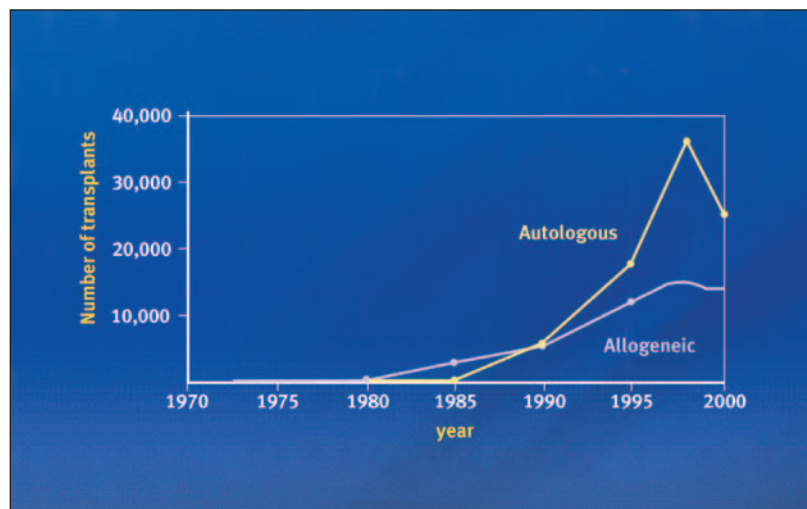


# Can Haematopoietic Progenitor Cell (HPC) Enumeration with the SYSMEX XE-2100 guide further Flow Cytometric CD34+ Measurements and Timing of Leukapheresis?

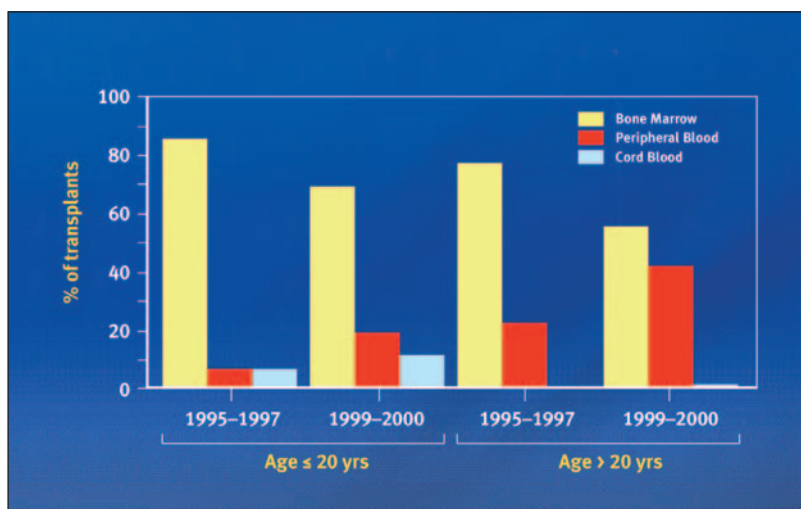
Christian Thiede

## Introduction

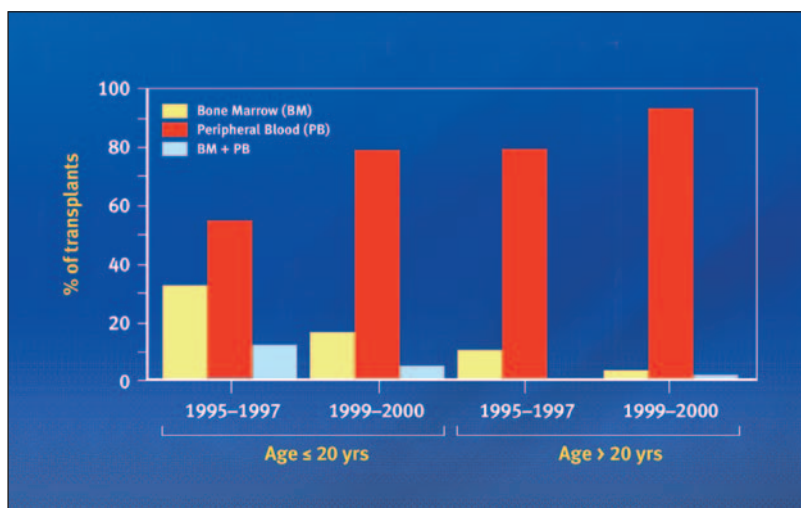
Stem cell transplantation is the only curative treatment for a number of haematological and oncological diseases including lymphomas, leukaemias and several solid tumours. Recently the indications for this kind of treatment have been extended into other fields of internal medicine, e.g. autoimmune diseases. For stem cell transplantation it is necessary to have a graft consisting either of autologous or allogeneic cells. The principal sources include bone marrow cells, peripheral blood stem cells or cord blood cells. During the past 30 years the number of bone marrow (BMT) and peripheral blood stem cell transplantations (PBSCT) has increased enormously as shown in **figure 1** using data from the International Bone Marrow Transplantation Registry (IBMTR). From IBMTR data it can also be seen that for allogeneic transplants, the source of stem cells increasingly is the peripheral blood for both children and adults (**figure 2**) and the same holds true for autologous transplants (**figure 3**) where in adult practice the figure approaches 90%.



**Figure 1**  
Annual numbers of blood and bone marrow transplants worldwide in the period 1970–2000. Data derived from the International Bone Marrow Transplant Registry (IBMTR).



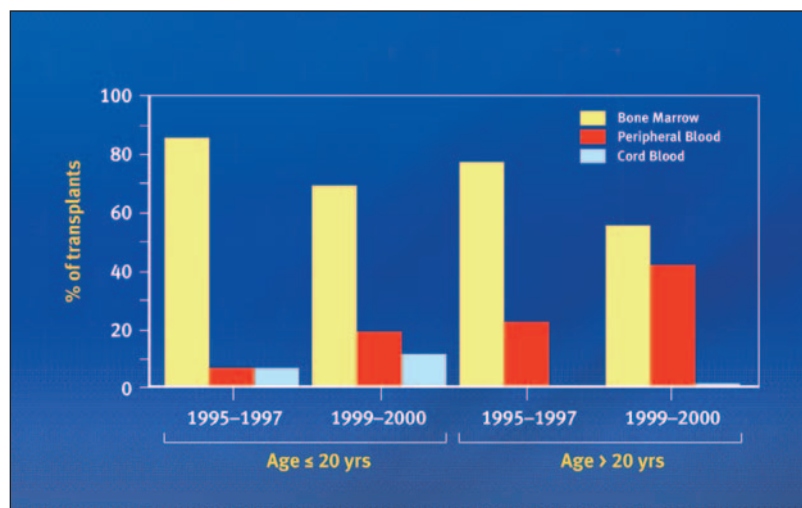
**Figure 2**  
Allogeneic stem cell sources by age in the period 1995-2000. Data derived from the International Bone Marrow Transplant Registry (IBMTR).



**Figure 3**  
Autologous stem cell sources by age in the period 1995-2000. Data derived from the International Bone Marrow Transplant Registry (IBMTR).

Because of this marked increase in the use of peripheral blood stem cells, the ability to detect stem cells in the circulating blood becomes paramount. There are three methods of achieving this. First there are flow cytometry assays for the quantitation of CD34+ cells. This procedure, which is the most widely used, requires experienced personnel, is time-consuming and expensive. Standardisation of the technique is of vital importance and a number of groups are working on this issue. Secondly there is the colony forming unit (CFU-GM) assay which, since it requires 14 days to complete, is not a practical technique for standard clinical monitoring. Thirdly there is the relatively new potential marker, the Haematopoietic Progenitor Cell (HPC) as measured by a number of blood cell analysers manufactured by the SYSMEX Corporation. These include the SE-9000, the SE-9500 and the XE-2100. The HPC measurement is therefore a rapidly generated, inexpensive parameter produced by a routine haematology blood count analyser. The technique is, by now, quite well known and is based on the differential lysis

of mature cell membranes leaving the more immature cells relatively intact due to differences in the lipid content of cell membranes. Measurement occurs in the IMI (Immature Information) channel of the analyser using capacitance (RF) and impedance (DC) signals (**figure 4**). Many groups have published the results of studies and some are summarised in **table 1**.



**Figure 4**  
IMI scattergram identifying the location of the HPC and other cell types.

Author	Instrumental	Number	Analyses	Results
Pollard et al, 1999 [1]	SE-9500	58 patients 9 donors	114	Corr for CD34+ and Aph yield
Yu et al, 2000 [2]	SE-9500	55 patients 11 donors	112	Corr for CD34+ and Aph yield
Peng et al, 2001 [3]	SE-9500	21 patients 13 donors	112	Corr for CD34+ and Aph yield

**Table 1**  
Selected HPC literature.  
(Corr = correlation;  
Aph = apheresis)

Most workers have used the SE-9500 analyser and each series is relatively small particularly with reference to the number of healthy donors studied. Most of these studies showed that there is a good correlation between the HPC count and the CD34+ count and that there is also a correlation with the apheresis yield.

Our department started transplantation in 1994 and we now perform about 140 transplants per year, 80–90 of which are allogeneic transplants and 50–60 autologous. More than 90% of transplants are performed using peripheral blood stem cells. We also have an accredited Good Manufacturing Practice (GMP) facility for stem cell processing which is enforced by German law and guarantees production under standards and conditions similar to those in the pharmaceutical industry.

When we took delivery of the XE-2100 at the end of 2000 we were very interested in evaluating the HPC parameter since it offered an opportunity to reduce our running costs. Our evaluation aimed to compare HPC with CD34+ assays in their ability to predict the optimum time for apheresis. We were also interested in its ability to predict apheresis yield. A further interest was to observe any differences between pre-treated patients scheduled for autologous transplantation and healthy donors.

## Apheresis Procedures

The protocol used for autologous transplants is as follows: starting at Day 5 following the last pulse of chemotherapy, patients are given a daily dose of 5–10 µg/G-CSF per kg body weight, the growth factor which induces the proliferation and liberation of stem cells into the circulation. We have, so far, studied a group of 62 patients (145 analyses) comprising 27 cases of non-Hodgkin's lymphoma (NHL), 12 cases with multiple myeloma, 11 patients with acute myeloid leukaemia (AML), 2 cases of acute lymphoblastic leukaemia (ALL) and 10 cases with solid tumours.

The protocol for allogeneic transplants was much simpler. We have studied mobilisation in 94 healthy volunteers (106 analyses). At Day 0 the volunteer donor was given 7.5 µg/kg body weight G-CSF and subsequently a single similar daily dose. At Day 5 apheresis was performed and if this did not yield sufficient cells ( $5 \times 10^6$  per kg of recipient in our institution) a second apheresis on Day 6 was undertaken to increase the total cell number.

## Methods

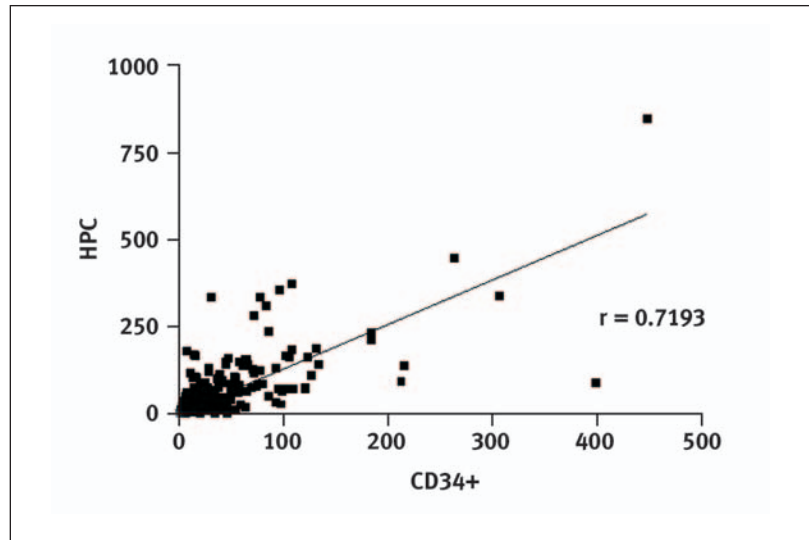
For flow cytometry we used either the FACScan or the more recent FACSCalibur (both by Becton Dickinson) with a CD34-PE (HPCA-2; Becton Dickinson) antibody and a second CD45-FITC (T29/33; Dako) antibody. The protocol used was that of the European Working Group for Clinical Cell Analysis (EWGCCA) [4].

For HPC analysis we used the SYSMEX XE-2100 with standard settings, the cells being measured in the Immature Myeloid Information (IMI) channel.

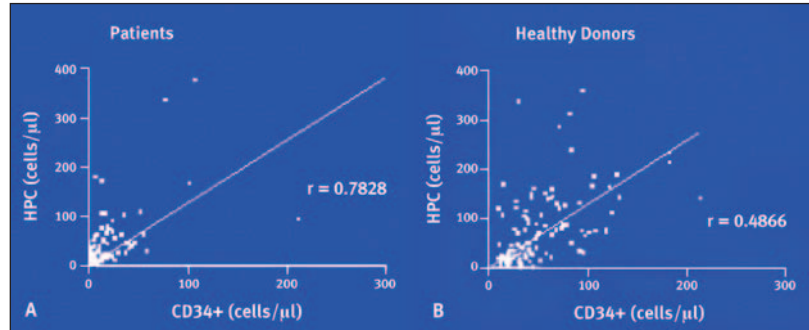
## Results

The correlation between HPC and CD34+ cells is shown in **figure 5** for all 251 analyses. While there is a correlation ( $r = 0.7193$ ) it is not perfect. However, when the overall group is subdivided into two, a different picture emerges. In the pre-treated group, the correlation between HPC and CD34+ improves to  $r = 0.7828$  (**figure 6A**) while the healthy volunteer donor group falls to  $r = 0.4866$  (**figure 6B**). What is interesting about the latter group is that there are several subjects who show no HPC at all but considerable numbers of CD34+ cells. On the other hand, reverting to figure 6 it is obvious that there are some patients with

considerable numbers of HPC but very few CD34+ cells. These last two observations may limit the usefulness of the HPC count as a marker for identification of candidate times for apheresis.



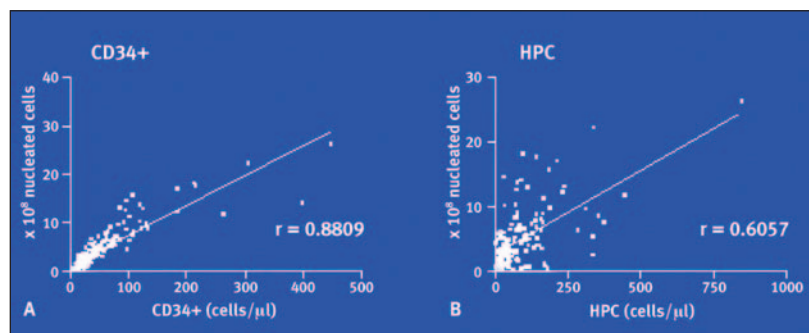
**Figure 5**  
Correlation HPC/CD34+;  
 $n = 251$ . Counts/ $\mu$ L



**Figure 6A**  
Correlation HPC/CD34+;  
patients identified  
separately. Counts/ $\mu$ L

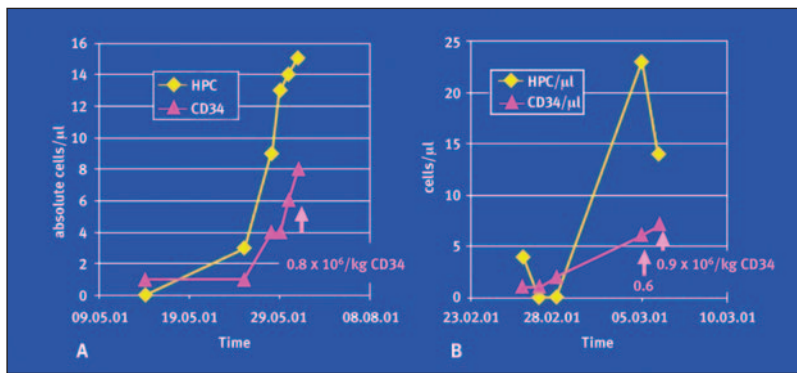
**Figure 6B**  
Correlation HPC/CD34+;  
healthy donors identified  
separately.

With regard to prediction of apheresis yield, CD34+ gives an excellent correlation of  $r = 0.8809$  (**figure 7A**). HPC on the other hand shows a greater degree of dispersion with  $r = 0.6057$  (**figure 7B**).



**Figure 7**  
Correlation with apheresis  
yield.  
(A) CD34+  $n = 160$ ;  
(B) HPC  $n = 157$ .

**Figure 8** shows examples of two patients considered to be poor mobilisers for autologous transplantation where we would like to harvest at least  $2.0 \times 10^6$  CD34+ positive cells per kg body weight. In the first patient (**figure 8A**) there is an increase in HPC but the CD34+ response is disappointing not reaching the threshold of  $10/\mu\text{L}$ . Note that the yield of CD34+ cells was only  $0.8 \times 10^6/\text{kg}$  body weight. In the second case (**figure 8B**) the CD34+ mobilisation was even lower and the apheresis yield was only  $0.9 \times 10^6/\text{kg}$  body weight.



**Figure 8**  
A and B. HPC and CD34+  
in 'poor' mobilisers.

## Conclusions

Some observations can be made on the data gathered during the past year. The HPC counts obtained are consistently higher than CD34+ counts to the extent of 3.5fold in patients and 1.7fold in volunteer donors. There is a highly significant correlation between HPC counts and CD34+ counts particularly in patients but less so in volunteer donors.

HPC counts  $\leq 10/\mu\text{L}$  in pre-treated patients predict low probability of adequate CD34+ counts and low apheresis yield with a negative predictive value of 81%. In healthy donors low HPC counts did not sufficiently predict low CD34+ counts, i.e., in  $12/10^6$  measurements with HPC counts below  $10/\mu\text{L}$ , median CD34+ counts were  $23/\mu\text{L}$ . In our view, while promising in pre-treated patients, HPC counts are not very useful in the setting of healthy donors.

So how are we using the HPC count currently? In those patients undergoing apheresis prior to autologous transplantation, we observe the total white blood cell count and when it increases above  $1 \times 10^9/\text{L}$  then we use the HPC count and wait until its value rises above  $10/\mu\text{L}$ . Then we prefer CD34+ measurements. Using this strategy for the last 57 patients, we have performed 107 HPC analyses, presumably thus saving 107 CD34+ assays and therefore saving some € 5000.

As final conclusions, HPC may be a useful screening parameter for scheduling apheresis in pre-treated patients but it does not seem to be a good stem cell measure for previously untreated individuals, i.e. healthy volunteer donors.

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- [3] **Peng L., Yang J., Yang H. *et al* (2001)**  
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## Acknowledgements

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