Journal of Thrombosis and Haemostasis, **11**: 1583–1592 DOI: 10.1111/jth.12315

ORIGINAL ARTICLE

Activated platelets correlate with mobilization of naïve $CD34^+$ cells and generation of $CD34^+/KDR^+$ cells in the circulation. A meta-regression analysis

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To cite this article: de Boer HC, van Oeveren-Rietdijk AM, Rotmans JI, Dekkers OM, Rabelink TJ, van Zonneveld AJ. Activated platelets correlate with mobilization of naïve $CD34^+$ cells and generation of $CD34^+/KDR^+$ cells in the circulation. A meta-regression analysis. *J Thromb Haemost* 2013; **11**: 1583–92.

Summary. *Background:* Bone marrow-derived circulating $CD34^+$ progenitor cells participate in remodeling and repair of the vasculature. Coexpression of the kinase-insert domain-containing receptor (KDR) has been proposed to identify the regenerative capacity. Recently, we provided evidence that the major fraction of circulating $CD34^+/KDR^+$ cells is not mobilized from bone marrow, but is generated at sites of vascular injury through interaction with platelets. *Objectives:* To determine the relationship between platelet activation, the recruitment of naïve $CD34^+$ cells and the generation of $CD34^+/KDR^+$ progenitor cells in a broad range of (patho)physiologic conditions, a detailed meta-regression analysis was conducted. *Methods/Results:* Twenty-eight conditions were found in which the numbers of $CD34^+$ and/or $CD34^+/KDR^+$ cells and the levels of soluble P-selectin, as a marker for in vivo platelet activation, were documented. To combine heterogeneous data from 214 selected articles, results were standardized to a uniform scale by calculating standardized mean differences (SMDs) obtained from patient and control cohorts. Subsequently, a random-effects meta-regression analysis was performed on pooled SMDs. *Conclusions:* Our systemic survey supports a model in which activated platelets are a determinant for mobilization of $CD34^+$ cells from the bone marrow and the generation of $CD34^+/KDR^+$ cells in the circulation.

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Received 6 December 2012
Manuscript handled by: S. Machin
Final decision: D. Lane, 16 May 2013

Keywords: aspirin, circulation, platelets, stem cells, vasculature.

Introduction

Numerous studies have described the participation of circulating endothelial progenitor cells (EPCs) in remodeling and repair of the vasculature. Coexpression of the kinase-insert domain-containing receptor (KDR) has been put forward as a determinant for the regenerative properties of progenitor cells, and reduced numbers of $CD34^+/KDR^+$ cells were shown to correlate with an increased risk for development of atherosclerotic disease [1,2]. Furthermore, several studies have shown a positive relationship between $CD34^+/KDR^+$ [3] and $CD133^+/KDR^+$ [4] cells and endothelial function determined by measuring endothelium-dependent flow-mediated dilation. It is believed that $CD34^+/KDR^+$ cells are mobilized from bone marrow (BM) by chemokines or growth factors, released in response to hypoxia [5] or vascular injury [6]. However, $CD34^+$ cell fractions in human BM, or $CD34^+$ cells mobilized by granulocyte colony-stimulating factor (G-CSF) into the peripheral blood of healthy subjects [7,8], contain very low numbers of $CD34^+$ cells that coexpress KDR (< 0.5% of $CD34^+$ cells) [9–11].

Recently, we provided evidence that the major fraction of circulating $CD34^+/KDR^+$ cells are not mobilized from the BM as predestined EPCs, but are generated at sites of vascular injury through interaction with activated platelets adhering to the endothelium [7]. Platelets are immobilized within minutes after infliction of a mechanical injury [6] or upon ischemic injury [5]. This injury-associated microenvironment is permissive for the homing of circulating cells by expressing vascular endothelial growth factor (VEGF) [12] and stromal cell-derived factor 1α (SDF-1α) [13,14], which are major determinants of the

response to an injury [13]. Activated platelets abundantly express P-selectin, which supports the tethering and rolling of $CD34^+$ cells, leading to the expression of adhesion molecules and subsequent firm adhesion of the $CD34^+$ cells [7]. Upon firm adhesion, the cells become subject to shear, and translocate KDR [7] and CXCR4 [15] from intracellular storage pools to the cell membrane, which allows them to enter a VEGF-susceptible and SDF-susceptible pro-vasculogenic state. Thus, this platelet-driven sequence of events makes the injury-mediated microenvironment a very specialized local 'fertile soil' for the generation of $CD34^+/KDR^+$ cells.

Whereas most of the $CD34^+/KDR^+$ cells will remain involved in the regenerative process, a fraction of these cells may be released back to the circulation and contribute to the $CD34^+/KDR^+$ cell fraction that was previously thought to be BM-derived.

This could imply that the expression of KDR by $CD34^+$ cells is a direct reflection of platelet activation in the circulation. It could also imply that platelet inhibition, which is frequently used in cardiovascular disease management, modulates progenitor cell activity. In fact, in atherosclerosis-prone patients with diabetes mellitus type 2 (DM2), we showed that treatment with aspirin (300 mg day^{-1}) resulted in 47% fewer $CD34^+/KDR^+$ cells in the circulation [7].

To further clarify the relationship between platelet activation, mobilization of naïve $CD34^+$ cells from BM, and the generation of circulating $CD34^+/KDR^+$ progenitor cells, we performed a detailed random-effects meta-regression analysis in a variety of (patho)physiologic conditions associated with tissue injury and/or ischemia.

Methods

Definition of (endothelial) progenitor cells

In the literature, a consensus has been reached concerning the minimal antigenic profile for progenitor cells as expressing CD34 and/or CD133 and for EPCs as cells coexpressing KDR. As our own observations (data not shown) and data from the literature [16] show that practically all $CD34^+$ cells coexpress CD133, we consider the markers CD34 and CD133 to be analogous. Therefore, we defined cells expressing CD34 and/or CD133 as naïve progenitor cells, and KDR-expressing $CD34^+$ and/or $CD133^+$ cells as EPCs.

Study selection

To be able to show a relationship between in vivo platelet activation and circulating (endothelial) progenitor cells, we first had to identify (patho)physiologic conditions associated with tissue injury and/or ischemia, in which soluble P-selectin (sP-selectin) levels and/or cell numbers were measured. Besides our study [7], no studies could be retrieved in which both parameters were measured simultaneously. Therefore, study conditions were identified by the use of PubMed from 1985 (the first year in which such a study was published) until May 2011 in which (endothelial) progenitor cells were enumerated, yielding 41 study conditions (see the flow diagram in Fig. S1). For 28 of these conditions, studies reporting on sP-selectin levels were found.

In a second step, we linked these 28 study conditions, defined by a dedicated keyword (Table S2), to a predefined search for progenitor cells, represented by search code A (endothelial progenitor). When < 20 references were retrieved with this strategy, search code B (CD34) was added. Similarly, for sP-selectin references, the dedicated keywords for the 28 study conditions were coupled to search C ('soluble P-selectin') or search D ('soluble adhesion molecules') when < 20 references were retrieved.

Data extraction was performed by two investigators independently, and yielded, in total, 891 articles for circulating progenitor cells (search code A, 652; search code B, 239). For sP-selectin, 619 articles were found (search code D, 465; search code D, 154).

All retrieved articles were screened for eligibility according to specified inclusion and exclusion criteria (Table S1). In total, 214 articles were included (89 for $CD34^+$ cells, 107 for $CD34^+/KDR^+$ cells, and 123 for sP-selectin). Note that a single article could yield more than one set of data, as some articles reported on more than one study condition. Therefore, the numbers mentioned in the column 'data sets (n)' (Table S2) do not necessarily correspond to the numbers of eligible articles in the columns of search codes A, B, C, or D.

Table S3 shows details of study conditions for circulating (endothelial) progenitor cells, with a description of study vs. control subjects, reference numbers for types of cell measured, number of subjects, absolute values and standard errors of the study and control group(s), and unit of measurement. Table S4 shows data for sP-selectin.

Standardization of results

Critical evaluation of the retrieved articles showed that many different methodologies and acronyms were utilized to enumerate (endothelial) progenitor cells (Table S3) and sP-selectin data (e.g. ELISA or automated analyzer). These heterogeneous data hampered direct comparison of absolute values. To standardize the data to a uniform scale, SMDs and 95% confidence intervals (CIs) were calculated from the differences in means between a study group and a reference group, divided by a pooled standard deviation, making the values independent of the measuring scales. When a study condition yielded more than one SMD, pooled SMDs (± 95% CI) were calculated, and these are shown in Table S5 for circulating (endothelial) progenitor cells ($CD34^+$ as cell type 1, and $CD34^+/KDR^+$ as cell type 2) and in Table S6 for sP-selectin.

Meta-regression analysis and statistics

For the final quantitative analysis, random-effects meta-regression analysis was performed (Stata12 Software, StataCorp LP, College Station, TX, USA) on pooled SMDs (± 95% CI) for circulating (endothelial) progenitor cells and sP-selectin. In any meta-analysis from multiple studies, heterogeneity is inevitable. To obtain an idea of the degree of heterogeneity, we performed statistical analysis for heterogeneity, and measured I^2 and τ^2 (Table S7) [17]. Regression coefficients (β) and accompanying P-values are reported.

Results

Meta-regression analysis

A survey was conducted to identify reports on (patho) physiologic conditions in which numbers of circulating (endothelial) progenitor cells and sP-selectin, as a marker for in vivo platelet activation [18], were documented. Although EPCs were studied in many (clinical) conditions (n = 41), for only 28 conditions were sP-selectin data also reported. To enable comparison of the information selected from many different reports, all data were converted to a uniform scale by calculating SMDs. Note that SMDs do not correspond to absolute values, but constitute an arbitrary measure of the effect size in study subjects relative to control subjects.

One of the inclusion criteria was that the reference group should consist of age-matched and gender-matched subjects (Table S1). We consider this to be important, as age and gender are confounders for the number of progenitor cells (see condition 26 in the Supporting Information).

We noticed that reference groups could consist of disease-matched controls or healthy controls; for example, the reference group for patients with acute myocardial infarction (AMI) were either subjects with stable angina pectoris (SAP) or healthy individuals. To assess the influence of these different reference groups on SMD values, we examined the condition 'AMI' (condition 4) in detail. No significant differences were observed for $CD34^+$ cells, $CD34^+/KDR^+$ cells or sP-selectin when we measured SMDs from the AMI cohorts vs. healthy controls or the AMI cohort vs. disease-matched controls (data not shown). Therefore, we included studies describing both reference groups, to compile as many studies as possible.

Platelet activation is associated with increased numbers of circulating CD34⁺ and CD34⁺/KDR⁺ cells

Random-effects meta-regression analysis showed highly significant associations of sP-selectin with the number of circulating cells, as shown in Fig. 1A for sP-selectin in relation to $CD34^+$ cells ($P < 0.001$, $\beta = 0.79$) and in Fig. 1B for sP-selectin in relation to $CD34^+/KDR^+$ cells ($P < 0.001$, $\beta = 0.88$).

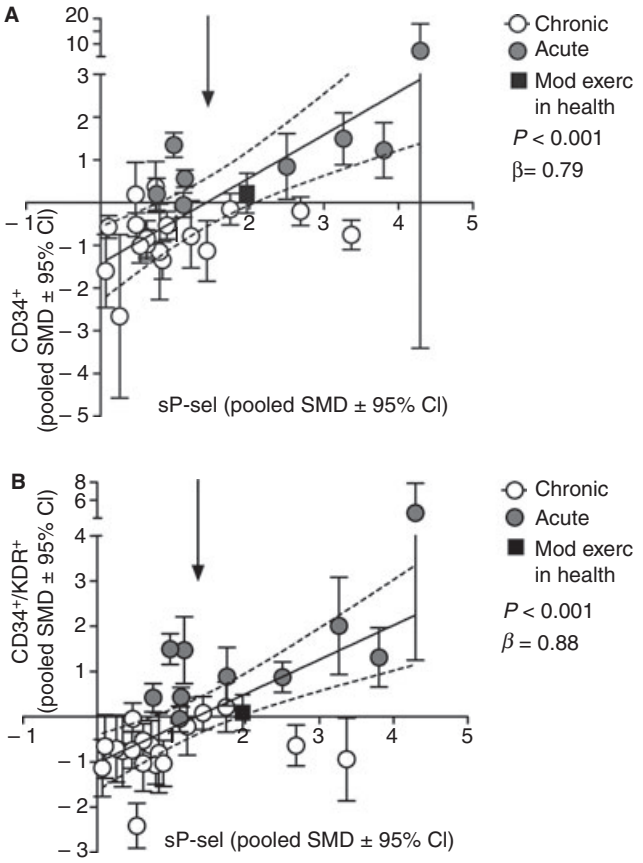

Fig. 1. Linear regression analysis for soluble P-selectin and circulating progenitor cells. Linear regression plots are shown with pooled standardized mean differences (SMDs) for soluble P-selectin (sP-sel) as continuous variable, in association with pooled SMDs for $CD34^+$ cells (A) and for $CD34^+/KDR^+$ cells (B) obtained from study conditions as compared with reference control conditions. A subdivision for 'acute' (gray circles) and 'chronic' (white circles) conditions is shown. For comparison, the study condition 'moderate exercise in health' (Mod exerc in health, ■) is shown, which was not included in the final quantitative analysis. The arrows indicate the intercept of the regression curve with the x-axis. CI, confidence interval; KDR, kinase-insert domain-containing receptor.

These data are in line with a role for activated platelets in the mobilization of naïve $CD34^+$ cells and in the conversion to $CD34^+/KDR^+$ cells. This observation is confirmed in Fig. 2, which depicts a highly significant association ($P < 0.0001$, $\beta = 0.92$) in all selected study conditions, showing that an increase in the number of $CD34^+$ cells in the study group as compared with the reference group (SMD > 0) coincides with an increase in the number of $CD34^+/KDR^+$ cells.

'Chronic' and 'acute' conditions show different profiles of circulating cells and sP-selectin

Studies have demonstrated that acute clinical conditions lead to the mobilization of EPCs, and that chronic condi-

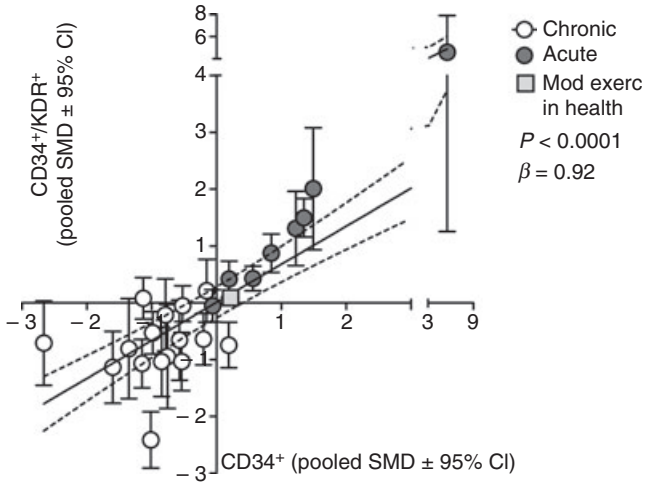

Fig. 2. Linear regression analysis for $CD34^+$ cells and $CD34^+/KDR^+$ cells. Linear regression plots are shown comparing pooled standardized mean differences (SMDs) for $CD34^+$ cells and $CD34^+/KDR^+$ cells obtained from study conditions as compared with reference control conditions. A subdivision for 'acute' (gray circles) and 'chronic' (white circles) conditions is shown. For comparison, the study condition 'moderate exercise in health' (Mod exerc in health, rectangle) is shown, which was not included in the final quantitative analysis. The black solid line represents the regression curve with correlation coefficient $\beta = 1.0$. CI, confidence interval; KDR, kinase-insert domain-containing receptor.

tions are associated with reduced numbers of circulating EPCs. For example, a temporary increase in the number of KDR^+ cells was reported in acute trauma [19] and AMI [20], whereas, during 'chronic' conditions, such as chronic heart failure (CHF) [21] and metabolic syndrome (MetS) [22], a decrease in the number of circulating KDR^+ cells was observed. To investigate whether this phenomenon also applies for other (patho)physiologic conditions, we subclassified the 28 conditions selected in this meta-analysis as follows: the 'chronic' conditions rheumatoid arthritis/rheumatoid disease (RA/RD), chronic kidney disease/chronic renal failure (CKD/CRF), hypertension (HT), CHF, coronary artery disease (CAD), DM2, obesity, MetS, SAP, subclinical atherosclerosis (subclin athero), sickle cell anemia (SCA), smoking, atrial fibrillation (AF), diabetes mellitus type 1 (DM1), chronic obstructive pulmonary disease (COPD), erectile dysfunction (ED), aging, systemic sclerosis (SSc), and insulin resistance (IR); and the 'acute' conditions cardiopulmonary bypass/coronary artery bypass grafting (CPB/CABG), cardiopulmonary resuscitation (CPR), severe trauma, AMI, cardiac syndrome X (CSX), acute stroke, maximal exercise in health or disease, percutaneous coronary intervention (PCI), and sepsis. The pooled SMDs (mean ± 95% CI) for circulating naïve progenitor cells, KDR^+ progenitor cells and sP-selectin are shown in Forest plots (Fig. 3). One condition, 'moderate exercise in health' (condition 7B), was not included in the final meta-regression analysis, as this condition does not induce ischemia and was thus considered as a normal situation.

This subclassification showed that SMDs for the 'chronic' conditions were located predominantly below zero for both $CD34^+$ cells (Fig. 3B) and KDR^+ cells (Fig. 3D), indicating decreases in the numbers of these cell types as compared with reference subjects, whereas in the 'acute' conditions, SMDs for both cell types (Fig. 3A, C) were located mainly above 0, indicating increases in the numbers of these cell types as compared with reference subjects. Interestingly, the regression curves for $CD34^+/KDR^+$ cells (Fig. 1A) and $CD34^+$ cells (Fig. 1B) intercept the *x*-axis at SMD 1.4 ± 0.6 for sP-selectin (see arrows), suggesting that a minimal arbitrary threshold of SMD > 0.8 for sP-selectin between study subjects and control subjects was required for mobilization of $CD34^+$ cells and generation of $CD34^+/KDR^+$ cells.

These data indicate that, in 'chronic' conditions, a decreased number of circulating $CD34^+$ cells coincides with the propensity to generate fewer $CD34^+/KDR^+$ cells, even at high levels of platelet activation (SMD > 2.0), as was the case for RA/RD (SMD = 3.4, condition 10) and CKD/CRF (SMD = 2.7, condition 11). On the other hand, subjects with SSc (condition 27), who also show reduced numbers of CD34+ cells (SMD = − 1.013), but then in combination with reduced levels of sP-selectin (SMD = − 0.536), showed an even more pronounced reduction in the number of $CD34^+/KDR^+$ cells (SMD = − 2.414). Likewise, in patients with DM2 (condition 15), we confirmed a reduction in the number of circulating $CD34^+$ cells and additionally showed that in vivo inhibition of platelet activation by aspirin reduced the number of $CD34^+/KDR^+$ cells [7].

In contrast, in conditions where the number of $CD34^+$ cells is increased in combination with a substantial increase in the sP-selectin level (SMD > 0.8), as is the case in most 'acute' conditions, the number of circulating $CD34^+/KDR^+$ cells was increased. Apparently, for the generation of pro-vasculogenic cells, substantial platelet activation is a primary determinant, and efficient mobilization of $CD34^+$ cells from BM is a secondary requirement. This implies that platelet inhibitors may affect the generation of $CD34^+/KDR^+$ cells, and subsequently the vasculo-protective function of these cells.

Profiles of circulating cells after G-CSF-mediated mobilization

Our concept would predict that G-CSF-mediated mobilization of progenitor cells from BM should yield mainly naïve $CD34^+/KDR^-$ cells. Indeed, when healthy subjects were treated with G-CSF for transplantation purposes, the number of $CD34^+$ cells increased significantly (SMD = 2.684), whereas the number of $CD34^+/KDR^+$ cells did not [7] (SMD = 0.264; Table S8). This prominent recruitment of $CD34^+/KDR^-$ cells in excess of the generation of $CD34^+/KDR^+$ cells in healthy subjects was also observed by Powell *et al.* [8].

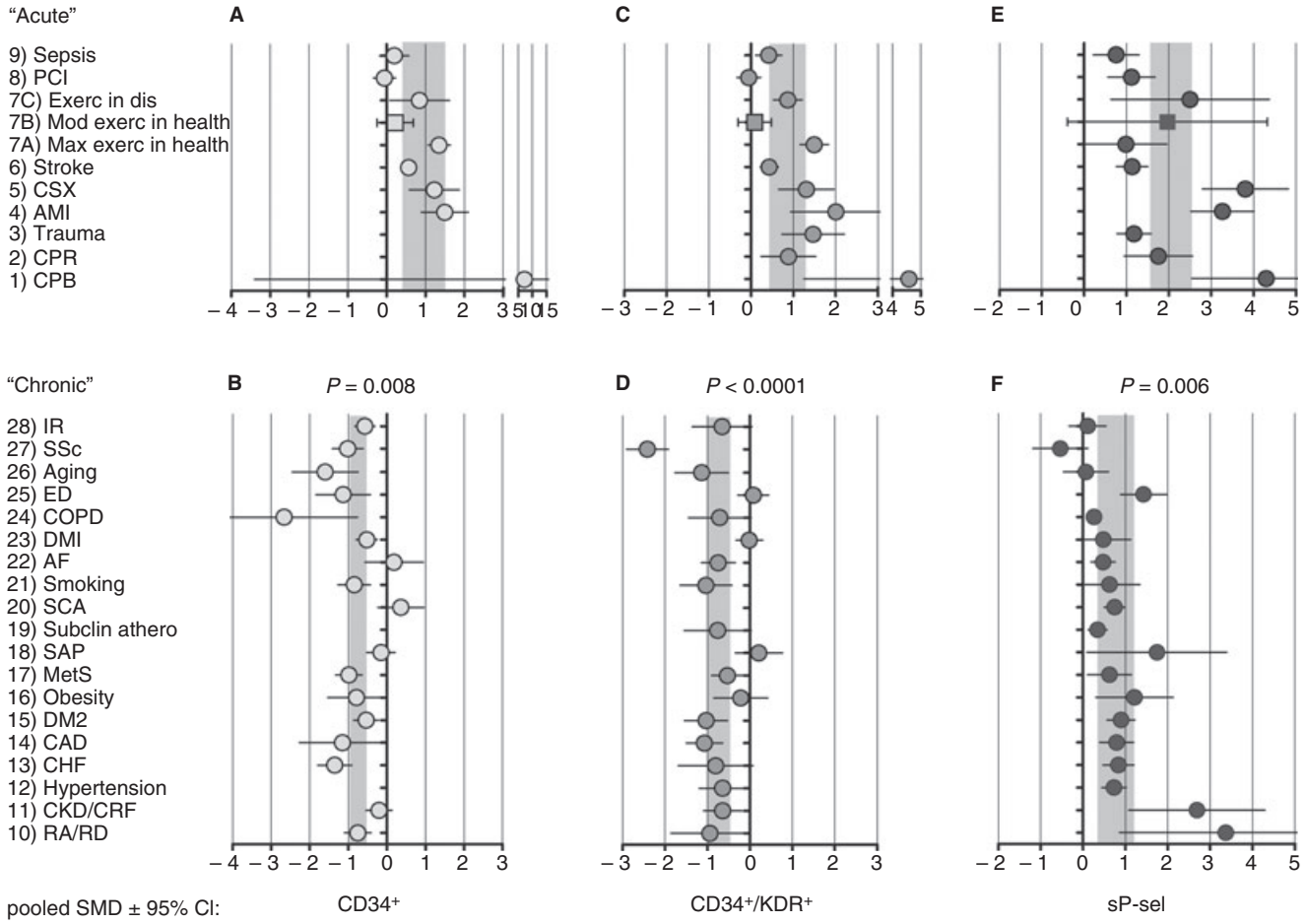

Fig. 3. Forest plots of the selected study conditions, devided in 'acute' and 'chronic' conditions. Forest plots show pooled standardized mean differences (SMDs) (± 95% confidence interval [CI]) for all selected study conditions, subdivided into 'acute' conditions (A, C, E) and 'chronic' conditions (B, D, F), showing data for $CD34^+$ cells (A, B), $CD34^+/KDR^+$ cells (C, D) and soluble P-selectin (sP-sel) (E, F). The gray area in each panel represents the standard error of the mean (B, E) or interquartile ranges of the median (A, C, D, E). *P*-values represent the significance between levels of $CD34^+$ or $CD34^+/KDR^+$ cells and sP-sel in 'acute' vs. 'chronic' conditions. AF, atrial fibrillation; AMI, acute myocardial infarction; CAD, coronary artery disease; CHF, chronic heart failure; CKD/CRF, chronic kidney disease/chronic renal failure; COPD, chronic obstructive pulmonary disease; CPB, cardiopulmonary bypass; CPR, cardiopulmonary resuscitation; CSX, cardiac syndrome X; DM1, diabetes mellitus type 1; DM2, diabetes mellitus type 2; ED, erectile dysfunction; Exerc in dis, exercise in disease; IR, insulin resistance; KDR, kinase-insert domain-containing receptor; Max exerc in health, maximal exercise in health; MetS, metabolic syndrome; Mod exerc in health, moderate exercise in health; PCI, percutaneous coronary intervention; RA/RD, rheumatoid arthritis/rheumatoid disease; SAP, stable angina pectoris; SCA, sickle cell anemia; SSc, systemic sclerosis; Subclin athero, subclinical atherosclerosis.

In contrast, when G-CSF-induced progenitor cell mobilization was studied in patients with acute ischemia resulting from AMI in comparison with patients recovering from myocardial infarction (myocardial infarction older than 14 days; old myocardial infarction [OMI]) [23], an excess of $CD34^+/KDR^+$ cells (SMD = 0.757) over BM-recruited $CD34^+/KDR^-$ cells (SMD = 0.268) was generated. Thus, this study confirms that the 'acute' (ischemic) stage of disease is associated with the generation of KDR^+ cells.

Interestingly, under the same conditions, in vivo platelet activation showed a similar tendency, namely a significant increase in sP-selectin levels in AMI patients as compared with OMI patients (SMD = 21.544) or healthy controls (SMD = 22.617) [24], whereas OMI patients showed no (SMD = −0.011) [25] or a slightly increased level (SMD = 0.742) [24] of platelet activation as compared with healthy controls, suggesting that the acute stage of myocardial infarction is also associated with in vivo platelet activation (Table S8). These data independently support our hypothesis that circulating $CD34^+/KDR^+$ cells are generated in the periphery, rather than being mobilized from the BM as predestined KDR^+ EPCs, and that this generation is associated with in vivo platelet activation.

Aspirin may hamper the generation of $CD34^+/KDR^+$ cells

In many of the 'acute' cardiac conditions, such as AMI and PCI, or in 'chronic' cardiac conditions, such as SAP,

CHF, and CAD, patients may use aspirin or other platelet inhibitors, which may suppress platelet activation and thus the numbers of circulating $CD34^+$ and $CD34^+/KDR^+$ cells. This may explain why, in the 'acute' condition of PCI (Fig. 3, condition 8) [26], in which most patients receive aspirin, a minor SMD of − 0.059 for $CD34^+$ cells (after vs. before PCI) and an SMD of − 0.051 for $CD34^+/KDR^+$ cells (after vs. before PCI) was obtained, which is clearly different from the situation in other 'acute' conditions. For comparison, healthy individuals without any medication, representing the best-case scenario, who were subjected to 'acute' maximal exercise-induced ischemia (condition 7A), showed a mean SMD of 0.986 for sP-selectin, a mean SMD of 1.347 for $CD34^+$ cells, and a mean SMD of 1.496 for $CD34^+/KDR^+$ cells, indicating efficient naïve progenitor cell mobilization and sufficient platelet activation to generate KDR^+ progenitor cells.

Discussion

During the last decade, numerous articles have reported on the characterization and enumeration of circulating EPCs, on the relationship of their numbers with the risk of occurrence of cardiovascular events and progression of atherosclerosis, on their relationship with endothelial function, and on their potential to repair vascular injury. Every study by itself has contributed to a better understanding of the ontogeny and biology of EPCs. However, because of controversies regarding cell definition and methods of enumeration, a clear consensus on the relevance of EPCs for diagnostic or even therapeutic use has been lacking.

Recently, we provided evidence that $CD34^+/KDR^+$ cells are generated upon peripheral, platelet-dependent activation of multipotent $CD34^+/KDR^-$ cells in DM2 patients. Consequently, we postulated that in (clinical) conditions associated with vascular injury/ischemia and subsequent platelet activation, the circulation would be supplemented with pro-vasculogenic cells. With platelet activation as the primary determinant, a clear definition of (endothelial) progenitor cells, and predefined selection criteria for study conditions, we conducted a comprehensive systematic review to test this hypothesis. To enable comparison of all selected studies, data were transformed to a uniform scale by the use of SMD calculations.

Our random-effects meta-regression analysis confirmed a highly significant correlation between in vivo platelet activation and $CD34^+/KDR^+$ cells, but also with naïve $CD34^+$ cells in the circulation.

The correlation between platelet activation and the number of circulating progenitor cells is corroborated by several investigations. For examples, activated (P-selectin-positive) platelets express or release SDF-1α [14], leading to mobilization of BM-derived $CD34^+$ progenitors into the periphery [27], as was shown in an acute injury model in mice [28] and in acute coronary syndrome in humans [27]. Furthermore, platelets not only release SDF-1α but also secrete VEGF [29] and sphingosine-1-phosphate (S1P) [30] upon activation. In patients with heart failure [31] and HT, there were elevated plasma levels of VEGF, which showed a positive correlation with sP-selectin levels and were reduced upon aspirin treatment [32]. Additionally, plasma levels of S1P have been shown to lead to trafficking of stem cells from BM to peripheral blood [33]. As platelets are a rich source of VEGF, SDF-1α, and S1P [30], these factors may act synergistically in attracting stem cells from BM to the peripheral blood.

Interestingly, our data suggest that a minimal threshold of platelet activation is needed before naïve stem cells exit from the BM compartment. Apparently, the concentration gradient of mobilizing factors in the circulation needs a certain minimal elevation to overcome the concentration of these factors in BM. Hence, we propose that platelet activation beyond a minimal threshold can lead to the delivery of sufficient amounts of mobilizing factors to attract naïve stem cells to the periphery. Subsequently, activated platelets establish an adhesive platform to support the conversion of $CD34^-$ to $KDR^+/CD34^+$ cells [7]. This implies that measuring the absolute number of $CD34^+$ cells is more informative about vessel repair capacity than measuring $CD34^+/KDR^+$ cells, which is consistent with a study by Fadini *et al*. [34].

The fact that in many 'chronic' conditions, such as CHF, DM1, and DM2, lower levels of $CD34^+$ cells have been reported may be consistent with 'exhaustion' of BM resulting from chronic demand from the periphery [35] or defective mobilization to the periphery caused by endothelial dysfunction in BM following oxidative stress or hyperglycemia [36].

Our findings suggest that patients with cardiovascular disease, who take inhibitors of platelet aggregation, are hampered in their capacity to generate $CD34^+/KDR^+$ cells. As these cells have been shown to contribute to re-endothelialization, endothelial function, and vascular remodeling, the long-term use of platelet inhibitors may have consequences for the capacity to maintain vascular homeostasis.

In this meta-analysis, we did not document the use of aspirin in detail, as aspirin use was either not mentioned or aspirin was very inconsistently administered among patient cohorts. In a previous study, however, we performed a randomized double-blind, placebo-controlled cross-over trial in which DM2 patients underwent a placebo phase and an aspirin phase, after which sP-selectin levels and the numbers of circulating cells were measured [7]. Our cohort yielded paired results, making this study particularly suitable to determine the effect of the extent of in vivo platelet activation and platelet inhibition by aspirin in relation to levels of circulating (endothelial) progenitor cells. In Fig. 4, our findings are combined in a three-compartment model:

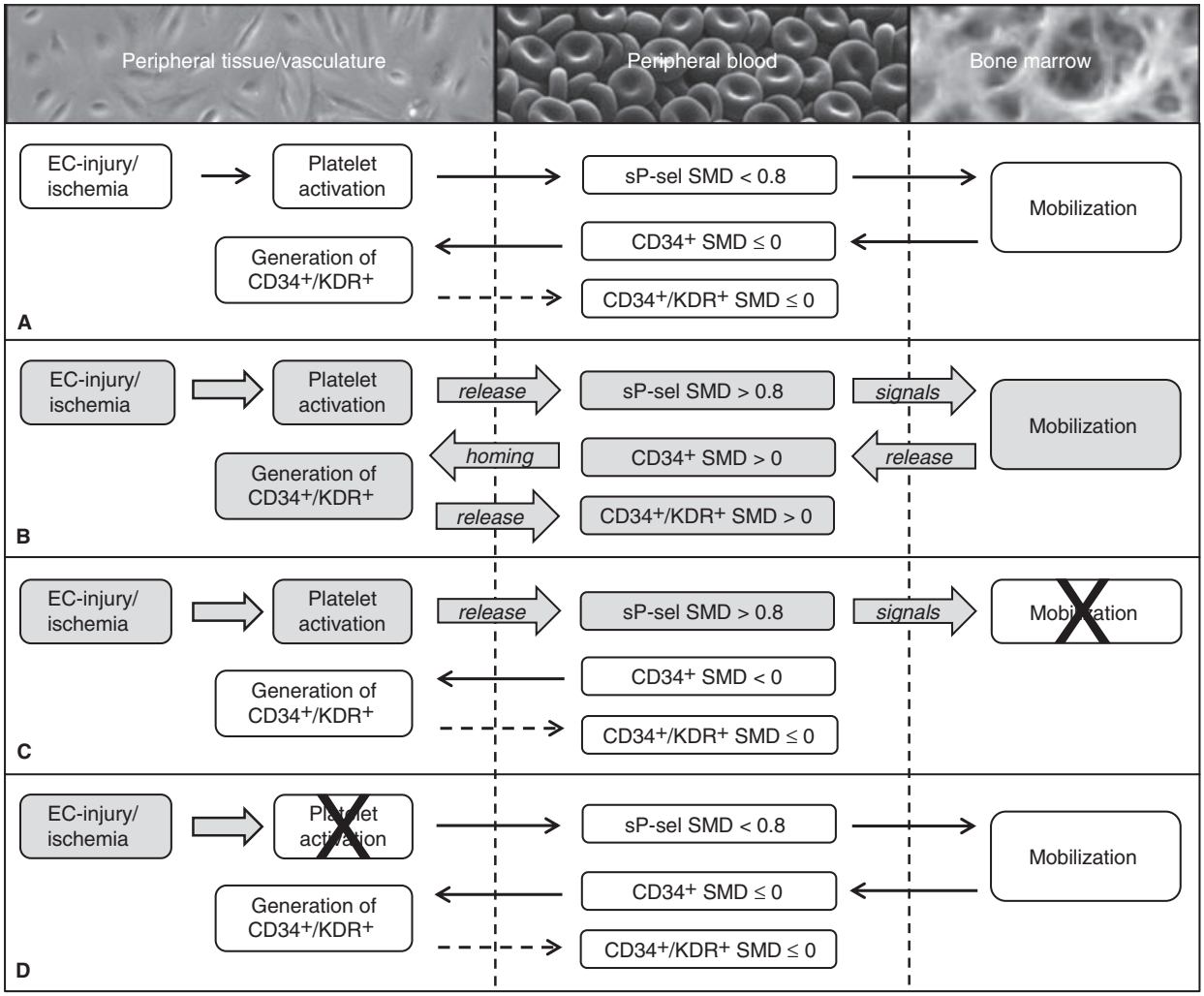

Fig. 4. Proposal of a three-compartment model. (A) Minor ischemia/injury in peripheral tissues leads to platelet activation below a minimal threshold (standardized mean difference $[SMD]_{sP\text{-}sel} < 0.8$), which is insufficient to attract fresh $CD34^+$ cells from bone marrow (BM), while already circulating $CD34^+$ cells may home to the injury site ($SMD_{CD34+} \leq 0$). (B) Pronounced tissue damage/ischemia provokes platelet activation beyond a minimal threshold ($SMD_{sP\text{-}sel} > 0.8$), leading to signals for recruitment of fresh $CD34^+$ cells ($SMD_{CD34+} > 0$) from the BM compartment to the peripheral blood compartment. $CD34^+$ cells home to the platelet thrombi deposited at the site of the vascular injury, where they are converted to $CD34^+/KDR^+$ cells, some of which are released to the peripheral blood ($SMD_{CD34+/KDR+} > 0$). (C) When pronounced tissue damage induces platelet activation beyond a minimal threshold ($SMD_{sP\text{-}sel} > 0.8$), but BM is dysfunctional, the peripheral blood compartment will lose $CD34^+$ cells to the injury site without supplementation from BM ($SMD_{CD34+} < 0$). (D) When platelet activation is inhibited, e.g. by aspirin, pronounced tissue damage will not result in platelet activation beyond a minimal threshold ($SMD_{sP\text{-}sel} < 0.8$) and will not lead to supplementation of peripheral blood $CD34^+/KDR^-$ cells ($SMD_{CD34+} \leq 0$) or generation of $CD34^+/KDR^+$ cells ($SMD_{CD34+/KDR+} \leq 0$). EC, endothelial cell; KDR, kinase-insert domain-containing receptor; sP-sel, soluble P-selectin.

A Minor ischemia/injury in peripheral tissues leads to platelet activation below a minimal threshold (illustrated by the arbitrary indicator $SMD_{sP\text{-}sel} < 0.8$), which is insufficient to attract fresh $CD34^+$ cells from BM, while already circulating $CD34^+$ cells may home to the injury site ($SMD_{CD34+} \leq 0$).

B Pronounced tissue damage/ischemia provokes platelet activation beyond a minimal threshold ($SMD_{sP\text{-}sel} > 0.8$), leading to signals for recruitment of fresh $CD34^+/KDR^-$ cells ($SMD_{CD34+} > 0$) from the BM compartment to the peripheral blood. $CD34^+$ cells home to the platelet thrombi deposited at the injury site, where they are converted to $CD34^+/KDR^+$ cells, some of which are released to the peripheral blood ($SMD_{CD34+/KDR+} > 0$).

C When pronounced tissue damage induces platelet activation beyond a minimal threshold ($SMD_{sP\text{-}sel} > 0.8$), but BM is dysfunctional, the peripheral blood compartment will lose $CD34^+$ cells to the injury site without supplementation from BM ($SMD_{CD34+} < 0$).

D When platelet activation is inhibited, e.g. by aspirin ($SMD_{sP\text{-}sel} < 0.8$), supplementation with naïve $CD34^+$ cells from BM is hampered ($SMD_{CD34+} \leq 0$) and the

generation of $CD34^+/KDR^+$ cells is suppressed ($SMD_{CD34+/KDR+} \leq 0$).

Thus, our model proposes platelet activation as a primary requirement for the recruitment of $CD34^+/KDR^-$ cells from BM and for the generation of $CD34^+/KDR^+$ cells in the periphery, provided that BM is able to respond sufficiently to the recruitment signals.

Whereas many interventions have shown a beneficial effect in humans on the number of $CD34^+$ cells, e.g. insulin-like growth factor 1 [37], erythropoietin [38], and CXCR4 antagonists [39], aspirin may well have long-term adverse effects on vasculo-protection mediated by (endothelial) progenitor cells.

In line with the role of activated platelets in vascular maintenance and repair, Amano *et al*. [40] showed that infusion of platelets enhanced the angiogenic response after ischemia, and that this response was blunted in thrombopoeitin$^{-/-}$ mice. In addition, Feng *et al*. [41] showed that platelet depletion, which may mimic pharmacologic inhibition of platelet activity, resulted in decreased blood flow and vessel density in a mouse model of hindlimb ischemia. Of course, these animal models are non-compromised with respect to cardiovascular risk factors. Nevertheless, when circulating progenitor cell levels are measured as a prognostic marker for future cardiovascular events, the use of platelet inhibitors should be taken into account.

To our knowledge, this is the first time that a general relationship has been shown between in vivo platelet activation and the levels of circulating $CD34^+$ and $CD34^+/KDR^+$ cells in human subjects. With this survey, we aim to put the research on the ontogeny of circulating (endothelial) progenitor cells and the use of platelet inhibition into a new scientific and clinical perspective.

Addendum

H.C. de Boer: designed the study, performed data extraction, analyzed and interpreted the data, performed statistical calculations, and wrote the manuscript; A.M. van Oeveren-Rietdijk: performed data extraction; J.I. Rotmans and T.J. Rabelink: provided helpful discussions and reviewed the manuscript; O.M. Dekkers: performed and supervised statistical calculations; A.J. van Zonneveld: supervised the research and wrote the manuscript.

Acknowledgements

This work was supported by the Netherlands Institute of Regenerative Medicine (NIRM) (program: controlled release of endothelial progenitor cells from the bone marrow niche).

Disclosure of Conflicts of Interest

The authors state that they have no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Flow diagram.
Table S1. Inclusion and exclusion definitions for study selection.
Table S2. Yields of papers during the screening, eligibility and inclusion phase.
Table S3. Included studies reporting on circulating cells.
Table S4. Included studies reporting on soluble P-selectin.
Table S5. Standardized mean differences (SMD) and pooled SMDs with 95% confidence intervals (lower vs. upper limits) calculated for circulating progenitor cells.
Table S6. Standardized mean differences (SMD) and pooled SMDs with 95% confidence intervals (lower vs. upper limits) calculated for sP-selectin values.
Table S7. Heterogeneity statistics.
Table S8. The effect of G-CSF treatment on numbers of circulating progenitor cells and sP-selectin values.

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