Circulating endothelial progenitor cell levels and function in patients who experienced late coronary stent thrombosis

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Aims

The pathogenesis of late coronary stent thrombosis may be related to impaired arterial healing. Endothelial progenitor cells (EPCs) have been shown to play an important role in repair and re-endothelialization following vascular injury. We hypothesized that patients who develop late stent thrombosis may have reduced or dysfunctional EPCs, and aimed to compare EPC level and function in patients who experienced stent thrombosis vs. matched controls.

Methods and results

Patients who developed late (>30 days) stent thrombosis within the past 3 years were compared with matched patients who underwent stenting and did not develop stent thrombosis. All patients had blood samples taken ≥3 months from the stent thrombosis or index procedure. The proportion of peripheral mononuclear cells (PMNCs) expressing vascular endothelial growth factor receptor 2 (VEGFR-2), CD133, and CD34 was evaluated by flow cytometry. Endothelial progenitor cell colony forming units (CFUs) were grown from PMNCs, characterized and counted following 7 days of culture. The two groups (n = 30 each) were well-matched (93.3% men, mean age 60–62 years, 33.3% diabetes, 73–80% DESs). The proportion of cells co-expressing VEGFR-2, CD133, and CD34 was evaluated by flow cytometry. Endothelial progenitor cell colony forming units (CFUs) were grown from PMNCs, characterized and counted following 7 days of culture. The two groups (n = 30 each) were well-matched (93.3% men, mean age 60–62 years, 33.3% diabetes, 73–80% DESs). The proportion of cells co-expressing VEGFR-2, CD133, and CD34 was lower in the stent thrombosis group compared with the control [VEGFR-2+CD133+: 0.18% (0.03–0.41%) vs. 0.47% (0.16–0.66%), P = 0.01; VEGFR-2+CD34+: 0.32% (0.22–0.70%) vs. 0.66% (0.24–1.1%), P = 0.03]. The number of EPC CFUs was also lower in the stent thrombosis group [3.9% (3.2–5.5%) vs. 8.3% (6.5–13.4%) colonies/well, respectively, P < 0.0001].

Conclusion

Patients who suffered late coronary stent thrombosis appear to have reduced levels of circulating EPCs and impaired functional properties of the cells. These findings require validation by further studies, but may contribute to understanding the pathogenesis of late stent thrombosis.

Keywords

Stents • Thrombosis • Endothelium • Progenitor cells

Introduction

Stent thrombosis is a rare but life-threatening complication of coronary stent implantation. It most often presents as an acute myocardial infarction (AMI) and is associated with poor outcome.1–4 Early stent thrombosis can occur following implantation of either bare-metal stents (BMS) or drug-eluting stents (DES), whereas late (>30 days) and very late (>1 year) stent thrombosis occur more commonly with DES.3–4 Research of the possible mechanisms of stent thrombosis has focused on several areas. First, procedure and stent-related factors, such as stent under-expansion or mal-apposition, persistent dissections, and bifurcation stenting.1,2,5–7 Second, humoral and platelet-related factors mainly enhanced platelet reactivity caused by low response to anti-platelet drugs, or pre-mature cessation of anti-platelet therapy.2,8–12 An additional factor that contributes to the risk of thrombosis following coronary stent implantation is the rate of stent re-endothelialization—especially in DES. An intact endothelium...
layer possesses both anti-platelet and anti-thrombotic properties and is, therefore, highly important for preventing late stent thrombosis. Joner et al. have shown that implantation of DES results in delayed arterial healing compared with BMS, characterized by poorer endothelialization and persistent fibrin deposition. Furthermore, when comparing human autopsies of DES with vs. without evidence of late stent thrombosis, the most powerful histological predictor of late stent thrombosis was the degree of endothelial coverage. 

The exact mechanism of stent re-endothelialization in humans has not been fully uncovered, but endothelial progenitor cells (EPCs) may play an important role. Endothelial progenitor cells are bone marrow-derived cells that are mobilized to the circulation in response to tissue or vessel injury and incorporate into the sites of injury. Circulating EPCs co-express CD133, CD34, and vascular endothelial growth factor receptor 2 (VEGFR-2) antigens on their surface and have the potential to differentiate into mature endothelial cells. Recent evidence suggests these cells participate in the process of vascular (arterial) repair, by promoting re-endothelialization following injury. Endothelial progenitor cells have been shown to mobilize and incorporate into denuded parts of the vessel wall after balloon injury; a process associated with accelerated endothelial regeneration. Moreover, transplantation of isolated peripheral blood EPCs into balloon-injured arteries led to rapid re-endothelialization of the vessels. In humans, a rapid increase in the levels of circulating EPCs was observed following acute vascular insults or coronary artery stenting, implying that focal endothelial injury may trigger mobilization of EPCs into the peripheral circulation.

Despite the potential role of EPCs in re-endothelialization following coronary stenting, there is no information on their level or function in patients who develop late stent thrombosis. We hypothesized that patients who develop late stent thrombosis may have reduced EPC numbers and/or dysfunctional EPCs, as a factor contributing to their risk of thrombosis. Accordingly, our aim was to evaluate the level and function of circulating EPCs in patients who developed late stent thrombosis, compared with a matched group of patients who underwent coronary stenting and did not develop stent thrombosis. A secondary aim was to compare platelet reactivity among the two groups.

### Methods

#### Patients

The study group included 30 patients who presented at our institution with definite late or very late stent thrombosis within the past 3 years (March 2006–May 2009). Index procedures were performed between January 2005 and March 2009. The patients were identified from our database, which includes all patients (consecutive) who undergo percutaneous coronary intervention (PCI) at the catheterization laboratories in the Rabin Medical Center, which is a tertiary medical centre. All patients who were admitted with a definite diagnosis of late stent thrombosis were enrolled. Enrolment and testing of the control group was performed concurrently to the study group—between February 2008 and August 2009. The control group included 30 matched patients who underwent PCI with stenting, between June 2007 and May 2009, and did not develop stent thrombosis, at least 3 months from the procedure. Enrolment and testing of the control group was matched to the study group by the following criteria: sex, age ± 10 years, diabetes status, and type of stent (DES vs. BMS—whether or not stent thrombosis occurred in a DES, a control patient with at least one implanted DES was selected). In both groups only patients currently treated with aspirin, clopidogrel, and statins were included. Contraindications to inclusion in both groups were anaemia (haemoglobin <10 g/dL), renal insufficiency (creatinine ≥2.5 mg/dL), history of an ACS or revascularization in the past three months, any type of malignant, or haematologic disorder.

The study was approved by the investigational review board (ethics committee) of the Rabin Medical Center, Israel; and all subjects provided written informed consent.

#### Blood sampling

Following overnight fasting, venous blood was drawn from an antecubital vein for EPC testing (blood drawn in heparinized tubes) and platelet reactivity testing (blood drawn in citrated tubes). Blood samples were processed within 1 h of blood collection.

#### Circulating endothelial progenitor cell level and function

Circulating EPC levels were quantified by measurement of surface markers—VEGFR-2, CD34, and CD133 by flow cytometry. Functional aspects of EPCs were evaluated by measurement of colony forming units (CFU), and by migration and the MTT assays of the cultured cells after 7 days of culture.

#### Isolation of mononuclear cells

Peripheral mononuclear cells (PMNCs) were fractionated using Ficoll density-gradient centrifugation. The mononuclear cells were isolated and washed with phosphate-buffered saline after red cell lysis.

#### Flow cytometry

Aliquots of PMNCs were incubated with monoclonal antibodies against VEGFR-2 (FITC labelled) (R&D, Minneapolis, USA), CD45-CYT5.5 (Dako, Denmark), and either CD133 (PE-labelled) (Miltenyi Biotech, Auburn, CA, USA), or CD34 (PE-labelled, Miltenyi Biotech). Isotype-identical antibodies were used as controls. After incubation, cells were washed with phosphate-buffered saline and analysed with a flow cytometer (FACSCalibur, Becton Dickinson). Each analysis included 100,000 events, after selection for CD45-positive cells (including low-intensity CD45+ cells) and exclusion of debris. In the next step gated CD34 or CD133-positive cells were examined for the expression of VEGFR-2 (example shown in Figure 1). Analyses were performed in duplicates. Results are presented as the percentage of PMNCs (after selection for CD45+ and exclusion of debris) co-expressing either VEGFR-2 and CD133, or VEGFR-2 and CD34.
Colony forming unit quantification

Isolated PMNCs were re-suspended with Medium 199 (Invitrogen, Carlsbad, CA, USA) supplemented with 20% foetal calf serum (Gibco BRL Life Tech, Gaithersburg, MD, USA). Isolated cells were re-suspended in Medium 199 and plated on 6-well plates coated with human fibronectin at a concentration of $5 \times 10^6$ cells per well. After 48 h, the non-adherent cells were collected and re-plated onto fibronectin-coated 24-well plates ($10^6$ cells per well). Endothelial progenitor cell colonies were counted using an inverted microscope 7 days after plating. An EPC colony was defined as a cluster of at least 100 flat cells surrounding a cluster of rounded cells, as previously described. A central cluster alone without associated emerging cells was not counted as a colony. To confirm endothelial cell lineage, indirect immunostaining of randomly selected colonies was performed with antibodies directed against VEGFR-2, CD31 (Becton Dickinson, NJ, USA), and Tie-2 (Santa Cruz, Biotechnology, CA, USA). Results are expressed as mean number of CFUs per well.

MTT assay

The MTT assay was performed in 15 randomly selected patients in each group, to evaluate viability of the cultured EPCs. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) measures mitochondrial activity in living cells. After 7 days of culture, MTT (Sigma, St. Louis, USA) 1 mg/mL was added to the EPC medium culture, and incubated for an additional 3–4 h. After the incubation, the medium was removed and the cells were solubilized in isopropanol. (Mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring, yielding purple MTT crystals, which can be dissolved in isopropanol). The amount of the dye released from the cells was measured with a spectrophotometer at 570 nm and subtracted background at 690 nm. An increase in the number of viable cells results in an increase in the amount of MTT formed and, therefore, in absorbance. Therefore, optical density is directly correlated with viable cell quantity.

Migration assay

Migration of EPCs was measured by the modified Boyden migration assay (Greiner bio-one, Germany) in 15 randomly selected patients in each group. After 7 days in culture the cells were detached using Trypsin EDTA, harvested by centrifugation, re-suspended in 500 mL of endothelial basal medium (EBM), counted, and $1 \times 10^5$ cells were placed in the upper part of a modified Boyden chamber. The chamber was placed in a 24-well culture dish containing EBM and human recombinant vascular endothelial growth factor (50 ng/mL) (PeproTech, Asia). After 24 h of incubation at 37°C, the lower side of the filter was washed with PBS and fixed with 2% paraformaldehyde. For quantification, cell nuclei were stained with DAPI (Santa Cruz, CA, USA). Migrating cells into the lower chamber were counted manually in three random microscopic fields. This methodology has been previously described, and shown to be a useful functional assay for estimating EPC migration in response to various stimuli.
Platelet reactivity

Platelet reactivity was evaluated by turbidimetric light transmittance aggregation, performed in platelet-rich plasma with a platelet count adjusted to $250 \times 10^8$/mm$^3$. Platelets were stimulated with 0.5 mg/mL ($\sim 1.5$ mM) arachidonic acid (AA), 5 and 10 $\mu$m adenosine diphosphate (ADP). Aggregation was performed with a BioData PAP-4 platelet aggregometer (BioData Corp., Horsham, PA). Platelet-poor plasma was used as a reference. The extent of aggregation was defined as the maximal amount of light transmission reached ≤6 min after addition of the agonist.

Statistical analysis

Endothelial progenitor cell parameters (flow cytometry determined levels, number of EPC CFUs, and results of the functional assays) were non-normally distributed (as determined by the Shapiro–Wilk normality test). Therefore, EPC data are presented as median (25–75th percentile), and comparisons between the two groups were performed using Wilcoxon matched-pairs signed-rank test, two-tailed test (except for the MTT and migration assays, performed in subgroups and compared by Wilcoxon non-matched test). Other parameters in the study—platelet reactivity tests and clinical variables—were normally distributed and, therefore, they are presented as mean ± SD. Comparisons of the continuous normally distributed variables were performed by paired Student’s $t$-tests (two-tailed). Categorical variables were compared using chi-square tests. Analyses were performed using SPSS version 15 statistical software (SPSS Inc., Chicago, IL), and $P < 0.05$ was considered statistically significant.

Results

The study group included 30 patients who suffered a stent thrombosis event an average of 16.9 ± 10 months following the index procedure (13 late and 17 very late stent thrombosis). The stent thrombosis episode presented as an ACS in all cases (60% ST elevation MI, 30% non-ST elevation MI, and 10% unstable angina). Anti-platelet treatment just prior to the stent thrombosis episode consisted of aspirin and clopidogrel in 10 patients (33.3%), aspirin alone in 14 patients (46.7%), and no anti-platelet treatment in 6 patients (20%, among them one patient discontinued anti-platelet treatment 3 days before the stent thrombosis episode). Testing for the current study was performed 10.4 ± 9 months following the stent thrombosis episode. To these 30 patients, 30 control patients who underwent PCI with stenting and did not develop stent thrombosis were matched. In the control group testing for the current study was performed 14.1 ± 6 months following the PCI.

Clinical characteristics and current medications were similar among the two groups (Table 1). It should be noted that prior MI rates—defined as occurring before the index procedure—were similar among the two groups. However, when taking into account the clinical presentation of the stent thrombosis episode in the stent thrombosis group, 90% of the patients in this group had an MI occurring before the current testing, compared with 60% in the control group ($P = 0.01$). Aspirin was taken at a dose of 100 mg daily, except for two patients in each group who took 75 mg daily. Clopidogrel was taken at a dose of 75 mg daily, except for five patients in the stent thrombosis group who received 150 mg daily. Procedural characteristics and indication for the index procedure were similar among the two groups (Table 2). It should be noted that despite our matching attempt the rate of DES use (at least one) was not identical among the two groups (80% and 73.3% in the stent thrombosis and control groups).

Comparison of endothelial progenitor cells among the two groups

Figure 2 depicts the proportion of PMNCs that co-expressed VEGFR-2 and CD133, or VEGFR-2 and CD34, as determined by flow cytometry. Patients with stent thrombosis had a lower proportion of cells that were VEGFR-2+CD133+ and a lower proportion of VEGFR-2+CD34+ cells than their control counterparts, reflecting lower levels of circulating EPCs [VEGFR-2+CD133+: 0.18% (0.03–0.41%) vs. 0.47% (0.16–0.66%), $P = 0.01$; VEGFR-2+CD34+: 0.32% (0.22–0.70%) vs. 0.66% (0.24–1.1%), respectively, $P = 0.03$]. The median number of EPC CFUs following 7 days of culture was also lower among patients with a history of stent thrombosis compared with the control patients [3.9% (3.2–5.5%) vs. 8.3% (6.5–13.4%) colonies per well, respectively, $P < 0.0001$, Figure 3]. Figure 4 displays representative EPC colonies stained with antibodies directed against CD31, Tie-2, and VEGFR-2 (to confirm endothelial cell lineage). Functional aspects of the cultured cells were tested by the MTT and migration assays (Figure 5). Viability of the cells (MTT assay) was lower among patients with a history of stent thrombosis compared with the control group (panel 5A). Although the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical characteristics and current medications</th>
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<tr>
<td></td>
<td>Stent thrombosis (n = 30)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59.6 ± 10</td>
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<tr>
<td>Men (%)</td>
<td>28 (93.3)</td>
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<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>27.7 ± 4</td>
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<tr>
<td>Diabetes mellitus (%)</td>
<td>10 (33.3)</td>
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<tr>
<td>Hypertension (%)</td>
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<td>Current smoker (%)</td>
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<tr>
<td>Family history of CAD (%)</td>
<td>15 (50)</td>
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<tr>
<td>Prior myocardial infarction* (%)</td>
<td>20 (67.7)</td>
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<tr>
<td>Prior CABG (%)</td>
<td>6 (20)</td>
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<tr>
<td>Current medications</td>
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<tr>
<td>Aspirin (%)</td>
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<td>Clopidogrel (%)</td>
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<td>Statins (%)</td>
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<td>ACE inhibitors/ARBs (%)</td>
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<tr>
<td>Beta blockers (%)</td>
<td>22 (73.3)</td>
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<tr>
<td>Calcium channel blockers (%)</td>
<td>3 (10)</td>
</tr>
<tr>
<td>Proton pump inhibitors (%)</td>
<td>8 (26.7)</td>
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*Prior myocardial infarction before the index procedure (in the stent thrombosis group before the stent thrombosis presentation).
migratory capacity also appeared to be numerically lower in the stent thrombosis group, the differences were not statistically significant (panel 5B).

Analysis of the subgroup of patients who received some antiplatelet treatment prior to the stent thrombosis episode (n = 24) vs. their matched controls (n = 24) revealed similar results to the whole group [VEGFR-2+CD133+ cells: 0.20% (0.04–0.42%) vs. 0.49% (0.20–0.63%), P = 0.008; VEGFR-2+CD34+ cells: 0.44% (0.25–0.97%) vs. 0.72% (0.42–1.15%), P = 0.04; EPC CFUs: 4.3% (3.3–5.3%) vs. 8.3% (7.5–12.8%) colonies/well, respectively, P < 0.0001].

Table 2  Procedural characteristics (index procedure)

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<tr>
<th></th>
<th>Stent thrombosis (n = 30)</th>
<th>Control group (n = 30)</th>
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<tbody>
<tr>
<td>Indication for index procedure</td>
<td></td>
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<tr>
<td>ST elevation MI (%)</td>
<td>18 (60)</td>
<td>15 (50)</td>
</tr>
<tr>
<td>Non-ST elevation MI (%)</td>
<td>2 (6.7)</td>
<td>3 (10)</td>
</tr>
<tr>
<td>Stable/unstable angina (%)</td>
<td>10 (33.3)</td>
<td>12 (40)</td>
</tr>
<tr>
<td>Coronary vessel treateda</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD (%)</td>
<td>17 (56.7)</td>
<td>15 (50)</td>
</tr>
<tr>
<td>LCX (%)</td>
<td>7 (23.3)</td>
<td>6 (20)</td>
</tr>
<tr>
<td>RCA (%)</td>
<td>8 (26.7)</td>
<td>11 (36.7)</td>
</tr>
<tr>
<td>Multi-vessel disease (%)</td>
<td>21 (70)</td>
<td>18 (60)</td>
</tr>
<tr>
<td>Drug-eluting stent (%)</td>
<td>24 (80)</td>
<td>22 (73.3)</td>
</tr>
<tr>
<td>Total stent length (mm)</td>
<td>21.6 ± 8</td>
<td>20.3 ± 7</td>
</tr>
<tr>
<td>Minimal stent diameter (mm)</td>
<td>3.1 ± 0.7</td>
<td>3.1 ± 0.5</td>
</tr>
<tr>
<td>Number of stents/patient</td>
<td>1.4 ± 0.6</td>
<td>1.5 ± 0.7</td>
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*Two patients in each group had percutaneous coronary intervention performed in two coronary vessels during the index procedure.
MI, myocardial infarction; LAD, left anterior descending; LCX, left circumflex; RCA, right coronary artery.

Figure 2  Proportion of peripheral mononuclear cells that co-expressed vascular endothelial growth factor receptor 2 and CD133, or vascular endothelial growth factor receptor 2 and CD34, among the stent thrombosis group (n = 30) vs. control group (n = 30). Patients who experienced stent thrombosis had a lower proportion of cells that were VEGFR-2+CD133+ and a lower proportion of VEGFR-2+CD34+ cells than their control counterparts, reflecting lower levels of circulating endothelial progenitor cells. Results expressed as median (25–75th percentile).

Figure 3  Quantification of endothelial progenitor cell colony forming units following 7 days of culture among the stent thrombosis group (n = 30) vs. control group (n = 30). The mean number of CFUs per well was lower among the patients who experienced stent thrombosis. Representative EPC colonies are depicted below the respective bars (magnification ×20). Results expressed as median (25–75th percentile).

**Comparison of platelet reactivity**

Table 3 presents platelet aggregation in response to ADP and AA among the two groups. Patients in the stent thrombosis group had higher platelet aggregation in response to 5 μM ADP than the control group (40.5 ± 17% vs. 31.8 ± 13%, P = 0.02), and tended to have higher aggregation in response to 10 μM ADP (47.8 ± 16% vs. 40.5 ± 15%, P = 0.06). Aggregation in response to AA did not differ significantly between the two groups.

**Discussion**

To our knowledge this is the first study to assess the level and function of EPCs in patients who experienced late stent thrombosis. We have found that compared with a matched control group of patients who underwent stenting and did not develop stent thrombosis, patients who experienced late stent thrombosis had a lower level of circulating EPCs (mononuclear cells that co-expressed VEGFR-2 and either CD133 or CD34) and impaired functional properties of the EPCs after 7 days of culture. In addition, patients who experienced stent thrombosis tended to have higher platelet reactivity, assessed by aggregation in response to ADP.

The mechanisms contributing to the development of late and very late stent thrombosis have not been fully delineated. Stent
thrombosis appears to be a complex process involving the interplay of several stent-related and patient-related factors predisposing to increased thrombogenicity. Recently Cook et al. have demonstrated that very late DES stent thrombosis is associated with histopathological signs of inflammation and intravascular ultrasound evidence of vessel remodeling. Studies of autopsy-proven stent thrombosis have indicated that delayed arterial healing and incomplete endothelialization, as well as hypersensitivity reactions and inflammation are the main pathological processes involved in stent thrombosis. Lack of complete and uniform stent endothelialization may lead to incomplete neointimal coverage, which has been shown to be associated with thrombus formation in stented segments. Since EPCs are presumed to have an important role in the process of re-endothelialization following stenting, a reduction in the level and/or function of EPCs, as observed in our study group, may be an important contributing factor to the development of stent thrombosis. It should be noted that DES specifically (in contrast to BMS) have been shown to have attenuating effects on EPC level and function. In vitro, rapamycin inhibits proliferation, migration, and differentiation of human EPCs. Accordingly, implantation of sirolimus-eluting stents has been reported to suppress mobilization of CD34+ mononuclear cells and their differentiation to endothelium-like cells, compared with BMS.

The definition of EPCs has been controversial, and various methodologies have been used to characterize these cells. Circulating EPCs have been characterized by the expression of several surface antigens—CD133, CD34, and/or VEGFR-2 (17-21). We, therefore, quantified all three antigens and presented data on their co-expression (VEGFR-2 and CD133, or VEGFR-2 and CD34). We also employed several methodologies to assess functional aspects of the cells after growth in culture conditions—colony forming capacity, viability, and migration capacity. The majority of the quantitative and functional assays demonstrated a reduction in the level and function of EPCs in the patients who experienced stent thrombosis, most importantly the dysfunction in EPC CFUs supported by reduced cell viability.

The two groups in our study also differed in platelet reactivity, as assessed by aggregation in response to ADP. This finding is in line with previous case-control studies that compared patients who experienced stent thrombosis vs. a control group, and found increased platelet reactivity and reduced response to anti-platelet drugs among the patients with history of stent thrombosis. Our study may have underestimated the differences in platelet reactivity between the two groups, since five patients in the stent thrombosis group received 150 mg of clopidogrel when tested, thus further inhibiting ADP-induced platelet aggregation (compared with 75 mg) and possibly narrowing the gap between the two groups. It should be noted that platelets have been shown to play an important role in the process of recruitment and homing of EPCs to the site of arterial injury in the acute phase following the injury. Whether the interaction between platelets and EPCs also has physiologic importance in the ‘chronic’ long-term phase after arterial injury and repair remains to be established.

Our study has several limitations. First, it is a case-control study with the limitations inherent to retrospective identification of subjects in the groups examined. The case-control design was chosen because of the rarity of stent thrombosis occurrence. It should be

![Figure 4](https://example.com/figure4.png)

**Figure 4** Representative EPC colonies after 7 days of culture stained with antibodies directed against CD31, Tie-2, and vascular endothelial growth factor receptor 2 to confirm endothelial cell lineage (magnification ×20).
emphasized that the two groups in our study were matched for the important relevant factors that have been shown to affect EPC level and function: age, gender, diabetes, statin treatment, and use of DES. The two groups were also well-balanced in the majority of other clinical, pharmacological, and procedural variables. Second, EPC profile was assessed months after the stent thrombosis episode or index procedure, in order to study these cells in the chronic ‘quiescent’ phase and avoid the temporary acute changes noted previously following PCI and AMI.\cite{26,27,38}

In the case of the control group this period also allowed follow-up to preclude stent thrombosis development. Third, since stent thrombosis is associated with a relatively high mortality rate,\cite{1,2,3,4} there may have been bias introduced by the selection and identification of living patients to the stent thrombosis group (‘survival bias’). Fourth, the MTT and migration assays were performed in only 15 patients in each group. Finally, anti-platelet treatment just prior to the stent thrombosis episode was heterogeneous, ranging from dual anti-platelet treatment to no anti-platelet treatment. Discontinuation of anti-platelet treatment has been shown to be a strong predictor of stent thrombosis development.\cite{12} It is likely, however, that several complex processes contribute to stent thrombosis development, including platelet reactivity status and stent endothelialization status; the latter potentially dependent on EPC level and function.

Despite the limitations, the current study has important potential clinical implications. If, indeed, EPCs have an essential role in stent endothelialization, and attenuated EPC level or function contributes to stent thrombosis development, via impaired endothelialization, then augmenting EPC status may reduce the risk of stent thrombosis. In this regard, the development of the ‘EPC capture stent’, coated with an antibody directed against CD34, is a promising step.\cite{41,42} In preliminary human studies use of this stent, aimed at ‘capturing’ circulating EPCs, has been shown to be feasible and safe.\cite{41,42} Another stent coated with cyclic Arg–Gly–Asp peptide has been shown in porcine model to enhance endothelial coverage, possibly by increased early recruitment of EPCs.\cite{43} Other approaches may be pharmacological EPC augmentation after PCI, by drugs such as high-dose statins\cite{22,31,38} or erythropoietin,\cite{44} or by cell-based vascular therapy employing EPCs (autologous or other).\cite{24} Further research is required to validate our findings of attenuated EPC level and function in patients who experienced stent thrombosis, and to explore methods to promote EPCs and arterial healing after coronary stenting.

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