Plasma Angiopoietin-1, Angiopoietin-2, Angiopoietin Receptor Tie-2, and Vascular Endothelial Growth Factor Levels in Acute Coronary Syndromes

Kaeng W. Lee, MRCP; Gregory Y.H. Lip, MD, FRCP; Andrew D. Blann, PhD

Background—Angiopoietin (Ang) -1 and -2, their receptor Tie-2, and vascular endothelial growth factor (VEGF) regulate angiogenesis and may be important in myocardial collateral development. Elevated levels of growth factors and their receptors are reported in myocardial infarction (MI), but changes after an acute coronary event are unknown.

Methods and Results—Plasma Ang-1, Ang-2, Tie-2, and VEGF levels were measured on admission (baseline) and at 48 hours (acute stage) in 126 patients with acute coronary syndrome (82 MI, 44 unstable angina pectoris). Baseline levels were compared with those of 40 patients with stable angina and 40 healthy controls. Measurements were repeated in 38 MI patients at 6 and 18 weeks (chronic stage). Baseline Ang-2 and Tie-2 levels were highest in MI patients (P < 0.001). Patients with MI and unstable angina pectoris had higher VEGF levels compared with stable angina patients and healthy control subjects (P < 0.001). Ang-1 levels were unchanged from baseline to 6 weeks but were elevated at 18 weeks. Ang-2 changes followed a biphasic pattern, being higher at baseline and 6 weeks but lower at 48 hours and 18 weeks. Tie-2 levels increased from baseline and remained elevated in the chronic phase. VEGF peaked at 6 weeks and then decreased toward baseline at 18 weeks.

Conclusions—Plasma Ang-2, Tie-2, and VEGF levels but not Ang-1 levels were increased in patients with acute coronary syndrome. Serial changes in the plasma levels and interrelationships among Ang-1, Ang-2, Tie-2, and VEGF levels from the acute to the chronic stages in MI may reflect the progressive stages of angiogenesis activity in the ischemic-necrotic myocardium in vivo. (Circulation. 2004;110:2355-2360.)

Key Words: acute coronary syndromes ■ angiogenesis ■ angiopoietins ■ vascular endothelial growth factor ■ receptor, Tie-2

Myocardial necrosis-ischemia can trigger a response to improve myocardial perfusion by the formation of new capillaries (angiogenesis) and by the enlargement of preexisting collateral vessels (arteriogenesis).1 Angiogenesis is a highly regulated process that requires the orchestrated interaction of endothelial cells, extracellular matrix, and surrounding cells mediated by a cascade of growth factors, their receptors, and intracellular signals.2 One such growth factor is vascular endothelial growth factor (VEGF). This potent endothelial cell–specific mitogen in vitro and in vivo is able to induce endothelial cell migration, proliferation, and blood vessel formation.2 VEGF mRNA, protein, and its receptors’ expression can be rapidly upregulated in the myocardium within minutes of ischemia (or hypoxia).3 Indeed, VEGF levels are substantially increased in vivo in ischemic human and animal myocardium.4,5 Furthermore, increased VEGF can be detected in the peripheral blood of patients with acute myocardial infarction (MI)6-10 or refractory angina.11

Recently, angiopoietin (Ang)-1 and -2, the ligands for the Tie-2 receptor, have been identified and interact with VEGF.12,13 For example, Ang-2 and VEGF act synergistically to produce a stable and functional microvasculature. Indeed, coronary angiogenesis is a coordinated event involving the coexpression of both VEGF and Ang-2.5,14,15 Interestingly, a recent in vivo study has suggested that Ang-1 can also be antiangiogenic, offsetting VEGF-induced angiogenesis.14 Thus, changes in the local balance of the angiopoietins and VEGF and the temporal pattern of their expression and interaction may be of pathophysiological importance in determining vessel stability, maturation, and angiogenesis at different stages of neovascularization.

Angiogenesis is upregulated not only in the acute but also the subacute to chronic phases after acute MI (AMI). VEGF is raised in patients with acute myocardial ischemia and infarction,6,9-11 and serial studies indicate a gradual increase from day 1 of AMI, reaching a maximum level a few weeks afterward and then falling toward baseline in about 6 months. Raised levels of circulating Tie-2 have also been demonstrated in patients with coronary artery disease, with higher

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levels found in AMI patients than in healthy control subjects. However, serial changes in Ang-1, Ang-2, and Tie-2 and their relationships with VEGF and Tie-2 in patients after acute coronary syndromes (ACS), including AMI or angina pectoris, are unknown. We therefore hypothesized increasing levels of Ang-1 and Ang-2, along with raised VEGF and Tie-2, in patients with different stages of cardiovascular disease, ie, stable angina (SA), unstable angina, and AMI, compared with healthy subjects. The testing was done in a cross-sectional study. We subsequently hypothesized significant serial changes in these molecules from the acute phase to the recovery phase in patients with AMI by measuring levels at defined points up to 18 weeks after the acute event.

**Methods**

We prospectively recruited patients presenting with their first AMI to our coronary care unit. The clinical diagnosis of AMI was based on the concurrence of prolonged (>30 minutes) chest pain or discomfort, elevated myocardial enzymes (total creatine kinase [CK] and CK-MB more than twice the upper normal limit and/or raised troponin I levels to at least the high-risk level) and ECG changes (ST-T segment elevation or depression) occurring within 24 hours of symptom onset. We also recruited patients presenting with unstable angina pectoris (UAP), defined as the presence of typical angina at rest or on minimum exertion associated with acute and transient ST-T segment ECG changes but with normal cardiac enzymes, including troponin levels. All patients received standard therapy, including aspirin, low-molecular-weight heparin, intravenous nitrates, a statin, β-blocker, and ACE inhibitor, when appropriate. To avoid possible influences of percutaneous coronary intervention (PCI) or catheter-related acute-phase reactions on the measured parameters, patients requiring urgent PCI or coronary angiographic investigation within the first 24 hours of admission were excluded from this study. Other exclusion criteria were age >75 years, Killip class III or IV heart failure, significant valvular heart disease, previous history of MI, atrial fibrillation, peripheral vascular disease, chronic inflammatory diseases, and known history of neoplastic diseases.

Baseline values of the acute patients were compared with those of age- and sex-matched patients with clinically SA and healthy control subjects. Patients with SA were recruited from those attending for elective day-case coronary angiography and found to have ≥1 coronary stenoses (>50% severity) in major coronary arteries. These patients had no previous history of MI, coronary bypass surgery, and/or PCI. Healthy control subjects—defined by careful history, examination, and basic blood tests—were recruited from members of the hospital staff and from patients attending the hospital for nonacute minor surgical procedures such as cataract or hernia repair surgery. None of the acute or stable patients or healthy control subjects had a history of renal or liver disease, malignancy, connective tissue disease, deep vein thrombosis or pulmonary embolism, recent infections, or inflammatory disorders, and none were taking regular nonsteroidal antiinflammatory drugs or anticoagulants. The local ethics committee approved the study, and all subjects gave informed consent.

**Blood Samplings**

Venous blood samples were taken from the acute patients within 24 hours of admission and 48 hours thereafter between 8 and 9 AM while in a fasting condition. Samples from AMI patients during follow-up (at 6 and 18 weeks after the index event), from the SA patients (in the morning before coronary angiography), and from healthy control subjects were taken after overnight fasting and abstinence from tobacco and alcoholic or caffeine-containing beverages the evening before. All samples were collected into trisodium-citrated tubes and were immediately placed on ice. Within 30 minutes of collection, samples were centrifuged at 3000 rpm (1000g) for 20 minutes, divided into aliquots, and stored at −70°C until batch analysis.

**Laboratory**

VEGF, Tie-2, Ang-1, and Ang-2 were measured by commercial ELISA as previously described in detail. All assays were performed in duplicate. Intra-assay and interassay coefficients of variation for all ELISA assays were <5% and <10%, respectively.

**Power Calculations**

Expecting growth factor data to be nonnormally distributed, we hypothesized an ordered linear trend in the growth factors of 25% across each of 4 stages of heart disease (eg, median, 100 arbitrary units; interquartile range [IQR], 22 to 215 in healthy control subjects rising to 125 in SA, 156 in UAP, and 195 in AMI patients) that would give values of P for trend = 0.005 and P = 0.004 for the entire data set. Logarithmic transformation, followed by ANOVA and Tukey’s tests, gave P = 0.045 overall and P < 0.05 for a difference between healthy control subjects and patients with AMI, with 40 subjects per group. A data set of this number provides the 1−β power of 0.9 to detect a difference of one half an SD at P < 0.01. Our second planned analysis was changes in growth factors over 4 time points (baseline, 48 hours, 6 weeks, and 18 weeks) in the AMI patients. Because we expected a high dropout rate, we recruited 82 subjects at baseline but collected full data sets on 38. Friedman’s ANOVA provides the power to detect a median increase of 15% with P < 0.01 at 1 time point and of P < 0.05 at a second time point.

**Statistical Analysis**

Data were analyzed by the Shapiro-Wilk test to determine distribution and are expressed as mean±SD or as medians with IQRs. Comparisons between groups were performed by the Kruskal-Wallis or 1-way ANOVA with (after log transformation) Tukey’s post hoc test set at a fixed P < 0.05 or P < 0.01. Categorical variables were compared by use of the χ² test. Serial data were analyzed by Friedman’s (2-way) repeated-measures ANOVA to compare variables at baseline, 48 hours, 6 weeks, and 18 weeks. Correlations were sought with Spearman’s rank correlation. A value of P < 0.05 was considered statistically significant. All statistical analyses were performed with SPSS version 11 (SPSS Inc); power calculations were done on Minitab 13 (Minitab Inc, State University, Philadelphia, Pa).

**Results**

The baseline clinical and demographic details are summarized in Table 1. Of the 82 AMI patients recruited, 55 patients presented with ST-segment elevation AMI (STEMI); the remainder were non-STEMI. All but 5 patients with STEMI received thrombolytic therapy on admission. Thirty-eight AMI and 10 UAP patients had inpatient PCI.

Table 2 shows the cross-sectional results. There were no difference in Ang-1 levels between groups (P = 0.132) and no significant linear trend across the groups (P = 0.059). Overall, there were significant differences in the Ang-2 data between groups (P < 0.001), and Tukey’s post hoc test showed this to be between AMI and the other 3 groups (P < 0.05). Ang-2 was also raised in UAP compared with healthy control subjects. There was a significant increasing linear trend from healthy control subjects to AMI (P for linear trend = 0.025). Similarly, Tie-2 levels (P < 0.001 overall) were significantly higher in AMI patients compared with UAP patients, SA patients, and healthy control subjects, and levels in patients with UAP were higher than in the control subjects (all P < 0.05). However, the ordered linear trend in Tie-2 levels was P = 0.058. VEGF data were also significantly different between groups (P < 0.001), with levels in AMI greater than those in UAP patients, SA
patients, and control subjects and UAP levels greater in SA patients and control subjects (all $P<0.05$). Significance of the ordered linear trend had a value of $P=0.011$. Among AMI patients, the median levels of Ang-1, Ang-2, Tie-2, and VEGF at baseline or 48 hours were not significantly different between the STEMI and non-STEMI patients (data not shown).

Overall, Ang-1 correlated weakly with VEGF ($r=0.15, P<0.05$), whereas Ang-2–Tie 2, Ang-2–VEGF, and Tie-2–VEGF correlations were strong ($r=0.58, 0.62,$ and 0.74, respectively; all $P<0.001$; Figure 1). In all AMI patients, peak total CK levels were significantly correlated with 48-hour Ang-2, Tie-2, and VEGF levels ($r=0.3,$ all $P<0.05$).

Although baseline data were obtained on 82 patients with AMI, subsequent other clinical disease, cardiovascular events (death, second MI), interventions (PTCA, CABG), withdrawal of consent, and major changes in drugs resulted in complete data from only 38 patients. Significant serial changes in all measured indexes from baseline to the 18-week follow-up were observed (each $P<0.001$ overall) (Table 3 and Figure 2). Compared with baseline, Ang-1 levels were significantly elevated at 18 weeks (but for the 4 time points, $P$ for linear trend=$0.227$, indicating that this was not simply a linear increase). Ang-2 levels were higher at baseline and at 6 weeks but were lower at 48 hours and 18 weeks ($P$ for linear trend=$0.742$). Tie-2 levels increased steadily from baseline but were significant only at 18 weeks ($P$ for linear trend=$0.063$). VEGF also increased from baseline, reaching the highest levels at 6 weeks and then decreasing toward baseline at 18 weeks ($P$ for linear trend=$0.286$).

**Discussion**

Angiogenesis is a highly coordinated process. VEGF, its receptors, and the Ang/Tie-2 systems are the predominant coordinators for angiogenesis. In vitro experimental studies have shown that VEGF, Ang-2, and Tie-2 but not Ang-1

### Table 1. Baseline Demographic and Clinical Characteristics of All Subjects

<table>
<thead>
<tr>
<th></th>
<th>AMI (n=82)</th>
<th>UAP (n=44)</th>
<th>SA (n=40)</th>
<th>Control Subjects (n=40)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>60±10</td>
<td>61±11</td>
<td>59±10</td>
<td>59±8</td>
<td>0.66</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>59 (72)</td>
<td>30 (68)</td>
<td>26 (65)</td>
<td>23 (58)</td>
<td>0.45</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28±4</td>
<td>28±5</td>
<td>27±4</td>
<td>28±5</td>
<td>0.9</td>
</tr>
<tr>
<td>Active smoker, n (%)</td>
<td>36 (44)</td>
<td>10 (23)</td>
<td>5 (13)</td>
<td>5 (13)</td>
<td>0.001</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>132±21</td>
<td>132±15</td>
<td>136±23</td>
<td>135±16</td>
<td>0.38</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.8±0.9</td>
<td>5.6±0.8</td>
<td>3.6±0.7</td>
<td>5.6±0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.0±0.1</td>
<td>1.0±0.2</td>
<td>0.9±0.2</td>
<td>1.2±0.1</td>
<td>0.003</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>91±26</td>
<td>86±16</td>
<td>88±17</td>
<td>79±17</td>
<td>0.024</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>7.3±2.7</td>
<td>7.4±3.1</td>
<td>6.2±2.6</td>
<td>5.0±0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>20 (24)</td>
<td>14 (32)</td>
<td>8 (20)</td>
<td>...</td>
<td>0.45</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>34 (42)</td>
<td>19 (43)</td>
<td>19 (48)</td>
<td>...</td>
<td>0.82</td>
</tr>
<tr>
<td>Medications, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>77 (94)</td>
<td>37 (84)</td>
<td>37 (93)</td>
<td>...</td>
<td>0.21</td>
</tr>
<tr>
<td>β-Blocker</td>
<td>57 (70)</td>
<td>24 (55)</td>
<td>25 (63)</td>
<td>...</td>
<td>0.24</td>
</tr>
<tr>
<td>Statin</td>
<td>57 (70)</td>
<td>26 (60)</td>
<td>30 (75)</td>
<td>...</td>
<td>0.27</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>32 (39)</td>
<td>14 (32)</td>
<td>20 (50)</td>
<td>...</td>
<td>0.23</td>
</tr>
<tr>
<td>Nitrates</td>
<td>27 (33)</td>
<td>30 (68)</td>
<td>7 (18)</td>
<td>...</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CCB</td>
<td>8 (10)</td>
<td>15 (34)</td>
<td>11 (28)</td>
<td>...</td>
<td>0.002</td>
</tr>
<tr>
<td>Insulin</td>
<td>8 (9.8)</td>
<td>6 (14)</td>
<td>3 (7.5)</td>
<td>...</td>
<td>0.64</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; BP, blood pressure; HDL-C, HDL cholesterol; and CCB, calcium channel blocker. Values are mean±SD when appropriate.

### Table 2. Baseline Levels of Research Indexes Among the 4 Study Groups

<table>
<thead>
<tr>
<th></th>
<th>AMI (n=82)</th>
<th>UAP (n=44)</th>
<th>SA (n=40)</th>
<th>Control Subjects (n=40)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ang-1, ng/mL</td>
<td>14.3 (6.6–20)</td>
<td>10.9 (7.8–17.4)</td>
<td>11 (6.4–17)</td>
<td>8.2 (5.2–17.2)</td>
<td>0.132</td>
</tr>
<tr>
<td>Ang-2, ng/mL</td>
<td>9.2 (5.4–13.8)†‡§</td>
<td>6.2 (3.7–9.0)†</td>
<td>5.5 (3.8–8.5)†</td>
<td>3.3 (2.5–5.3)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Tie-2, ng/mL</td>
<td>11.5 (8.7–15.3)†‡§</td>
<td>7.7 (6.2–11.3)†</td>
<td>6.9 (6.2–8.1)</td>
<td>5.6 (4.2–7.7)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>VEGF, pg/mL</td>
<td>126 (83–217)†‡§</td>
<td>86 (48–118)†‡§</td>
<td>46 (28–67)</td>
<td>27 (21–30)</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Values are median (IQR). $*P$-values are from one-way ANOVA after log transformation. Tukey’s post hoc test: †$P<0.05$ vs healthy control subjects; ‡$P<0.05$ vs UAP; §$P<0.05$ vs SA.
expression is upregulated in ischemic myocardium, whereas clinical studies in patients with ACS (AMI patients) have shown increased peripheral blood VEGF. The present study shows that in addition to raised VEGF and Tie-2 levels, Ang-2 levels were also raised in ACS patients within 24 hours of presentation, although Ang-1 levels were not different compared with those of healthy control subjects and SA patients. Patients with evidence of myocardial damage (ie, AMI) had the highest levels of Ang-2, VEGF, and Tie-2 compared with other groups.

A recent analysis of serum from >1000 ACS patients reported not only that VEGF elevations significantly correlated with clinical evidence of myocardial ischemia but also that high VEGF was a powerful independent significant predictor of death and nonfatal MI. Two studies have shown significant correlations between VEGF and peak CK levels, suggesting that the extent of myocardial damage may be linked to elevated circulating VEGF levels. We confirm this aspect in the present study, adding the novel finding that Tie-2 and Ang-2 are also significantly correlated with peak total CK levels in patients with AMI. In addition, VEGF, Ang-2, and Tie-2 levels were significantly correlated during the acute phase and in the recovery phase after the acute coronary events, although this may simply be mathematical in that patients with the most severe disease seem likely to have the highest levels of all growth factors. Nevertheless, the prognostic significance of Ang-2 and Tie-2 warrants further investigation.

The formation of a normal functioning vasculature requires the collaborative interactions of the VEGF and the Ang/Tie-2 systems. Indeed, recent in vitro ischemia-reperfusion studies in animal models have suggested that the local balance of growth factors and the temporal pattern of their expression and interactions may be of pathophysiological importance in angiogenesis toward either vessel stability and maturation or angiogenesis and new vessel growth or even vessel regression and apoptosis. However, our serial measurements of these angiogenic indexes in the peripheral circulation in patients after AMI do not always reflect patterns observed in these animal models. For example, myocardial ischemia in adult rats significantly upregulated Ang-2 and Tie-2 mRNA expression and protein levels 48 to 72 hours after reperfusion, whereas Ang-1 and Tie-1 mRNA and protein were unchanged after ischemia-reperfusion. We found no increase in plasma Ang-1 or Tie-2 and a decrease in Ang-2. Repetitive brief episodes of myocardial ischemia in a canine heart model increased VEGF (consistent with our data) and Ang-2 (at variance with our data) expression in myocardial interstitial fluid, reaching a maximum level at day 3, whereas Ang-1 remained relatively constant at all times (consistent with our data).

Recent studies have suggested a dual role of Ang-2 in angiogenesis, depending on the availability of VEGF. In the presence of VEGF, Ang-2 acts as an agonist and stimulates angiogenesis. In the absence of VEGF, Ang-2 competitively antagonizes Ang-1-induced Tie-2 phosphorylation and hence leads to vessel regression. Thus, Ang-2 and VEGF act synergistically to produce a stable and functional microvasculature. Our data show a remarkable correlation among the baseline levels of VEGF, Ang-2, and Tie-2, suggestive of enhanced angiogenesis in vivo during the acute phase of myocardial ischemia-necrosis.

The present study is the first to report sequential changes in circulating levels of Ang-1, Ang-2, and Tie-2 during the acute and recovery phases in AMI patients. Several other clinical studies have investigated the short- and/or long-term changes...
in circulating VEGF levels after AMI.6–10 For example, Hojo et al7 demonstrated a gradual rise in VEGF from day 1 of AMI, reaching a peak at 2 weeks and decreasing thereafter. Kranz et al8 also showed that after AMI, serum VEGF levels gradually increased, reaching a maximum at day 10 and remaining elevated at 3 weeks, with a return toward baseline only after 6 months. Our data on plasma VEGF appear to follow a similar pattern: progressively increasing over time from the acute phase, reaching a peak at 6 weeks, and returning to baseline at 18 weeks. Another study in 19 patients10 also reported serum VEGF levels were elevated in the early phase of AMI and level fell sharply to the normal control range within 30 minutes of successful reperfusion by early PCI, suggesting that circulating levels of VEGF may acutely reflect the myocardial ischemia state. Such a dramatic drop in VEGF levels after PCI has not been substantiated by other workers.7 Some debate also remains as to whether serum (rather than plasma) is an appropriate medium for measurement of VEGF (and perhaps other growth factors).26,27 given the influence of platelet activation in ACS, and the administration of various antithrombotic drugs in this setting.6,8

Given the evidence discussed, our findings of raised Ang-2, VEGF, and Tie-2 and their temporal changes in the peripheral blood may have pathophysiological relevance to the ischemic-induced angiogenesis in the myocardium, although we have no direct evidence that increased plasma levels of our measured indexes actually reflect the angiogenesis activity in the ischemic myocardium in these patients. We recognize that, except for the demonstration of a visible collateral circulation on high-resolution coronary angiography, there is as yet no clinical method available to detect angiogenic activity in infarcted myocardium in real time. Nonetheless, the serial changes in plasma levels and correlations among circulating levels of VEGF, Ang-2, Tie-2, and Ang-1 in our AMI patients probably indicate the progressive stages of angiogenesis in the ischemic myocardium in vivo. Indeed, our findings provide further insight into the pathophysiology of disease and raise very important research questions that will be the subject of future study (eg, relation to coronary anatomy, residual ischemia) as we seek to understand the role of angiogenesis in cardiovascular disease.

Acknowledgments

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References


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