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The New Sysmex XN-2000 Automated Blood Cell Analyzer More Accurately Measures the Absolute Number and the Proportion of Hematopoietic Stem and Progenitor Cells Than XE-2100 When Compared to Flow Cytometric Enumeration of CD34$^+$ Cells

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The gold standard to count circulating hematopoietic stem and progenitor cells (HPCs) is to enumerate CD34$^+$ cells by using flow cytometry [1, 2]. XE-2100 (Sysmex, Kobe, Japan), a currently used hematology analyzer, can be used to quantify HPCs, generating counts that reportedly correlate with those obtained using flow cytometric analysis of CD34$^+$ cells; however, XE-2100 HPC counts were 1.86-fold higher than those calculated by flow cytometry [3]. The recently launched XN-2000 (Sysmex) can also be used to quantify HPCs, but its performance on HPC counts has not been evaluated. We assessed the HPC counting performance of XE-2100 and XN-2000 and compared them to CD34$^+$ cell counts by flow cytometry.

A total of 120 peripheral blood samples collected from 31 patients who had undergone stem cell transplantation (SCT) at Asan Medical Center, Seoul, Korea between June 2013 and October 2013 were randomly selected. The CD34$^+$ cell proportion (%) was measured in each sample by using flow cytometric enumeration of CD34$^+$ HPCs [1], performed using FACScanto II (Becton-Dickinson, Sunnyvale, CA, USA) and phycoerythrin (PE)-conjugated CD34 and fluorescein isothiocyanate (FITC)-conjugated CD45 (Becton-Dickinson). CD34$^+$ cell numbers obtained using XE-2100 and XN-2000 analyzers were calculated by multiplying their respective white blood cell (WBC) counts with their CD34$^+$ cell proportion (%). As a reference for the HPC counts, the CD34$^+$ cell counts, defined as the means of XE-2100 and XN-2000 CD34$^+$ cell numbers, were generated. HPC numbers and proportion (%) were measured by both XE-2100 (immature myeloid information [IMI] channel, XE-2100 HPC numbers, and proportion [%]) and XN-2000 (white precursor cell [WPC] channel, XN-2000 HPC numbers and proportion

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[%]). Linear regression and Bland-Altman analyses were applied to compare XE-2100 and XN-2000 HPC proportion (%) and the $CD34^+$ cell proportion obtained using flow cytometry. An identical analysis was performed comparing XE-2100 and XN-2000 HPC numbers and $CD34^+$ cell counts.

HPC count-related parameters for 120 samples obtained using the two automated blood cell analyzers were compared. XN-2000 HPC numbers were significantly lower than those obtained using XE-2100 (0.005×10^9/L vs. 0.007×10^9/L, $P<0.001$). The regression analysis of XE-2100 HPC and $CD34^+$ cell proportion (%) showed poor correlation ($\gamma=0.187$). In contrast, the proportion (%) of XN-2000 HPCs yielded correlations that were significantly improved ($\gamma=0.590$). XE-2100 HPC and $CD34^+$ cell proportion (%) showed a mean bias of 0.2%, suggesting that HPC proportion (%) measured using XE-2100 is overestimated by a mean of 0.2% when compared to those measured using flow cytometry (Fig. 1A). In contrast, HPC proportion (%) measured using XN-2000 produced an improved mean bias of 0.0% (Fig. 1B).

Subsequent analysis of HPC numbers confirmed that XN-2000 showed improved performance over XE-2100. The regression analysis between HPC numbers measured using the two automated blood cell analyzers and $CD34^+$ cell numbers showed that XN-2000 had improved correlation ($\gamma=0.652$) compared to XE-2100 ($\gamma=0.548$). The analysis also showed that bias was reduced when XN-2000 was used instead of XE-

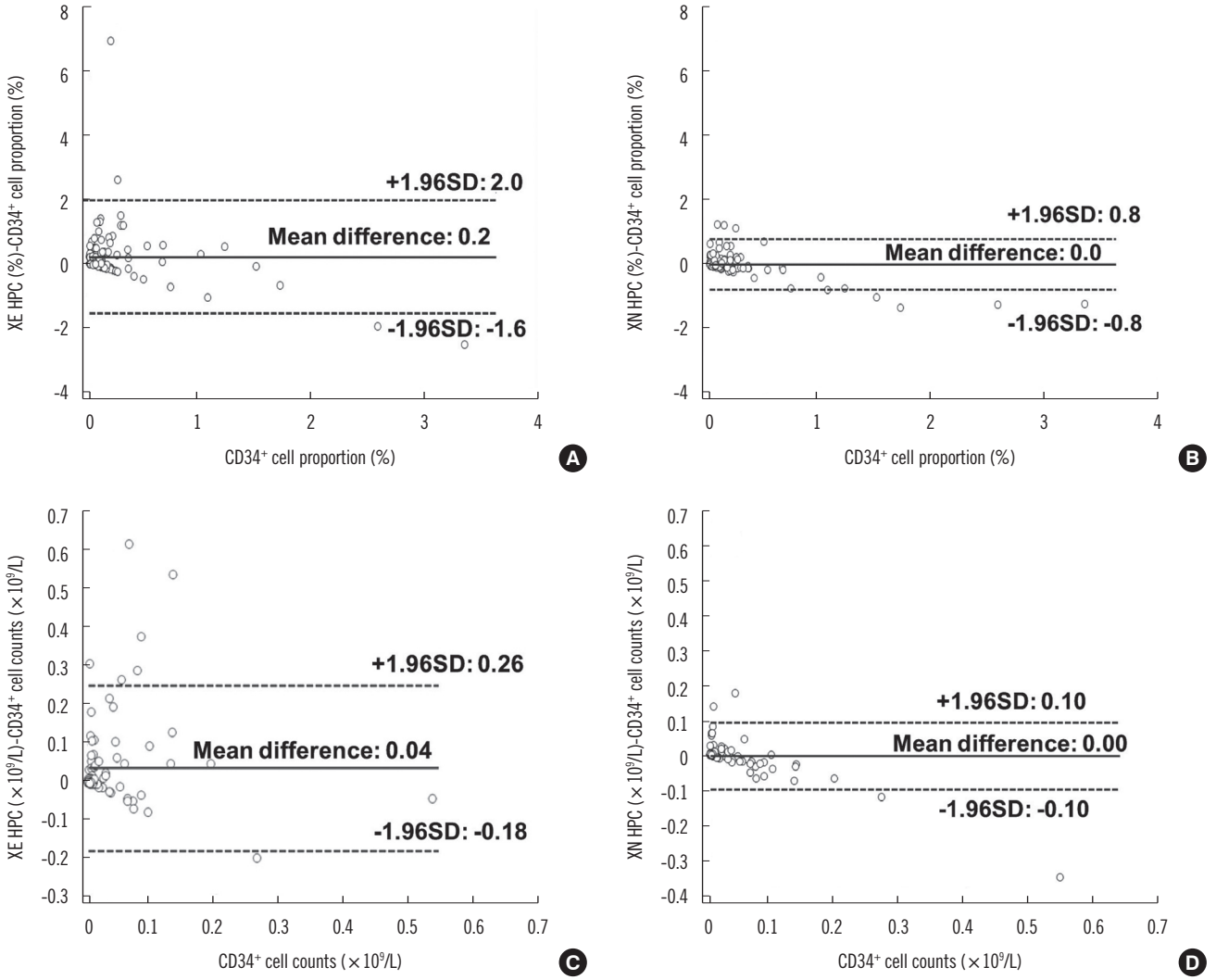

Fig. 1. Bland-Altman analysis results for HPC proportion (%) measured using XE-2100 (A), XN-2000 (B) automated blood cell analyzers, and the $CD34^+$ cell proportion (%) measured using flow cytometry are shown. Bland-Altman analysis results comparing absolute HPC numbers measured using XE-2100 (C) and XN-2000 (D) analyzers and $CD34^+$ cell counts are also shown.
Abbreviations: CD, cluster of differentiation; HPC, hematopoietic stem and progenitor cells.

2100 (0.00×10^9/L vs. 0.04×10^9/L) (Fig. 1C and D). The cutoff point in the $CD34^+$ cell counts for deciding stem cell collection time in SCT is reported to be 0.01×10^9/L-0.02×10^9/L [4-6]. Thus, identical stem cell collection cutoff counts in HPC numbers for XN-2000 can be calculated as 0.017×10^9/L-0.021×10^9/L by using the regression equation determined in our study (XN-2000 HPC count=0.4006×$CD34^+$ cell count+0.0133, γ=0.652).

In our study, correlations (γ=0.548) between XE-2100 HPC numbers and those obtained using flow cytometry were weaker than those obtained in a previous study (γ=0.7482) [3]. This discrepancy might be attributed to the small sample size in our study (120 vs. 236), or higher proportion of samples with low or high WBC counts ($<5.0\times10^9$/L or $>40.0\times10^9$/L) in our study (40.8%) than in the previous study (34.3%), since the discrepancy between XE-2100 HPC numbers and those obtained using flow cytometry were more frequently reported in samples with low or high WBC counts than in normal samples [3]. Because our study population included patients with hematologic malignancy, i.e., unhealthy donors for allogeneic SCT, selection bias may also contribute to this discrepancy. In this study, we demonstrated that HPC counts are overestimated when XE-2100 was used when compared to flow cytometry, and the degree of overestimation is significantly reduced by using XN-2000 instead. Therefore, these results suggest that XN-2000 generates more accurate HPC counts than XE-2100, when compared with flow cytometric enumeration of $CD34^+$ cells.

In conclusion, XN-2000 can provide more accurate HPC-related data than XE-2100, providing more accurate information to clinicians in terms of the timing of stem cell collection for SCT.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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