15.

Haemopoietic Progenitor Cell (HPC) Counts on the SYSMEX XE-2100: A New Tool for Peripheral Blood Stem Cell (PBSC) Harvest Monitoring

Florence Cymbalista and Remi Letestu

Introduction

A crucial point in optimising peripheral blood stem cell (PBSC) collection is determining precisely when apheresis has to begin. The decision to harvest PBSC is frequently based on the enumeration of CD34+ cells by flow cytometry. The method is expensive, complex, time consuming and creates organizational difficulties as regards bed occupancy and staff availability. Awaiting the result of the CD34+ assay frequently delays the harvesting procedure.

The aim of this study was to evaluate an alternative method for circulating haemopoietic stem cell quantification, namely by means of the Haemopoietic Progenitor Cell (HPC) parameter generated by the SYSMEX XE-2100 automated haematology analyser. The objective was to determine how HPC could be introduced into clinical practice to minimise the number of CD34 determinations routinely performed, determine its cut-off value for predicting a good yield and evaluate its overall impact and cost.

Patients

Two centres participated in this study over a period of one year. Patients were included in the study provided HPC and CD34 were determined simultaneously at least on the presumed day of the harvest and, in many cases, the days prior. Some 101 patients with haematological malignancies and 10 healthy donors were studied. Diagnoses were available for 91 patients and included 27 patients with Non-Hodgkin's lymphoma, 11 patients with Hodgkin's disease, 7 patients with chronic lymphocytic leukaemia, 10 patients with macroglobulinaemia, 16 patients with multiple myeloma and 17 patients with acute myeloid leukaemia.

The mobilization protocols consisted of chemotherapy plus G-CSF for 72 patients and G-CSF alone for 19 patients and the 10 healthy donors. Out of 101 mobilization procedures, PBSC were harvested in 77 patients. In the remaining 24 patients, the decision not to harvest was made on the fact that the CD34+ counts were below 10/mm^3.

Counting Methods

HPC Count: HPC are identified in the IMI-HPC area of the IMI channel of the SYSMEX XE-2100 on the basis of their resistance to the lysing reagent, their volume (direct current) and their internal structure (radio-frequency). HPC, like all immature cells, are resistant to the lytic reagent used and are located within a specific gated area of the scattergram.

CD34+ Count: CD34+ cells were quantified using flow cytometry by different procedures in the two centres:

- Using ProCount (Becton Dickinson) kits on a FACScalibur (BD) flow cytometer by a single platform method.
- Using a combination of antibodies including anti-CD45 and anti-CD34 antibodies on a FACScalibur (BD) flow cytometer by a dual platform method.

Results were analysed using Cell-Quest® software.

Results

Statistical analyses were performed using Statview® software. Using Spearman's rank test a strong correlation was found between CD34+ and HPC count (r_s = 0.669, p < 0.0001). Less significant correlations were observed with mononuclear cell count, IMI parameter, IG count and qualitative flags. Neither HPC nor CD34 counts were correlated with total WBC counts (**figure 1**). The global correlation between CD34+ cells and HPC was very close to that shown in other studies with r^2 = 0.651 (**figure 2**). In order to gain better insight into the relationship between the two parameters both technical issues and patient characteristics were examined further.

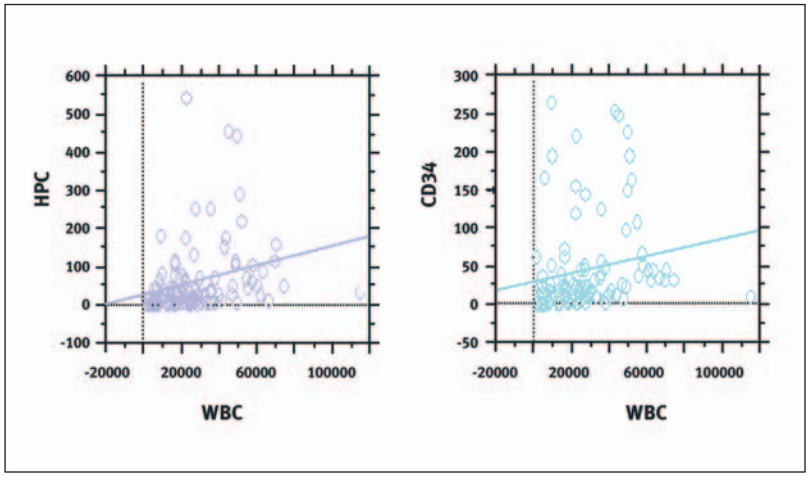

Figure 1
Correlation between HPC and CD34+ with WBC. All counts expressed/mm³.

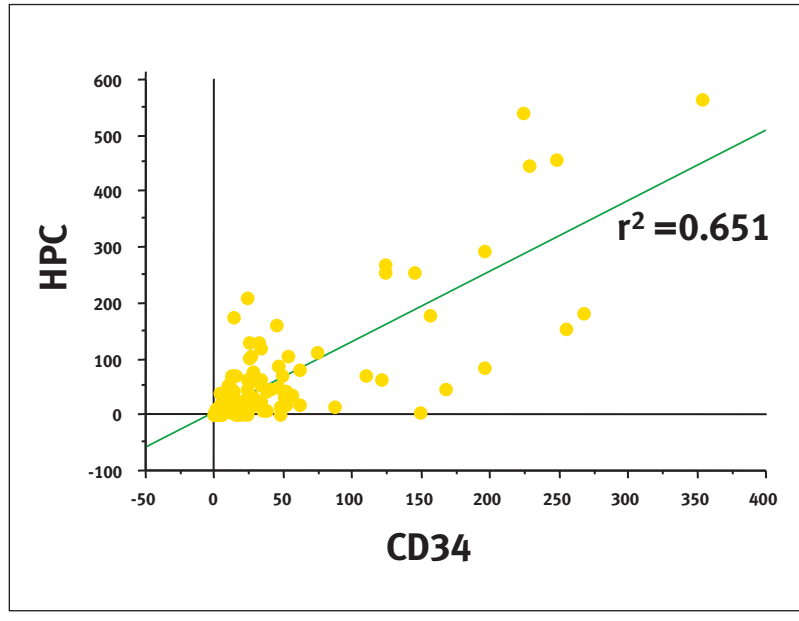

Figure 2
Correlation between HPC and CD34+. All counts expressed as/mm³.

Technical Issues:

Reproducibility was assessed by performing repeatability studies on 10 specimens, each being analysed 30 times. The coefficient of variation (CV) for higher counts was 17% but was up to 30% for lower counts of HPC (**figure 3**).

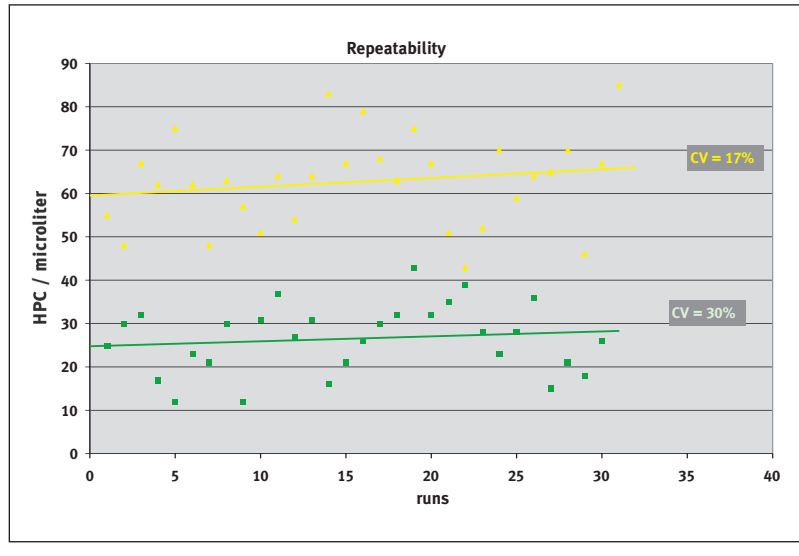

Figure 3
Repeatability studies.

Doubling dilution tests were performed to evaluate linearity. We observed a good median coefficient of correlation (r = 0.9944, SD = 0.0207), › 0.98 in 76% cases and › 0.95 in 94% cases. In eight cases with impaired linearity, the discrepancy was always observed between the pure sample IMI# (count of immature cell) and the first dilution. There was no significant correlation between linearity and the count from Immature Information (IMI), IMI coefficient (defined as the ratio IMI# to IMI-total) or initial HPC number. Our results demonstrate that HPC count is linear even in the presence of high number of immature myeloid cells but can be impaired in some patients or in samples with high WBC count.

The two different methods for CD34+ enumeration had no impact on the correlation between HPC and CD34+ (**figure 4**) with $r^2 = 0.676$ in Centre 1 and $r^2 = 0.606$ in Centre 2.

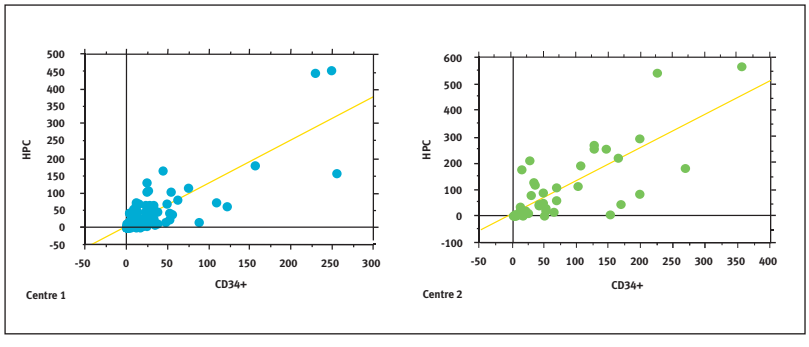

Figure 4
Comparison of the CD34+ methods in the two centres. Correlation between HPC and CD34+.

Patient Issues:

A number of different correlation studies were performed with different patient groups to assess the effect of disease related issues. In the first instance HPC and CD34+ assays were compared in healthy donors undergoing mobilization with G-CSF (**figure 5**). The correlation ($r^2 = 0.923$) appears stronger for healthy donors than for patients, however it must be conceded that the number of cases is small (n = 10). Analysis by diagnosis proved to be very interesting when comparing a group of non-Hodgkin's and Hodgkin's lymphoma (**figure 6**), acute myeloid leukaemia (**figure 7**) and chronic lymphoproliferative disease and multiple myeloma (**figure 8**). The correlation between HPC and CD34+ was highest for the group of non-Hodgkin's lymphoma and Hodgkin's lymphoma ($r^2 = 0.761$). It was less good for acute myeloid leukaemia ($r^2 = 0.544$) and really poor for the group containing chronic lymphoproliferative disorders and multiple myeloma ($r^2 = 0.182$).

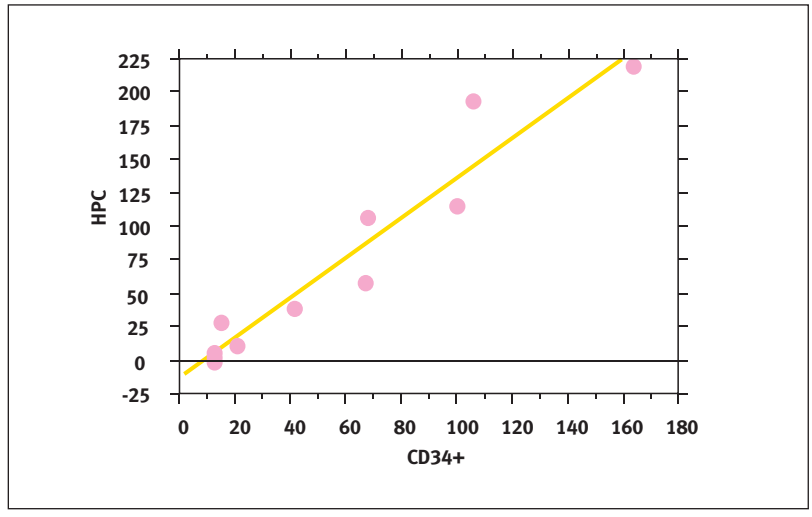

Figure 5
Correlation between HPC and CD34+ in healthy donors. (r^2 = 0.923)

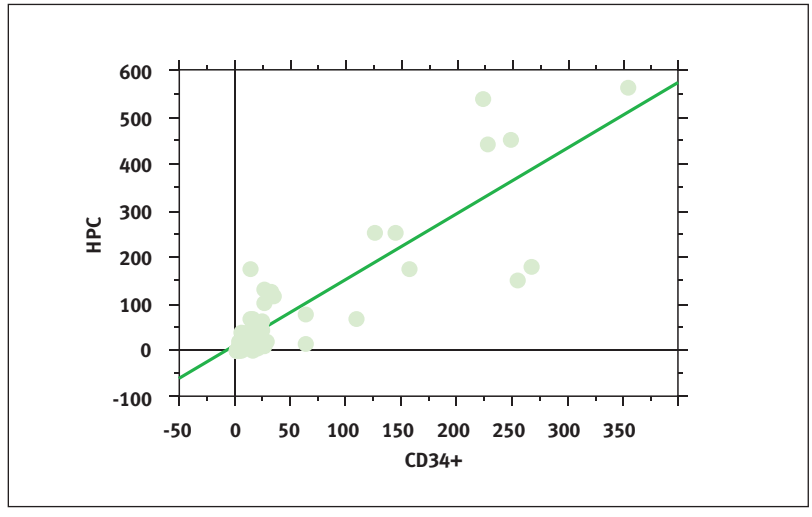

Figure 6
Correlation between HPC and CD34+ in a group (n = 38) of Hodgkin and non-Hodgkin lymphomas. (r^2 = 0.761).

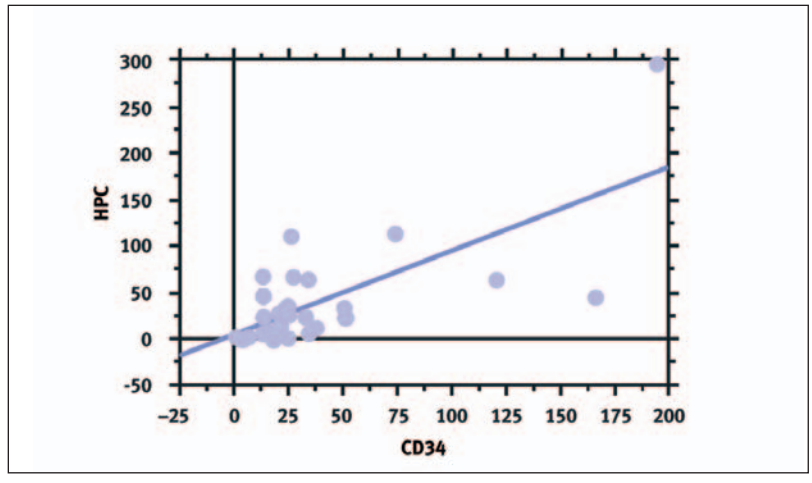

Figure 7
Correlation between HPC and CD34+ in a group (n = 17) of patients with acute myeloid leukaemia (r^2 = 0.544).

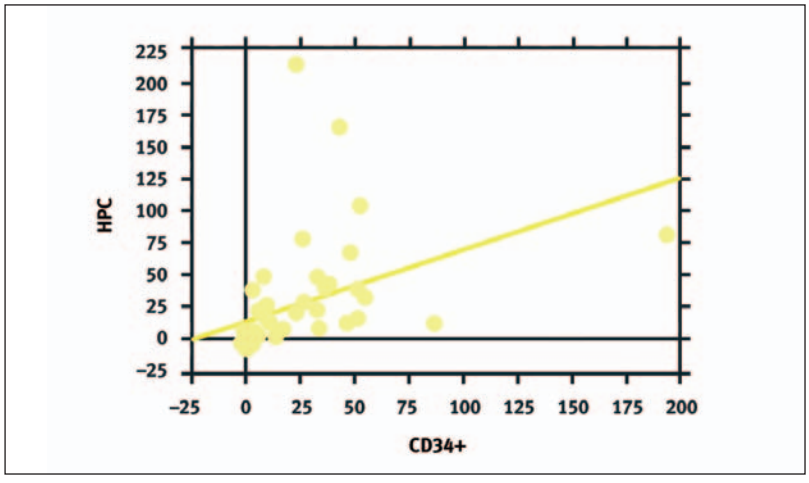

Figure 8
Correlation between HPC and CD34+ in a group (n = 33) of patients with chronic lymphoproliferative disease and multiple myeloma (r^2 = 0.182).

A further study compared the effect of the different mobilising regimens on the HPC/CD34+ correlations. The correlation was good for patients receiving chemotherapy plus G-CSF (**figure 9**) with r^2 = 0.675. However the HPC and CD34+ numbers did not correlate so well for patients receiving G-CSF alone (figure 10) with r^2 = 0.49. It must be noted that the study illustrated in **figure 10** was restricted to patients and did not include healthy donors.

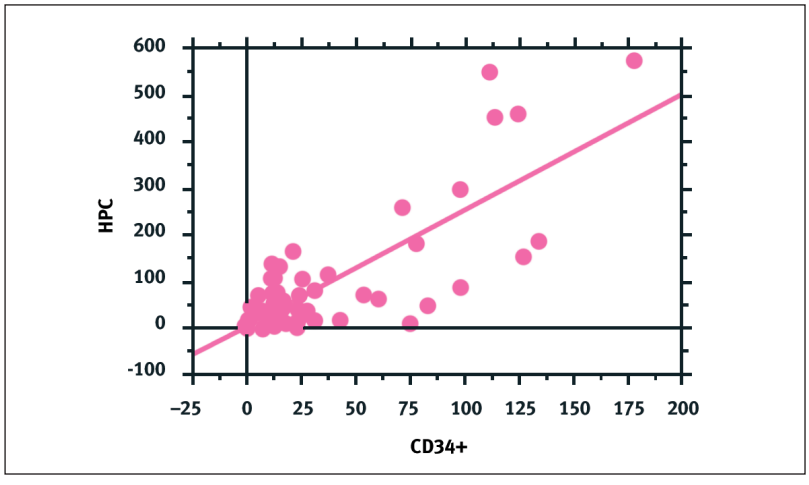

Figure 9
Correlation between HPC and CD34+ in patients receiving chemotherapy plus G-CSF for mobilization (r^2 = 0.675).

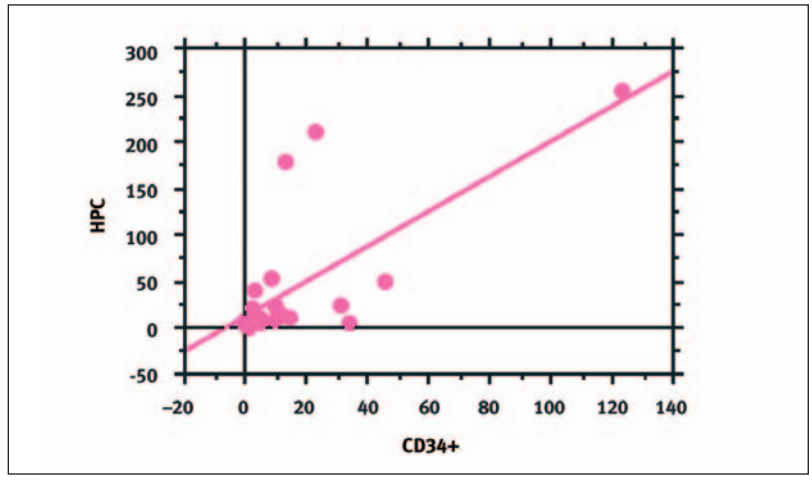

Figure 10
Correlation between HPC and CD34+ in patients receiving G-CSF alone for mobilization (r^2 = 0.49).

The linear correlation was good between the number CFU-GM and either peripheral blood CD34+ cells count (r^2 = 0.81) or HPC count (r^2 = 0.71, n = 112) as shown in **figure 11**. The correlation was even stronger between HPC and CFU-GM than it was between HPC and CD34+. Consequently we may hypothesize that HPC include CD34 negative precursors with colony forming unit potential.

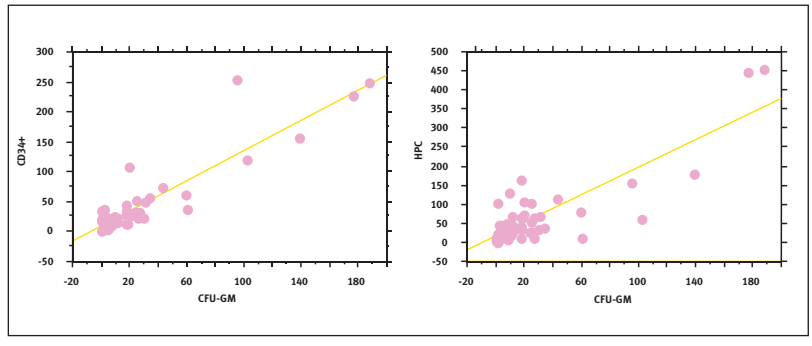

Figure 11
Correlation of CFU-GM with CD34+ and HPC.

Decision limits:

The next step was to determine a lower cut-off for the HPC parameter. There were 26 occasions when the HPC count was zero and in 25/26 the CD34+ count was ‹ 10/mm³. One patient had a CD34+ count › 10/mm³ but was not harvested until the following day by which time the HPC count had risen to 62/mm³ and an excellent yield was obtained (CD34+ = 121/mm³ and CFU-GM = 102 x 10^4/kg). In 30 patients it was possible to determine both HPC and CD34+ levels in consecutive samples from Day 5 before harvesting. In all cases except the precited one, in which the appearance of HPC was delayed by 24 hours, HPC preceded or was concomitant with the CD34+ increase. It therefore seemed reasonable not to determine the CD34+ count when HPC = 0.

The next step was to determine an upper cut-off above which it was unnecessary to await the result of a CD34+ assay. The HPC count was › 30/mm³ in 56 of 164 determinations. Of these 56 patients, 53 had CD34+ levels › 10/mm³ and were harvested on that same day. Details of the three discordant cases are given in **table 1**.

Patient	Mobilization	WBCx10⁹/L	HPC/mm³	CD34+/mm³	Harvest
NHL	Chemo+GCSF	27.5	47	4	Not done
WM	GCSF	47.5	52	9	Not done
CLL	GCSF	23.0	41	4	Done

Table 1
Details of the three patients with HPC counts › 30/mm³ and CD34+ counts ‹ 10/mm³.
NHL = non-Hodgkin's lymphoma,
WM = Waldenstrom's macroglobulinaemia,
CLL = chronic lymphocytic leukaemia.

The CLL patient with an HPC count of 41/mm³ and a CD34+ count of 4/mm³ was harvested on the same day as the assays and produced a good yield. Given these results it seems entirely reasonable to begin harvesting without awaiting for the results of the CD34+ assay when the HPC count is › 30/mm³. The relative incidence of HPC counts between 10 and 30/mm³ and low CD34+ counts on harvest day could not be evaluated properly in this study because the decisions not to harvest were made exclusively on CD34+ counts and the patients.

Conclusions

From this study HPC determination has been shown to be helpful in two situations:

- during the follow-up of patients after mobilisation, the CD34+ count does not need to be assessed before HPC are detectable,
- if the HPC count is › 30/mm³, it is reasonable not to await the CD34+ results before harvesting.

These two situations account for more than half the CD34+ determinations carried out in our practice, so already that is a substantial gain.
For the future, some technical improvements are required to extend the use of HPC counts. Improving the reproducibility of the determination of HPC would most probably increase the performance of the test between 0 and 30 HPC/mm³.

Since this present study seems to raise more questions than it answers, it may be of benefit to study more patients to assess the effect of prior marrow involvement, the influence of the mobilization procedure, the relationship of HPC counts to CFU-GM and recovery from transplantation.

Acknowledgements

I would like to take this opportunity to express my thanks to the many colleagues who assisted in this study.

CHU Necker, Paris: F. Audat, V. Baccini, L. Garcon, C. Persiaux, L. Dal Cortivo, F. Lefrère and F. Valensi.

CHU Hotel-Dieu, Paris: C. Marzac, R. Belhocine, D. Louisy and JY. Perrot
Roche Diagnostics: JP. Pérol, F. Schillinger.