\pm$ 0.57	3.23 $\pm$ 1.51	58.33 $\pm$	18.76 $\pm$ 8.30	1.81 $\pm$ 0.59	4.26 $\pm$ 1.24
Patients with splenectomy and adequate harvest	n=8	35.25 $\pm$ 6.51	3.07 $\pm$ 1.33	0.8 $\pm$ 0.15	0.96 $\pm$ 1.10	28.77 $\pm$ 10.18	12.37 $\pm$ 3.65	0.57 $\pm$ 0.35	1.05 $\pm$ 0.81
	<i>p-value</i>	0.02	0.07	0.0002	0.01	0.002	0.08	0.0007	0.002

Abbreviations: Lymph: lymphocytes; RET: reticulocytes; lymph/neu ratio: lymphocyte/neutrophil ratio. Data are expressed as mean $\pm$ SD. *p*-values are derived by the unpaired Student's *t*-test.