Consumptive reduction following increased production of CD34-positive cells and carotid intima-media thickness in non-hypertensive elderly Japanese men

Yuji Shimizu1,2*, Hirotomo Yamanashi3, Yuko Noguchi1, Jun Koyamatsu4, Kairi Kiyoura1, Shin-Ya Kawashiri1 and Takahiro Maeda1,3,4

Abstract: Background: Increase in the number of circulating CD34-positive cells has been shown to have a protective effect against atherosclerosis, owing to an inverse association with the disease. However, increased hematopoietic activity is also reported in atherosclerotic participants. Thus, this study investigated the association between circulating CD34-positive cells and carotid intima-media thickness (CIMT) in elderly men.

Methods: We conducted a cross-sectional study in 335 non-hypertensive elderly men (aged 60–69 years), as hypertension masks the beneficial effects of circulating CD34-positive cells.

Results: In case of non-overweight subjects, no association was found between the CD34-positive cell count and CIMT (adjusted-standardized parameter estimate (β) between natural log-transformed CD34-positive cells and CIMT was β = −0.03, p = 0.630), while a significant inverse correlation was observed between CD34-positive cell count and CIMT in case of overweight subjects (β = −0.26, p = 0.029). As overweight subjects had significantly higher levels of CD34-positive cells than non-overweight subjects (p = 0.005), we analyzed the results further by stratifying.

ABOUT THE AUTHOR
The authors are engaged in the research on the prevention of cardiovascular disease. Our present work uses epidemiological method to focus on the possible mechanism of vascular structure maintenance among a general older population. Integrating the concepts of geriatric medicine and regenerative medicine is our main strategy for the present study. This novel strategy also contributes to broadening the view of epidemiological study. Therefore, this is an important first step to develop cardiovascular disease prevention methods which account for the aging process such as decreasing hematopoietic activity.

PUBLIC INTEREST STATEMENT
Bone marrow activity has recently been revealed to be closely associated with vascular maintenance since hematopoietic stem cells (immature cells such as CD34-positive cells) derived from bone marrow reportedly play a major role in vascular homeostasis. And a previous study showing an association between obesity and decreased numbers of circulating CD34-positive cells and increased CIMT. However, CD34-positive cells have been seen in human atherosclerotic lesions, and increased hematopoietic activity has also been reported in atherosclerosis.

In the present study, we found that aggressive endothelial repair not only causes the elevation of circulating CD34-positive cells by increasing the activity of CD34-positive cell production but also causes consumptive reduction of circulating CD34-positive cells since many of them become mature cells (CD34-negative cells) such as endothelial cells, mural cells, and foam cells.
the levels of CD34-positive cells. Results showed that the significant association between the CD34-positive cell count and CIMT is limited to subjects with a high CD34-positive cell count ($\beta = -0.09, p = 0.242$ for subjects with low CD34-positive cell count; and $\beta = -0.22, p = 0.007$ for subjects with a high CD34-positive cell count).

**Conclusion:** The inverse correlation between CD34-positive cells and CIMT was limited to overweight subjects and individuals with a high CD34-positive cell count as a result of endothelial injury that stimulates CD34-positive cell production.

**Subjects:** Bioscience; Health and Social Care; Medicine, Dentistry, Nursing & Allied Health

**Keywords:** CD34-positive cells; consumptive reduction; CIMT

1. Introduction

Bone marrow-derived endothelial progenitor cells (CD34-positive cells) contribute to maintenance of the vascular endothelium (Asahara et al., 1997; Takahashi et al., 1999). Endothelial dysfunction is one of the initial processes that lead to the onset of atherosclerosis (increased arterial stiffness) (Endemann & Schiffrin, 2004). Therefore, increased levels of circulating CD34-positive cells may have a beneficial effect in preventing atherosclerosis.

However, CD34-positive cells have been observed in human atherosclerotic lesions (Moreno et al., 2004; Torsney, Mandal, Halliday, Jahangiri, & Xu, 2007) and increased hematopoietic activity has also been reported in patients with atherosclerosis (van der Valk et al., 2017). Furthermore, CD34-positive cells are known to differentiate into endothelial cells as well as foam cells (Daub et al., 2006). These observations indicate that increased levels of circulating CD34-positive cells are positively associated with active atherosclerotic lesions.

Thus, we speculated that hematopoietic activity should be a measure for the association between circulating CD34-positive cells and atherosclerosis. Carotid intima-media thickness (CIMT) is considered to an indicator of arterial stiffness, and it is correlated with body mass index (BMI) (Ciccone et al., 1999; Leite et al., 2012; Rashid & Mahmud, 2015). In this study, we adopted 25 kg/m$^2$ as the BMI cutoff point, adhering to the conventions as per World Health Organization (WHO) which classifies BMI $\geq 25$ kg/m$^2$ as overweight (WHO Expert Consultation, 2004).

As hypertension is a well-known vascular impairment risk-factor, the necessity of vascular repair is much higher in hypertensive subjects than in non-hypertensive subjects. In hypertensive subjects, the threshold activity of CD34-positive cells is easily achieved, followed by exhaustive reduction of these cells. This results in masking of beneficial effects of circulating CD34-positive cells such as preventing atherosclerosis in such subjects (Shimizu et al., 2017, 2016a, 2015). Therefore, delineating the association between circulating CD34-positive cells and CIMT in relation to other potential determinants in non-hypertensive elderly subjects could be an efficient tool to understand the mechanisms of endothelial repair.

Additionally, platelets are shown to play a role in endothelial repair in conjunction with circulating CD34-positive cells (Shimizu, Sato, & Koyamatsu, et al., 2016a). The damaged vascular endothelial cells are thought to release sub-endothelial components such as collagen (Nakamura, Kambayashi, Okuma, & Tondon, 1999) and von Willebrand factor (Zaffran, Meyer, Negrescu, Reddy, & Fox, 2000) to activate platelets. Activated platelets then induce an increase in circulating CD34-positive cells (Stellos et al., 2009) and their differentiation into endothelial cells and foam cells (Daub et al., 2006). As the aggressive endothelial repair causes exhaustion of CD34-positive cells at a much higher rate than replenishment by platelets (Shimizu, Sato, & Koyamatsu, et al., 2016a; Shimizu et al., 2019a,
the correlation between levels of circulating CD34-positive cells and platelets could be informative in evaluating the mechanisms underlying the present results.

Thus, we conducted a cross-sectional study to elucidate the association between circulating CD34-positive cells and CIMT in non-hypertensive elderly Japanese participants.

2. Materials and methods

2.1. Study population

This cross-sectional study was conducted in men (n = 617; age 60–69 years) using the data collected between 2013 and 2015 from the annual medical check-up of the general population living in Goto city and Saza town in western Japan.

Subjects with high (≥10,000 cells/μL, n = 8) and low (<1,000 cells/μL, n = 2) white blood cell counts were excluded to avoid the influence of inflammatory or hematological disease. Additionally, subjects with a low BMI (<18.5 kg/m², n = 21) were excluded from the study to avoid the effects of undernutrition; subjects with missing data were also avoided (n = 6). Subjects with hypertension (n = 245) were not included in the study as hypertension can mask the beneficial effects of circulating CD34-positive cells (Shimizu, Sato, & Koyamatsu, et al., 2015, 2016a). Thus, a total of 335 men with a mean age of 65.3 years (SD: ± 2.5; range: 60–69) were enrolled in this study.

All the procedures involving human participants were in accordance with the ethical standards of the institution research committee and, 1964 Declaration of Helsinki and its amendments. This study was approved by the Ethics Committee of Nagasaki University Graduate School of Biomedical Sciences (project registration number 14,051,404). Written consent forms were made available in Japanese to ensure that the participants understood the objective of the study and informed consent was obtained from them.

2.2. Data collection and laboratory measurements

The information on clinical characteristics, current drinking/smoking status, and use of anti-hypertensive medication was obtained by trained interviewers.

Body height and height with bare feet and light clothing were measured using an automatic body composition analyzer (BF-220; Tanita, Tokyo, Japan), and BMI (kg/m²) was calculated. Systolic and diastolic blood pressure were recorded at rest. Hypertension was defined as a systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg, as stated in earlier studies (Shimizu, Sato, & Koyamatsu, et al., 2015, 2016a).

Fasting blood samples were collected in an EDTA-2K tube, a heparin sodium tube, a siliconized tube, and a sodium fluoride tube. Samples from the EDTA-2K tube were used to measure the levels of reticulocytes, white blood cells, and platelets using an automated procedure at SRL, Inc. (Tokyo, Japan).

Fresh samples from the heparin sodium tube were used to quantify the circulating CD34-positive cells using the BD (Beckton Dickinson Biosciences) Trucount™ technology—an accurate and reproducible single platform assay that conforms to the guidelines recommended by the International Society of Hematotherapy and Graft Engineering (ISHAGE) (Sutherland, Anderson, Keeney, Nayar, & Chin-Yee, 1996)—and automated software of the BD FACSCanto™ II system.

Samples from a sodium fluoride tube were used to measure hemoglobin A1c (HbA1c) by the latex coagulation method. Serum was used to measure the concentration of HDL-cholesterol (HDL-C) using the direct method, while triglycerides (TG) and creatinine were each measured enzymatically. Glomerular filtration rate (GFR) was estimated by an established method proposed by a group
working on the Japanese Chronic Kidney Disease Initiative (Imai et al., 2009). According to this protocol, GFR (mL/min/1.73 m\(^2\)) was equal to 194 × [serum creatinine (enzyme method)] \(^{-1.094}\) × [age] \(^{-0.287}\).

Measurement of CIMT was done by an experienced technician using ultrasonography of common carotid arteries by LOGIQ Book XP with a 10-MHz transducer (GE Healthcare, Milwaukee, WI, USA). Maximum values for the left and right CIMT were calculated using Intimascope software (MediaCross, Tokyo, Japan). The protocol of CIMT evaluation has been described in detail elsewhere (Hara et al., 2006).

### 2.3. Statistical analysis

All the evaluated characteristics of the study population by BMI status were expressed as mean ± SD, except TG and circulating CD34-positive cells.

Since TG and circulating CD34-positive cells showed a skewed distribution, it was expressed as median [the first quartile, the third quartile], followed by logarithmic transformation. Simple correlation analysis and multiple linear regression analysis of CIMT with relevant factors adjusted for confounding factors by BMI status were calculated. In multiple linear regression analysis, adjustments were made for age, systolic blood pressure (mmHg), current drinker or smoker status (yes/no), BMI (kg/m\(^2\)), HDL-C (mg/dL), TG (mg/dL), HbA1c (%), and GFR (mL/min/1.73 m\(^2\)). We used continuous variables for age, systolic blood pressure, BMI, TG, HDL-C, HbA1c, and GFR, as well as categorized variables for current drinker status and current smoker status. Since the circulating CD34-positive cell count reflected the endothelial maintenance activity, further analyses were conducted based on stratification of circulating CD34-positive cell levels.

Since bone marrow-derived endothelial cells such as CD34-positive cells play an important role in maintaining the vascular endothelium (Asahara et al., 1997; Takahashi et al., 1999), and the level of circulating CD34-positive cells may serve as a direct indicator of vascular maintenance activity (Shimizu, Sato, & Koyamatsu et al., 2015, 2017, 2016a), we also evaluated the association between CD34-positive cells and CIMT, stratified by circulating CD34-positive cell levels.

Both platelets and CD34-positive cells are known to be involved in vascular repair (Daub et al., 2006). Even if the platelet as well as CD34-positive cell numbers are stimulated by endothelial injury, aggressive repair (a cause of atherosclerosis) might result in consumptive reduction of CD34-positive cells but not of platelets (Shimizu, Sato, & Koyamatsu et al., 2016a). Therefore, evaluating the correlation between platelets and circulating CD34-positive cells would help us understand the mechanism underlying present results. We thus made BMI status-specific and CD34-cell level-specific correlations between platelets and circulating CD34-positive cells using simple correlation analysis and multiple linear regression analysis adjusted for known confounding factors.

All statistical analyses were performed using the SAS system for Windows (version 9.4: SAS Inc., Cary, NC). The p-value <0.05 was considered statistically significant.

### 3. Results

#### 3.1. Characteristics of the study population by BMI status

Table 1 shows the characteristics of the study population based on BMI status. We found significantly higher circulating CD34-positive cells in overweight subjects (1.17 [0.76–1.84] cells/μL) compared to non-overweight subjects (0.93 [0.62–1.38] cells/μL; p= 0.005). Similarly, BMI, TG, HbA1c, serum creatinine, reticulocytes, and white blood cells were all significantly higher, whereas HDL-C was significantly lower in overweight subjects compared to non-overweight subjects.
3.2. Association between circulating CD34-positive cells and CIMT based on BMI status

Table 2 shows a simple correlation analysis and multiple linear regression analysis of CIMT with adjustment for confounding factors, and Figure 1 shows a scatter plot of circulating CD34-positive cells and CIMT in a) non-overweight and b) overweight subjects. Results showed a significant inverse correlation between circulating CD34-positive cells and CIMT in overweight subjects, but no significant association was observed in non-overweight subjects.

3.3. Characteristics of the study population stratified by levels of circulating CD34-positive cells

Since circulating CD34-positive cell count should be a direct indicator of endothelial maintenance activity, we performed further analysis focusing on levels of circulating CD34-positive cells. Table 3 shows the characteristics of the study population based on levels of circulating CD34-positive cells. Compared to subjects with low levels of circulating CD34-positive cells, subjects with high levels of circulating CD34-positive cells showed significantly higher values with regard to BMI, TG, HbA1c, reticulocyte count, white blood cell count, and platelet count; these individuals also had significantly lower HDL-C.

3.4. Association between circulating CD34-positive cells and CIMT based on the levels of circulating CD34-positive cells

Table 4 shows a simple correlation analysis and multiple linear regression analysis of CIMT with relevant factors stratified by the circulating CD34-positive cell count. Figure 1 shows scatter plot of circulating CD34-positive cells and CIMT among subjects with c) low levels of circulating CD34-positive cells, and d) high levels of circulating CD34-positive cells. We found a significant inverse correlation between circulating CD34-positive cells and CIMT in subjects with high levels of...
Table 2. Simple correlation analysis and multiple linear regression analysis of carotid intima-media thickness (CIMT) with relevant factors adjusted for confounding factors

|                                      | Non-overweight (BMI < 25 kg/m²) | Overweight (BMI ≥ 25 kg/m²) |
|--------------------------------------|---------------------------------|-----------------------------|
|                                      | r (p)  | B   | β   | p   | r (p)  | B   | β   | p   |
| Participants                         | 252    | 83  |     |     |        |     |     |     |
| Age                                  | 0.15 (0.015) | 0.011 | 0.14 | 0.023 | 0.16 (0.155) | 0.010 | 0.15 | 0.181 |
| Systolic blood pressure              | 0.14 (0.022) | 0.003 | 0.18 | 0.005 | -0.04 (0.712) | -0.002 | -0.10 | 0.396 |
| Current drinker                      | -0.07 (0.242) | -0.026 | -0.07 | 0.304 | -0.07 (0.509) | -0.028 | -0.09 | 0.464 |
| Current smoker                       | -0.07 (0.292) | 0.025 | 0.04 | 0.500 | -0.09 (0.444) | -0.035 | -0.07 | 0.563 |
| Body mass index (BMI)                | -0.01 (0.848) | -0.001 | -0.01 | 0.841 | -0.01 (0.909) | -0.003 | -0.03 | 0.810 |
| Serum HDL-cholesterol (HDL-C)        | -0.04 (0.524) | -0.001 | -0.09 | 0.197 | -0.07 (0.537) | -0.001 | -0.07 | 0.564 |
| Serum triglycerides (TG)             | -0.11 (0.070) | -0.060 | -0.16 | 0.024 | -0.03 (0.775) | 0.002 | 0.01 | 0.966 |
| Hemoglobin A1c (HbA1c)               | 0.04 (0.480) | 0.015 | 0.04 | 0.511 | 0.08 (0.493) | 0.013 | 0.06 | 0.618 |
| Glomerular filtration rate (GFR)     | 0.08 (0.229) | 0.001 | 0.07 | 0.235 | 0.06 (0.564) | 0.001 | 0.15 | 0.233 |
| Circulating CD34-positive cell       | -0.05 (0.437) | -0.009 | -0.03 | 0.630 | -0.25 (0.025) | -0.060 | -0.26 | 0.029 |

r (p): simple correlation coefficient (p value). B: parameter estimate. β: standardized parameter estimate. p: p value for multivariable linear regression models. TG and circulating CD34-positive cells are calculated in logarithmic values.
circulating CD34-positive cells, but we did not find any significant association between circulating CD34-positive cells and CIMT in subjects with low levels of CD34-positive cells.

3.5. Association between circulating CD34-positive cells and CIMT based on circulating CD34-positive cell levels and stratified by overweight status

To elucidate the overweight status-specific impact of the level of circulating CD34-positive cells on the association between circulating CD34-positive cells and CIMT, we conducted a fully adjusted partial correlation analysis. Results showed that among non-overweight subjects, the partial correlation coefficients (r) for low levels of CD34-positive cells (n = 135) and high levels of CD34-positive cells (n = 117) were −0.06 (p = 0.522) and −0.24 (p = 0.013), respectively. Among overweight subjects, the corresponding values were −0.21 (p = 0.345) (n = 32) and −0.23 (p = 0.140) (n = 51), respectively.

3.6. Association between circulating CD34-positive cells and CIMT by circulating CD34-positive cell levels limited to subjects not taking anti-hypertensive medication

Since antihypertensive medication can potentially influence the association between circulating CD34-positive cells and CIMT, owing to an increase in the number of circulating CD34-positive cells (Pelliccia et al., 2010), we conducted a further analysis limited to subjects not taking antihypertensive medications. Results showed essentially the same associations; B = −0.08, β = −0.16, p = 0.076 for subjects with low levels of circulating CD34-positive cells (n = 113), and B = −0.10, β = −0.26, p = 0.017 for subjects with high levels of circulating CD34-positive cells (n = 95).

3.7. Correlation between circulating CD34-positive cells and platelets

BMI status-specific and CD34-positive cell levels-specific correlations between circulating CD34-positive cells and platelets are shown in Table 5. Using multiple linear regression analysis, no significant correlations between platelets and circulating CD34-positive cells were observed for overweight and subjects with high CD34-positive cells, while significant positive correlations were observed for non-overweight and subjects with low CD34-positive cells.
4. Discussion

The major findings of the present study were a significant inverse correlation between circulating CD34-positive cells and CIMT in overweight subjects and subjects with high levels of circulating CD34-positive cells; however, no such association was observed in non-overweight subjects and subjects with low levels of circulating CD34-positive cells.

A previous study on asymptomatic men reported an inverse association between circulating CD34-positive cells and subclinical atherosclerosis (Bielak et al., 2009). Although increased hematopoietic activity has been reported in subjects with atherosclerosis (van der Valk et al., 2017), the mechanism underlying this paradoxical phenomenon has not yet been resolved.

Carotid atherosclerosis is reported to be associated with silent ischemic brain damage (Moroni et al., 2016), and elevated risk for ischemic stroke and subclinical atherosclerosis is genetically determined (Liu et al., 2013). As BMI >25 kg/m² is an independent risk factor for ischemic stroke (Yonemoto et al., 2011) and endothelial dysfunction leading to atherosclerosis (Endemann & Schiffrin, 2004) in Japanese men, overweight subjects might have a higher risk of endothelial dysfunction. However, in the present study, CIMT between non-overweight and overweight subjects was comparable, although the hematopoietic activity was significantly higher in the latter group, as evaluated by levels of CD34-positive cells, reticulocytes, and white blood cells.

In the current study, overweight men showed a significantly higher CD34-positive cell count than non-overweight men. Previous studies have reported that bone marrow-derived endothelial progenitor cells such as CD34-positive cells support the integrity of the vascular endothelium (Asahara et al., 1997; Takahashi et al., 1999). This implies that overweight subjects have a higher endothelial

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| Table 3. Characteristics of study population based on levels of circulating CD34-positive cells |
|-----------------------------------------------|
| Low CD34-positive cells (<1.02 cells/μL) | High CD34-positive cells (≥1.02 cells/μL) | p |
|-----------------------------------------------|
| No. of participants | 167 | 168 |
| Age, years | 65.5 ± 2.4 | 65.1 ± 2.5 | 0.143 |
| Systolic blood pressure, mmHg | 122 ± 11 | 123 ± 9 | 0.224 |
| Diastolic blood pressure, mmHg | 73 ± 9 | 74 ± 7 | 0.261 |
| Body mass index (BMI), kg/m² | 22.8 ± 2.7 | 23.8 ± 2.7 | <0.001 |
| Current drinker, % | 46.1 | 51.2 | 0.354 |
| Current smoker, % | 12.0 | 14.3 | 0.533 |
| Serum HDL-cholesterol (HDL-C), mg/dL | 58 ± 14 | 54 ± 13 | 0.005 |
| Serum triglycerides (TG), mg/dL | 87 [62–119] | 112 [82–148] | 0.027 |
| Hemoglobin A1c (HbA1c), % | 5.6 ± 0.5 | 5.8 ± 0.7 | 0.01 |
| Serum creatinine, mg/dL | 0.86 ± 0.16 | 0.91 ± 0.82 | 0.403 |
| Glomerular filtration rate (GFR), mL/min/1.73m² | 71.8 ± 14.0 | 72.6 ± 15.4 | 0.608 |
| Circulating CD34 positive cells, cells/μL | 0.65 [0.49–0.80] | 1.46 [1.24–2.04] | <0.001 |
| Reticulocytes, % | 11.4 ± 3.9 | 12.4 ± 4.1 | 0.044 |
| White blood cells, cells/μL | 4889 ± 1195 | 6180 ± 1270 | <0.001 |
| Platelets, ×10⁴/μL | 204 ± 5.5 | 230 ± 5.0 | <0.001 |
| Carotid intima-media thickness (CIMT), mm | 0.91 ± 0.18 | 0.92 ± 0.19 | 0.651 |

Values: mean ± standard deviation. *1: Values are median (the first quartile, the third quartile). Regression model for mean values was used for determining p values. *2: Logarithmic transformation was used for evaluating p.
Table 4. Simple correlation analysis and multiple linear regression analysis of carotid intima-media thickness (CIMT) with relevant factors adjusted for confounding factors by levels of circulating CD34-positive cells

|                      | Low CD34-positive cells (<1.02 cells/μL) | High CD34-positive cells (≥1.02 cells/μL) |
|----------------------|-----------------------------------------|------------------------------------------|
|                      | \( r (p) \) | \( B \) | \( \beta \) | \( p \) | \( r (p) \) | \( B \) | \( \beta \) | \( p \) |
| Participants         | 167         | 168       |                      |              |                      |              |
| Age                  | 0.16 (0.043) | 0.012     | 0.16               | 0.046       | 0.15 (0.057) | 0.010      | 0.14       | 0.083       |
| Systolic blood pressure | 0.10 (0.196) | 0.003     | 0.16               | 0.049       | 0.12 (0.115) | 0.002      | 0.12       | 0.128       |
| Current drinker      | -0.15 (0.058) | -0.037    | -0.10              | 0.235       | -0.003 (0.973) | -0.015      | -0.04      | 0.625       |
| Current smoker       | 0.04 (0.606) | 0.009     | 0.02               | 0.850       | 0.03 (0.721) | 0.029      | 0.05       | 0.512       |
| Body mass index (BMI)| 0.06 (0.479) | 0.002     | 0.03               | 0.702       | -0.03 (0.710) | 0.001      | 0.02       | 0.812       |
| Serum HDL-cholesterol (HDL-C) | -0.10 (0.198) | -0.002    | -0.14              | 0.111       | 0.005 (0.953) | 0.0001     | 0.01       | 0.950       |
| Serum triglycerides (TG) | -0.17 (0.029) | -0.083    | -0.23              | 0.009       | -0.03 (0.701) | 0.008      | 0.02       | 0.825       |
| Hemoglobin A1c (HbA1c) | 0.08 (0.327) | 0.016     | 0.04               | 0.613       | 0.04 (0.571) | 0.009      | 0.04       | 0.665       |
| Glomerular filtration rate (GFR) | 0.06 (0.428) | 0.001     | 0.06               | 0.405       | 0.07(0.335) | 0.001      | 0.05       | 0.507       |
| Circulating CD34-positive cell | -0.12 (0.132) | -0.042    | -0.09              | 0.242       | -0.21 (0.006) | -0.090     | -0.22      | 0.007       |

\( r (p) \): simple correlation coefficient (p value). \( B \): parameter estimate. \( \beta \): standardized parameter estimate. \( p \): p value for multivariable linear regression models. TG and circulating CD34-positive cells are calculated in logarithmic values.
Table 5. Simple correlation analysis and multiple linear regression analysis of circulating CD34-positive cells with platelets adjusted for confounding factors

|                     | Non-overweight (BMI<25 kg/m²) | Overweight (BMI≥25 kg/m²) |
|---------------------|-------------------------------|---------------------------|
|                     | r (p) | B  | β    | p    | r (p) | B  | β    | p    |
| Participants        |       | 252 |      |      | 83    |     |      |      |
| Circulating CD34-positive cells | 0.27 (<0.001) | 0.03 | 0.26 | <0.001 | 0.29 (0.007) | 0.03 | 0.24 | 0.060 |
| r (p): simple correlation coefficient (p value). B: parameter estimate. β: standardized parameter estimate. p: p value for multivariable linear regression models. BMI: body mass index. HDL-C: HDL-cholesterol. TG: triglycerides. GFR: glomerular filtration rate. TG and circulating CD34-positive cell are calculated in logarithm values. In multiple adjusted model, adjustments are made for age, systolic blood pressure, current drinker, current smoker, BMI, HDL-C, TG, HbA1c, and GFR.
repair activity than non-overweight subjects; however, we found no significant difference in CIMT values between the two groups.

We saw a significant inverse association between circulating CD34-positive cells and CIMT in overweight subjects. The consumptive reduction of circulating CD34-positive cells might be responsible for this inverse correlation. Aggressive endothelial repair, which induces the development of atherosclerosis, may cause consumptive reduction of circulating CD34-positive cells, as many undergo maturation (CD34-negative cells) to form endothelial cells, mural cells, and foam cells (Daub et al., 2006). A previous study shows an association of obesity with decreased numbers of circulating CD34-positive cells and increased CIMT, which gets altered on weight loss (Müller-Ehmsen et al., 2008). These findings support the above-mentioned mechanism, as weight loss lessens the influence of consumptive reduction of CD34-positive cells.

The association between CD34-positive cells and CIMT could be determined by the increased production as well as consumptive reduction of CD34-positive cells. Overweight non-hypertensive subjects may have a higher CD34-positive cells production rate vs. consuming reduction rate. Overweight subjects thus show higher levels of circulating CD34-positive cells than non-overweight subjects.

Since the association between CD34-positive cells and CIMT is likely to be determined by both the production rate and consumptive reduction rate of CD34-positive cells, the exact levels of circulating CD34-positive cells should determine these associations directly. We further conducted an analysis to investigate the association between CD34-positive cells and CIMT accounting for actual circulating CD34-positive cell levels. Results showed that this significant association was limited to subjects with a high CD34-positive cell count. Additionally, a fully adjusted partial correlation analysis of non-overweight subjects showed no significant correlation between circulating CD34-positive cells and CIMT in subjects with low levels of CD34-positive cells, and a significant inverse correlation for subjects with high levels of CD34-positive cells.

To evaluate the influence of consumptive reduction of circulating CD34-positive cells, we made further analysis of platelets and circulating CD34-positive cells stratified by BMI status and CD34-positive levels. By multiple linear regression analysis, positive correlation between platelet and circulating CD34-positive cells was observed in non-overweight and low CD34-positive cells participants; however, no significant correlation was observed for overweight and high CD34-positive cells participants. As consumptive reduction of CD34-positive cells results in diminishing the significant positive correlation between these two factors (Shimizu, Sato, & Koyamatsu, et al., 2016a; Shimizu et al., 2019a, 2019b, 2019c), no significant correlation indicated that the influence of consumptive reduction of CD34-positive cells was strong in participants with overweight and high CD34-positive cells. Furthermore, for overweight subjects, the simple correlation analysis between platelets and circulating CD34-positive cells showed significant positive correlation. After adjustment of known cardiovascular risk factors, this correlation became non-significant. For high CD34-positive cell participants, both by simple correlation analysis and multiple linear regression analysis, those correlations showed no significance. Weaker influence of consumptive reduction in overweight subjects could be blamed for this phenomenon.

The present study is the first to report the impact of being overweight on the association between CD34-positive cells and CIMT among non-hypertensive elderly men. It also showed that CD34-positive cell number is an important determinant for this association. Our present results also revealed that higher levels of CD34-positive cells do not indicate a lower risk of atherosclerosis, but they do indicate a likelihood of appropriate endothelial repair, as the circulating CD34-positive cell count is determined by both production and consumptive reduction.

In the present study, overweight subjects had significantly higher numbers of circulating CD34-positive cells than non-overweight subjects. Subjects with a high level of circulating CD34-positive
cells had a significantly higher BMI than those with a lower level of circulating CD34-positive cells. Height has been shown to have a possible influence on hematopoietic activity (Shimizu, Sato, & Koyamatsu, et al., 2016b) and the productivity of circulating CD34-positive cells (Shimizu, Sato, & Koyamatsu, et al., 2017; Shimizu et al., 2019b). An inflammatory disadvantage related to a single nucleotide polymorphism (SNPs) (rs3782886) that is specific to Asians was also revealed to be associated with short stature (Shimizu, Sato, & Noguchi, et al., 2017). Additionally, short stature is reported to be positively associated with low-grade inflammation (Shimizu, Yoshimine, & Nagayoshi, et al., 2016a) and subclinical atherosclerosis (Shimizu et al., 2013) for subjects with high BMI, but not for those with low BMI. Furthermore, subjects of short stature with low BMI, but not high BMI, may have a risk of stroke (Shimizu et al., 2014), anemia (Shimizu, Nakazato, & Sekita, et al., 2015) and dyslipidemia (Shimizu, Yoshimine, & Nagayoshi, et al., 2016b). As Japanese individuals are known to have a low BMI and short stature, the present results cannot be extrapolated to other ethnic groups without conducting similar studies.

Potential limitations of this study warrant consideration. Although the level of circulating CD34-positive cells was inversely associated with CIMT, no data is available with regards to the evaluation of endothelial function. Further analysis that includes endothelial function-related evaluation would be necessary. Although an inverse association between circulating CD34-positive cells and CIMT was observed in overweight subjects, we could not reach meaningful levels with regard to the number of circulating CD34-positive cells for the stratified analysis owing to small sample size. Conducting a larger study with more overweight subjects would be necessary. As this was a cross-sectional study, causal relationships were not established.

5. Conclusions
In conclusion, a significant inverse correlation between circulating CD34-positive cells and CIMT was observed in overweight subjects and individuals with high levels of circulating CD34-positive cells, while no such association was seen in non-overweight subjects and individuals with low levels of circulating CD34-positive cells. Therefore, consumptive reduction of CD34-positive cells following increased production of these cells might serve as a determinant for the association between the circulating CD34-positive cell count and CIMT. These results show that a high CD34-positive cell count does not indicate a lower risk of atherosclerosis but does indicate a likelihood of appropriate endothelial repair.

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Competing interests
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Author details
Yuuji Shimizu,1,2
E-mail: shimizu@asaka-ganjun.jp
Hirotomo Yamanashi1
E-mail: yhirotomo@yahoo.co.jp
Yuka Noguchi1
E-mail: y-noguti@nagasaki-u.ac.jp
Jun Koyamatsu1
E-mail: jun_koyamatsu@yahoo.co.jp
Kairi Kiyoura1
E-mail: kiyoura@nagasaki-u.ac.jp
Shin-Ya Kawashiri1
E-mail: shin-ya@nagasaki-u.ac.jp
Takahiro Maeda1,3,4
E-mail: tmaeda@nagasaki-u.ac.jp

1 Department of Community Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan.
2 Department of Cardiovascular Disease Prevention, Osaka Center for Cancer and Cardiovascular Disease Prevention, Osaka, Japan.
3 Department of General Medicine, Nagasaki University Hospital, Nagasaki, Japan.
4 Department of Island and Community Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan.

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