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Influence of preinfarction angina on the release kinetics of endothelial progenitor cells and cytokines during the week after infarction.

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NO conflict of interest.

Structured Abstract

Background: Preinfarction angina, a possible form of ischemic preconditioning, improves the prognosis in patients who experience a major ischemic event, though the associated pathophysiology is not yet fully understood. The aim of this study was to determine the possible involvement of endothelial progenitor cells (EPC), the vascular endothelial growth factor (VEGF) and the hepatocyte growth factor (HGF) in the development of preinfarction angina.

Methods and results: We studied 41 patients (60.5±12 years; 34% women) and 14 healthy controls; 43.9% of the patients had preinfarction angina. No differences were found in the baseline characteristics of the two groups. Although the EPC, VEGF and HGF were raised as compared with the control group, no significant differences were found according to the presence or absence of preinfarction angina in the levels of EPC (baseline, p=0.25; day 3, p=0.11; day 7, p=0.32), VEGF (baseline, p=0.96; day 3, p=0.06; day 7, p=0.57), or HGF (baseline, p=0.18; day 3, p=1; day 7, p=0.86). An association was seen in the patients who had preinfarction angina between the EPC levels at baseline and on days 3 and 7 and the HGF on admission with the time from the angina to the STEMI (β=-0.070; β=-0.066; β=-0.081; β=-80.16; P<0.05), showing a reduction in the level of EPC cells for each hour passed since the event.

Conclusions: No differences were found in the release kinetics of endothelial progenitor cells, VEGF or HGF after a first infarction according to whether or not the patients had angina during the week before the infarction.

Key words: Acute myocardial infarction; preinfaction angina; vascular endothelial growth factor; hepatocyte growth factor; endothelial progenitor cells.

INTRODUCTION

Ischemic preconditioning, initially reported by Murry et al (1), is a protective phenomenon of the heart in response to short consecutive episodes of ischemia prior to a later major ischemic event. Preinfarction angina has been described as a clinical form of ischemic preconditioning (2,3), and is associated with fewer cardiovascular complications in patients who have it just before an infarction (4,5). Preinfarction angina is also associated with a smaller infarct, fewer patients with heart failure, a lower cardiac mortality, fewer malignant ventricular arrhythmias and even greater recovery of ventricular function after the infarction. However, this beneficial effect does not appear to be permanent (6), and various circumstances related with its clinical effect, such as age, are under discussion (7,8).

Multiple mechanisms are involved in the pathophysiology of ischemic preconditioning: phosphorylation of protein kinase, K-dependent membrane channels, nitric oxides (eNOS, iNOS), free oxygen radicals, COx-2 or HIF-1 (hypoxia-inducible Factor-1) (9,10,11), though the mechanisms have yet to be fully defined. Several theories have been put forward over recent years concerning the possible role played by endothelial progenitor cells (EPC) and cytokines in the pathophysiology of ischemic preconditioning (12,13,14). The role of cells and cytokines has been studied in animals (12) or under experimental conditions (13,14), but no evidence currently exists concerning the pathophysiology in patients with prior angina and acute myocardial infarction, despite the growing interest in new forms of cardioprotection in patients with ischemic heart disease (15,16). The aim of this study, therefore, was to determine the possible existence of a different cell activation (EPC and the cytokines vascular

endothelial growth factor, VEGF and hepatocyte growth factor, HGF) that could be involved in the pathophysiology of preinfarction angina.

MATERIAL AND METHODS

Patients

From August 2006 to June 2007 we studied 41 patients admitted with a diagnosis of acute myocardial infarction, defined as an acute coronary syndrome with ST elevation (STEMI) with suggestive chest pain and an elevation of at least 3 mm in the ST segment in at least three precordial leads, with no history of ischemic heart disease and within 8 h of symptoms. Preinfarction angina was defined as the presence of at least one chest pain less than 30 min, the week before the onset of the infarction (8). A control group was composed of 14 persons with similar demographic characteristics who attended the hospital to provide blood samples. These persons provided a short medical history relating to risk factors and the reason for the blood extraction, and were excluded if they had prior cardiac disease. Patients were excluded if they had had chest pain compatible with angina for more than one week before the infarction or if they had underlying structural heart disease (cardiomyopathy or important valve disorders) (8). Cardiovascular risk factors were defined according to the ACC/AHA standards (17). A history of smoking was considered to exist if the person smoked at the time of blood extraction or had quit smoking within the previous month. The study conformed to the norms of the Declaration of Helsinki and was approved by the hospital Ethics Committee. All the patients gave written informed consent for inclusion in the study. The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology

Immunophenotype characterization and quantification of the circulating progenitor cells.

Three blood samples were taken from the patients with STEMI (on arrival at the emergency department (first 8 hours since symptoms beginning), and on days 3 and 7) and one sample in the control group. The samples were processed by flow cytometry and stored for later analysis after verifying that all the patients had normal blood test results. The blood samples from all the participants were collected in tubes with EDTA (ethylenediamine tetraacetic acid) and processed within 24 h after extraction. The quantification of the CD34+ cell subset in peripheral blood was done following the ISHAGE guidelines (18). The cells were stained with the following anti-human antibodies: anti-CD34 labeled with fluorescein isotiocianate (FITC, Becton Dickinson clone, Pharmigen, San Jose, CA), anti-CD133 labeled with ficoerythrine (PE, Miltenyi Biotech clone, Germany), anti-CD45 labeled with protein pyridine chlorophyll (PerCP, Becton Dickinson clone) and anti-KDR labeled with aloficocianina (APC, R&D Systems clone, Minneapolis, MN). To rule out nonspecific antibody binding, isotopic controls were used (Becton Dickinson and R&D systems). The blood samples were acquired with a FACScalibur flow cytometer and analyzed using Cell-Quest (Becton Dickinson) software. Cell residues were excluded with forward-sideways scatter signals. Measurements were made of the absolute number of CD34+, CD34+CD133+, CD34+KDR+ and CD45+weak CD34+CD133+KDR+ (EPC) in the population of circulating mononuclear cells and of the percentage of KDR+ and CD133+KDR+ (EPC) cells in the CD45+weak CD34+ subset.

Immunophenotype characterization of cytokines

Venous blood samples were obtained at the time of admission and on day 3 and day 7 and collected in sterile tubes containing EDTA as anticoagulant. Plasma was obtained

within 1 hour by centrifugation at 2000*g* at room temperature for 10 min. All plasma samples were aliquotted and frozen at –80ºC until analysis.

Measurement of vascular endothelial growth factor and hepatocyte growth factor concentrations

Plasma vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) concentrations were determined using commercially available quantitative sandwich enzyme-linked immunosorbent assay kits (ELISA, R&D System, Minneapolis, MN) according to the manufacturer's guidelines. Inter- and intra-assay variation was found to conform to the information provided by the supplier. Laboratory measurements were made by researchers who were blinded to clinical data.

Statistical analysis

The quantitative data are expressed as mean ± standard deviation and the qualitative data as percentages. The Shapiro-Wilk test was made first to verify normality. The Student *t* test was used to compare means in independent groups if the variables complied with normality and the Mann-Whitney U test if not. To determine the presence of significant differences in the levels of each cell subset between baseline and days 3 and 7 according to whether the patient had previous angina or not, we used two-way ANOVA for repeated measurements. For the group of patients with prior angina, a logistic regression model was constructed, where the dependent variable was the number of cells and the independent variable was the time since the previous angina. Differences were considered to be statistically significant if the $P<0.05$. The calculations were made with SPSS 12.0 (SPSS Inc., Chicago, IL).

RESULTS

Baseline characteristics

The mean age of the 41 consecutive patients admitted to our hospital who fulfilled the inclusion criteria was 60.5±12.3 years, with 34.1% women; 68.3% had hypertension, 29.3% diabetes, 41.5% dyslipidemia, 63.4% were smokers and 43.9% had had angina the week before the infarction (Table 1).

Groups with and without previous angina

Of the 41 study patients, 43.9% (18 patients) had preinfarction angina. In comparison with the group of patients with no previous angina (Table 1), only hypertension was associated with the presence of previous angina (88.9% vs. 52.2%; P=0.012). No differences were seen in age, sex, diabetes mellitus or previous treatment. Nor were significant differences found concerning post-infarction ventricular dysfunction, maximum elevation of CPK or start of revascularization therapy (Table 1).

Influence of baseline characteristics and presence of preinfarction angina

With the exception of hypertension (OR=7.33; 95% CI, 1.36-39.43), the risk of preinfarction angina was not significantly increased by any of the other baseline characteristics studied, i.e., diabetes (OR=1.41; 95% CI, 0.36-5.47), dyslipidemia (OR=1.87; 95% CI, 0.53-6.61), or number of vessels affected (OR=2.5; 95% CI, 0.60-10.26).

Kinetics of endothelial progenitor cells, vascular endothelial growth factor and hepatocyte growth factor.

Analysis of the kinetics of the EPC showed a progressive increase over the different time points, reaching a maximum on day 7 after onset of symptoms (Table 2). However, comparison of the EPC kinetics according to whether the patient had preinfarction angina or not showed no significant differences between the two groups in the number of EPC mobilized in plasma (Table 2) or in their kinetics (Fig. 1). Significant

differences were found in the level of EPC on admission, and on days 3 and 7 as compared with the control group (P<0.001), but not with the status of previous angina (P=0.61).

The plasma levels of VEGF rose to a peak on day 3, later falling on day 7. The differences were significant between days 1 and 3, and nearly significant between days 1 and 7. As with the EPC, there were no differences according to whether the patients had had preinfarction angina or not, either in their plasma concentration or in the kinetics of the curve (Table 2; Fig. 2). The differences between those with and without preinfarction angina in the levels of VEGF at baseline and on days 3 and 7 were not significant at any of the 3 points (P=0.27), nor was the interaction with the status of prior angina significant (P=0.36).

The highest plasma concentrations of HGF were seen on admission, with no differences according to whether the patients had preinfarction angina or not (Table 2; Fig. 3). Significant differences were found in the HGF levels between admission, days 3 and 7 at each point (P<0.001) compared with the control group, but not with the status of previous angina (P=0.65).

Relation between time since angina and EPC levels

The baseline level of EPC in the patients who had preinfarction angina was significantly associated with the time from the angina to the STEMI (β=-0.070; 95% CI, -0.123 to -0.017; P=0.013), with a reduction in EPC levels for each hour that had passed. The same was seen for the EPC cells on day 3 (β=-0.066; 95% CI, -0.122 to -0.011; P=0.022) and day 7 (β=-0.081; 95% CI, -0.146 to -0.017; P=0.017). The baseline level of the cytokine HGF was significantly associated with the time from the angina to the STEMI (β=-80.16; 95% CI, -145.69 to -14.63; P=0.021) (Table 3)

DISCUSSION

The results of this study show the lack of differences in the release kinetics of endothelial progenitor cells and cytokines (VEGF and HGF) according to the presence or absence of preinfarction angina in a group of patients with a first myocardial infarction.

Hypoxia-ischemia, especially the acute form, plays an important role as an attractant of EPC and growth factors. It also has a potentiation effect in experimental situations, increasing the differentiation and migration capacity of the EPC (13,18), as well as their sensitivity to chemoattractants, such as HGF (18).

Small studies in patients have found a greater increase in VEGF in patients with acute myocardial infarction (AMI) and prior angina as compared with those without prior angina (19,10), whereas HGF levels were no different (20). Likewise, an experimental study found a higher number of EPC in those with previous ischemia (12). This rise in VEGF in states of preinfarction angina has not only been attributed to a direct angiogenic and chemotactic role exerted by the ischemia, but also to the setting in motion of classic pathophysiological mechanisms related with the development of the preconditioning, such as the activation of protein kinase (21,22). Both EPC (12) and VEGF (23) may thus be important mediators in the cascade of ischemic preconditioning, such that after a first minor ischemic event that activates this cascade, a later major ischemic event again activates the preconditioning mechanism, though this time much more efficiently, producing a greater and faster release of EPC, as well as chemotactic growth factors. However, as commented, this different response was not found in our study between those patients with preinfarction angina and those without.

As with earlier studies (5), this study shows that preinfarction angina is an entity that confers a certain degree of protection in patients who experience it, and shows how

these persons tend to have a milder AMI (greater post-AMI EF) in comparison with those who do not have preinfarction angina. Given the limited sample size we saw no significant differences in the number of cardiovascular events. We did, however, detect a greater number of cells in those patients whose time from the angina to the onset of STEMI was shorter, which shows that the efficacy of the mechanisms of preinfarction angina is greater in the first part of the double window period described by Yellon et al (24), thus suggesting that the activation of the various pathophysiological mechanisms involved in ischemic preconditioning is more effective the closer the major ischemic event is to the minor event. These data agree with those reported by Kloner et al (4), who described a greater benefit of previous angina when this occurred just a few days before the major ischemic event.

Accordingly, although the presence of ischemic preconditioning and preinfarction angina is accepted and its benefits in patients who have it are recognized, we still do not fully understand its physiology and cannot yet use it for therapeutic purposes.

Due to sample size and other study limitations, we should be carefull stating that this study rules out the possibility of the direct involvement of EPC and cytokines in the pre-infarction angina. But even so, the aim of this study was to generate or discard a hypothesis that could explain the protective effect of preinfarction.

Conclusions

Following a first infarction there occurs a significant increase in EPC, as well as in HGF and VEGF in comparison with the controls. This increase was more marked on day 7 for the EPC, on day 3 for the VEGF and clearly on day 1 for the HGF. However, no significant differences were detected in the release kinetics and peripheral blood concentrations of EPC or the growth factors VEGF and HGF depending on the presence

or absence of preinfarction angina, and we could not therefore determine their participation in the pathophysiology of preinfarction angina.

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Table 1. Baseline characteristics of the whole group, the groups with and without previous angina, and the control group.

	Whole group N=41	***With previous angina n=18 (43.9%)***	***Without previous angina n=23 (56.1%)***	*Controls (N=14)*
Baseline characteristics				
Age (yrs)	60.5±12	60.7±9.5	60.3 ±14	63.4±4.9
Women	34.1%	5 (27.8%)	9 (39.1%)	7 (50%)
Hypertension	28 (68.3%)	16 (88.9%)	12 (52.2%)*	7 (50%)
Diabetes Mellitus	12 (29.3%)	6 (33.3%)	6 (26.1%)	4(28.6%)
Dyslipidemia	17 (41.5%)	9 (50%)	8 (34.8%)	6(42.9%)
Smokers	26 (63.4%)	11 (61.1%)	15 (65.2%)	4(28.6%)†
Prior kidney failure	2 (4.9%)	1 (5.6%)	1 (4.3%)	-
Angiography				
Lesion in LM	2 (5.6%)	1 (6.7%)	1 (4.8%)	-
Lesion in ADA	18 (50%)	7 (46.7%)	11 (52.4%)	-
Number of vessels	1.49 ±0.7	1.71 ±0.8	1.33 ±0.5	-
LVD post AMI	4 (10%)	1 (5.9%)	3 (13%)	-
EF post AMI	51.70 ± 11	54.3 ±8.6	49.7 ±13.5	-
CPK max (UI/ml)	1986±2115	1746 ±2144	2181 ±2122	-
Time since previous first angina (hours)	27.51 ± 2	27.51 ± 27	-	-
Time to start of revascularization (min)	171.68±96	155.8 ±88.4	184.6 ±106	-

* P<0.05 vs. previous angina; † P<0.05 vs. overall group

AMI: acute myocardial infarction; LVD: left ventricular dysfunction; EF: ejection fraction; LM: Left main coronary disease; ADA: Anterior Descending Artery.

The quantitative variables are expressed as the mean ± standard deviation

Table 2. Values of EPC and cytokines at the different times after extraction.

	First 8 hours	*Day 3*	*Day 7*	*Control Group*
EPC (EPC/10^3 leuc)	6.83±4.49	6.50±4.21	8.03±5.17	0.50±0.22
Without PA	8.01±5.15	7.65±4.73	9.24±5.93	
With PA	5.48±3.26	5.26±3.29	6.65±3.89	
VEGF (µgr/ml)	86.75±115.43	114.53±114.02	101.17±73.10	57.01±26.65
Without PA	71.06±43.83	83.57±52.28	91.61±55.59	
With PA	110.89±177.34	159.78±160.46	115.13±93.89	
HGF (µgr/ml)	10175.59±4561	3743.59±4367	2655.43±4022	859.14±607.83
Without PA	9272±4759	3658±4578	2087±2296	
With PA	11459±4072	3868±4218	3486±5712	

PA: preinfarction angina; EPC: endothelial progenitor cells; HGF: hepatocyte growth factor; VEGF: vascular endothelial growth factor.

Statistical differences depending of de presence of preinfarction angina or not:

- EPC: First 8 hrs: 0,249; Day 3: p: 0,155; Day 7: p: 0,317
- VEGF: First 8 hrs: 0,956; Day 3: p: 0,057; Day 7: p: 0,578
- HGF: First 8 hrs: 0,258; Day 3: p: 1,000; Day 7: p: 0,863

The quantitative variables are expressed as the mean ± standard deviation

Table 3. Relation between the time passed (hours) since the previous angina and the numbers of the mononuclear cell subsets and cytokines studied.

	Baseline			Day 3			Day 7		
	β	95% CI	p	β	95% CI	p	B	95% CI	p
EPC	-0.07	[-0.12.-0.02]	0.013 (*)	-0.07	[-0.12.-0.01]	0.022 (*)	-0.08	[-0.15.-0.02]	0.017 (*)
HGF	-80.16	[-145.69.-14.63]	0.021 (*)	-51.57	[-161.78. 58.64]	0.328	63.62	[-42.65. 169.90]	0.217
VEGF	3.95	[1.37. 6.55]	0.006 (*)	3.63	[1.56. 5.71]	0.002 (*)	-0.58	[-2.37. 1.21]	0.495
CD34	0.10	[-0.27. 0.46]	0.574	-0.01	[-0.44. 0.41]	0.946	0.07	[-0.31. 0.44]	0.701
CD3CD4133	-0.04	[-0.11. 0.04]	0.318	-0.03	[-0.11. 0.04]	0.340	-0.05	[-0.14. 0.03]	0.208
CD34KDR	-0.03	[-0.10. 0.03]	0.322	-0.05	[-0.14. 0.04]	0.292	-0.05	[-0.13. 0.03]	0.221

Independent variable: time since angina.

(*) P< 0.05

Figure 1. Kinetics of the EPC according to the presence or absence of preinfarction angina.

Figure 2. Kinetics of the VEGF according to the presence or absence of preinfarction angina.

Figure 3. Kinetics of the HGF according to the presence or absence of preinfarction angina.

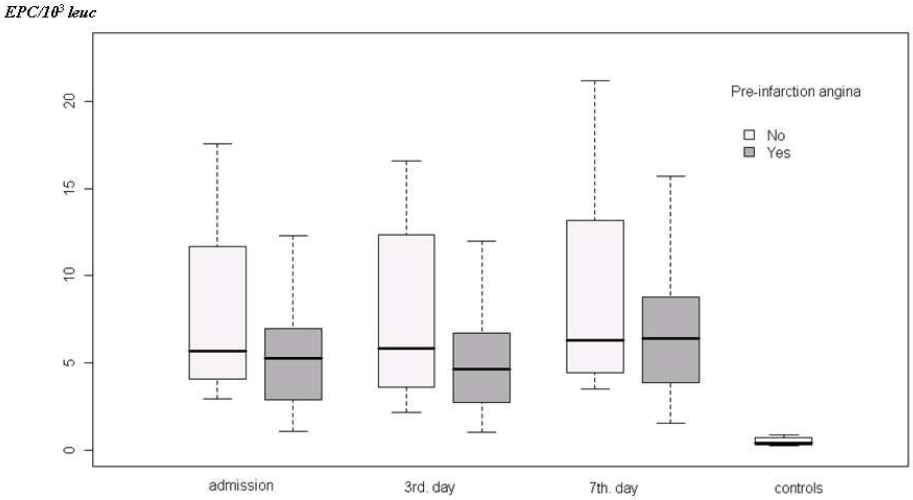

Figure 1. Kinetics of the EPC according to the presence or absence of preinfarction angina.

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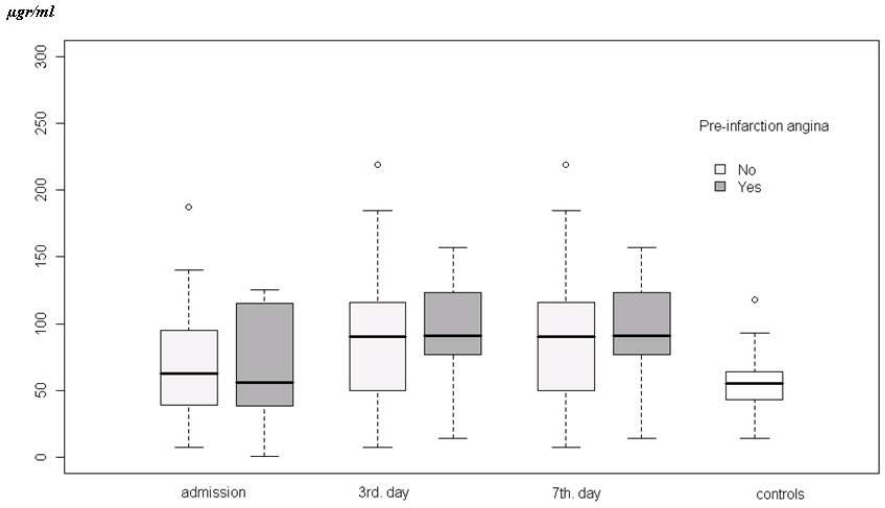


Figure 2. Kinetics of the VEGF according to the presence or absence of preinfarction angina.

254x190mm (96 x 96 DPI)

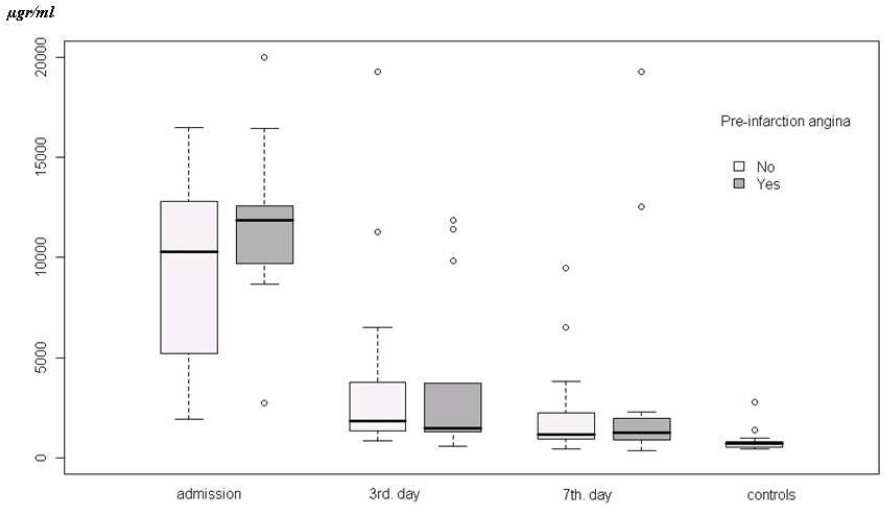
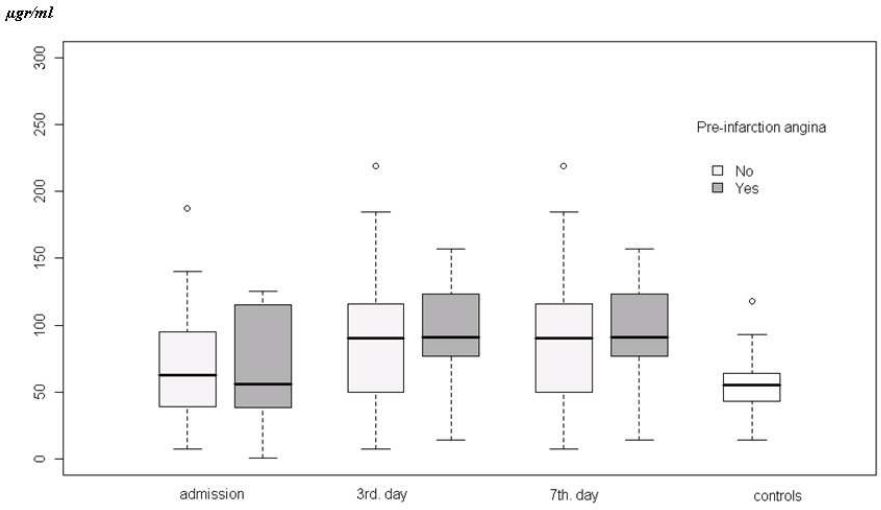


Figure 3. Kinetics of the HGF according to the presence or absence of preinfarction angina.

254x190mm (96 x 96 DPI)



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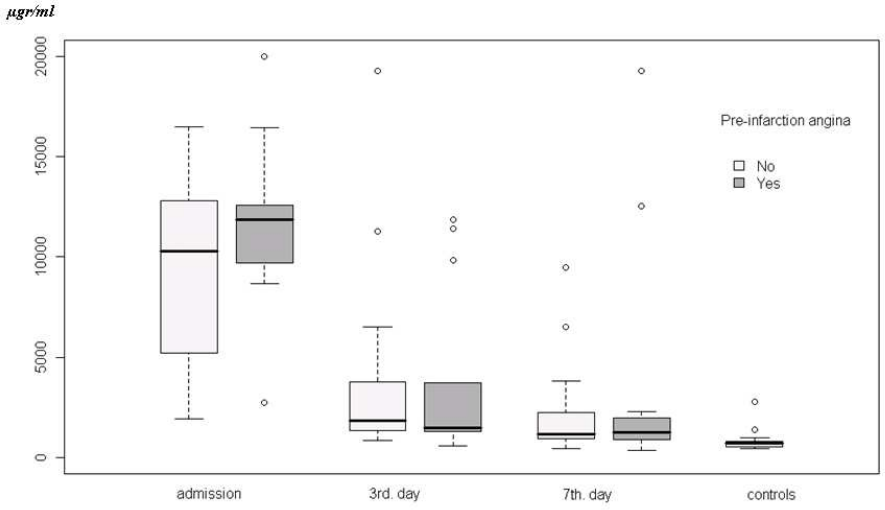

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