Circulating Endothelial Progenitor Cells Exhibit Diurnal Variation

Honey E. Thomas, Rachael Redgrave, Michael S. Cunnington, Peter Avery, Bernard D. Keavney, Helen M. Arthur

Endothelial progenitor cells (EPCs) are circulating bone marrow–derived mononuclear cells that have the potential to promote postnatal neovascularization and endothelial repair. Reduced numbers of EPCs have been demonstrated in patients with coronary artery disease (CAD) and those with cardiac risk factors including smoking, hyperlipidemia, hypertension, diabetes mellitus, and increasing age. EPCs are mobilized in the peripheral blood of patients after acute coronary syndromes, percutaneous coronary intervention, and vascular trauma/surgery, suggesting that the numbers of circulating EPCs may be a useful biomarker of cardiovascular risk and that endogenous vascular repair may be an important modulator of the clinical course of CAD.

Present data suggest that CD133+KDR+ cells in the bone marrow mature to CD133−CD34+KDR+ cells with subsequent loss of CD133 and CD34 reflecting transformation into a differentiated mature endothelial cell. It is also known that the hematopoietic system has a circadian rhythm and numbers of circulating blood cells show diurnal variations. However, there have been no published studies to date that investigate this property in EPCs.

We recruited 15 healthy male Caucasian adult volunteers aged between 23 and 45 (average age 30.5 years) who were free from cardiovascular disease or known cardiac risk factors, and were nonsmokers. Volunteers were also requested to refrain from caffeine during the study. We obtained an EDTA peripheral blood sample at 8 AM, 3 PM, and 10 PM on the same day from each individual. 100 μL of peripheral blood was added to Trucount tubes (BD biosciences) containing fluorescent beads to permit calculation of absolute numbers of EPCs. A FACScalibur was used to record the presence of cells expressing the surface marker combinations that are commonly used to define EPCs: CD34, CD133, and kinase domain receptor (KDR). CD45 expression was used as an additional gating criterion for CD34+ cells. Fluorescent antibodies, anti–CD45–fluorescein isothiocyanate (FITC), anti–CD34–PercP-cy5.5 (BD biosciences), anti–VEGR–2–PE (R&D Systems), and anti–CD133–activated protein C (APC) (Miltenyi Biotec), or fluorescent antibody isotype controls were added to 3 replicate blood samples at the 3 time points for each individual. After red blood cell lysis (Pharmlyse, BD biosciences), 60 000 cells in the lymphocyte region (defined on a forward and side scatter plot) were recorded using CellQuest software. EPC counts were normally distributed and differences in EPC numbers across the 3 time points were assessed using 2-way analysis of variance (ANOVA), with individual assigned as a blocking factor. Paired t tests were then performed on data that showed significant differences (P<0.05) in the ANOVA and were used to compare EPC numbers between time points. A 2-sided probability value of <0.05 was used to define statistical significance. All volunteers gave informed consent for the study and procedures were approved by the regional ethics committee.

The mean cell counts with 95% confidence intervals for EPCs (defined using 6 cell surface marker combinations) for 15 individuals at the 3 time points are displayed in the Figure. The overall mean value for all individuals across all time points for EPCs is similar to those seen in other studies of healthy individuals. There was a striking and significant increase in the numbers of circulating EPCs at 10 PM compared with 3 PM which was consistent across the 6 EPC populations (Figure). The highest fold increase was observed for CD34+CD133−KDR− cells which showed a 42% (P=0.012) increase at 10 PM compared with 3 PM, whereas the smallest increase was 17% (P=0.013) for CD34+CD45+ cells. The CD34+ cells which are thought to represent the broadest definition of EPCs showed a 28% increase over the same time period (P<0.001) and were the only cell type to show a significant drop (16%; P=0.031) between 8 AM and 3 PM.

The evening peak of circulating EPCs resembles the circadian pattern exhibited by neutrophil, monocyte, and lymphocyte numbers in peripheral blood and may be a direct consequence of the marked diurnal patterns in circulating levels of factors known to influence EPC mobilization, eg, GM-colony stimulating factor (CSF), G-CSF, and glucocorticoids. Given the potential therapeutic utility of EPCs, identification of the responsible factor(s) in future studies would be of major interest. The fluctuations in EPC numbers seen during a 14-hour time period suggest that the turnover of EPCs may be relatively high, even in healthy individuals with no specific vascular insult. It is not clear whether this might be attributable to either the ongoing consumption of EPCs as part of low level vascular repair processes or the removal of circulating EPCs which are not currently required.

Our results have important implications for other studies in this field. Any systematic difference in the time of day when case and control groups are sampled could be a major

From the Institute of Human Genetics (H.E.T., R.R., M.S.C., B.D.K., H.M.A.) and the School of Mathematics and Statistics (P.A.), Newcastle University, UK.

Correspondence to Helen M. Arthur, Institute of Human Genetics, International Centre for Life, Central Parkway, Newcastle University, NE1 3BZ, UK. E-mail helen.arthur@newcastle.ac.uk


© 2008 American Heart Association, Inc.

Arterioscler Thromb Vase Biol is available at http://atvb.ahajournals.org

DOI: 10.1161/ATVBAHA.107.160317
The numbers of circulating EPCs at 8 AM, 3 PM, and 10 PM show diurnal variation. Mean EPC numbers per μl blood from 15 volunteers with 95% confidence intervals are shown for each cell type (defined using 6 cell surface marker combinations) at the 3 sample times. Horizontal bars and probability values identify results of paired t tests between the time points.

Confounding factor in case-control studies relating EPC numbers to cardiovascular risk. There are also implications for studies involving serial sampling, such as after MI, where sampling times would not be controlled with respect to the circadian clock.

It is tempting to speculate whether the diurnal variations in EPC numbers might have clinical consequences. MI, acute coronary syndrome, sudden cardiac death, and ischemic stroke manifest a marked circadian variation with a peak in the early morning. This may result from a variety of different processes, including increased vascular and sympathetic nervous system tone, higher arterial blood pressure, and relative hypercoagulability. It is also possible that the lower levels of EPCs seen in the early and middle parts of the day compared with the late evening might lead to prolonged exposure of endothelial basement membrane at sites of vascular repair and subsequent thrombotic cardiovascular events. Further work on the mechanisms of EPC recruitment to repair sites and contribution to reendothelialization are needed to investigate this possibility.

Acknowledgments
The authors thank Ian Dimmick for flow cytometry support.

Sources of Funding
This research was supported by the Newcastle upon Tyne NHS hospitals trust and the British Heart Foundation.

References
Circulating Endothelial Progenitor Cells Exhibit Diurnal Variation
Honey E. Thomas, Rachael Redgrave, Michael S. Cunnington, Peter Avery, Bernard D. Keavney and Helen M. Arthur

doi: 10.1161/ATVBAHA.107.160317
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/28/3/e21

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/