Migratory activity of circulating progenitor cells and serum SDF-1α predict adverse events in patients with myocardial infarction

Orazio Fortunato1, Gaia Spinetti1, Claudia Specchia2, Elisa Cangiano3, Marco Valgimigli3, and Paolo Madeddu4*

1IRCCS MultiMedica, Milan, Italy; 2Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy; 3University of Ferrara, Ferrara, Italy; and 4Experimental Cardiovascular Medicine, Bristol Heart Institute, School of Clinical Sciences, University of Bristol, Level 7, Bristol Royal Infirmary, Upper Maudlin Street, Bristol BS2 8HW, UK

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1. Introduction

Current biomarkers for the assessment of secondary risk after an acute myocardial infarction (AMI) reflect the underlying inflammatory background, as in case of C-reactive protein,1 or the severity of myocardial damage/dysfunction, as for cardiac troponin,2 creatine phosphokinase,3 and B-type natriuretic peptide.4 Little attention has been paid so far to markers of the early reparative response to myocardial ischaemia.9,10 Furthermore, the expression of CXCR4, the receptor for stromal cell-derived factor 1α (SDF-1α), identifies a therapeutically active population of bone marrow-derived MNCs.14

PC mobilization and homing are driven by cytokines released from the ischaemic tissue, including vascular endothelial growth factor (VEGF)15 and SDF-1α.16 SDF-1α is up-regulated in the myocardium after ischaemia17 and modulates the survival, proliferation, and differentiation of stem cells.18 Furthermore, the serum levels of SDF-1α correlate with CD31/CD34pos and kinase insert domain receptor (KDR)/CD34pos PC counts in patients with AMI.19

Importantly, pro-angiogenic PCs have been recently considered for their value as clinical biomarkers. In longitudinal studies, a reduced CD34/KDRpos PC count has been shown to independently predict

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Aims

Following acute myocardial infarction (AMI), pro-angiogenic progenitor cells (PCs) are released from the bone marrow into the circulation and home to the ischaemic site attracted by a chemokine gradient. It is unknown if components of this early homeostatic response might help forecast the long-term clinical outcome. This study investigates if the number and migratory activity of circulating PCs predict adverse events in patients with AMI (clinical trial: NCT01271309).

Methods and results

Basal counts and in vitro migratory activity of CD34/CD45/CD133/CXCR4 PCs and serum cytokine levels were assessed during the first 5 days after AMI in a consecutive series of 172 patients. Clinical outcomes of the study were death, repeat AMI, and new-onset heart failure at a 1-year follow-up. The association between PC counts and cytokine levels with the incidence of clinical outcomes was assessed by multivariable regression models. AMI patients who underwent an event showed higher serum stromal cell-derived factor 1α (SDF-1α) levels and reduced spontaneous motility of PCs in an in vitro migration assay when compared with event-free subjects. After adjustment for age, gender, the presence or absence of ST elevation, or diabetes, the percentage of PCs non-migrated towards vehicle or SDF-1α were both independent predictors of death or repeat AMI and new-onset heart failure (odds ratio [OR] = 2, P = 0.015 and OR = 1.90, P = 0.018, respectively). Moreover, serum SDF-1α levels predict adverse events (OR = 3.8, P = 0.007).

Conclusion

Biomarkers reflecting the migratory activity of circulating PCs may aid the assessment of secondary risk in AMI patients.

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the risk of adverse events in patients with cardiovascular disease.\textsuperscript{20,21} Moreover, reduced circulating levels of CD34/\textsuperscript{KDR}pos PCs were independently associated with atherosclerotic disease progression.\textsuperscript{22} In contrast, a recent study in mice showed that haematopoietic progenitors liberated from the bone marrow following AMI could seed the spleen, yielding a sustained production of atherogenic monocytes.\textsuperscript{23} To the best of our knowledge, no data are available on the utility of PC assessment for forecasting secondary risk in AMI patients. A small-size clinical study from Leone et al.\textsuperscript{24} showed that circulating CD34pos PC counts are an independent predictor of global and regional improvement of ventricular function in AMI patients at a 1-year follow-up. However, the study was not designed to provide information on clinical outcomes. Furthermore, it is not known if functional measures of circulating pro-angiogenic PCs could help in risk stratification.

We hypothesize that quantitative and functional deficits of circulating PCs might implicate a reduced reparative response and consequently, infer a higher threat for later adverse events in patients with AMI. The primary aim of this study was therefore to determine whether abundance and migratory activity of antigenically defined PCs, namely CD34/CD45/CD133/CXCR4pos cells, predict all-cause mortality, re-infarction, or new-onset heart failure in patients with AMI. The association between cytokines related to PC migration and clinical outcomes was also evaluated as secondary aim of the study.

Figure 1  Flow cytometry characterization of circulating PCs and assessment of migratory activity \textit{in vitro}. (A) Representative scattergrams and gating strategy used to detect different PC populations within the CD45\textsuperscript{dim} MNC fraction. (B) Box plots showing the percentage of CD45\textsuperscript{dim}CD34, CD45\textsuperscript{dim}CD133, and CD45\textsuperscript{dim}CD34/CD133/CXCR4-positive MNCs in PB of patients with AMI and controls. (C) Dispersion graphs showing the number of migrated cells towards SDF-1\textsubscript{α} or vehicle. (D) Box plots illustrating the migration index, i.e. the ratio between the number of SDF-1\textsubscript{α}-migrated and vehicle-migrated MNCs of patients with AMI and controls. Data in (B and D) are represented as boxes bordered at the 25th and the 75th percentiles of the predictor variable and a median line at the 50th percentile. Whiskers extend from the box to the upper and lower adjacent values and are capped with an adjacent line.
Methods

See Supplementary material online for expanded methods.

Study design

An observational prospective study was designed to assess the association between basal PC counts and migratory activity (primary aim) with adverse events, including all-cause mortality, repeat AMI, or new-onset heart failure at the 1-year follow-up in a consecutive cohort of 172 patients admitted to the Ferrara University Hospital with AMI (142 with ST segment elevation AMI [STEMI] and 30 non-STEMI [NSTEMI]). Cytokines known to be implicated in PC recruitment were also analysed (secondary aim). Since normal criteria were not available for PC number and migratory ability in our population, PC counts and cytokines were also determined in a group of randomly selected controls referred to the same institution with non-ischaemic arrhythmias or electrophysiology studies (C, n = 20) or stable angina pectoris (SA, n = 12). The study adhered to the principles of the Declaration of Helsinki, approved by the Ethnic Committee of the University of Ferrara, and registered as a clinical trial (NCT01271309).

Table 1 Characteristics of enrolled subjects

<table>
<thead>
<tr>
<th></th>
<th>AMI (N = 172)</th>
<th>C (N = 20)</th>
<th>P-value*</th>
<th>SA (N = 12)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male, %)</td>
<td>75.0</td>
<td>65.0</td>
<td>0.335</td>
<td>66.7</td>
<td>0.522</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.7 ± 11.1</td>
<td>69.4 ± 7.1</td>
<td>0.152</td>
<td>65.3 ± 9.1</td>
<td>0.897</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>15.7</td>
<td>17.7</td>
<td>0.834</td>
<td>20.0</td>
<td>0.718</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.0 ± 4.2</td>
<td>27.4 ± 2.3</td>
<td>0.763</td>
<td>28.5 ± 4.2</td>
<td>0.341</td>
</tr>
<tr>
<td>Glycaemia (mg/dL)</td>
<td>147.8 ± 54.8</td>
<td>111.8 ± 22.4</td>
<td>0.005</td>
<td>110.4 ± 27.3</td>
<td>0.020</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>14.0 ± 1.4</td>
<td>13.6 ± 1.5</td>
<td>0.239</td>
<td>14.1 ± 1.4</td>
<td>0.859</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.0 ± 0.4</td>
<td>1.1 ± 0.2</td>
<td>0.384</td>
<td>0.9 ± 0.2</td>
<td>0.560</td>
</tr>
<tr>
<td>WBC count (×10³/mL)</td>
<td>12.8 ± 4.2</td>
<td>11.1 ± 3.7</td>
<td>0.089</td>
<td>9.0 ± 2.5</td>
<td>0.003</td>
</tr>
<tr>
<td>Troponin I (ng/L)</td>
<td>5.72 ± 7.82</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CPK-MB (ng/mL)</td>
<td>36.51 ± 59.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD.
BMI, body mass index; AMI, acute myocardial infarction; C, controls; SA, stable angina; WBC, white blood cells; CPK-MB, creatine phosphokinase-muscle band.

*AMI vs. C.
**AMI vs. SA.

Figure 2  SDF-1α induces PC enrichment in the migration assay. (A) Schematic diagram showing the methodology of the migration assay: migrated and non-migrated MNCs were analysed for the expression of CD34, CD133, CD45, and CXCR4 markers by flow cytometry. (B–D) Graphs illustrating the PC enrichment induced by the stimulation of migration with SDF-1α: data are fold change relative to spontaneous migration in the presence of vehicle with regard to CD34 (B), CD133 (C), and CD34/CD45/CD133/CXCR4-positive cells (D). Boxes are bordered at the 25th and 75th percentiles of the predictor variable and a median line at the 50th percentile. Whiskers extend from the box to the upper and lower adjacent values and are capped with an adjacent line.
2.2 Flow cytometry characterization of circulating PCs

Peripheral blood (PB) MNCs were separated on a Ficoll (Amersham Biosciences) gradient. The antigenic profile of flow cytometry was determined in a fluorescence-activated cell sorting (FACS) Canto flow cytometer using the FACS Diva software [both Becton, Dickinson and Company (BD)] according to the ISHAGE guidelines. (Figure 1A) using anti-CD34-PE, anti-CD45-FITC, anti-CXCR4-PE-Cy5 (all from BD), and anti-CD133/2-APC (Miltenyi) antibodies.

2.3 Cell migration

MNC migration was studied in the transwell chamber assay (Corning, pore size: 3 μm). SDF-1α (100 ng/mL) or the SDF-1α vehicle (EBM-2 medium containing 0.1% bovine serum albumin) was added to the lower compartment of the migration chamber and migrated MNCs counted after 18 h. Moreover, non-migrated and migrated cells were analysed by FACS.

2.4 Cytokine measurements

Serum levels of SDF-1α, VEGF-A, platelet-derived growth factor-BB (PDGF-BB), basic-fibroblast growth factor (b-FGF), and insulin growth factor (IGF) were measured using commercially available ELISA kits (R&D).

2.5 Statistical analysis

The Student’s t-test and the Wilcoxon–Mann–Whitney test were used to compare symmetric and skewed continuous variables distributions between cases and control groups, respectively. Categorical variables were compared across groups using the chi² test. A combined event was defined as the occurrence of death or repeat AMI or new-onset heart failure during the 1-year follow-up. To evaluate the association between known risk factors for combined event occurrence (i.e. age, gender, ST elevation, and diabetes) and another including known prognostic factors for combined event occurrence (i.e. age, gender, ST elevation, and diabetes) and another including known prognostic factors plus the biomarker, as independent predictors of risk of events. We used the area under the receiver operating characteristic curve (AUC) to determine the discriminatory capability of the two models. AUCs were compared using a non-parametric approach.

The sole reliance on the AUC to assess whether a new marker offers an additional value in risk estimation is often not useful. Therefore, the increased discriminative value of the studied biomarker was further examined using the integrated discrimination improvement (IDI) index, described by Pencina et al. This index is based on the difference between two models in the individual estimated risks. An increased risk for an observed case and a decreased risk for an observed non-case implies better prediction ability, whereas the opposite implies worse prediction ability. IDI considers the improvement from one model to the other in average sensitivity, that is the integral of sensitivity over all possible cut-off values, offset by any average decrease in specificity.

Time to the combined event during the 1-year follow-up was defined as the time from AMI diagnosis to combined event occurrence (the date of death or the date of the earliest event between re-AMI and new-onset heart failure was considered in the time-to-event analysis). Patients who were free of events at the end of the 1-year follow-up were censored at that time. The cumulative incidence was estimated using the Kaplan–Meier method. The log-rank test was used to evaluate the differences in incidence between subgroup of patients.

Continuous data are expressed as mean ± SD. All reported P-values are two-sided. A P-value of < 0.05 was considered significant.

3. Results

3.1 Clinical characteristics

Baseline characteristics of patients are summarized in Table 1. No difference among the groups was detected with regard to age, gender, and the presence of diabetes. When compared with controls, AMI patients showed significantly higher fasting glycaemia (P = 0.005 vs. C and P = 0.02 vs. SA) and leucocyte counts (P = 0.003 vs. SA).

Table 1. Baseline characteristics of patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>AMI (n=50)</th>
<th>C (n=50)</th>
<th>SA (n=50)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64 ± 11</td>
<td>63 ± 10</td>
<td>62 ± 10</td>
<td></td>
</tr>
<tr>
<td>Gender (male)</td>
<td>35</td>
<td>25</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Diabetes (yes)</td>
<td>20</td>
<td>15</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Smoking (yes)</td>
<td>15</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

3.2 AMI increases the number of circulating PCs

Single-stained CD34 and CD133 MNCs were more frequent in AMI (P = 0.067 and 0.011 vs. C, respectively); however, no difference was observed when comparing AMI with SA (Figure 1A and B). Likewise, the abundance of pro-angiogenic PCs defined as CD45dimCD34/CD133/CXCR4-positive MNCs was increased in AMI patients,

Figure 3 Comparison of predictor distribution in AMI patients with or without events. (A–F) Box plots show the distribution of CD34/CD45/CD133/CXCR4 cell abundance (A), the percentage of CD34/CD45/CD133/CXCR4 cells non-migrated towards vehicle or SDF-1α (B and C, respectively), the percentage of CD34/CD45/CD133/CXCR4 cells migrated towards vehicle or SDF-1α (D and E, respectively), and the serum SDF-1α levels (F) in the event-free and event-positive groups. Boxes are bordered at the 25th and 75th percentiles of the predictor variable and a median line at the 50th percentile. Whiskers extend from the box to the upper and lower adjacent values and are capped with an adjacent line. Outliers are displayed as markers.
compared with controls \((P = 0.08 \text{ vs. } C \text{ and } P = 0.05 \text{ vs. } SA)\). No group differences were observed with regard to CXCR4 fluorescence intensity in this MNC fraction (Supplementary material online, Figure S1).

### 3.3 MNCs from AMI patients show impaired directed migratory activity

We assessed the directed migratory activity of MNCs using SDF-1α as a stimulus and vehicle as a control of spontaneous cell motility. Individual values of migrated cells are shown in Figure 1C. We also calculated a migration index by dividing the number of cells that migrate towards SDF-1α by the number of cells that migrate spontaneously. The index was significantly reduced in AMI patients when compared with C and SA controls \((P = 0.0096 \text{ and } 0.0053, \text{ respectively, Figure 1D})\).

In addition, migrated and non-migrated cells were separately analysed to compute the percentage of antigenically defined PCs in each fraction (Figure 2A). When considering MNCs from control subjects (C), we found that SDF-1α significantly increases the relative abundance of migrated CD34, CD133, and CD34/CD45/CD133/CXCR4-positive cells when compared with the vehicle \((P = 0.009, 0.007, \text{ and } 0.049, \text{ respectively})\). In contrast, SDF-1α -induced migration was reduced in both the AMI and SA groups (Figure 2B–D).

### 3.4 Serum cytokine levels

We measured the PB levels of different cytokines and growth factors that are implicated in PC mobilization. Results indicate significantly lower levels of SDF-1α and b-FGF in AMI patients when compared with C \((P = 0.024 \text{ and } < 0.001, \text{ respectively})\), whereas no difference was detected with regard to IGF, VEGF-A, and PDGF-BB (Supplementary material online, Table S1).

### 3.5 Clinical outcomes

During the 1-year follow-up, a total number of 27 single events were registered in the AMI group, including eight deaths \((4.7\%)\), four repeat AMI \((2.3\%)\), seven new-onset heart failure \((4.1\%)\), one death after new-onset heart failure \((0.6\%)\), and three with both repeat AMI and new-onset heart failure \((1.7\%)\). Since single-event incidence was underpowered to reach statistically valuable conclusions, we considered combined events (death or repeat AMI or new-onset heart failure) in the

| Table 2 Cell counts in patients with or without combined events |
|------------------|--------------|------------------|
| Cell counts                  | Event (N = 23) | Event-free (N = 149) |
| %CD34/CD45/CD133/CXCR4 cells | Median | Inter-quartile range | Median | Inter-quartile range | P-value |
| %CD34/CD45/CD133/CXCR4 cells migrated towards vehicle | 26.23 | 18.9–38.57 | 25.60 | 15.05–38.58 | 0.614 |
| %CD34/CD45/CD133/CXCR4 cells migrated towards SDF-1α | 26.40 | 17.65–37.1 | 25.00 | 15.05–38.58 | 0.614 |
| %CD34/CD45/CD133/CXCR4 cells non-migrated towards vehicle | 23.75 | 17.86–45.45 | 23.79 | 9.57–26.53 | 0.012 |
| %CD34/CD45/CD133/CXCR4 cells non-migrated towards SDF-1α | 22.12 | 15.06–30.81 | 16.53 | 8.3–27.26 | 0.072 |

| Table 3 Univariate and multivariable analyses of experimental parameters and the risk of a combined event |
|------------------|--------------|------------------|
| Unit                  | Univariate OR | 95% CI          | P-value | Multivariable OR | 95% CI          | P-value |
| %CD34/CD45/CD133/CXCR4 cells | 1 U increase in log2 | 1.27 | 0.86–1.88 | 0.232 | 1.21 | 0.78–1.85 | 0.393 |
| %CD34/CD45/CD133/CXCR4 cells migrated towards vehicle | 1 U increase in log2 | 1.46 | 0.90–2.37 | 0.121 | 1.64 | 0.92–2.95 | 0.096 |
| %CD34/CD45/CD133/CXCR4 cells non-migrated towards vehicle | 1 U increase in log2 | 1.89 | 1.10–3.26 | 0.021 | 2.00 | 1.15–3.49 | 0.015 |
| %CD34/CD45/CD133/CXCR4 cells migrated towards SDF-1α | 1 U increase in log2 | 1.57 | 0.77–2.02 | 0.365 | 1.55 | 0.86–2.77 | 0.142 |
| %CD34/CD45/CD133/CXCR4 cells non-migrated towards SDF-1α | 1 U increase in log2 | 1.55 | 0.96–2.51 | 0.072 | 1.90 | 1.12–3.22 | 0.018 |
| SDF-1α (pg/mL) | For 1000 U increase | 3.40 | 1.60–9.99 | 0.003 | 3.83 | 1.44–10.19 | 0.007 |
| VEGF-A (pg/mL) | Presence vs. absence | 1.07 | 0.41–2.84 | 0.887 | 1.28 | 0.46–3.60 | 0.637 |
| b-FGF (pg/mL) | Presence vs. absence | 1.83 | 0.45–7.39 | 0.395 | 1.14 | 0.25–5.22 | 0.868 |
| IGF (pg/mL) | For 100 U increase | 0.40 | 0.07–2.35 | 0.311 | 0.49 | 0.08–3.14 | 0.45 |
| PDGF-BB (pg/mL) | Presence vs. absence | 0.62 | 0.21–1.79 | 0.376 | 0.70 | 0.22–2.20 | 0.54 |
| Troponin I (ng/L) | 1 U increase in log2 | 1.26 | 0.99–1.59 | 0.052 | 1.32 | 1.02–1.72 | 0.035 |
| CPK-MB (ng/mL) | 1 U increase in log2 | 1.11 | 0.88–1.41 | 0.373 | 1.24 | 0.94–1.63 | 0.12 |

*Adjusted by age, sex, the presence of ST elevation, and diabetes.
association analysis with cell counts, migration-associated variables, and cytokines at baseline.

We categorized the AMI population into two groups based on the presence or the absence of a combined event. As expected, we observed that the age of patients with an event was significantly higher than that of event-free subjects (73.2 ± 11.7 vs. 64.6 ± 10.6 years, \( P = 0.0004 \)). A combined event incidence was not different when considering gender distribution (12.4% males and 16.3% females, \( P = 0.5 \)), the presence or absence of diabetes (14.8 vs. 13.1%, \( P = 0.81 \)), or ST elevation (12.7 vs. 16.7%, \( P = 0.56 \)). Moreover, the mean time of blood collection from AMI was not associated with the occurrence of a combined event (3.73 ± 1.32 for patients with an event vs. 3.32 ± 1.32 days for event-free subjects, \( P = 0.184 \)). We found no difference between the two subgroups with regard to PC abundance (Figure 3A and Table 2).

We also compared event-positive and event-free patients with regard to indexes of migration, including the percentage of PCs remaining in the upper chamber (non-migrated PCs) or relocating to the lower chamber (migrated PCs) following exposure to SDF-1α or vehicle. Of the four variables considered, only the percentage of PCs non-migrated towards vehicle was significantly higher in patients with an event when compared with event-free subjects (\( P = 0.012 \)) (Figure 3B and Table 2), whereas no group difference was found for the percentage of PCs non-migrated or migrated towards SDF-1α (Figure 3C–E and Table 2). Furthermore, patients with an event have significantly higher serum levels of SDF-1α (2237 ± 216 vs. 1595 ± 59 pg/mL, \( P = 0.0002 \)) (Figure 3F).

Cell counts in event-positive and event-free patients are reported in Table 2. Separate analyses of these parameters in STEMI and NSTEMI patients are reported in Supplementary material online, Table S2.

### 3.6 Univariate analysis of cell and cytokine variables as predictors of event

Univariate analysis of experimental parameters confirms that the abundance of circulating PCs is not a predictor of adverse effects in AMI patients (Table 3). In contrast, among indices of migratory activity, the percentage of PCs non-migrated towards vehicle was confirmed to be a predictor of a combined event (\( \text{OR} = 1.89, 95\% \text{ CI} 1.10–3.26, \ P = 0.021 \)). Given that biomarkers have been expressed in a log2 scale, this means that, for a doubling of these cell counts, the odds of a combined event increase by 89%.

Among cytokines, SDF-1α levels were positively associated with the risk of a combined event (\( \text{OR} = 3.40, 95\% \text{ CI} 1.60–9.99, \ P = 0.003 \)) for each 1000 U increase in the cytokine level.

### 3.7 Multivariable analysis of cell and cytokine variables as predictors of events

Multiple logistic regression, considering age, gender, the presence of STEMI (which is associated with larger infarct and thus potentially higher cell mobilization), or diabetes as factors known to increase the incidence of adverse cardiovascular events, \( \text{OR} = 1.89, 95\% \text{ CI} 1.10–3.26, \ P = 0.021 \), revealed that the percentage of PCs non-migrated towards vehicle and SDF-1α are predictors of death or repeat AMI or heart failure (towards vehicle \( \text{OR} = 2, 95\% \text{ CI} 1.15–3.49, \ P = 0.015 \); towards SDF-1α \( \text{OR} = 1.90, 95\% \text{ CI} 1.12–3.22, \ P = 0.018 \)). Moreover, SDF-1α levels were predictive of a combined event (\( \text{OR} = 3.8, 95\% \text{ CI} 1.44–10.19, \ P = 0.007 \) for each 1000 U increase in the cytokine levels, Table 3).

Figure 4 illustrates the relationship between predictive variables at multiple regression analysis and the risk of a combined event. We confirmed a direct relationship between the risk of a combined event and levels of PCs non-migrated towards vehicle (Figure 4A) or SDF-1α (Figure 4B), as well as serum SDF-1α levels (Figure 4C).

Since troponin I and creatine phosphokinase-muscle band (CPK-MB) are acknowledged biomarkers of AMI, \( \text{OR} = 1.32, 95\% \text{ CI} 1.02–1.72, \ P = 0.035 \), Table 3). Furthermore, troponin I and CPK-MB were highly correlated (\( r = 0.91 \), \( P < 0.001 \)), but neither of them was associated with SDF-1α (\( r = 0.08, P = 0.3822 \); and \( r = 0.02, P = 0.8035 \), respectively).
The predictive value measured by AUC of a reference model including the known prognostic factors for combined events (i.e., age, gender, the presence of STEMI, or diabetes) was 0.724. The inclusion in the above-reference model of the variable ‘%CD34/CD45/CD133/CXCR4 cells non-migrated towards vehicle’ yielded a gain in the AUC of 0.053, with the difference being not statistically significant ($P = 0.090$). Inclusion of the variable ‘%CD34/CD45/CD133/CXCR4 cells non-migrated towards SDF-1α’ yielded a gain in the AUC of 0.048 ($P = 0.047$), while inclusion of SDF-1α yielded a gain in the AUC of 0.090 ($P = 0.092$).

The IDI was 0.136 [standard error (SE) = 0.043] for %CD34/CD45/CD133/CXCR4 cells non-migrated towards vehicle ($P = 0.002$), 0.121 (SE = 0.042) for %CD34/CD45/CD133/CXCR4 cells non-migrated towards SDF-1α ($P = 0.003$), and 0.097 (SE = 0.049) for SDF-1α ($P = 0.047$).

Taking into account the time to event, the cumulative incidence of combined events was estimated at 5.2, 9.3, 12.2, and 13.4% at 3, 6, 9, and 12 months, respectively (Figure 5A). No patient was lost to follow-up. Time-to-event analyses confirmed the associations suggested by the multivariable logistic models between the percentage of PCs non-migrated towards vehicle (log-rank $P$-value = 0.02) (Figure 5B), SDF-1α (log-rank $P = 0.07$) (Figure 5C), and serum SDF-1α (log-rank $P = 0.01$) (Figure 5D) and the risk of combined events. The effect of each predictive variable did not change when they were simultaneously included in a multivariable model (data not shown).

4. Discussion

Markers with the ability to forecast post-AMI complications are required to identify those patients who need special medical attention. This study newly investigates the predictive value of cellular biomarkers known to be implicated in the early reparative process. Results indicate that PC migratory activity and circulating SDF-1α levels independently predict the clinical outcome of patients with AMI.

Circulating PCs have been proposed to take part in cardiac healing through direct and indirect mechanisms. Hence, a reduced number of mobilized PCs may suggest a failed reparative response, eventually resulting in more complicated clinical outcomes. A previous report by Leone et al. showed a significant correlation between PCs and left ventricular remodelling at 1 year from AMI. In our study, however, a multivariable regression model using circulating PCs as a predictive variable failed to find any association with post-AMI complications. Data from animal and human studies indicate that chemo-attractant factors released by the ischaemic myocardium stimulate the recruitment and homing of pro-angiogenic PCs. We found that circulating PCs from AMI patients are insensitive to SDF-1α stimulation in an in vitro migration assay. This result is in accordance with the finding that PCs from patients with cardiovascular risk factors have an impaired migratory capacity. Hence, a reduced sensitivity of PCs to SDF-1α may mirror an impaired recruitment of healing cells to the infarcted heart, leading to poorer recovery and late complications. In our study, we tested different indices of migration, including the percentage of migrated and non-migrated PCs following stimulation with SDF-1α (indicative of directed migratory activity) or vehicle (indicative of spontaneous motility). We found that the percentage of non-migrated PCs is a predictor of adverse events at the 1-year follow-up. Although the PC fraction migrated in the lower chamber is generally used to assess directional motility, some migrated cells may be retained within the filter resulting in under-estimation of

**Figure 5** Cumulative incidence of combined events. Line graphs showing (A) cumulative incidence and (B–D) incidence in patients’ groups categorized according to (B) CD34/CD45/CD133/CXCR4 cells non-migrated towards vehicle (log-rank $P = 0.02$), (C) CD34/CD45/CD133/CXCR4 cells non-migrated towards SDF-1α (log-rank $P = 0.07$), and (D) serum SDF-1α levels (log-rank $P = 0.01$). Medians were used as the cut-off to categorize patients.
migration. Therefore, the assessment of non-migrated cells could represent a more accurate estimation of the functional deficit and hence, a better predictor of the clinical outcome. The fact that non-migrated PCs forecast adverse events independently of whether they are stimulated with plasma SDF-1 or vehicle suggests that spontaneous motility rather than chemokine-directed migration is relevant as a prognostic predictor. Moreover, we show that SDF-1α is higher in AMI patients with an adverse event than in event-free patients, and that non-migrated PCs and SDF-1α levels are predictors of adverse events independent of age, gender, the presence of STEMI, or diabetes.

We could not find any association between SDF-1α and cardiac enzymes or ST elevation, thus discounting the possibility that the higher incidence of adverse events associated with SDF-1α is attributable to larger infarcts. The pathophysiological link between SDF-1α and adverse outcomes remains obscure particularly in the light of the fact that this chemokine reportedly promotes reparative angiogenesis.38,39 On the other hand, a recent report indicates that SDF-1α/CXCR4 signalling exerts detrimental effects in a rat model of ischaemia.40 Moreover, SDF-1α could facilitate plaque formation41 and contribute to the pathogenesis of atherosclerosis and thrombotic events.42 The levels of PCs and cytokines generally peak between 3 and 5 days post-AMI.44–46 In line with recommendations from the ethical committee, we performed a single venepuncture within this timeframe rather than multiple collections to avoid additional unjustified stress to the patient. We verified that the time of blood collection is not different between event-positive and event-free patients. Furthermore, the time of blood collection is not a confounder of the association between predictors and the incidence of events. In fact, for each predictor, adjustment for the time of blood collection changes neither the ORs nor the significance in multivariate analysis. Hence, a single determination of the predictor represents a practical means to recognize patients at risk.

In conclusion, investigation of the migratory activity of antigenically defined circulating PCs and measurement of serum SDF-1α help identify AMI patients at high risk for late adverse events. Validation in a large cohort of patients is warranted before introduction of these biomarkers into clinical use.

5. Study limitations

The consistent use of primary PCI might account for the low number of adverse events observed in our cohort. As a consequence, revascularization was not a confounding factor in our study. On the other hand, we had to consider the value of predictors towards combined end-points including all-cause mortality, re-infarction, or new-onset heart failure. A larger cohort is required to assess the predictor value for each specific adverse event. Another relevant open question pertains to the capacity of the studied predictors to anticipate late adverse effects, when other factors could affect the outcome and thus, confuse the associations. Nevertheless, predictors of early events are of indisputable importance in establishing life-saving care and treatment.

Supplementary material

Supplementary material is available at Cardiovascular Research online.

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