A Look at the Bone Marrow Predicts the Global Outcome

Gian Paolo Fadini

There is no part of the millet that does not make a sound [...].

—Zeno’s paradox of the Grain of Millet

Just 15 years ago, the amount of hematopoietic stem cells (HSC) circulating in peripheral blood (PB) was considered >0. In a healthy subject, out of 6000 leukocytes per microliter, HSCs expressing the typical marker CD34 account for 3 cells/μL (≈0.05% of total). In hematology, increased PB CD34+ cells indicate the timing for HSC collection after mobilization, and helps in the diagnosis of leukemia. However, there was no interest in understanding the clinical meaning of half-of-zero—a reduction in circulating CD34+ cells below normal values—until a role for bone marrow (BM)-derived cells in vascular homeostasis hypothesized. The discovery of endothelial progenitor cells (EPC) has fostered a new era in cardiovascular research, focused on regeneration versus damage. Although still awaiting definite clinical demonstration, a wealth of experimental data indicates that EPCs promote vascular repair and angiogenesis.1

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CD34 is a sialomucin adhesion molecule with unknown function, which identifies HSC in the BM. Small amounts of CD34+ HSC also circulate in PB and through peripheral organs. Although sources other than BM have been hypothesized, >90% of circulating CD34+ cells originate from the BM.2 Experimental and proof-of-concept human studies suggest that BM-derived HSC can repopulate the vasculature and parenchymatous organs beyond hematopoietic tissues.3 In fact, the function and biological meaning of steady-state PB CD34+ HSC is largely unknown. Far from being the result of a passive shedding from the BM, HSCs (and EPCs) are continuously released from the BM to PB after a circadian rhythm regulated by the autonomous nervous system.4 Probably, this recirculation ensures appropriate relocation of HSC in preferred niches. What happens when this highly coordinated homeostatic process fails?

While studying CD34+VEGFR2+ EPCs, it was noted that PB CD34+ cells are reduced with aging and in the presence of cardiovascular risk factors.1 Together with the notion that EPC maintain cardiovascular health, this suggested that an impaired endogenous regeneration by BM-derived cells can promote onset and progression of cardiovascular disease, and it supported the design and conduction of cell-therapy trials for cardiovascular disease. Then, half-of-zero CD34+ cells became of great interest.

In this issue of Circulation Research, Patel et al5 report that low levels of circulating CD34+ cells predict all-cause and cardiovascular mortality in patients undergoing coronary angiography over 2 years. It should be noted that 4 previous longitudinal studies assessed the prognostic role of PB stem/progenitor cell phenotypes: of these, 2 reported an association between reduced CD34+ cells and all-cause mortality6,7 (Table). Combining those data with Patel et al’s8 finding yields a pooled hazard ratio of death from any cause of 2.78 (95% CI, 1.29–7.37) for low versus high CD34+ cell count in a total of 1335 patients (Figure).

The work by Patel et al8 has important strengths as compared with what already available in the literature: (1) large sample size; (2) consistent patient phenotype; (3) inclusion of 2 cohorts for test validation; and (4) specific focus on mortality. An important finding was that, among different phenotypes, only CD34+ cells significantly predicted mortality and improved risk stratification. Although the coexpression of CD133 does not provide any incremental phenotypic information (most CD133+ cells are CD34+ HSCs and not EPCs), CD34+VEGFR2+ cells showed no association with the outcomes, despite being representative of a more specific population of EPCs. This is most likely attributable to the lower coefficient of variation of CD34+ as compared with the CD34+VEGFR2+ cell count. This was already known because, according to the Poisson distribution of rare events, CV% (coefficient of variation) increases as absolute cell count decreases.1 In addition, although anti-CD34 monoclonal antibodies are clinical grade, anti-VEGFR2 monoclonal antibodies are for research use only and lot-by-lot variations occur. The relatively unstable expression of VEGFR2 also contributes to the low intra-assay reproducibility of VEGFR2 staining.

This methodological issue limits the use of CD34+VEGFR2+ cells as prognostic biomarkers, but does not deny the role of CD34+VEGFR2+ EPCs in vascular biology.

The test-validation approach used in Patel et al’s5 work took advantage from the availability of 2 independent cohorts of similar patients. Despite a change in the protocol to enumerate PB CD34+ cells, the authors found that the optimal absolute cutoff derived from cohort 1 (0.737 cells/μL) could be applied successful to cohort 2 to predict mortality. This first preliminary attempt to validate a threshold of less-than-zero CD34+ cells needs to be replicated in different population, as discrepancies exist on the threshold below which a CD34+ cell count is indicative of excess mortality risk.6,7 The possibility to pool percentile-normalized cell counts in survival analysis...
was already shown, but may be problematic when differences in the average CD34+ cell level is expected to occur among patient population, such as in those with coronary artery disease or chronic renal failure as compared with those without.

Patel et al’s work has yet important limitations. First, the inclusion of high-risk patients allowed statistical significance in mortality analysis over a short-time course, but prognostic markers always perform better in high-risk population than in low-risk population. The presence of coronary artery disease (and of acute events in some cases) probably masked the well-known effects of traditional cardiovascular risk factors on CD34+ cells, possibly overfitting the model. Finally, the relatively short follow-up duration allowed a limited number of deaths to be collected, leading to wide uncertainties in the hazard ratio estimates.

Notwithstanding these limitations and keeping in mind that causality cannot be inferred from association studies, these novel clinical data support the biological role of BM-derived stem cells in cardiovascular biology and aging. Indeed, reduction of PB CD34+ cells must be necessarily traced back to the BM. According to a theoretical 3-compartment model, the level of circulating CD34+ cells results from a combination of replenishment by the BM, half-life in PB, and homing to target tissues. Failure of the BM to mobilize CD34+ cells to the PB is the only plausible explanation for low steady-state PB CD34+ cells. There is a growing interest in the BM status in patients with cardiovascular disease or risk factors. For instance, acute myocardial infarction induces BM metabolic activation, and failure of the BM to mobilize CD34+ cells post-acute myocardial infarction is associated with a poor outcome. In diabetes mellitus, especially with vascular disease, the BM undergoes extensive remodeling and stem cell mobilization is impaired. Generally, by providing stem cells potentially involved in the repopulation of many organs and tissues, the BM can be seen as a central housekeeper of organismal global health, whereas syndromes characterized by pathological BM failure are associated with premature aging.

Years ago, it was unbelievable that less than what was considered zero produced any signal (see the Zeno’s paradox). Now, the measure of PB CD34+ cells, routinely done by most hematology laboratories, is getting closer to become a marker of cardiovascular and general health. Multicenter, long-term studies in low-risk population are warranted to confirm this view. Understanding the mechanisms leading to shortage in BM-derived CD34+ cells is the next challenge and may indicate ways to replenish them. In addition, a deeper understanding of BM dysfunction in cardiovascular disease is of great interest and mobilization tests may provide additional information over and beyond the steady-state CD34+ cell count.

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### Disclosures
None.

### References

### Table. Summary of the Studies Available Reporting an Association Between Circulating Stem/Progenitor Cells and Hard End Points

<table>
<thead>
<tr>
<th>Study</th>
<th>Cell Phenotype</th>
<th>N Patients</th>
<th>Follow-Up Duration</th>
<th>Hardest Significant End Point</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Werner et al</td>
<td>CD34+VEGFR2+ (EPCs)</td>
<td>519</td>
<td>12 mo</td>
<td>Cardiovascular death</td>
<td>3.23 (1.59–6.25)</td>
</tr>
<tr>
<td>Fadini et al</td>
<td>CD34+ (HSCs)</td>
<td>214</td>
<td>34 mo</td>
<td>All-cause mortality</td>
<td>2.83 (1.14–7.02)</td>
</tr>
<tr>
<td>Maruyama et al</td>
<td>CD34+ (HSCs)</td>
<td>216</td>
<td>23 mo</td>
<td>All-cause mortality</td>
<td>5.02 (1.08–23.25)</td>
</tr>
<tr>
<td>Schmidt-Lucke et al</td>
<td>CD34+VEGFR2+ (EPCs)</td>
<td>120</td>
<td>10 mo</td>
<td>Cardiovascular events</td>
<td>6.33 (1.80–21.85)</td>
</tr>
</tbody>
</table>

Only the hardest statistically significant end point is reported. CI indicates confidence interval; EPC, endothelial progenitor cell; HR, hazard ratio; and HSC, hematopoietic stem cell.

### Figure. Forest plot for hazard ratios (HR) of death from any cause for low vs high CD34+ cell count in the available studies reporting this association (from Table). Data are combined with results from the study by Patel et al. The overall weighted mean effect is then calculated. CI indicates confidence interval.

### Death from any cause

- **Fadini et al.**
- **Maruyama et al.**
- **Patel et al.**
- **OVERALL**

*0 2 4 6 8 10 20 25 HR (95% CI) for low vs high CD34+ cells*


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