The number and function of circulating $CD34^+CD133^+$ progenitor cells decreased in stable coronary artery disease but not in acute myocardial infarction

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ABSTRACT

Objective Circulating $CD34^+CD133^+$ cells are one of the main sources of circulating endothelial progenitor cells (EPCs). Age is inversely related to the number and function of $CD34^+CD133^+$ progenitor cells in stable coronary artery disease (CAD), but the relationship remains unclear in acute myocardial infarction (AMI). The authors aimed to clarify how ageing affects the number and function of mobilised $CD34^+CD133^+$ progenitor cells in AMI.

Design and results Circulating $CD34^+CD133^+$ progenitor cells were measured by flow cytometry. Measurements were made at admission for CAD, or on day 7 after the onset of AMI. In stable CAD (n=131), circulating $CD34^+CD133^+$ cells decreased with age ($r=-0.344$, $p<0.0001$). In AMI, circulating $CD34^+CD133^+$ cells did not correlate with age (n=50), and multivariate analysis revealed that the decreased number of circulating $CD34^+CD133^+$ cells was associated with male sex and higher peak creatinine kinase. The ability to give rise to functional EPCs, which show good migratory and tube-forming capabilities, deteriorated among stable CAD subjects (n=10) compared with AMI subjects (N=6).

Conclusions In stable CAD, the number and function of circulating $CD34^+CD133^+$ progenitor cells decreased with age, whereas those mobilised and circulating in AMI did not.

Bone marrow-derived endothelial progenitor cells (EPCs) are known to maintain cardiovascular homeostasis.[1–3] In a non-ischaemic condition, circulating EPCs constitute a pool of progenitor cells that repair the injured endothelium.[3] In an ischaemic condition, EPCs are mobilised from bone marrow to peripheral blood, home to the ischaemic sites, and proliferate at the local foci.[4 5] Studies have demonstrated that circulating EPC levels are reduced by the accumulation of cardiovascular risk factors such as diabetes,[6] smoking,[7] hypertension[8] and hypercholesterolaemia,[8] and that the number and function of circulating EPCs are inversely correlated with the endothelial dysfunction.[2] Furthermore, circulating EPC levels are reported to predict future cardiovascular events.[9 10] Therefore, EPC is now considered to be a biomarker of cardiovascular diseases.

Ageing is one of the strongest risk factors for cardiovascular disease. Previous investigations demonstrated that age is associated with a reduced number and function of EPCs, circulating $CD34^+/KDR^+$ cells, or $CD133^+$ cells in healthy individuals and patients with coronary artery diseases (CAD).[8 11] However, there are very few reports concerning the effects of ageing on $CD34^+CD133^+$ progenitor cells in acute myocardial infarction (AMI) subjects.[12]

Therefore, we aimed to clarify the potential effects of ageing on circulating $CD34^+CD133^+$ progenitor cells in patients with stable CAD and in patients with AMI. We also sought to examine whether the functions of $CD34^+CD133^+$ cell-derived EPCs differ between CAD and AMI. We examined only never-smoking subjects, because smoking exerts detrimental effects on circulating $CD34^+CD133^+$ cells and EPCs.[7]

METHODS

Study patients

Patients were recruited from Nagoya University Hospital, Japanese Red Cross Nagoya Daiichi Hospital or Ogaki Municipal Hospital from January 2004 to December 2006. We examined circulating $CD34^+CD133^+$ cell levels in 131 consecutive non-smoking patients with stable CAD and circulating $CD34^+CD133^+$ cell levels in 50 AMI non-smoking patients. We previously demonstrated that circulating EPC and $CD34^+$ cell levels peaked at day 7 after the onset of AMI.[13] Therefore, we investigated circulating EPC and $CD34^+CD133^+$ cell levels on day 7 after the onset of AMI.[14] Definitions of stable CAD or AMI and criteria for coronary risk factors were written in detail in the online data supplement files (please see http://heart.bmj.com/).

Blood sampling and medical treatment

In subjects with stable CAD, peripheral blood (PB) samples were collected on the day before the day of coronary angiography. In subjects with AMI, PB samples were collected 7 days after the onset of AMI. Detailed description regarding blood examination and medical treatment are written in the online supplement files (please see http://heart.bmj.com/).

Circulating $CD34^+CD133^+$ progenitor cells and functional assessments of endothelial progenitor cells

Methods regarding quantification of $CD34^+CD133^+$ progenitor cells and functional assessments of endothelial progenitor cells (EPCs) are described in detail in the online supplement files (please see http://heart.bmj.com/).

Statistical analysis

Data are presented as the mean±SD or median (25–75th percentile) for continuous variables, and as percentages for categorical variables. Correlations between the variables were assessed using the Pearson correlation analysis (r) or the Spearman rank correlation coefficient (ρ) as appropriate. Multiple stepwise regression analyses were performed to determine the factor to affect the circulating $CD34^+CD133^+$ cell levels in AMI patients. Comparisons between the in vitro experimental groups were performed using ANOVA followed by Tukey–Kramer test. In all analyses, p values <0.05 were considered statistically significant. All statistical analysis was performed using Statview 5.0-J (SAS Institute, Cary, North Carolina).

RESULTS

The characteristics of the study patients are shown in tables 1, 2. The number of circulating $CD34^+CD133^+$ cells was significantly lower in subjects with stable IHD (734 counts/ml) compared with subjects with AMI (1655 counts/ml) ($p<0.0001$).

$CD34^+CD133^+$ cells decreased with age in subjects with stable CAD

In subjects with stable CAD, the number of circulating $CD34^+CD133^+$ cells decreased linearly with age ($\rho=-0.360$, $p<0.0001$) (figure 1A). Multiple regression analysis, using diabetes mellitus, hypertension, sex, 'statin use' and age as dependent variables, revealed that age was the only and significant factor determining the circulating $CD34^+CD133^+$ cell levels in subjects with CAD.

Relationship between $CD34^+CD133^+$ cell levels and age in patients with AMI

In contrast to subjects with stable CAD, circulating $CD34^+CD133^+$ cell levels on day 7 after the onset of AMI did not correlate with age (figure 2B). Multiple stepwise regression analyses using sex, age, hypertension, diabetes mellitus, 'statin use,' peak CK values, BNP, CRP and VEGF as dependent variables indicated that only sex (male) and high peak CK value were the significant and negative factors to predict circulating $CD34^+CD133^+$ cell levels on day 7 after the onset of AMI. There were no interaction effects between sex and peak CK levels.

Functional assessment of $CD34^+CD133^+$ cell-derived EPCs

EPCs were obtained after cultivation of circulating $CD34^+CD133^+$ cells. The migratory activity of EPCs from male subjects with AMI, whether younger or elder, was significantly enhanced compared with that of EPCs from the corresponding age male subjects with stable CAD ($p<0.05$, respectively) (figure 2A). Similarly, the number of EPCs incorporated into the endothelial network formation was significantly increased in older adult subjects with AMI compared with older adult subjects with stable CAD ($p<0.05$) (figure 2B).

Table 1 Characteristics of patients with stable coronary artery disease

Age (years)	64±11
Male/female	102/29
BMI (kg/m^2)	24.2±3.6
Low-density lipoprotein cholesterol (mmol/l)	2.96±0.9
High-density lipoprotein cholesterol (mmol/l)	1.31±0.3
$CD34^+CD133^+$ cells (counts/ml)	615 (409–868)
Hypertension (%)	38.2
Diabetes mellitus (%)	35.9
Statin use (%)	47.3

Table 2 Characteristics of patients with acute myocardial infarction

Age (years)	65±9
Male/female	37/13
BMI (kg/m^2)	24.5±3.1
Status before admission	
Hypertension (%)	48
Diabetes mellitus (%)	30
Statin use (%)	12
Site of infarction	
Anteroseptal, no (%)	21 (42.0)
Inferior, no (%)	23 (46.0)
Lateral, no (%)	4 (8)
Posterior, no (%)	0 (0)
Peak creatine kinase (U/l)	2450 (1500–2930)
Brain natriuretic peptide (pg/ml)	146 (90–380)
C-reactive protein (mg/l)	65.0 (12.5–198)
Vascular endothelial growth factor (pg/ml)	32.3 (15.6–62.3)
$CD34^+CD133^+$ cells (counts/ml)	1930 (1400–2950)

DISCUSSION

Major findings in this study were as follows: (1) In subjects with stable CAD, circulating $CD34^+CD133^+$ cell levels decreased with age. (2) In subjects with AMI, (a) there was no significant relationship between the number of mobilised $CD34^+CD133^+$ cells and age, and (b) peak CK level and sex (male) were negatively associated with the number of mobilised $CD34^+CD133^+$ cells. (3) As for the functions of $CD34^+CD133^+$ cell-derived EPCs, both the migratory activity and the network-forming ability with HUVECs were superior in subjects with AMI to those with stable CAD.

Circulating EPCs are known to play an important role not only in ischaemia-induced angiogenesis but also in healing the injured endothelium.[1 3 4] However, the results from clinical trials involving administration of autologous bone marrow cells to patients with critical limb ischaemia or myocardial infarction are not consistent.[15–18] One of the reasons for this discrepancy may be related to the fact that most patients were targeted at older subjects, whereas in animal models, most experiments were performed in young mice.[19–21]

Circulating $CD34^+$ or $CD133^+$ cells are reported to decrease with age in subjects with stable CAD.[3] This explains, in part, the varying efficacy of autologous bone marrow cell transplantation into critical ischaemic limb or ischaemic myocardium. However, in the present study, the inverse relationship between age and circulating $CD34^+CD133^+$ cells was not confirmed in subjects with AMI. Our findings are consistent with the description about $CD34^+/CD117^+$ and $CD34^+/CXCR4^+$ cells in AMI by Wojakowski *et al*.[22] These findings imply that, although circulating $CD34^+CD133^+$ cells decrease in patients with stable CAD, some patients still have the capacity to release $CD34^+CD133^+$ cells into the circulation from bone marrow in the event of AMI. Signals from ischaemic tissue may be responsible for stimulating the release of $CD34^+CD133^+$ cells. So far, VEGF is known to be one of the

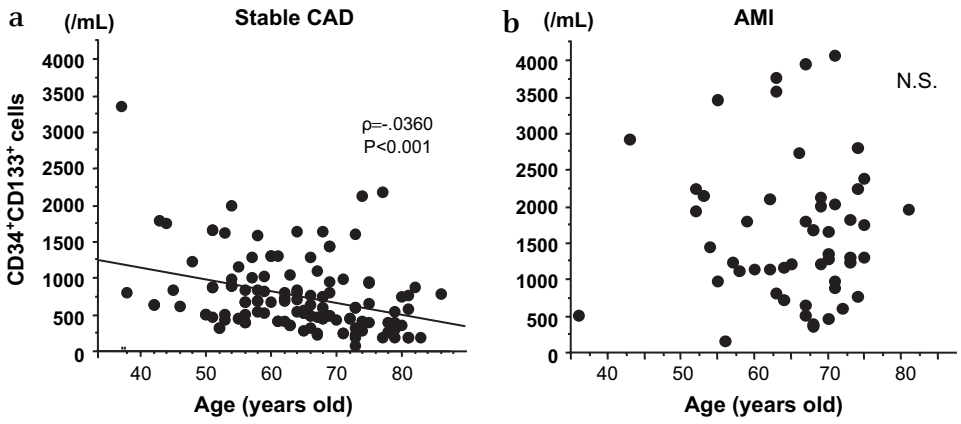

Figure 1 (A) Relationship between circulating $CD34^+CD133^+$ cell levels and age in subjects with stable coronary artery disease (CAD). The number of circulating $CD34^+CD133^+$ decreased linearly with age ($\rho=-0.0360$, $p<0.001$). (B) Relationship between circulating $CD34^+CD133^+$ cell levels and age in subjects with acute myocardial infarction (AMI). The number of circulating $CD34^+CD133^+$ cells 7 days after AMI was not associated with age.

major angiogenic cytokines released from ischaemic tissues.[3] However, VEGF did not correlate with circulating $CD34^+CD133^+$ levels in the present study. Because VEGF levels increase quickly after the onset of AMI and return to the normal range,[23] the relationship between $CD34^+CD133^+$ levels and VEGF could not be observed as late as day 7. Alternatively, other angiogenic chemokines such as Angiopoietin-1[24] and SDF-1[3] may be a dominant factor affecting the mobilisation of $CD34^+CD133^+$ cells around 7 days after the onset of AMI.

In the present study, the migratory activity of EPCs and network-forming ability of EPCs were superior in subjects with AMI compared with those with stable CAD. In AMI, increased inflammatory and angiogenic cytokine/chemokine, such as VEGF[13] and SDF-1,[3] might improve the functions of the EPCs mobilised from bone marrow. A similar phenomenon was reported by Vöö *et al*, who found an enhanced chemotactic response of $CD133^+$ circulating progenitor cells following AMI.[25]

Recently, the precise origin of these EPCs has become the subject of debate.[26] Nonetheless, the importance of EPCs in maintaining vascular homeostasis remains unchanged, because they have been used clinically to induce angiogenesis,[15–18] We can safely state that circulating $CD34^+CD133^+$ cells and these cell-derived EPCs are maintained in at least some AMI subjects.

The peak CK level and sex (male) were negatively associated with $CD34^+CD133^+$ cell mobilisation in AMI. Our findings are consistent with the study by Wojakowski *et al* indicating that the number of mobilised stem cells is negatively correlated with cardiac necrosis markers (Troponin I, CK-MB), and that the peak number of CD34 cells was shown to be significantly lower than in patients with LVEF≤40%.[22] The high value of peak CK in the present study was associated with lower LVEF, which made for the mobilisation of $CD34^+CD133^+$ cells after AMI decreased.

The fact that the number of mobilised $CD34^+CD133^+$ was lower in men was also consistent with the report by Hoetzer *et al*, which clarified that the EPC colony-forming capacity and migratory activity were higher in women than in men.[27] Although oestrogen is known to mobilise EPC from bone marrow, our female subjects were all in postmenopause. Thus, it is unlikely that oestrogen affected the mobilisation of EPCs in the present study. The circulating EPC level might be intrinsically higher in women than in men, which would explain one mechanism of slow atherosclerotic progression in women.

Limitations of our study include the fact that we measured $CD34^+CD133^+$ cell levels and EPC levels at a single time after the onset of AMI. However, we previously confirmed that EPC and $CD34^+$ levels peaked during 4–7 days after the onset.[13] Therefore, the number measured on day 7 after the onset could be used to indicate the circulating $CD34^+CD133^+$ cell level in subjects after AMI. Second, other types of EPCs derived from myeloid and mesenchymal were not taken into consideration. Serial blood samplings would be warranted to elucidate the behaviour of $CD34^+CD133^+$ cell and EPC levels in both CAD and AMI. Additionally, different types of EPCs should also be investigated.

In conclusion, the number of circulating $CD34^+CD133^+$ cells decreased with age in non-smoking subjects with stable CAD,

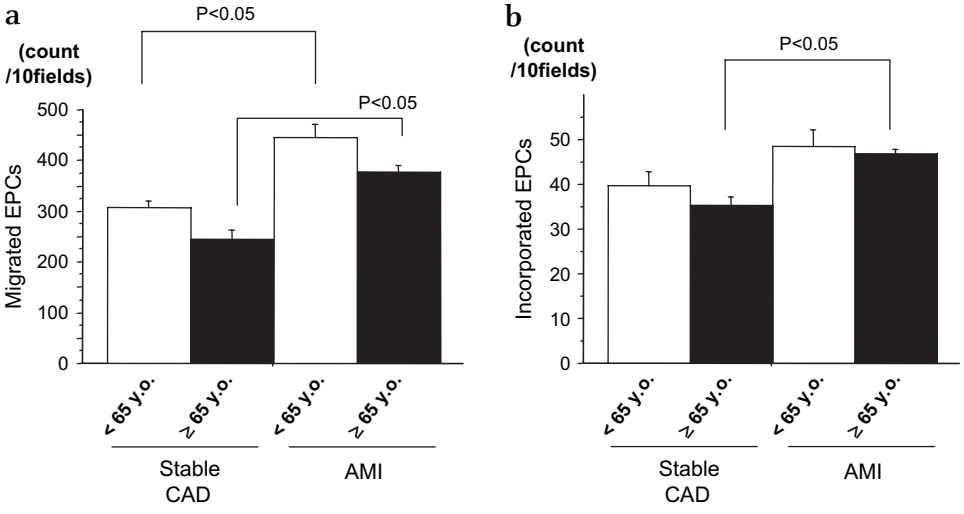

Figure 2 Migratory activity and network forming activity of endothelial progenitor cells (EPCs) differing between subjects with chronic coronary artery disease (CAD) and subjects with acute myocardial infarction (AMI). (A) Migratory activity of $CD34^+CD133^+$ cell-derived EPCs assessed by modified Boyden chamber methods. Both in subjects <65 years old and in subjects ≥65 years old, migratory activity was significantly enhanced in EPCs from AMI compared with EPCs from stable CAD ($p<0.05$). Interestingly, enhanced migratory activity of EPCs was observed even in AMI subjects ≥65 years old. (B) Similar to migratory activity, the ability of network-forming activity of $CD34^+CD133^+$ cell-derived EPCs with human umbilical vein endothelial cells was improved in AMI compared with stable CAD. The statistically significant difference was only observed in subjects ≥65 years old.

but not in subjects with AMI. Furthermore, the functions of $CD34^+CD133^+$ cell-derived EPCs in subjects with AMI did not deteriorate, even in older people. Taken together, some older adults with CAD may well have the capacity to mobilise and utilise residual EPCs in the event of AMI, even if the circulating $CD34^+CD133^+$ cell level is low.

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Competing interests None.

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REFERENCES

1. **Asahara T,** Murohara T, Sullivan A, *et al*. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997;**275**:964—7.
2. **Hill JM,** Zalos G, Halcox JP, *et al*. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med* 2003;**348**:593—600.
3. **Dimmeler S,** Leri A. Aging and disease as modifiers of efficacy of cell therapy. *Circ Res* 2008;**102**:1319—30.
4. **Shintani S,** Murohara T, Ikeda H, *et al*. Augmentation of postnatal neovascularization with autologous bone marrow transplantation. *Circulation* 2001;**103**:897—903.
5. **Yamamoto K,** Kondo T, Suzuki S, *et al*. Molecular evaluation of endothelial progenitor cells in patients with ischemic limbs: therapeutic effect by stem cell transplantation. *Arterioscler Thromb Vasc Biol* 2004;**24**:e192—6.
6. **Tepper OM,** Galiano RD, Capla JM, *et al*. Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. *Circulation* 2002;**106**:2781—6.
7. **Kondo T,** Hayashi M, Takeshita K, *et al*. Smoking cessation rapidly increases circulating progenitor cells in peripheral blood in chronic smokers. *Arterioscler Thromb Vasc Biol* 2004;**24**:1442—7.
8. **Vasa M,** Fichtlscherer S, Aicher A, *et al*. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 2001;**89**:E1—7.
9. **Schmidt-Lucke C,** Rossig L, Fichtlscherer S, *et al*. Reduced number of circulating endothelial progenitor cells predicts future cardiovascular events: proof of concept for the clinical importance of endogenous vascular repair. *Circulation* 2005;**111**:2981—7.
10. **Werner N,** Kosiol S, Schiegl T, *et al*. Circulating endothelial progenitor cells and cardiovascular outcomes. *N Engl J Med* 2005;**353**:999—1007.
11. **Heiss C,** Keymel S, Niesler U, *et al*. Impaired progenitor cell activity in age-related endothelial dysfunction. *J Am Coll Cardiol* 2005;**45**:1441—8.
12. **Wojakowski W,** Kucia M, Kazmierski M, *et al*. Circulating progenitor cells in stable coronary heart disease and acute coronary syndromes: relevant reparatory mechanism? *Heart* 2008;**94**:27—33.
13. **Shintani S,** Murohara T, Ikeda H, *et al*. Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. *Circulation* 2001;**103**:2776—9.
14. **Numaguchi Y,** Sone T, Okumura K, *et al*. The impact of the capability of circulating progenitor cell to differentiate on myocardial salvage in patients with primary acute myocardial infarction. *Circulation* 2006;**114**(1 Suppl):I114—19.
15. **Tateishi-Yuyama E,** Matsubara H, Murohara T, *et al*. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. *Lancet* 2002;**360**:427—35.
16. **Rosenzweig A.** Cardiac cell therapy—mixed results from mixed cells. *N Engl J Med* 2006;**355**:1274—7.
17. **Kajiguchi M,** Kondo T, Izawa H, *et al*. Safety and efficacy of autologous progenitor cell transplantation for therapeutic angiogenesis in patients with critical limb ischemia. *Circ J* 2007;**71**:196—201.
18. **Krause K,** Jaquet K, Schneider C, *et al*. Percutaneous intramyocardial stem cell injection in patients with acute myocardial infarction: first-in-man study. *Heart* 2009;**95**:1145—52.
19. **Rauscher FM,** Goldschmidt-Clermont PJ, Davis BH, *et al*. Aging, progenitor cell exhaustion, and atherosclerosis. *Circulation* 2003;**108**:457—63.
20. **Edelberg JM,** Tang L, Hattori K, *et al*. Young adult bone marrow-derived endothelial precursor cells restore aging-impaired cardiac angiogenic function. *Circ Res* 2002;**90**:E89—93.
21. **Shimada T,** Takeshita Y, Murohara T, *et al*. Angiogenesis and vasculogenesis are impaired in the precocious-aging klotho mouse. *Circulation* 2004;**110**:1148—55.
22. **Wojakowski W,** Tendera M, Zebzda A, *et al*. Mobilization of CD34(+), CD117(+), CXCR4(+), c-met(+) stem cells is correlated with left ventricular ejection fraction and plasma NT-proBNP levels in patients with acute myocardial infarction. *Eur Heart J* 2006;**27**:283—9.
23. **Seko Y,** Imai Y, Suzuki S, *et al*. Serum levels of vascular endothelial growth factor in patients with acute myocardial infarction undergoing reperfusion therapy. *Clin Sci (Lond)* 1997;**92**:453—4.
24. **Kobayashi K,** Kondo T, Inoue N, *et al*. Combination of in vivo angiopoietin-1 gene transfer and autologous bone marrow cell implantation for functional therapeutic angiogenesis. *Arterioscler Thromb Vasc Biol* 2006;**26**:1465—72.
25. **Vöö S,** Eggermann J, Dunaeva M, *et al*. Enhanced functional response of CD133+ circulating progenitor cells in patients early after acute myocardial infarction. *Eur Heart J* 2008;**29**:241—50.
26. **Yoder MC,** Mead LE, Prater D, *et al*. Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. *Blood* 2007;**109**:1801—9.
27. **Hoetzer GL,** MacEneaney OJ, Irmiger HM, *et al*. Gender differences in circulating endothelial progenitor cell colony-forming capacity and migratory activity in middle-aged adults. *Am J Cardiol* 2007;**99**:46—8.