Hepatocyte growth factor — the earliest marker of myocardial injury in ST-segment elevation myocardial infarction

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Abstract

Background: Hepatocyte growth factor (HGF) concentration increases in the first few hours of myocardial infarction (MI).

Aim: (1) To illustrate human HGF (hHGF) plasma concentration during the first 24 h of ST segment elevation myocardial infarction (STEMI); (2) To estimate the odds ratio of STEMI in the context of hHGF measurements; (3) To describe the normal concentration of hHGF in healthy subjects.

Methods: The study groups consisted of 73 STEMI patients and 11 healthy volunteers. In all the patients, we took blood samples for hHGF twice, i.e. on admission to hospital and 24 h later.

Results: The median value of hHGF in healthy volunteers was 666 pg/mL (576; 760 pg/mL). In STEMI, the highest values of hHGF were observed in the first measurement. An increase of 1 pg/mL in hHGF level increased STEMI odds ratio by 0.2%.

Conclusions: In acute MI, of the known biomarkers, hHGF rises the earliest and very promptly returns to normal values.

Key words: myocardial infarction, STEMI, biomarkers, hepatocyte growth factor

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INTRODUCTION

Hepatocyte growth factor (HGF) was originally identified and cloned as a potent mitogen for hepatocytes [1–3]. HGF exhibits also mitogenic, motogenic, and morphogenic activities for a wide variety of cells [1–5]. Recent studies have indicated that HGF has multiple activities in various tissues during the development and course of various diseases. The list of the diseases in which HGF increases is very long and includes: hepatic disorders, pulmonary diseases, renal diseases, pneumonia, pancreatitis, ulcerative colitis, Crohn's disease, rheumatoid arthritis, Alzheimer's disease, Parkinson's disease and many others. Circulating HGF levels are closely correlated with a disease stage and parameters. Biological activities of HGF have been found to be initiated by autophosphorylation of proto-oncogene c-Met, the receptor tyrosine kinase for HGF (cMet/HGF) [6]. Receptor for HGF is induced in the embryonic heart and constitutionally expressed in the adult heart, especially in coronary endothelial [7, 8]. Ueda et al. [9] revealed that both HGF and c-Met/HGF receptor mRNAs were upregulated in response to myocardial ischaemic injury, and that HGF is likely to have a cytoprotective effect on cardiac tissue, presumably through the c-Met/HGF receptor. In 1996 Matsumori et al. [10], and in 1997 Sato et al. [11], in small groups of patients (10–12 subjects) with acute myocardial infarction (MI), revealed the usefulness of HGF as a sensitive method for early diagnosis of acute MI and prognostic indicator of clinical course in this heart disease. We revealed the importance of HGF concentration measurement in an early stage of acute coronary syndrome in the results of the study we published in 2010 [12]. This showed that in the first few hours of MI, the HGF concentration reached the highest values and preceded an increase in troponin I, a myocardial necrosis marker assessed routinely [12]. Comparison between patients with major, moderate and minor myocardial damage in ST segment elevation MI (STEMI) and non ST segment elevation MI (NSTEMI) revealed differences in peak HGF concentration [12]. We also found that HGF was a significant prognostic factor whose levels during acute MI (concentrations at admission)

correlated with the presence of serious cardiovascular events which made up a primary composite end point, and were observed in a three-month follow-up in our study patients [12]. In 2011, Lamblin et al. [13] published results of a study which confirmed our results because they revealed that circulating HGF levels measured at discharge in patients with first acute MI correlate with all markers of left ventricular remodelling and are associated with rehospitalisation for heart failure.

Due to the very promising results of the first part of our study, we decided to continue research in patients with STEMI. The aims of the next phase of the study were: (1) to illustrate HGF plasma concentration during the first 24 h of STEMI; (2) to estimate the odds ratio of STEMI in the context of HGF measurements; and (3) to detect the normal HGF concentration in healthy subjects.

METHODS

Our study comprised a total of 73 patients admitted between January 2007 and September 2009 to the Coronary Care Unit at the Institute of Cardiology (mean age: 57 ± 11 years, range: 31–84 years, 15% women) with a first episode of acute coronary syndrome in their lives, who were eligible for coronary angiography and, eventually, percutaneous coronary intervention (PCI). All the subjects agreed to participate in the study after being informed of its nature and purpose. Patients' informed consent and the protocol of the study were approved by the Institutional Local Ethics Committee. The inclusion criteria were: (1) typical criteria of STEMI according to European Society of Cardiology guidelines, (2) indication for coronary angiography (with PCI, if necessary), (3) signed informed consent form. The exclusion criteria were: (1) known history of MI, and (2) refusal to sign a consent form.

The group of healthy volunteers consisted of 11 subjects (mean age: 42 ± 6 years, range: 32–54 years, 55% women), without any symptoms of diseases in actual history or in physical examination. None of the volunteers was currently being treated with any drugs. They agreed to participate in the study and each signed an informed consent approved by the Institutional Local Ethics Committee. In the group of healthy volunteers, human HGF (hHGF) levels were measured once to establish the normal value of hHGF for our laboratory and to estimate the receiving operation curve.

In the group of 73 patients, we took blood samples for hHGF twice, i.e. on admission to hospital and 24 h later.

All patients were additionally assessed routinely for such biomarkers of myocardial injury as cardiac troponin I (cTnI), creatinine kinase MB isoenzyme (CK-MB) activity, N-terminal prohormone B-type natriuretic peptide (NT-proBNP) and high sensitive C-reactive protein (hsCRP). These measurements were performed twice, i.e. on admission to hospital due to STEMI and 24 h later. Siemens assay was used to determine cTnI (diagnostic cut-point 0.1 ng/mL) and CK-MB activity (diagnostic cut-point was 6 U/L). NT-proBNP (cut-point 125 pg/mL) and hsCRP with cut-point 0.5 mg/dL were established with Roche assay. All these measurements were performed in a standard diagnostic laboratory, while plasma concentration of hHGF was determined in the research laboratory of the Institute of Cardiology. Blood was collected into tubes with 5% EDTA and centrifuged at 2,000 g for 15 min at 4°C. Separated plasma was stored at –80°C until assay. Human HGF was determined in plasma using Quantikine Elisa kit (R&D System, Minneapolis, MN, USA). Assay sensitivity was 40 pg/mL. Intra- and inter-assay coefficients of variation were 7.0% and 8.4%, respectively. Mean value of hHGF evaluated in EDTA plasma using Quantikine Elisa kit and presented in the manufacturer's brochure as a reference value was 787 pg/mL (range 469–1113 pg/mL).

Statistical analysis

For non-normally distributed data, the results are expressed as the median and interquartile ranges. For normally distributed data, the results are shown as mean value and standard deviation. The logistic regression model was used to estimate the receiver operating characteristic (ROC) curve for hHGF.

RESULTS

Among 73 patients with STEMI, in 30 (41%) anterior or lateral or antero-lateral MI was diagnosed. In 43 (59%) patients, inferior MI and/or posterior or additionally lateral was present. The mean chest pain duration was 226 ± 185 min. The median values of examined biomarkers on the first and second days of STEMI are shown in Table 1. Except CK-MB, all the changes in plasma concentration of examined biomarkers were significant ($p < 0.0001$) (Table 1). The highest concentration of hHGF was observed in the first measurement, while hsCRP, NT-proBNP, CK-MB and cTnI level increased in the second measurement (Table 1). The median values of hHGF and other markers of myocardial injury patients with STEMI are presented in Table 1. The median value of hHGF in healthy volunteers was 666 pg/mL (576; 760 pg/mL). Receiver operating characteristic (ROC) curve, which illustrates the sensitivity and specificity for STEMI detection by measuring hHGF, was calculated by statistical analysis of values from the first hHGF measurements in patients with STEMI and values from healthy volunteers (Fig. 1). It was revealed that an increase of 1 pg/mL in hHGF level increased STEMI odds ratio by 0.2%. Table 2 presents the number of patients with negative and positive hHGF and cTnI observed in the first measurement. On admission, hHGF sensitivity for STEMI diagnosis was 49/54 (91%) while cTnI sensitivity was 49/64 (77%). At the same time, specificity for hHGF was 4/19 (21%) and 4/9 (44%) for cTnI.

DISCUSSION

Clinically, due to recommendations as to treatment choice, it is particularly important to diagnose MI as soon as possible [14, 15].

Table 1. Values of all examined markers of myocardial injury in first and second measurement in 73 patients with ST elevation myocardial infarction shown as median value and values from 25% and 75% interquartile ranges (in brackets)

Parameter/measurement	First: on admission	Second: 24 h later	Δ
hsCRP [mg/dL]	0.17 (0.105; 0.455)	1.09 (0.53; 2.4)	(+) 0.865 (0.345; 2.075)*
NT-proBNP [pg/mL]	239.4 (127.1; 443.8)	1,296 (680.3; 5,009)	(+) 915.8 (421.8; 2,115.6)*
CK-MB [U/L]	12.1 (5.2; 63)	41.6 (22.9; 77.5)	(+) 14.8 [(–) 13.1; 40.3]
cTnI [ng/mL]	0.34 (0.1; 2.14)	26.46 (10.895; 50.43)	(+) 19.915 (7.265; 48.02)*
hHGF [pg/mL]	3,666 (833; 8,000)	699 (478; 899)	(–) 2,701 [(–) 7,058; (–) 32]*

Δ difference between first and second measurement, (+) increase, (–) decrease; *p < 0.0001; hsCRP — high sensitive C reactive protein; cTnI — cardiac troponin I, CK-MB — creatinine kinase MB isoenzyme, NT-proBNP — N-terminal prohormone B-type natriuretic peptide; hHGF — human hepatocyte growth factor

Table 2. Number of patients with negative and positive hHGF and cTnI in the first measurement

	Number of patients (%) with cTnI negative	Number of patients (%) with cTnI positive	Total
Number of patients (%) with hHGF negative	4 (5%)	5 (7%)	9 (12%)
Number of patients (%) with hHGF positive	15 (21%)	49 (67%)	64 (88%)
Total	19 (26%)	54 (74%)	73 (100%)

hHGF — human hepatocyte growth factor; cTnI — cardiac troponin I; positive cTnI > 0.1 ng/mL, positive hHGF > 666 pg/mL

p = 0.1086
OR = 1.002, 95% CI = 0.999–1.005
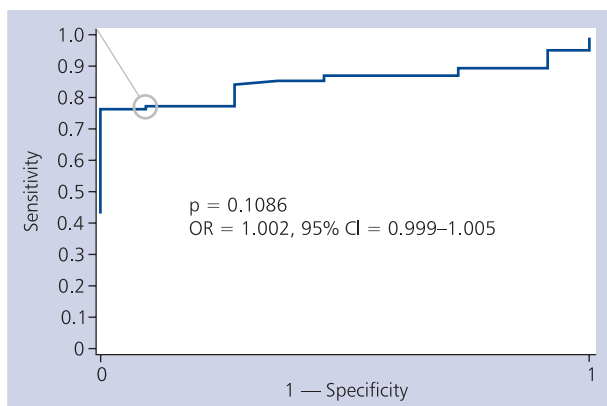
Figure 1. Receiver operating characteristic (ROC) curve for diagnostic importance of human hepatocyte growth factor (hHGF) in ST segment elevation myocardial infarction. ROC curve threshold point for hHGF was 831.1 pg/mL. This means that for this value of hHGF, maximum of Youden index was fulfilled. Threshold point is marked by a grey circle

In most patients, the diagnosis of acute coronary syndrome, especially STEMI, is based on such typical clinical syndromes as chest pain and ECG changes, and then treatment is introduced since the proper diagnosis need not be confirmed by an elevation of myocardial injury markers levels, such as troponins or CK-MB [14, 15]. During MI, an initial increase in troponins is seen about 4 h after the onset of acute coronary syndrome symptoms and may persist elevated for up to two weeks caused by proteolysis of contractile apparatus [15]. In the very early course of MI, the level of biomarkers is within,

or slightly over, normal limits. Postponing reperfusion therapy while waiting for MI to be confirmed by troponin level rise is an unnecessary waste of time and unfavourably affects the outcome of STEMI treatment. Unnecessary hospital delay in treatment reduces the positive results of fast and direct patient transport to the cath lab. The only situation which demands additional waiting for biomarker levels findings is an atypical course of MI when it is impossible to exclude diagnosis of other diseases with similar symptoms, such as acute aortic dissection, pulmonary embolisation, or myocarditis. This is the main reason why we still are looking for easy and fast diagnostic methods of STEMI.

The normal values of HGF in healthy females and males are known from the publications of Toi et al. [16] and range from 0.26 ± 0.17 to 0.39 ± 0.25 and from 0.29 ± 0.17 to 0.37 ± 0.22 ng/mL, respectively (after recalculation for pg/mL: 260 ± 170, 390 ± 250, 290 ± 170 and 370 ± 220). In 2000, Zhu et al. [17] measured HGF in 37 patients with MI and in 13 healthy subjects. They revealed that the mean value of HGF in the controls was 0.12 ng/mL [18]. In healthy volunteers examined in our study, the median value of hHGF was evaluated as 666 pg/mL. This concentration of hHGF was very similar to the reference value — 787 pg/mL described by producers of hHGF immunoassays kits used in the study and about two times higher than the values presented by Toi et al. [16] and about five times higher than those described by Zhu et al. [17]. These differences could be connected to different laboratory techniques or reagents. It was necessary to establish normal values for hHGF for our laboratory because we wanted to define the sensitivity and specificity of hHGF

as a STEMI detection marker. The ROC curve showed that an increase by 1 pg/mL in hHGF level increased STEMI odds ratio by 0.2%.

The study revealed that the highest values of hHGF were observed in the first assessment, i.e. performed on admission to hospital due to STEMI; they more than five times exceeded the concentration of 666 pg/mL which was estimated in healthy subjects. Moreover, the mean chest pain duration was < 4 h. Matsumori et al. [10] found that serum HGF was elevated within 3 h in 80% of patients with MI after the onset of chest pain. The mean value of HGF concentration was 9.4 ng/mL = 9,400 pg/mL, while the values in healthy subjects were < 0.39 ng/mL (< 390 pg/mL) [10]. Other important findings from the Matsumori et al. study [10] revealed that elevated HGF levels were significantly more frequent than those of creatinine kinase within 3 h, and elevated levels correlated well with those of serum creatinine kinase at 6–9 h after onset of acute MI. The authors did not find an increase in serum HGF value in patients with angina pectoris or other heart diseases [10]. For this reason, they concluded that HGF was a sensitive method for the early diagnosis of acute MI [10]. Zhu et al. [17] in 37 patients with acute MI also revealed the peak serum HGF concentration on admission (mean 0.54 ng/mL). Moreover, they found that HGF concentration had decreased by the 21st day after the onset of infarction [17]. The authors did not measure HGF concentration 24 h after the onset of MI as we did [17]. The intervals between assessments lasted seven days, so it is impossible to conclude when the maximal reduction of HGF level occurred [17].

We found hHGF 91% sensitivity for diagnosis of STEMI on admission, while at the same time cTnI sensitivity was lower (77%). Due to the concentration of HGF increasing in many different diseases, we expected low hHGF specificity. In our opinion, the advantage of hHGF compared to cTnI was high sensitivity on admission. This establishes hHGF's usefulness as a marker for early STEMI diagnosis confirmation.

HGF concentration can be influenced by heparin administration [17, 18]. In rats, heparin administration was found to increase blood HGF levels by 2.5–5 times compared to the control rats which received saline alone [18]. Zhu et al. [17] showed that intravenous administration of 3,000 units markedly, although transiently, increased circulating HGF levels by as much as 60-fold. HGF concentration had decreased to a negligible level 24 h latter [17]. Today, during the first medical contact, STEMI patients have to be pretreated with aspirin, clopidogrel and heparins. For this reason, it is impossible to include STEMI patients who did not receive medical treatment before hospital admission. Our previous study revealed significantly different HGF concentrations in STEMI patients: 3,398 (855; 8,000) and 3,239 (809; 7,516) and NSTEMI patients: 905 (614; 3,239) [12]. While STEMI and NSTEMI patients received heparins, the differences in HGF concentrations depended on the extent of the myocardial damage. The presented results of the hHGF measurement in STEMI patients suggest that hHGF is the earliest known marker of myocardial necrosis. In STEMI, neither troponins nor CK-MB increase as early as hHGF does. What is more, routinely measured markers of myocardial injury do not return to normal as quickly as hHGF does (Table 1). Independently of a concomitant disease, such as liver and lung diseases, renal failure, cancer, diabetes, hypertension or neurologic diseases in which blood hHGF concentration may also rise, the diagnosis in MI is confirmed by the dynamics of changes in hHGF level from high values to quickly reduced low concentrations. This feature of hHGF makes it possible to diagnose ischaemic complications during the early stage of STEMI, which would not be possible to confirm by troponins and CK-MB.

In our opinion, this is a very promising biomarker which can confirm the diagnosis of MI in a shorter and definitely faster way than by other known biomarkers. Now, the main aim of our ongoing study is to assess hHGF in a large group of patients with STEMI and confirm the usefulness of this parameter in short and long term prognosis in patients with STEMI.

CONCLUSIONS

In acute MI, of the known biomarkers, HGF rises the earliest and very promptly returns to normal values.

Conflict of interest: *none declared*

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Wątrobowy czynnik wzrostu — najwcześniejszy wskaźnik uszkodzenia miokardium w zawale serca z uniesieniem odcinka ST

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Streszczenie

Wstęp: Stężenie wątrobowego czynnika wzrostu (HGF) we krwi zwiększa się w pierwszych godzinach zawału serca.

Cel: Celem pracy były: (1) ocena stężenia ludzkiego HGF (hHGF) w osoczu w czasie pierwszych 24 godzin zawału serca z uniesieniem odcinka ST (STEMI); (2) określenie ilorazu szans wystąpienia STEMI w zależności od stężenia hHGF; (3) określenie stężenia hHGF u osób zdrowych.

Metody: Do badania włączono 73 chorych ze STEMI i 11 osób zdrowych. U chorych 2-krotnie (przy przyjęciu do szpitala i po 24 h) oznaczano we krwi stężenie hHGF.

Wyniki: U osób zdrowych mediana stężenia hHGF wynosiła 666 pg/ml (576; 760 pg/ml). U chorych najwyższą wartość hHGF obserwowano w pierwszym badaniu. Wzrost stężenia hHGF o 1 pg/ml zwiększał o 0,2% iloraz szans wystąpienia STEMI.

Wnioski: U osób z ostrym zawałem serca, w porównaniu z dotychczas stosowanymi biomarkerami, podwyższone stężenie hHGF pojawia się najwcześniej i bardzo szybko wraca do normy.

Słowa kluczowe: zawał serca, STEMI, biomarkery, wątrobowy czynnik wzrostu

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