Original Article

# Effects of Percutaneous Coronary Intervention on Serum Angiopoietin-2 in Patients with Coronary Heart Disease

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## Abstract

**Background:** Angiopoietin-2 (Ang-2) plays a crucial role in hypoxia-induced angiogenesis and is expressed only in sites of vascular remodeling. Ang-2 expression can be regulated by hypoxia inducible factors and other regulators with exposure to hypoxia. The objective of this study was to investigate the influence of percutaneous coronary intervention (PCI) on serum Ang-2 concentrations, and analyze the correlation between serum Ang-2 and the severity of coronary artery stenosis in patients with coronary heart disease (CHD).

**Methods:** Sixty-four patients with CHD were selected as the study group, each undergone PCI. Thirty-two healthy subjects were selected as the control group. Pre-PCI and post-PCI serum Ang-2 were measured by enzyme-linked immunosorbent assay. The severity of coronary artery stenosis was evaluated using angiographic Gensini scores, and the coronary collateral vessels were scored according to Rentrop's classification.

**Results:** Concentrations of pre-PCI serum Ang-2 in the study group were significantly higher than those in the control group (4625.06 ± 1838.06 vs. 1945.74 ± 1588.17 pg/ml, $P < 0.01$); however, concentrations of post-PCI serum Ang-2 were significantly lower than those of pre-PCI (3042.63 ± 1845.33 pg/ml vs. 4625.06 ± 1838.06 pg/ml, $P < 0.01$). Concentrations of pre-PCI serum Ang-2 were significantly correlated with Gensini scores ($r = 0.488$, $P < 0.01$); however, the decrease in serum Ang-2 after PCI was not correlated with Gensini scores, coronary collateral vessel grading, or left ventricular ejection fraction.

**Conclusions:** Serum Ang-2 concentrations significantly increased in patients with CHD, and PCI treatment significantly decreased these concentrations. Serum Ang-2 concentrations, but not the decrease in serum Ang-2 concentrations, were significantly correlated with the severity of coronary artery stenosis. These results suggested that Ang-2 may be a biomarker of myocardial ischemia and vessel remodeling.

**Key words:** Angiogenesis; Angiopoietin-2; Collateral Vessels; Coronary Heart Disease; Gensini Score

## Introduction

Angiogenesis is the formation of new blood vessels. Several growth factors are closely associated with blood vessel formation and remodeling, such as angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2), and vascular endothelial growth factor (VEGF),[1] each of which plays a different role in angiogenesis. Ang-1 promotes vascular stabilization and counteracts VEGF-induced angiogenesis whereas Ang-2 promotes angiogenesis by reducing the destabilization of endothelial cell junctions to enhance new vessel branching and sprouting.[2-4] Ang-2 acts on angiogenesis differently in different microenvironments. For example, Ang-2 promotes more angiogenesis in the presence of VEGF[5] but inhibits angiogenesis in the absence of VEGF.[6] Ang-2 is expressed only in vascular remodeling sites, and its expression can be regulated by hypoxia inducible factors (HIFs) and other regulators exposed to hypoxia.[7-10]

As a compensatory mechanism, coronary angiogenesis forms and develops in response to ischemia from coronary stenosis or occlusion in coronary heart disease (CHD), and new coronary vessels provide blood to the ischemic myocardium.[11] Ang-2 plays a crucial role in this hypoxia-induced angiogenesis, and increased levels

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of Ang-2 concentrations can be detected in the peripheral blood of patients with CHD. For example, our previous study revealed that serum concentrations of Ang-2 increase in patients with unstable angina pectoris.[12] Wang *et al*.[13] also demonstrated that serum Ang-2 concentrations increase in patients with CHD. It has been shown that the formation of new vessels can be reversed when ambient oxygen concentration is restored;[14] therefore, ischemic recovery can reverse angiogenesis activity. Because Ang-2 is an important regulatory factor in this specialized revascularization, ischemic recovery most likely leads to reversible changes in Ang-2 concentrations.

Given this information, we hypothesized that serum Ang-2 concentrations in patients with CHD might decrease after myocardial blood reperfusion by percutaneous coronary intervention (PCI) and the greater decreases in Ang-2 after PCI accompany more severe coronary artery stenosis. The present study was designed to investigate the influence of PCI on serum Ang-2 concentrations and the correlation between serum Ang-2 and the severity of coronary artery stenosis in CHD.

## Methods

### Subjects

Sixty-four consecutive patients with CHD were selected as the study group, each undergone coronary angiography (CAG) and PCI. Thirty-two age-matched healthy people from the medical examination center were selected as the control group. Patients with diabetes, tumor, autoimmune diseases, acute congestive heart failure, valvular heart disease, systemic infectious diseases, or ST-elevation myocardial infarction were excluded from the study.

The clinical data comprising name, age, sex, weight, height, cigarette smoking, history of hypertension, and the results of laboratory tests were gathered by a review of the patient's medical records. Another data sheet containing patient's Gensini scores and collateral grades was completed by experienced angiographers. All patients received standard medical treatment for their clinical conditions. The study protocol was reviewed and approved by the Human Research Ethics Committee of the First Affiliated Hospital of Guangxi Medical University, China. All patients signed the informed consent.

### Blood sampling procedures

Venous blood samples were collected from all patients with CHD in the morning after admission and within 24–48 h after PCI. The blood samples from the control group were taken during medical examinations. All blood samples were collected into test tubes, remained without anticoagulant for 0.5 h at room temperature, and then centrifuged at 5000 r/min for 5 min. The serum supernatant was removed and stored at −80°C until analysis.

### Measuring angiopoietin-2

Ang-2 concentrations in the blood samples were measured using enzyme-linked immunosorbent assay kits according to the manufacturer's instructions (RayBiotech, Inc., Norcross, GA, USA). Briefly, 100 μl of each diluted standard and sample was added to appropriate wells and incubated while gently shaking for 2.5 h at room temperature. The solution was discarded and washed 4 times. A volume of 100 μl of the prepared biotinylated antibody was added to each well and incubated while gently shaking for 1 h at room temperature. The solution was discarded and washed 4 times. Total 100 μl of prepared streptavidin solution was added to each well and incubated while gently shaking for 45 min at room temperature. The solution was discarded and washed 4 times. Total 100 μl of 3,3′,5,5′-Tetramethylbenzidine one-step substrate reagent was added to each well and incubated in the dark while shaking gently for 30 min at room temperature. Finally, 50 μl stop solution was added to each well and immediately read at 450 nm. The mean absorbance for each set of duplicate standards and samples was calculated, and the average zero standard optical density was subtracted. Data were extracted using SigmaPlot (Systat Software Inc., San Jose, CA, USA).

### Measuring coronary artery stenosis and collateral vessels

CAG and PCI were performed by experienced interventional cardiologists. The angiographic Gensini score was used to determine the severity of coronary artery stenosis.[15,16] Rentrop's classification system was used to score the coronary collateral vessels.[17,18] Collateral filling was graded as follows: 0 = none, 1 = filling of side branches only, 2 = partial filling of the epicardial segment, or 3 = complete filling of the epicardial segment. The severity of coronary artery stenosis and collateral vessels was scored by an experienced angiographer and then reviewed by a separate angiographer who was blinded to the initial reading. When there was disagreement between the two, the angiograms were reviewed by a third angiographer who was also blinded to the initial two readings and served as an arbitrator.

### Statistical analyses

The numerical values are expressed as the mean ± standard deviation (SD), and the data were analyzed using SPSS 19.0 (IBM Corporation, Armonk, NY, USA). Comparisons between the study and control groups were performed using the independent-samples *t*-test. Comparisons between the pre-PCI and post-PCI study groups were performed using the paired *t*-test. The correlations among study parameters were analyzed using the Pearson's correlation test. Statistical significance was defined as $P < 0.05$.

## Results

### Patient characteristics and clinical data

The baseline clinical data are summarized in Table 1. No significant differences were found between groups in terms of age, sex, smoking habits, high-density lipoprotein (HDL)-cholesterol, and triglycerides. As

**Table 1: Clinical characteristics of all subjects at baseline**

| Characteristics | Control group ($n$ = 32) | Study group ($n$ = 64) | $P$ |
|---|---|---|---|
| Age, mean ± SD (years) | 47.6 ± 4.5 | 47.9 ± 6.5 | NS |
| Male, $n$ (%) | 24 (75) | 53 (83) | NS |
| Smoking, $n$ (%) | 19 (59) | 41 (64) | NS |
| Hypertension, $n$ (%) | 0 (0) | 47 (73) | <0.01 |
| HDL-cholesterol (mmol/L) | 1.03 ± 0.21 | 1.13 ± 0.35 | NS |
| LDL-cholesterol (mmol/L) | 2.00 ± 0.67 | 2.84 ± 1.04 | <0.01 |
| Triglycerides (mmol/L) | 1.49 ± 0.76 | 1.60 ± 0.90 | NS |
| Medications, $n$ (%) | | | |
| Aspirin | – | 64 (100) | – |
| Clopidogrel | – | 64 (100) | – |
| ACEI | – | 41 (64) | – |
| Beta-receptors blocker | – | 48 (75) | – |
| Nitroglycerin | – | 21 (34) | – |
| Statin | – | 64 (100) | – |

NS: No significance; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; ACEI: Angiotensin-converting enzyme inhibitors; SD: Standard deviation. –: not available.

expected, patients in the study group had a significantly higher rate of hypertension and higher levels of low-density lipoprotein (LDL)-cholesterol. There was no clinical evidence of interventional complications in patients with CHD.

### Changes in serum angiopoietin-2 concentrations in patients with CHD

Pre-PCI serum Ang-2 concentrations in the study group were significantly higher than those in the control group (4625.06 ± 1838.06 vs. 1945.74 ± 1588.17 pg/ml, $P < 0.01$). To observe the effects of PCI on serum Ang-2 concentrations, we compared the levels of pre- and post-PCI serum Ang-2 concentrations. The results showed that serum Ang-2 concentrations significantly decreased after PCI (3042.63 ± 1845.33 pg/ml vs. 4625.06 ± 1838.06 pg/ml, $P < 0.01$).

### Serum angiopoietin-2 correlation to other parameters

All 64 patients with CHD had undergone CAG. The values of Gensini score were 61.61 ± 32.67, and collateral grading were 0.56 ± 0.96. Echocardiographic parameters were as follows: Left ventricular end systolic diameter (LVESD) 35.11 ± 7.63 mm, left ventricular end diastolic diameter (LVEDD) 53.38 ± 6.37 mm, and left ventricular ejection fraction (LVEF) 60.64 ± 12.36%. By Pearson's correlation, pre-PCI serum Ang-2 levels were unrelated to total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, coronary collateral grading, LVESD, or LVEDD. Pre-PCI serum Ang-2 levels were significantly correlated with Gensini scores ($r$ = 0.488, $P < 0.001$) and LVEF [$r$ = −0.293, $P < 0.001$; Figure 1] whereas the decreasing amplitude of serum Ang-2 was not correlated with Gensini scores ($r$ = 0.158, $P > 0.05$), coronary collateral grading ($r$ = −0.138, $P > 0.05$), or LVEF [$r$ = −0.228, $P > 0.05$; Figure 1].

## Discussion

Angiogenesis is a highly complex interconnected process, and Ang-2 is one of its predominant regulators. Animal studies have shown that Ang-2 expression is upregulated in ischemic or necrotic myocardium,[1,9] and clinical studies have shown that peripheral blood Ang-2 concentrations increase in patients with CHD.[12,13,19] The results of the present study were consistent with those of previous studies in that serum Ang-2 concentrations increased in patients with CHD. It has also been demonstrated that hypoxic regulation of Ang-2 is HIF-dependent,[20] and upregulation of Ang-2 from hypoxia occurs widely in endothelial cells *in vitro* and *in vivo*.[21] Thus, we presumed that the response to ischemia or hypoxia might be the initiating factor for increased Ang-2. Further studies suggest that the early release of Ang-2 might be part of an acute phase response, but the late release of Ang-2 might contribute to the induction of the angiogenetic mechanisms involved in tissue reparation. For example, Matsunaga *et al*.[1] demonstrated that Ang-2 expression peaks at day 3 and although it wanes thereafter, it still maintains relatively high levels at day 7 whereas capillary density begins to increase at day 7 after occlusion of the coronary artery. Wakui *et al*.[22] showed that in early ischemia, Ang-2 expression increases but capillary density remains at low levels; however, in late ischemia, capillary density increases sharply accompanied by remaining high levels of Ang-2. In conclusion, these results suggest that Ang-2 levels might be used as evidence of myocardial ischemia in the acute phase but an indicator of Ang-2-promoted angiogenesis in the subacute or chronic phase of CHD.

In the present study, we demonstrated that serum Ang-2 concentrations in patients with CHD significantly decrease after PCI. We presumed that this change in Ang-2 concentrations was a result of reduced hypoxia after PCI. Hypoxia is an important stimulus for HIF-induced Ang-2, which subsequently promotes angiogenesis. After restoring the blood flow to the ischemic zone, Ang-2 returns to control levels and the changes in Ang-2 parallels those of capillary density, suggesting that Ang-2-promotes extenuated angiogenesis. From these results, it appears that Ang-2 expression is regulated by a negative feedback loop based on an autocrine mechanism as follows: hypoxia acts as the stimulus to the endothelium, leading to increased Ang-2 concentrations; Ang-2 improves the balance of the oxygen supply and demand by promoting angiogenesis; angiogenesis promotes the formation of new functional vessels to decrease hypoxia by providing oxygen; and Ang-2 concentrations subsequently decrease. On contrary, a study by Benderro *et al*.[9] showed that Ang-2 levels increase during hypoxia and remain elevated even after reoxygenation, and another study showed an increase in Ang-2 after myocardial reperfusion.[23] A possible reasonable explanation for this is that these are the results of a myocardial ischemia/reperfusion injury, which causes additional inflammation and cell death, subsequently leading to an increase in infarct size.[24] In this study, patients with elevated ST myocardial infarction were excluded, and

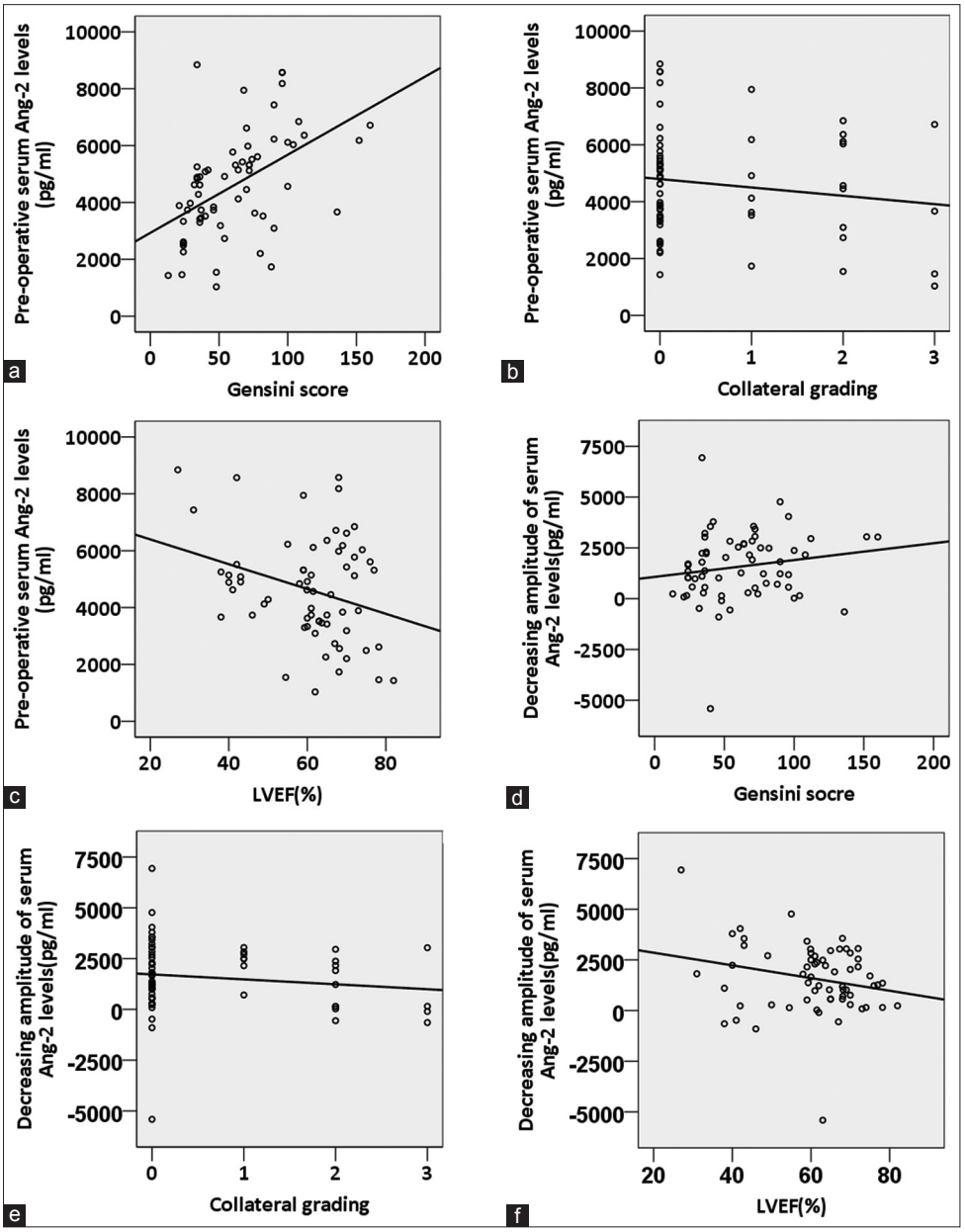


**Figure 1:** Serum angiopoietin-2 concentrations correlation to collateral grading, Gensini scores, and left ventricular ejection fraction. (a) Angiopoietin-2 and Gensini score (Pearson's $r = 0.488$, $P < 0.01$); (b) Angiopoietin-2 and collateral grading (Pearson's $r = -0.152$, $P > 0.05$); (c) Angiopoietin-2 and left ventricular ejection fraction (Pearson's $r = -0.293$, $P < 0.01$); (d) Decreasing amplitude of angiopoietin-2 and Gensini score (Pearson's $r = 0.158$, $P > 0.05$); (e) Decreasing amplitude of angiopoietin-2 and collateral grading (Pearson's $r = -0.138$, $P > 0.05$); (f) Decreasing amplitude of angiopoietin-2 and left ventricular ejection fraction (Pearson's $r = -0.228$, $P > 0.05$).

there was no clinical evidence of ischemia/reperfusion injury in the study subjects. Given that, the changes in serum Ang-2 concentrations after PCI might reflect the recovery of blood flow in the ischemic myocardium.

In this study, we also found that patients with coronary collateral vessels had higher Gensini scores than those without coronary collateral vessels. This observation might be explained by the higher HIF levels in patients with higher Gensini scores – higher Gensini scores mean more severe hypoxia. Before this study, we expected that the presence of collateral vessels might be correlated with higher levels of Ang-2; however, we found that serum Ang-2 levels were unrelated to coronary collateral grading. This might be explained by the different CHD phases among individuals that were recruited for our study, but it needs to be further investigated. A significantly positive correlation between Ang-2 and Gensini scores was found, and the result was consistent with those of our previous study.[12] Another significant finding was that the decreases in Ang-2 concentrations after PCI were not significantly correlated with the severity of coronary artery stenosis, which cannot be explained within the limits of our study; however, to our knowledge, our study is the first to demonstrate this temporal relationship in patients with CHD treated with PCI.

Several limitations need to be acknowledged. First, the sample size was small, which limits statistical power. Second, other clinical factors such as drugs and disease stage of individuals have not been analyzed. Third, the serial measurement of Ang-2 was limited in 24–48 h. In this study, we cannot rule out the possible changes of Ang-2 later than 48 h after PCI.

In conclusion, the major findings of this study are as follows: (1) serum Ang-2 concentrations increased in patients with CHD; (2) serum Ang-2 concentrations in patients with CHD decreased significantly after PCI; (3) pre-PCI serum Ang-2 concentrations, but not the decreases in serum Ang-2 concentrations, were significantly correlated with the severity of coronary artery stenosis. These results suggested Ang-2 may be a biomarker of myocardial ischemia and vessel remodeling.


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## Conflicts of interest

There are no conflicts of interest.

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