Increased Accuracy of HPC Quantification using the XE-HPC Master Technology

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We have evaluated the performance of the XE-HPC Master technology, an improved procedure for the quantification of haematopoietic progenitor cells (HPC) on the Sysmex XE-2100 automated haematology analyser. HPC values were determined in twenty healthy donors undergoing apheresis for peripheral blood stem cell collection using the standard HPC method (HPC res) and the improved method (XE-HPC Master) in parallel on two independent XE-2100 haematology analysers. The results indicate a substantially improved coefficient of variation using the XE-HPC Master (median 11%, range 5.1 – 30.2%) compared to the standard research HPC measurements (median 19.5%, range 4.2 – 35.7%) ($p < 0.0001$). Comparison with CD34 cell counts showed similar correlation coefficients for HPC values determined by both methods; however, the sample size is definitely too small to draw any conclusions. HPC values were found to decrease significantly over time when the samples were kept at room temperature. Dilution experiments did not indicate any effect of high WBC counts on the HPC determination. In conclusion, the XE-HPC Master technology offers significantly improved accuracy of HPC determination.

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INTRODUCTION

Apheresis of peripheral blood stem cells (PBSC) has become an important method for stem cell preparation for autologous and allogeneic blood stem cell transplantation. After mobilization of stem cells by haematopoietic growth factors, PBSC are harvested using continuous counter flow apheresis. In healthy donors, this is usually accomplished by a single apheresis. In contrast, for autologous transplantation, the optimal time point to obtain sufficient numbers of PBSC must be determined to avoid unnecessary manipulations. Measurement of $CD34^+$ cells using fluorescent activated cell sorting (FACS) represents the standard method for enumeration of stem cell content (reviewed in[1)]). However, this method requires specific and expensive equipment and experienced personnel. In addition, standardization of this method, a critical issue for preparation of stem cells under Good Manufacturing Practice (GMP) conditions, is still difficult[2)]. Recently, the use of the HPC parameter available on the Sysmex SE-9000 and the SE-9500 as well as the XE-2100 instruments (Sysmex Corporation, Kobe, Japan) has been described as an inexpensive and fast alternative for the quantification of circulating haematopoietic progenitor cells (HPC)[3)]. Most of the results indicate that HPC counts correlate with the number of $CD34^+$ cells, but data on the clinical applicability of this parameter are controversial[4-7)]. We have recently shown that the HPC values can be used to schedule apheresis in patients undergoing mobilization of peripheral blood stem cells for autologous transplantation[8)].

So far, the HPC parameter was available as a research tool only. More recently, an improved technique, called XE-HPC Master, has been developed. After installation of this optional module on the XE-2100, the HPC count is available as fully reportable parameter. The XE-HPC Master consists of software and hardware components. An additional thermistor monitors the reagent temperature and the software includes a special pre-heating sequence, which assures tight regulation of the IMI-reagent reaction temperature improving the reproducibility of the HPC analysis. The Sysmex Algorithm permits automatic gating. In addition, quality control is possible due to the availability of *e*-CHECK control reagents. The HPC technology has received FDA clearance, which allows marketing as a routine parameter.

In this study we have evaluated the XE-HPC Master technology in a total 30 healthy donors undergoing peripheral blood stem cell apheresis after PBSC mobilization with recombinant growth factors. We performed a direct comparison of values obtained with the standard HPC method (HPCres) parameter. In addition, we correlated the results with $CD34^+$ cell numbers and tested several parameters for their influence on HPC quantification.

PATIENTS, MATERIAL AND METHODS

Patients

HPC enumeration was performed on peripheral blood samples derived from 30 healthy volunteer donors undergoing apheresis after mobilization of PBSC. The mobilization protocol involved stimulation with 7.5µg/kg body weight/day recombinant granulocyte colony stimulating factor (G-CSF) for a total of 5 days. Blood specimens were collected into standard blood containers with K3-EDTA-anticoagulation (Sarstedt AG & Co, Nümbrecht, Germany). WBC and CD34 assays were performed on all specimens.

HPC measurements

HPC enumeration was performed on two independent XE-2100 instruments. One XE-2100 was equipped with the standard HPC software as research tool (HPC res), while on the second, the XE-HPC Master software and hardware extension (HPC mas) was installed. All measurements were performed in the same order; thus, the sample was measured on the HPC mas first and then, directly afterwards, on the HPC res instrument. The measurements were performed in the HPC analysis mode.

Flow cytometric CD34 enumeration

Quantification of $CD34^+$ cells was performed as outlined in detail recently[8]. Briefly, samples were incubated at room temperature with anti CD34 PE (HPCA-2, Becton Dickinson, Heidelberg, Germany) and anti CD45 FITC (T29/33; Dako Diagnostika, Hamburg, Germany) monoclonal antibodies. After red cell lysis and washing of cells, two-colour immunophenotyping was performed using the FACSCalibur flow cytometer (Becton-Dickinson, Franklin Lakes, NJ, USA). At least 50,000 events were registered. All analysis steps were performed according to published guidelines which have been validated in pan-European trials[9].

Statistical analysis

Comparison of differences between the two HPC quantification methods was performed using Student's t-test for matched observations. Linear regression was performed to compare HPC and CD34 values. All analyses were performed with the GraphPad PRISM 4.0 software package (GraphPad Software Inc., San Diego, CA, USA).

RESULTS AND DISCUSSION

We compared the HPC values obtained with both instruments (HPC res and HPC mas) in twenty donors. As shown in ***Fig. 1***, the values ranged from 15-597/µL for the HPC mas and from 18-368/µL for the HPC res. There was no clear trend for one instrument to measure consistently higher values than the other; in one half of the cases, HPC res values were higher than HPC mas while in the other half HPC mas values exceeded HPC res levels. The overall correlation of the results was good ($r = 0.8869$; $p < 0.0001$). To assess reproducibility, 10 consecutive measurements were performed on each analyser for all twenty donors. The median coefficient of variation (CV) was 11% for the HPC mas and 19.05% for the HPC res system. This difference was statistically highly significant ($p < 0.0001$; Student's t-test). Thus, the measurements on the HPC mas system showed a significantly greater reproducibility. This level of reproducibility is also superior to values recently reported by other groups[4].

For HPC analysis it is difficult to define accuracy, since no independent reference method assaying exactly the same population of cells exists. Flow cytometric quantification of $CD34^+$ cells represents the most widely accepted procedure to assess HPC content[2]. When HPC values were compared with CD34 measurements, Pearson's coefficient of correlation was $r = 0.71$ ($p = 0.0005$) (***Fig. 2(A)***) and $r = 0.76$ ($p = 0.0001$) (***Fig. 2(B)***) for the HPC mas and HPC res, respectively. Thus, there appears to be little difference in the correlation with the CD34 measurements; however, data sets are too small to draw any definite conclusions.

We also studied the effect of incubation time and absolute leukocyte counts on the HPC enumeration. Blood samples from 5 additional donors were aliquoted and incubated at room temperature for up to 6 hours. HPC values were measured in triplicate every 30 minutes. As shown in ***Fig. 3***, there was a reproducible decline of the HPC values over time, which ranged from 66.6% – 90.3% (median 76.2) of the initial values after 6

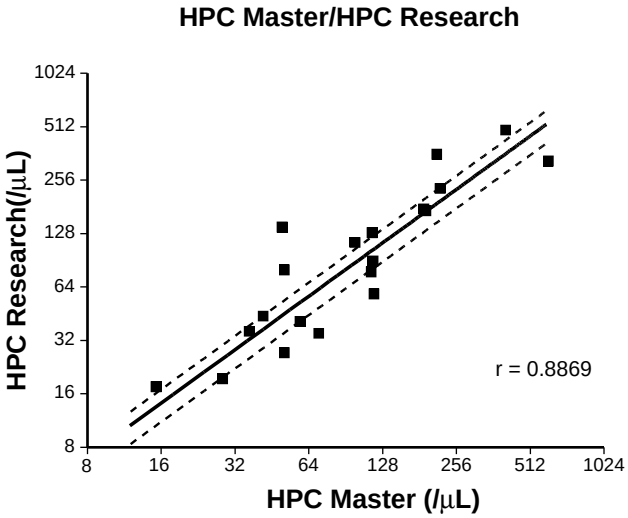

Fig. 1 *Correlation of HPC values determined with the XE-HPC Master and the HPC research instruments*

hours. Already after 3 hours the HPC values were only about 50% of the starting levels. Thus, the interval between sample collection and the measurement appears to be an extremely important factor influencing the final count. These results contrast with those recently reported by Creer, who stated that HPC measurements show <10% loss of cells in EDTA anticoagulated blood for up to 4 hours[10]. However, these data were reported from paediatric patients, so we cannot rule out differences due to different patient populations.

Recent results from healthy volunteer donors have led to the hypothesis that the high levels of mature cells mobilized in such donors might influence HPC measurements[8]. We therefore performed a series of dilutions of samples with initial WBC counts > 30×10^9/L. Samples were diluted 2-, 4-, 6-, 8-, 10- and 16-fold in phosphate buffered saline (PBS) and HPC values were measured in triplicate. As illustrated in ***Fig. 4***, higher values for HPC were seen in samples diluted 2- and 4-fold. However, these differences appear to be limited (maximum 30%)

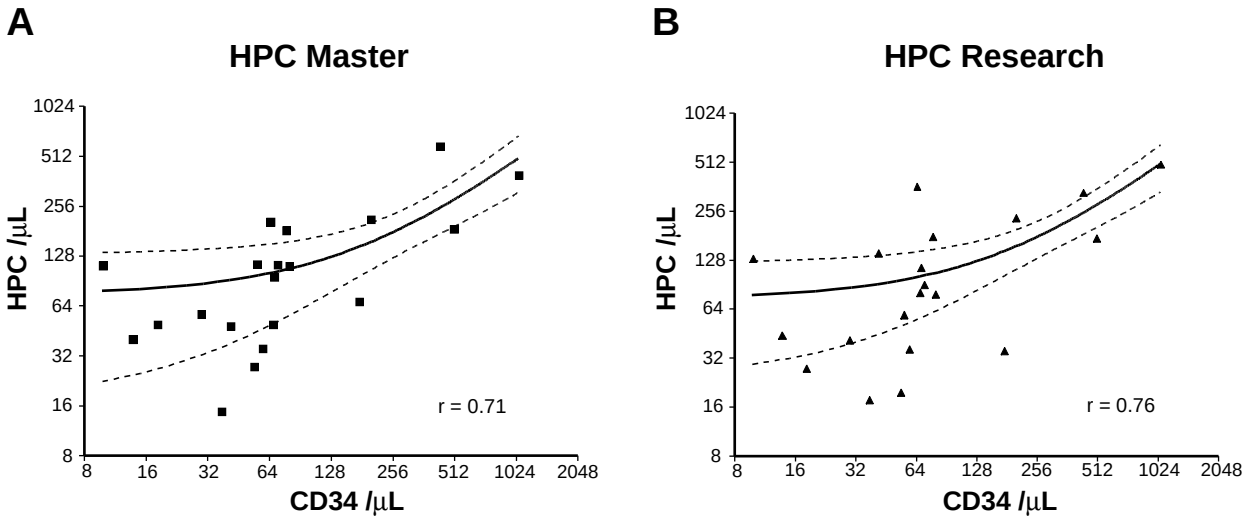

Fig. 2 *Linear regression of HPC values and CD34 counts*
(A) compares HPC mas with CD34 (B) compares HPC res with CD34

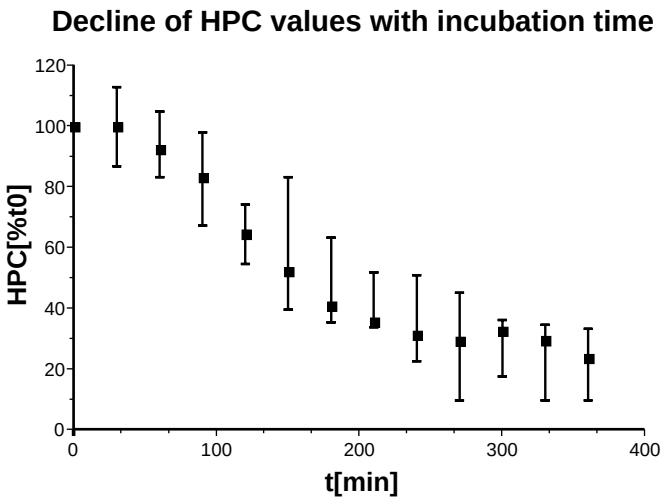

Fig. 3 *Effect of incubation time at room temperature on HPC values*
The graph shows the median and range.

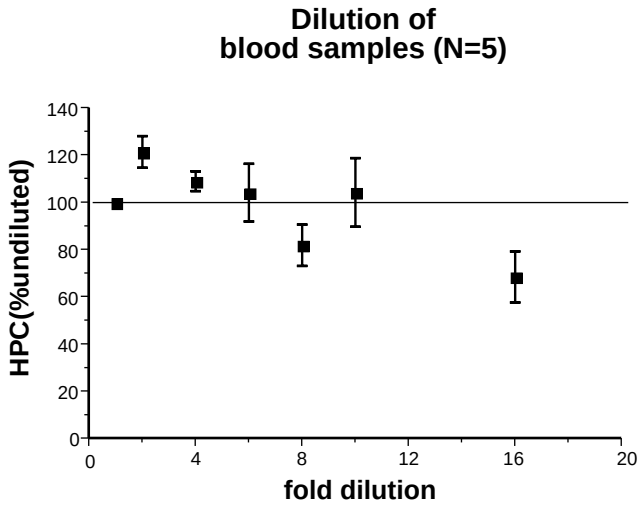

Fig. 4 *Determination of HPC values after dilution in cases with high WBC counts (> 30×10^9/L).*

given the considerable inherent variation of the method and the potential error induced by dilution. At the 16-fold dilution step, the lower limit of detection was reached in the majority of samples, which might explain the discrepancy at this level.

Taken together, this first clinical evaluation of the XE-HPC Master technology clearly showed a significantly increased reproducibility of HPC quantification compared to the standard procedure. However, the role of this novel procedure in the management of healthy donors as well as patients undergoing apheresis for autologous transplantation remains to be determined. The finding of a strong influence of the time interval between blood sampling and measurements on the HPC values deserves further attention and has to be taken into account in the interpretation of existing data as well as in the planning of further studies.

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