Early measurement of CD34+ cells in peripheral blood after cyclophosphamide and granulocyte colony-stimulating factor treatment predicts later CD34+ mobilisation failure and is a possible criterion for guiding "on demand" use of plerixafor

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Background. Early identification of predictive factors of failure to mobilise CD34+ cells could enable rational use of plerixafor during first mobilisation, avoiding the need for a second mobilisation course. However, "on demand" administration of plerixafor needs to be driven by established parameters to avoid inappropriate use.

Materials and methods. To address this issue, we studied the value of the peripheral blood CD34+ count, measured early (on days +10, +11, +12 and +13), in predicting the mobilisation outcome in the ensuing days. We retrospectively collected data from three Italian centres on 233 patients affected by multiple myeloma or lymphoma who underwent a first or second attempt at mobilisation with cyclophosphamide 4 g/m^2 and granulocyte colony-stimulating factor. To assess the diagnostic value of peripheral blood white blood cell and CD34+ cell counts with respect to "mobilisation failure", we considered failed mobilisation as "disease" and the CD34+ cell count in peripheral blood, on a specific day, as a "diagnostic test". For various thresholds, we measured sensitivity, false positive rate, specificity and positive predictive value (PPV) as well as the area under the receiver-operating characteristic curves (AUC).

Results. A CD34+ cell count $<10\times10^6$/L on day 13 had high sensitivity (1.00) and high specificity (1.00) for predicting subsequent mobilisation failure, with an AUC of 1.0. However, good prediction was also obtained using a lower threshold (CD34+ cell count: $<6\times10^6$/L) at an earlier time (day 12). The PPV of the day 13 threshold was 1.00 while that of the day 12 one was 0.87.

Discussion. We propose that patients with $<6\times10^6$/L CD34+ cells in peripheral blood on day 12 and $<10\times10^6$/L on day 13 following mobilisation with cyclophosphamide 4 g/m^2 and granulocyte colony-stimulating factor are candidates for "on demand" use of plerixafor, making the administration of this expensive agent more efficient and avoiding its inappropriate use.

Keywords: CD34+ cells, cyclophosphamide, PBSC mobilisation.

Introduction

Plerixafor is an active mobilising agent approved for use in association with granulocyte colony-stimulating factor (G-CSF)[1-4]. Plerixafor can also be used in association with chemotherapy and G-CSF[5-7], a triple combination which should have increased efficacy since it is known that mobilising agents, when combined together, can have a synergistic effect in peripheral blood stem cell (PBSC) mobilisation[8,9].

Given that plerixafor is very expensive, it is important that it is only given to appropriately selected patients. Patients requiring plerixafor treatment can be selected based on the fact that they have already failed a mobilisation (i.e., they are proven poor mobilisers)[10]. In this case a second mobilisation attempt can be made with plerixafor + G-CSF with or without chemotherapy, which in itself is relatively expensive. An alternative, which could

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have the advantage of avoiding a second mobilisation attempt, is to use plerixafor during first mobilisation in patients selected by having, at baseline, factors predictive of poor mobilisation (i.e. in predicted poor mobilisers)[10,11]. However, this way of using plerixafor is limited by the poor reliability of the factors predicting mobilisation in patients who have not been extensively pretreated[12-16]. A third way of using plerixafor is to administer it during first mobilisation, selecting patients based on early signs of mobilisation failure ("on demand" use). The criteria for judging mobilisation as "failed" have already been established for mobilisation induced by "G-CSF alone"[17,18], because the kinetics of G-CSF-induced mobilisation are well known[19,20] and reproducible. Thus patients with a CD34+ cell count below 10-20×10^6/L on day 5 are usually judged as "failed mobilisers". It has been proposed that plerixafor "on demand" treatment could be initiated in patients with CD34+ cell counts below this level on day 5[17,18,21,22]. However, mobilisation induced by G-CSF + chemotherapy has much more variable kinetics, in part because of the different types of chemotherapy used but also because of a wide inter-individual variability. The best time to use "on demand" plerixafor treatment in a poor mobiliser is not known, but timing may be of paramount importance because CD34+ mobilisation is a process which increases, peaks, decreases and ends, in spite of continuous administration of mobilising agents[19]. Indeed, early on demand use of plerixafor during poor mobilisation could have an enhanced mobilising effect compared to administration at a later time.

It is clear that, at present, on demand use of plerixafor after mobilisation induced by chemotherapy and G-CSF requires a better comprehension of the variability of CD34+ mobilisation kinetics and the establishment of an early time-point at which mobilisation may be judged to have failed. Furthermore, this information should be specific for each of the various chemotherapy schedules used in CD34+ mobilisation. Since cyclophosphamide and G-CSF is the schedule most frequently used both in multiple myeloma and in lymphoma, in a group of patients who underwent CD34+ mobilisation using this schedule we studied whether early CD34+ kinetics in peripheral blood can be used as an early indicator of failure of CD34+ mobilisation.

Materials and methods

Selection of patients

We retrospectively collected data from three centres that harvest PBSC. These centres were chosen because they have databases with prospectively entered information on CD34+ assays in peripheral blood for all patients who undergo mobilisation and because PBSC mobilisation was performed according to a pre-established protocol under the coordination of designated physicians (Riccardo Saccardi, Massimo Martino and Giuseppe Milone). The criteria for selecting patients were that they had a diagnosis of multiple myeloma or lymphoma, that they underwent a first or second mobilisation attempt during the years 2006-2011 and that the mobilisation schedule was cyclophosphamide at dose of 4 g/m^2 and G-CSF at a dose of 5-10 μg/kg.

The patients' characteristics are reported in Table I. Two hundred and thirty-three patients were studied of whom 181 had multiple myeloma and 52 had lymphomas (35 patients had non-Hodgkin's lymphomas and the other 17 had Hodgkin's lymphomas). Their mean age was 52 years and 217 were in their first attempt of mobilisation while 16 had a previous failed attempt.

Mobilisation using cyclophosphamide and granulocyte colony-stimulating factor

Cyclophosphamide was used at dose of 4 g/m^2, with the dose calculated on actual body weight or

Table I - Patients' characteristics.

Number	233
Age, years	Mean: 52.8 (19-69) Median: 55.0
Gender	Male 57.9% (n=135) female 42.0% (n=98)
Diagnosis	Multiple myeloma 76.6% (n=181) Lymphoma 22.3% (n=52)
Mobilisation attempt	First attempt 93.1% (n=217) Second attempt 6.8% (n=16)
G-CSF dosage	5 mg/Kg: 52.3% (n=122) 10 mg/Kg: 47.6% (n=111)
WBCx10^9/L at baseline Mean and range	5,770 (2,100-13,900)
Platelets x10^9/L at baseline Mean and range	212 (50-518)
Number of previous lines of chemotherapy administered	1=70% 2=24% >2=6%

corrected body weight, whichever was less[14]. The cyclophosphamide was administered by intravenous infusion over 1-3 hours on day 0 in association with an 80% dosage of Mesna administered in three divided doses at 8-hours intervals. G-CSF was administered subcutaneously, starting on day +1/+2, at a dose of 5-10 μg/kg and continued until harvesting was completed or, in patients who did not mobilise, until at least day +18 to +20.

Successful CD34+ mobilisation was defined as a CD34+ upsurge in peripheral blood to a level over 20×10^6/L. The same threshold was used as the criterion for starting apheresis. Target CD34+ values for harvesting were at least 2.0×10^6 cells/kg in patients with lymphomas and 4×10^6/kg in patients with multiple myeloma. G-CSF administration and monitoring of CD34+ cell counts was continued until day +18 to +20 in patients who did not mobilise CD34+ cells. CD34+ cell counts were assayed using a single platform method[23] and the PBSC harvest centres involved in this study were all participants in the CD34+ Quality Assessment (UK NEQUAS) programme.

Study design

We studied the value of CD34+ cell and white blood cell (WBC) counts in peripheral blood measured early, on days +10, +11, +12 and +13, for predicting the mobilisation outcome in the ensuing days. Peripheral blood CD34+ counts were sequentially monitored starting at day +9/+10 or later if the WBC in peripheral blood was below 2.0×10^9/L. Since the CD34+ count in peripheral blood after apheresis could be lowered by the apheretic procedure itself, CD34+ data obtained in peripheral blood after apheresis were not considered. In each patient, the CD34+ count in peripheral blood before apheresis was repeated on average 2.7 times (median 3; range 0-8).

Statistical analysis

We considered failed mobilisation as a "disease" and the WBC or CD34+ cell count in peripheral blood at pre-defined times as a "diagnostic test". The diagnostic performance of different thresholds of WBC or CD34+ cells, at different time-points, for identifying "failed mobilisation" was assessed by calculating sensitivity (number of patients who had a positive test and failed mobilisation/all patients who failed mobilisation); false positive rate (number of patients who had a positive test and successful mobilisation/all patients who had successful mobilisation); specificity (number of patients who had a negative test and successful mobilisation/all patients who had successful mobilisation) and positive predictive value (number of patients with a positive test and failed mobilisation/all patients who had a positive test).

The overall diagnostic performance of CD34+ and WBC counts in peripheral blood for predicting mobilisation failure was assessed by analysis of receiver operating characteristic (ROC) curves and the area under the curve (AUC). The association between variables was assessed by calculating the correlation coefficient (r) and the corresponding P values. The association of various factors with mobilisation outcome was studied using univariate and multivariate logistic regression analyses. Data were analysed using commercially available statistical software NCSS-2007 (NCSS, Kaysville, Utah, United States of America).

Results

CD34+ cell mobilisation success rate

Failed mobilisation, defined as a CD34+ peak below 20×10^6/L, was registered in 10.1% of all patients (5% of those with multiple myeloma and 25% of those with lymphomas) while an insufficient harvest (CD34+ cells harvested $<2\times10^6$/Kg) occurred in 18% of all patients (13.4% of those with multiple myeloma and 34% of those with lymphomas).

Factors associated with successful mobilisation were diagnosis of multiple myeloma (odds ratio 1.033; p =0.10) and WBC count at baseline (odds ratio 1.000; p =0.03), while age, dose of G-CSF and platelet counts were not significantly associated with mobilisation outcome. However, no factors resulted important when evaluated in multivariate analysis.

Variability in CD34+ upsurge

The CD34+ counts who had not already undergone apheresis varied, with a sharp increase on day +9 or +10; however, in some patients a significant upsurge of CD34+ cells appeared later on day +13, +14 or +15 (Figure 1). Accordingly, the day of first apheresis ranged from day +9 to day +17. Twenty percent of first aphereses were performed on day +10, 26% on day +11, 18% on day +12 and 19% on day +13, while 12.5% of all first aphereses were performed later.

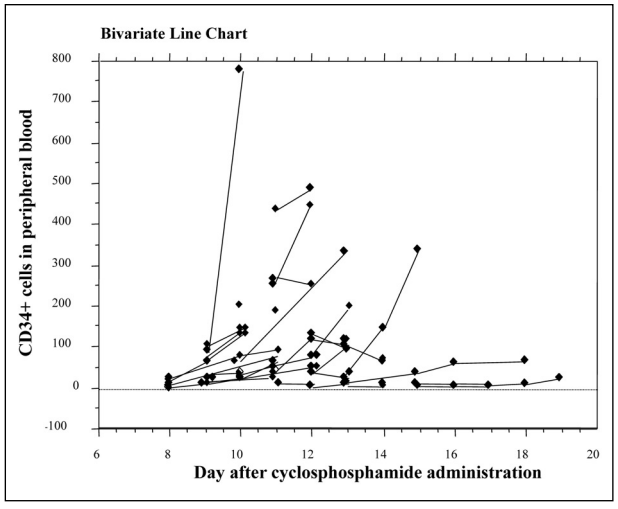


Figure 1 - CD34+ cell count in peripheral blood of a sample of patients mobilised using cyclophosphamide 4 g/m^2 and G-CSF.
There is evident variability in the final degree of CD34+ cell mobilisation and timing.

A late CD34+ upsurge is associated with lower degree of mobilisation

A later upsurge of CD34+ cells correlated with a low peak of CD34+ cells and there was an inverse correlation between day of first apheresis and total amount of CD34+ cells harvested (r= −0.237, p =0.0006). There was also an inverse correlation between day of first apheresis and CD34+ cells harvested per apheresis (r= −0.200, p =0.004). The median CD34+ cell counts in peripheral blood on days +10, +11, and +12, were 63.0×10^6/L, 53.6×10^6/L and 55.5×10^6/L, respectively. Thereafter, peaks values were lower and on day +13 the median CD34+ cell count was 38.8×10^6/L, on day +14 it was 31.8×10^6/L and on day +15 it was 22.1×10^6/L. In keeping with these data, the mean apheretic harvest was also higher in patients who started apheresis on day +10 (mean CD34+ cell aphereic harvest: 10.5×10^6/Kg) while it was 11.1×10^6/Kg in patients who started apheresis on day +11. The mean CD34+ cell apheretic harvest was 7.6×10^6/Kg in patients who started apheresis on day +12 and 5.9×10^6/Kg in those who started apheresis on day +13. In patients who began apheresis on day +14, the mean CD34+ cell apheretic harvest was 5.3×10^6/Kg. An evident inverse correlation (r =0.9; p =0.01) also existed between the day on which the first apheresis was performed and the CD34+ cell count in the peripheral blood on that day (Figure 2).

Amount of CD34+ cells in the peripheral blood on day +10 is correlated with the final peak of mobilisation

The day +10 CD34+ count in peripheral blood was correlated with the maximum CD34+ peak cell count registered later on the first day of harvest (r =0.871; p =0.0001). This correlation was maintained in both early mobilisers, defined as patients who had their first apheresis before day +13 (r =0.877; p =0.0001), and in late mobilisers defined as those who had their first apheresis on day +13 or later (r =0.772; p =0.001) (Figure 3).

Furthermore, the CD34+ cell counts in peripheral blood as early as day +10, when studied using logistic regression analysis, were associated with success of mobilisation (odds ratio 1.222, p =0.001).

CD34+ cell count in peripheral blood on day +10/+13 and failed mobilisation: raw data

On day +10, five patients had a CD34+ count below 5×10^6/L. Of these five patients, three (60%) failed to mobilise throughout the entire course of G-CSF administration while the other two (40%) did reach, at a later time, the threshold of 20×10^6/L and were, therefore, considered as mobilisers (Table II and III). Sixteen patients had a CD34+ cell count between 5 and 20×10^6/L and 31.2% of them (5/16) did not mobilise at later follow up.

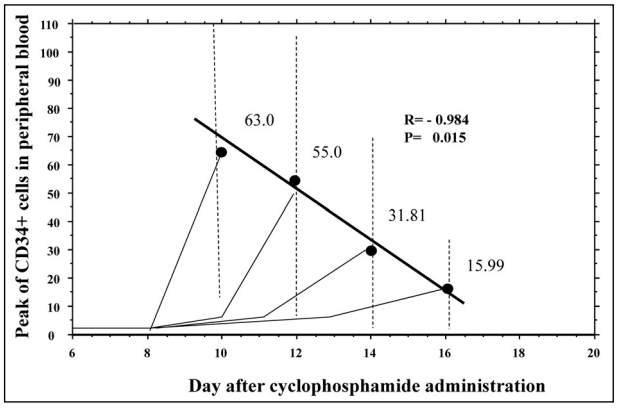


Figure 2 - Proposed model of CD34+ cell kinetics in the peripheral blood after mobilisation with cyclophosphamide 4 g/m2 and G-CSF.
Values of CD34+ cells in peripheral blood start to increase early, on days +8 to +10. The rate of increase is variable and the peak count is inversely proportional to the time the peak is reached. There is a clear inverse correlation between median CD34+ cell count in peripheral blood at time of first apheresis and the day of first apheresis. The dotted vertical lines represent the variance while the continuous black lines represent the hypothesised kinetics of the CD34+ cell upsurge in relationship to the time of first apheresis and to the degree of mobilisation.

Table II - CD34+ counts ($\times 10^6$/L) in all patients.

	Day +10 CD34 count	Day +11 CD34 count	Day +12 CD34 count	Day +13 CD34 count	Day +14 CD34 count	Day +15 CD34 count	Day +16 CD34 count
Mean	123.7	89.3	102.6	100.1	68.2	54.1	36.3
Count	115	91	84	65	32	14	6
Median	63.0	53.6	55.5	38.8	31.8	22.1	15.9
Range	1.5-1,033.1	0.3-712.5	1.2-484.0	1.8-642.0	1.4-339.0	0.9-334.0	2.9-138.6

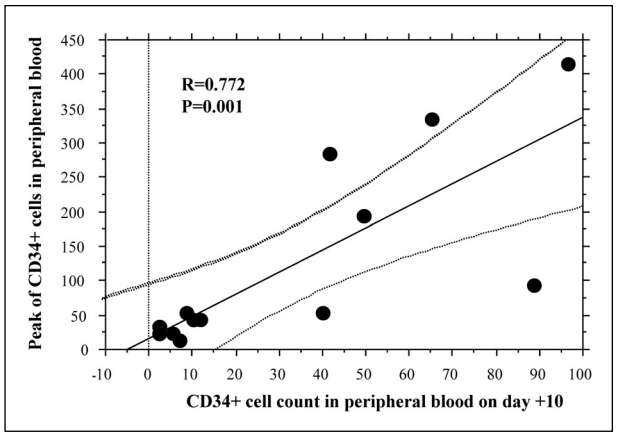


Figure 3 - Correlation between day +10 CD34+ cell count in peripheral blood and peak CD34+ cell counts. Day +10 CD34+ cell counts and the peak CD34+ cell counts reached later during mobilisation (only the data for patients who had their first apheresis on day +13 or later are shown).

Table III - CD34+ counts and mobilisation outcome (raw data).

Day	CD 34+ in PB (x10^6/L)	Failed mobilisation
+10	< 5	60%
	5-20	31%
+11	< 5	90%
	5-20	61%
+12	< 5	85%
	5-20	72%
+ 13	< 10	100%
	10-20	0%

On day +11, nine patients had a CD34+ cell count below 5×10^6/L and 88% of them (8/9) did not mobilise throughout the course of mobilisation while 13 patients had a count between 5 and 20×10^6/L and 61% (8/13) did not have successful mobilisation.

On day +12, seven patients had a CD34+ cell count below 5×10^6/L and 85% of them (6/7) did not mobilise while seven patients had a count between 5 and 20×10^6/L and 72% (5/7) did not mobilise.

On day +13, 12 patients had a CD34+ cell count below 10×10^6/L and none mobilised, while three patients had a count between 10 and 20×10^6/L and all of them mobilised. Results of CD34+ counts in all cases and in non-mobilising patients, at various days, are reported as Table II and III.

CD34+ cell counts in the peripheral blood on days +10 to +13 and failed mobilisation: receiver operating characteristic curves

Using "day +10 CD34+ cell count in peripheral blood" as a diagnostic test and considering failed mobilisation as a "disease", the area under the ROC curves was 0.96. The AUC observed for "day +12 CD34+ cell count" was 0.97, while the "day +13 CD34+ cell count" had a AUC of 1.00 (Figure 4).

Different thresholds of CD34+ cell counts in the peripheral blood (6×10^6/L and 10×10^6/L) at different time-points (days +10, +11, +12 and +13) gave the results shown in Table IV. In brief, a CD34+ cell count below 10×10^6/L on day +13 had a high sensitivity and specificity for predicting mobilisation failure. However, good predictive results were also obtained using a lower threshold (CD34+ cell count: 6×10^6/L) at an earlier time (day +11 or +12).

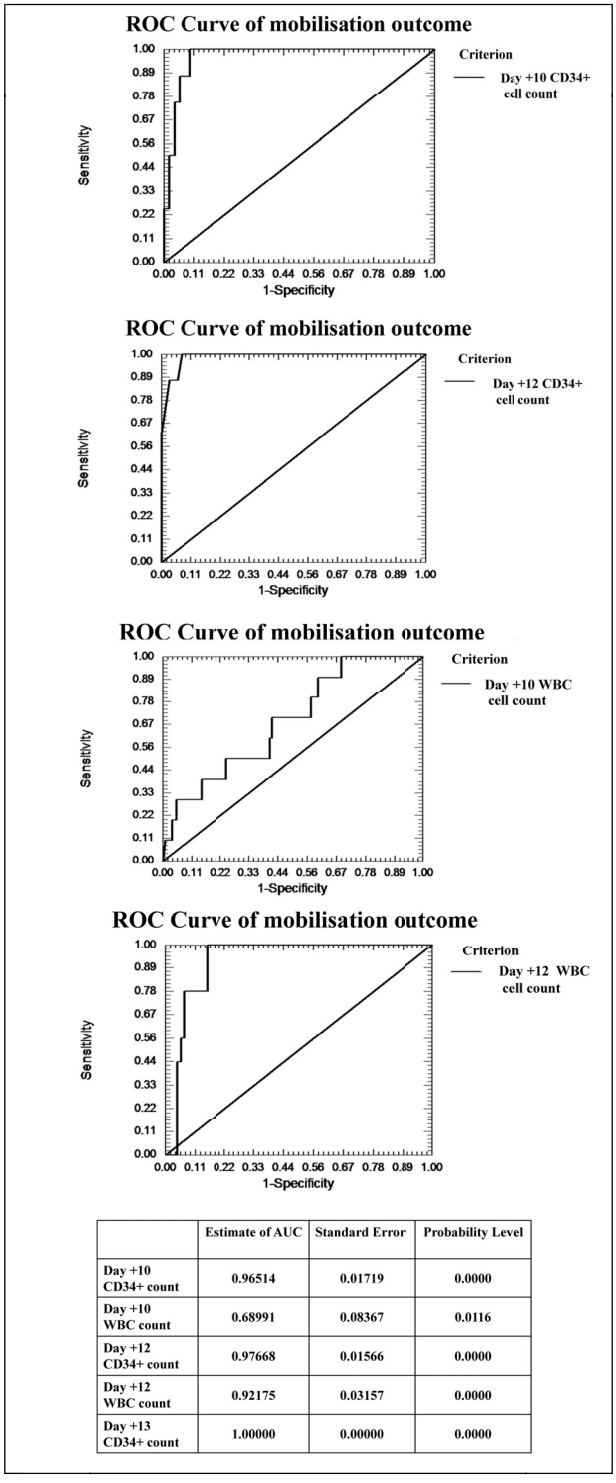
White blood cell count in peripheral blood on days +10 to +13 and failed mobilisation: receiver operating characteristic curves

A good correlation was found between WBC count on day +10 and amount of CD34+ cells harvested per single apheresis ($r = 0.519$, $p = 0.0001$) while WBC count on day +11 or +12 was weakly correlated with the amount of CD34+ cells harvested ($r = 0.186$, $p = 0.09$ and $r = 0.229$, $p = 0.04$, respectively).

The area under the ROC curve of WBC count on day +10 was 0.68 (Figure 4) and the cut-off of 2.0×10^9/L had a sensitivity of 0.30 a specificity of 0.87. However, the positive predictive value (PPV) was rather low (0.17) (Table V). The area under the ROC curves of WBC count on day +12 was 0.92 and the cut-off of 10.0×10^9/L had a sensitivity of 0.77 and a specificity of 0.83, but again the PPV was low (0.36).

Discussion

We studied the kinetics of mobilisation after cyclophosphamide 4 g/m^2 and G-CSF and found a great variability in the time of CD34+ upsurge and



	Estimate of AUC	Standard Error	Probability Level
Day +10 CD34+ count	0.96514	0.01719	0.0000
Day +10 WBC count	0.68991	0.08367	0.0116
Day +12 CD34+ count	0.97668	0.01566	0.0000
Day +12 WBC count	0.92175	0.03157	0.0000
Day +13 CD34+ count	1.00000	0.00000	0.0000

Figure 4 - Overall diagnostic results: receiver operating curves and the areas under the curves.
Legend: The scales on the right vertical axis refers to CD34+ cell count in peripheral blood at a given time. Each CD34+ cell count has a different sensitivity and specificity for predicting subsequent failure of mobilisation. From these data a receiver operating characteristic (ROC) curve is extrapolated and the area under the curve (AUC) can be calculated. The greater the value of the AUC, the better the performance of the diagnostic criteria.

Table IV - Results of early CD34+ cell count threshold for predicting mobilisation at a later time.

Day	CD34+ count in PB (x10^6/L)	Sensitivity (%)	False positive (%)	Specificity (%)	True PPV
+10	6	0.50	0.02	0.97	0.57
+11	6	0.69	0.01	0.98	0.90
+12	6	0.87	0.04	0.96	0.87
+13	6	0.75	0.00	1.00	1.00
+13	10	100	0.00	1.00	1.00

Legend
PB: peripheral blood; PPV: positive predictive value.

Table V - Results of white blood cell count for predicting mobilisation at a later time.

Day	WBC threshold	Sensitivity	False positive (%)	Specificity	True PPV
+10	2.0×10^9/L	0.30	0.12	0.87	0.17
+12	10.0×10^9/L	0.77	0.16	0.83	0.35

Legend
PPV: positive predictive value

in the extent of this upsurge. We also found that an early increase in CD34+ cell counts (>6×10^6/L) on day +10 or +11 occurs in mobilising patients and that this increase is predictive of the final, peak CD34+ cell count.

The data obtained in this study can be useful for determining, at an early time point, whether mobilisation will fail and, therefore, for starting plerixafor "on demand" already during the first mobilisation cycle. On demand use of plerixafor during a first mobilisation attempt may have many advantages over its use during a second course of mobilisation, since it avoids the expense[24-26] and clinical complications incurred by administration of a further chemotherapy course to mobilise PBSC[27,28].

There is no established criterion for the time at which to initiate "on demand" use of plerixafor because objective criteria which can be used to judge mobilisation as failed have not been identified in mobilisation courses based on chemotherapy[7,11,29]. Based on our study, we propose starting "on demand" use of plerixafor when the CD34+ cell count in peripheral blood is below 6×10^6/L on day +11/+12 or below 10 on day +13.

A recently published study addressed the issue of early, "on demand" administration of plerixafor in patients in whom mobilisation is failing[28]. The authors suggested a low level of CD34+ in peripheral blood on day +13 as a possible criterion for initiating

plerixafor administration. In the present study, we considered the overall usefulness of different CD34+ cell counts in peripheral blood at various time points as an indication for starting early, "on demand" plerixafor administraiton. Based on our data, a CD34+ cell count of 10×10^6/L or below, on day +13, could, indeed, be proposed: in fact no patients having fewer than 10×10^6/L CD34+ cells on day +13 reached higher values later on. This threshold was chosen to avoid inappropriate use of the drug and, in fact, the PPV of this threshold is almost 100%. The decision on whether to use plerixafor or not in a patient with a peripheral blood CD34+ cell count 10 and 20×10^6/L on day +13 is much more controversial and should be based on the collection efficiency that can be expected in this subgroup of patients in each centre and on the target CD34+ cell count. It would be interesting to study whether, in patients with a CD34+ cell count of 10-20×10^6/L on day +13, the use of plerixafor can significantly improve harvests and reduce the number of aphereses.

We cannot expect that "on demand" use of plerixafor using the proposed criteria will completely eliminate the need for a second course of mobilising treatment, because harvesting may be unsuccessful in some mobilised patients (defined as having a peak CD34+ cell count over 20×10^6/L) because of inefficiencies arising during the harvest (incorrect timing of apheresis, problems with venous access, low collection efficiency, etc.). These aspects also need to be optimised in order to reduce the need for a second mobilisation and to restrict the use of plerixafor to only those cases in which it is necessary. Both an adequate number of apheretic machines and extended operating hours throughout all the days of the week have been found to be factors effecting harvesting efficiency[30].

Our data need to be validated in an independent set of patients. A prospective study on early "on demand" use of plerixafor could assess the efficacy of this agent in patients selected on the basis of the criteria identified here.

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The Authors declare no conflicts of interest.

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