

Identification of serum endoglin as a novel prognostic marker after acute myocardial infarction

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Abstract

Endoglin is a proliferation-associated and hypoxia-inducible protein expressed in endothelial cells. The levels of soluble circulating endoglin and their prognostic significance in patients with acute myocardial infarction (AMI) are not known. In this observational prospective study serum endoglin levels were measured by ELISA in 183 AMI patients upon admission to hospital and 48 hrs later and in 72 healthy controls. Endoglin levels in AMI patients on admission were significantly lower than in healthy controls (4.25 ± 0.99 ng/ml *versus* 4.59 ± 0.87 ng/ml; $P = 0.013$), and decreased further in the first 48 hours (3.65 ± 0.76 ng/ml, $P < 0.001$). Upon follow-up (median 319 days), patients who died had a significantly greater decrease in serum endoglin level over the first 48 hrs than those who survived (1.03 ± 0.91 *versus* 0.54 ± 0.55 ng/ml; $P = 0.025$). Endoglin decrease was an independent predictor of short-term (30 days) (hazard ratio 2.33; 95% CI = 1.27–4.23; $P = 0.006$) cardiovascular mortality, and also predicts overall cardiovascular mortality during the follow-up (median 319 days) in AMI patients (hazard ratio 2.13; 95% CI = 1.20–3.78; $P = 0.01$). In conclusion, early changes in serum endoglin may predict mortality after AMI.

Keywords: endoglin • endothelial dysfunction • myocardial infarction • prognostic markers

Introduction

Several compensatory mechanisms take place at endothelial level after an acute cardiac event [1].

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Collateral circulation forms as a result of angiogenesis and endothelial cells, disrupted by plaque erosion or rupture, are regenerated [1]. Study of angiogenic markers has provided greater understanding of these adaptive processes. Furthermore, circulatory biomarkers may be of prognostic value in the setting of acute coronary syndromes (ACS) [2].

Endoglin is a homodimeric transmembrane glycoprotein, which is expressed mainly on the surface of

endothelial cells and is a part of the transforming growth factor β (TGF- β) receptor complex [3, 4]. Mutations of the endoglin gene are the cause of hereditary hemorrhagic telangiectasia type 1, a genetic disease characterized by arteriovenous malformations [5]. Tissue endoglin expression is increased during angiogenesis, wound healing, inflammation, hypoxia and other processes associated with altered vascular structure [6–8]. Elevated levels of serum endoglin in cancer patients correlate positively with neoangiogenesis, tumor metastasis and indicate poor prognosis [7, 8]. Endoglin null mice die *in utero* as a result of impaired angiogenesis in the yolk sac [9, 10]. Furthermore, cardiac defects in homozygous endoglin-deficient embryos have also been confirmed [10].

In view of endoglin's role in angiogenesis and stimulation by ischemic processes, we hypothesized that it may be important in the pathogenesis of acute coronary syndromes. To date however, neither its kinetics nor its potential as a prognostic factor in this setting have been studied. Hence, the purpose of the present study was to establish circulating levels of endoglin in patients with acute myocardial infarction (AMI) and to determine their prognostic significance.

Methods

Study subjects

In this prospective observational single-center study, we evaluated 183 consecutive patients with AMI admitted between December 2003 and July 2005. To establish the normal range of endoglin in serum, levels were also assayed in 72 healthy volunteers of similar age and sex. Written informed consent was obtained from all study participants and the study protocol was approved by our institutional review board.

AMI patients were included if they had chest pain >30 min in duration and presented within 24 hrs of symptom onset. Elevated Troponin I (TnI) or creatine kinase MB isoenzyme within 12 hrs of chest pain was also required. Creatine kinase (CK)-MB had to exceed two times the upper limit of normal in two samples and increase >50% over the preceding value to be significant. Patients with symptom onset more than 24 hrs prior to admission to the coronary care unit were excluded. Those with endometriosis, hereditary hemorrhagic telangiectasia, hemorrhoids, fibrotic disease and active infectious disease, receiving erythropoietin or an alternative angiogenic therapy or with

current or past neoplastic or immunological disorder were also ineligible. Patients in whom serum endoglin levels were not measured on admission and 48 hrs later were also excluded.

Serum endoglin enzyme-linked immunosorbent assay (ELISA)

Antecubital venous blood samples were collected from all patients on admission to the Coronary Care Unit and 48 hrs later. Blood samples were centrifuged at 15,000 g for 15 min and resulting supernatants were stored at -80°C until analyzed. Levels of endoglin in serum were measured by standard quantitative sandwich ELISA (Quantikine) kits (R&D System, Minneapolis, MN, USA) according to the manufacturers' instructions. Assay sensitivity was 0.007 ng/ml, intra-assay coefficient of variation (CV) was <3.2% and inter-assay CV was <6.7%. Endoglin levels on admission, at 48 hrs and change in serum endoglin level in the first 48 hrs were determined.

Biochemical analyses

Serum TnI and CK-MB levels were analyzed by immunometric technique. This involved reaction of TnI and CK-MB within the sample with a biotinylated antibody (mouse monoclonal anti-TnI and anti-CK-MB) and a horseradish peroxidase-labelled antibody conjugate (affinity-purified goat polyclonal anti-TnI and anti-CK-MB). The antigen antibody complex was captured by streptavidin on the wells. Unbound materials were removed by washing. The bound horseradish peroxidase conjugate was measured by a luminescent reaction.

Clinical events and follow-up

Clinical events assessed included cardiovascular death defined as death from myocardial infarction, stroke and sudden death. Short-term outcome was evaluated at 30 days and a minimum of 90 days follow-up was carried out in all patients to assess longer-term outcome.

Statistical analyses

Kolmogorov-Smirnov Test was used to confirm normal distribution. Normally distributed, parametric data are expressed as mean \pm standard deviation and compared by Student's *t*-test. Non-normally distributed variables were summarized by median and interquartile range and

compared by U Mann-Whitney test. Clinical and demographic variables and endoglin levels for the AMI cohort were entered individually into a Cox regression model, and a relationship with short- and long-term mortality was evaluated. Any variable identified as significant ($P < 0.05$) was included in the multivariate Cox regression analysis, which was performed in duplicate to determine relationship to short- and long-term mortality.

Receiver Operating Characteristic (ROC) area under the curve (AUC) analysis was performed to determine optimal cut-off values of endoglin decrease to predict cardiovascular death. The best cut-off value was defined as the point with the highest sum of sensitivity and specificity. Positive and negative predictive value and likelihood ratio were also obtained. Kaplan–Meier survival curves were generated using the cut-off value obtained in the ROCAUC analysis. Survival between groups was compared using log-rank test. A value of $P < 0.05$ was required for statistical significance. All analyses were performed using the Statistical Package for Social Scientists (12.0 for Windows SPSS Inc, Chicago, IL, USA).

Results

Baseline characteristics and assay results

AMI patients were 68% male and of mean age 70.6 ± 11.6 years. There were no differences in age and sex between the AMI patients and the control group (65% male and mean age 69 ± 10 years; $P > 0.05$). Median time from chest pain onset to admission among AMI patients was 300 min [IQ range 170–600].

Acute MI patients had significantly lower serum endoglin levels on admission compared to healthy controls (4.25 ± 0.99 versus 4.59 ± 0.87 ng/ml; $P = 0.013$). Forty-eight hours after admission endoglin levels in AMI patients were even lower (4.25 ± 0.99 versus 3.65 ± 0.76 ng/ml; $P < 0.001$) (Fig. 1). Diabetes, dyslipidemia and prior MI were associated with significantly lower admission endoglin levels. No demographic variable significantly influenced the extent of decrease in endoglin level in the first 48 hrs. (Table 1)

Clinical Events

Ten patients (5.4%) died during short-term (30 day) follow-up. Death occurred as a result of refractory cardiogenic shock ($n = 8$) and ventricular free wall

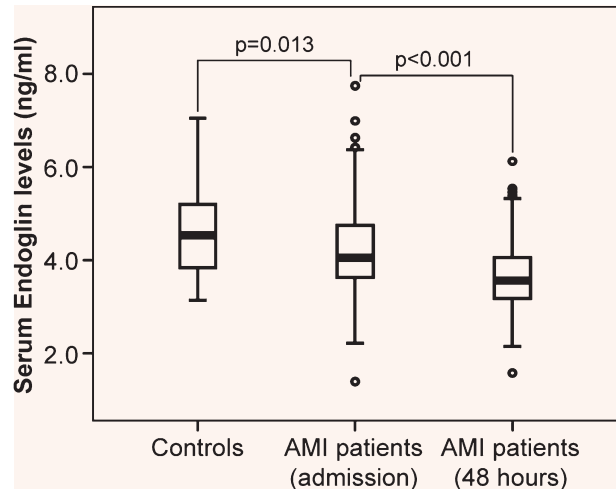


Fig. 1 Endoglin levels in healthy control subjects and in patients with acute myocardial infarction. Boxplot showing serum levels of endoglin in healthy control subjects and patients with acute myocardial infarction (AMI) on admission and after 48 hrs. The horizontal line in the center of the box represents the 50th percentile, the ends of the box represent the 25th and 75th percentiles, the tips of the whiskers represent the 5th and 95th percentiles and the closed circles indicate individual outliers.

rupture ($n = 2$). There was no significant association between endoglin level on admission or at 48 hrs and short-term mortality. The extent of decrease in serum endoglin level over 48 hrs, however, was significantly greater in patients who died compared to those who survived this period (median [IQ range]; 0.94 [0.53 – 1.97] versus 0.51 [0.18 – 0.87] ng/ml; $P = 0.028$). In Cox regression analysis Killip class (hazard risk [HR] 4.65 ; 95% CI = 1.18 – 18.2 ; $P = 0.027$), admission CK level (HR 1.0 ; 95% CI = 1.0 – 1.002 ; $P = 0.032$) and decrease in serum endoglin over the first 48 hrs (HR 2.33 ; 95% CI = 1.27 – 4.23 ; $P = 0.006$) were independent predictors of short-term cardiovascular mortality.

Twenty-one (11%) patients died (overall cardiovascular death) during the follow-up (median [IQ range]; 319 [143–451] days). No significant association between endoglin level on admission or at 48 hrs and rate of long-term mortality was detected. The decrease in serum endoglin level over the first 48 hrs, however, was significantly greater among patients who died compared to survivors (median [IQ range]; 0.73 [0.48 – 1.43] versus 0.49 [0.18 – 0.85]

Table 1 Baseline demographic and clinical characteristics of patients with acute myocardial infarction and relationship to serum endoglin

Variable	N = 183	Admission Endoglin level (ng/ml) <i>mean ± SD</i>	Decrease in Endoglin level over first 48 hrs (ng/ml) <i>median [interquartiles range]</i>
Men	68%	4.3 ± 0.9	0.5 [0.2–0.8]
Women	32%	4.2 ± 1.2	0.7 [0.3–1.0]
>70 years	59%	4.4 ± 0.9	0.5 [0.2–0.8]
<70 years	41%	4.2 ± 1.1	0.5 [0.2–0.9]
STEMI [†]	55%	4.2 ± 1.0	0.5 [0.3–0.9]
NSTEMI [‡]	45%	4.3 ± 1.0	0.5 [0.2–0.9]
Hypertension	59%	4.2 ± 1.1	0.5 [0.2–0.8]
No hypertension	41%	4.3 ± 0.8	0.5 [0.3–0.9]
Prior MI [§]	23%	4.0 ± 0.8*	0.5 [0.2–0.9]
No prior MI	77%	4.3 ± 1.0	0.4 [0.2–0.8]
Dyslipemia	56%	4.1 ± 0.8**	0.5 [0.2–0.9]
No dyslipemia	44%	4.4 ± 1.1	0.5 [0.2–0.8]
Diabetes	29%	4.0 ± 0.9***	0.5 [0.2–0.9]
No diabetes	71%	4.4 ± 1.0	0.4 [0.2–0.9]
EF >50% [¶]	64%	4.3 ± 0.9	0.5 [0.2–0.9]
EF <50%	36%	4.2 ± 1.0	0.5 [0.2–0.8]
CK		268.3 ± 409.3 (admission)	1353.3 ± 1622.8 (maximum)
CKMB		25.2 ± 55 (admission)	139.1 ± 492.9 (maximum)
Tnl		2.1 ± 7.8 (admission)	27.7 ± 34.28 (maximum)

[†]STEMI, ST segment elevation acute myocardial infarction; [‡]NSTEMI, non-ST segment elevation acute myocardial infarction; [§]MI, myocardial infarction; [¶]EF, ejection fraction. **P* = 0.035; ***P* = 0.023; ****P* = 0.038.

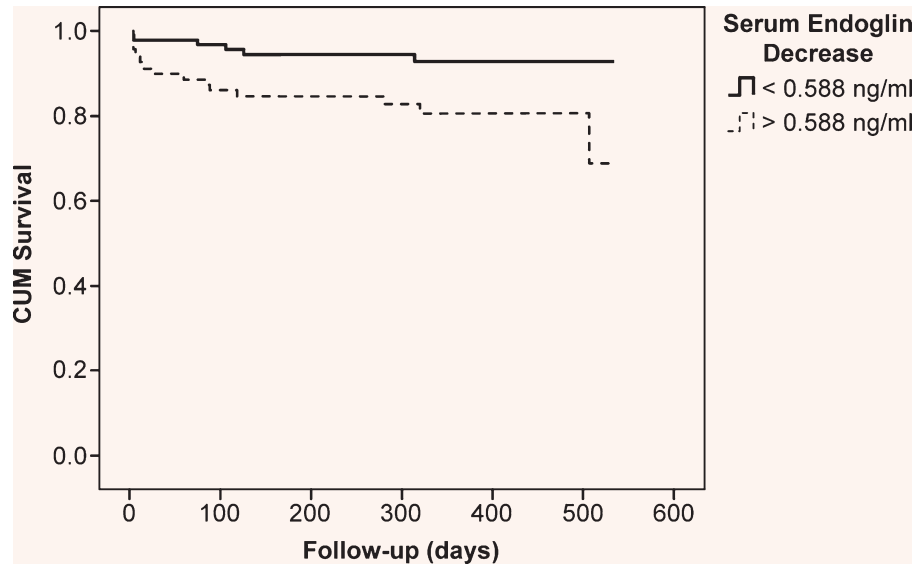
Study population characteristics and relationship with serum endoglin levels and endoglin decrease (endoglin on arrival minus endoglin at 48 hrs).

ng/ml; *P* = 0.007). Killip class at entry (HR 5.56; 95% CI = 1.9–16.4; *P* = 0.001), ejection fraction <30% (HHR 6.7; 95% CI 1.9–23.3; *P* = 0.003), peak CK (HR 1.0; 95% CI 1.0–1.001; *P* = 0.025) and endoglin decrease were independent predictors of long-term survival on Cox multivariable analysis (HR for endoglin decrease 2.13; 95% CI = 1.09–4.17; *P* = 0.027).

The ROCAUC relating decrease in serum endoglin over the first 48 hrs to overall mortality was 0.65 (95% CI 0.55–0.81; *P* = 0.007). The highest like-

lihood ratio corresponded to an endoglin decrease of 0.588 ng/ml (positive likelihood ratio 1.73, negative likelihood ratio 0.49). This value had a sensitivity of 0.72, a specificity of 0.60 and a negative predictive value of 0.94 (95% CI 0.89–0.98). Kaplan–Meier analysis confirmed that patients with an endoglin decrease greater than 0.588 ng/ml in the first 48 hrs had a significantly higher probability of cardiovascular death during follow-up than patients with lower values (*P* = 0.009) (Fig. 2).

Fig. 2 Overall survival in AMI patients stratified by early decrease in endoglin. Kaplan–Meier plot representing cumulative overall survival in patients with endoglin decrease (calculated as serum endoglin levels on admission minus serum endoglin levels 48 hrs later) above or below 0.588 ng/ml (value with the best sensitivity and specificity in the ROC analysis). (Log-rank test $P = 0.009$)



If we subtract the first 30 days from the overall follow-up period, the number of events is too low to perform multivariate studies. Thus, we cannot obtain conclusions about the predictive value of endoglin decrease on long-term mortality.

Discussion

In the present study, we found that early changes in serum endoglin level may be a novel independent prognostic indicator of cardiovascular mortality in patients with AMI. Early endoglin decrease may represent impaired endothelial function, which precedes subsequent adverse clinical outcome.

Although endoglin is a membrane protein, a soluble form can be also detected in serum. However, the nature of soluble endoglin, however, is poorly defined, which makes interpretation of the present data more challenging. Recently high levels of circulating endoglin were reported in patients with pre-eclampsia [11, 12]. The authors determined that circulating endoglin was an N-terminal cleavage product of full-length endoglin. Betaglycan, another TGF- β co-receptor with partial sequence homology to endoglin, can be shed by membrane-type metalloprotease-1 (MT1-MMP) [13]; hence, we would speculate that circulating endoglin may be generated similarly. Interestingly, metalloproteases play an integral role in the pathophysiology of AMI [14, 15].

Specifically, presence of MT1-MMP within the vessel wall has been linked to an unstable plaque phenotype, which is prone to rupture [16]. Furthermore, region- and type-specific changes in metalloproteases occur after experimental AMI in sheep. For example, MMP-1 and MMP-9 abundance was unchanged in a region remote from the infarct, fell to 3 % of the basal value in the transition zone and was undetectable in the AMI region. In contrast, MMP-13, MMP-8 and MT1-MMP increased by >300% in transition and AMI regions [17]. Thus, although not completely understood one might hypothesize that levels of circulating endoglin may be related to metalloprotease activation in AMI. A second possibility is that the decrease in circulating endoglin in the patients with the poorest prognosis is due to the decrease in endoglin membrane levels in the presence of constant levels of MMPs.

After an acute coronary event, compensatory mechanisms such as angiogenesis and endothelial regeneration take place, predominantly in response to tissue hypoxia resulting from myocardial ischemia. Myocardial perfusion is optimized through formation of new capillaries (angiogenesis) and by enlargement of pre-existing collateral vessels (arteriogenesis) [18]. Angiogenesis is a highly coordinated process in which vascular endothelial growth factor (VEGF), endoglin and hypoxia inducible factor 1 (HIF-1) play a pivotal role by coordinating interaction between endothelial cells, extracellular matrix and the surrounding cells [1, 19]. It is already known that

VEGF and HIF-1 expression are up-regulated in ischemic myocardium [20] and a recent study showed that elevated VEGF is a significant independent predictor of death and non-fatal myocardial infarction [2]. Indeed correlation between VEGF and peak CK levels had previously been determined, which suggests that VEGF expression may be linked to the extent of myocardial damage [21–23].

Our study reports for the first time, that serum endoglin levels on admission in patients with AMI were significantly lower than those of healthy subjects with the same age in average. Interestingly, prior myocardial infarction, hypercholesterolemia and diabetes mellitus were associated with lower admission endoglin levels among AMI patients. Since all three are associated with endothelial dysfunction [24] these data further support the hypothesis that reduced serum endoglin levels may be a marker of an impaired endothelial function. We and others previously demonstrated that tissue and cellular levels of endoglin are determinants of both endothelial-type nitric oxide synthase (eNOS) expression and NO production [25, 26]. Our study also demonstrated that serum endoglin levels decreased 48 hrs after admission in AMI patients and that early endoglin decrease may be a novel prognostic indicator of early cardiovascular death. Endoglin decrease could reflect attenuated endothelial response to ischemia or may be secondary to myocardial damage and consequent impaired angiogenesis in AMI patients. Consistent with this, we have recently demonstrated in an adult mouse hind limb ischemia model that decreased tissue levels of endoglin are associated with impaired angiogenesis [27, 28]. Therefore, changes in serum endoglin levels in AMI patients may reflect the effectiveness of angiogenesis in ischemic/necrotic myocardium and its relation with endothelial function.

In conclusion, our findings demonstrate an association between early decrease in serum endoglin level and short-term and overall outcome post-AMI. We have also provided further insight into the pathophysiology of myocardial ischemia and have raised important questions regarding the role of angiogenesis in cardiovascular disease.

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