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Growth differentiation factor 15 in different stages of heart failure: potential screening implications

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Abstract

Identification of individuals in the early stage of heart failure (HF) may allow earlier initiation of disease-modifying treatment. We evaluated concentrations of the growth differentiation factor (GDF)-15at different stages and its potential screening value in 208 subjects. Plasma GDF-15 was measured by using an enzyme-linked immunosorbent assay. GDF-15 was positively correlated with the stages of HF (r=0.804, p<0.001). In distinguishing patients with stage B HF, the area under the curve was 0.873 (p<0.001). These findings indicate that GDF-15 concentration was elevated with the progressing stages of HFand might have potential screening implications for stage B HF.

Keywords: *Growth differentiation factor 15; heart failure; biomarkers*

Introduction

Despite advances in treatment, the number of deaths from heart failure (HF) has increased significantly every year (Lloyd-Jones et al. 2010). The high mortality rate of HF is partly due to the lackof a screening strategy for detection at an early stage. In 2005 the American College of Cardiology (ACC)/American Heart Association (AHA) updated their guidelines for the management of chronic HF (CHF) and identified fourkey stages in the early stages of HF in patients (Hunt et al. 2005). Among patients without signs or symptoms of HF, those at high risk of HF were defined as stage A, and those who had structural heart disease were designated as stage B. Stage C included patients with current or past symptoms of HF, and stage D designated patients with truly refractory HF. Stage B might progress gradually to clinical HF (Hunt et al. 2005), and an adequate screening test for early detection of stage B patients might greatly improve HF survival. The identification of biomarkers of potential usefulness for the clinical handling of HF has been a prolific field in past years. Amino-terminal pro-brain natriuretic peptide (NT-proBNP) has already been developed as a diagnostic and prognostic biomarker in HF (Hunt et al. 2005, Pfister & Schneider2009, Baggish et al. 2010). However, NT-pro BNP has proved to be elusive as a screening tool for asymptomatic patients with cardiac structural heart disease (stage B) (Vasan et al. 2002, de Lemos et al. 2009). Growth differentiating factor (GDF)-15, a member of the transforming growth factor-β cytokine superfamily, is the latest marker to be identified in the heart. It was originally identified as macrophage inhibitory cytokine 1 (MIC-1) (Bootcov et al. 1997). GDF-15 has a number of overlapping pathways in the heart which makes it a good choice for evaluation. Despite being undetectable in normal cardiac myocytes, GDF-15 appears to be upregulated in response to experimental pressure overload in the mouse (Xu et al. 2006). In gene-targeted mice, GDF-15 has mitigating effects on hypertrophy, apoptosis and remodelling (Kempf et al. 2006, Xu et al. 2006). In view of the fact that GDF-15 might be a protective marker of multiple stress pathways in the heart, Kempf et al. reported that the concentration of GDF-15 was increased inpatients with CHF and was closely related to disease severity (Kempf et al. 2007a). Additionally, it has been reported that GDF-15 is a new biomarker of the risk of death in patients with CHF that provides prognostic information beyond established clinical and biochemical markers (Kempf et al. 2007b).

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Therefore, the present cross-sectional study was designed to analyse the plasma GDF-15 concentration at various stages of HF. In addition, we also investigated the potential clinical application of GDF-15 for early detection of stage B HF and compared its diagnostic value with NT-proBNP.

Methods

Study population

Patients were selected from the department of cardiology at the Peking University Third Hospital (Beijing, China) over the period July 2007 to June 2010. A total of 208 participants were divided into four groups which were composed of the different stages of HF (Hunt et al. 2005).

Stage A (n=49) included hypertensive patients (n=35) according to the 2007 European Society of Hypertension (ESH)/European Society of Cardiology (ESC) hypertension guidelines (Mancia et al. 2007) and stable angina pectoris patients (n=14) diagnosed according to ESH/ESC 2006 guidelines (Fox et al. 2006).

Stage B (n=58) included patients with old myocardial infarction (OMI, n=50) and left ventricular hypertrophy (LVH, n=8). OMI patients complied with the universal definition of myocardial infarction (Thygesen et al. 2007). LVH was confirmed with echocardiographic assessment criteria (left ventricular mass index, LVMI: male≥125 gm^{-2}, female≥110 gm^{-2})(Whitworth 2003).

Stage C (n=58) recruited previously symptomatic and currently asymptomatic CHF patients according to the ACC/AHA guideline (Hunt et al. 2005).

The control (Cont) group (n=53) included healthy volunteers without HF risk factors, cardiac structural lesion or HF symptoms.

Patients with acute myocardial infarction during the preceding 12 weeks, impairment of renal function or liver function, systemic inflammatory disease, infectious disease, cancer, acute cerebral infarction, pregnancy or who were using steroids that might affect the GDF-15 level were excluded.

The study was approved by the ethics committee of Peking University Health Science Center (document identification: IRB00001052-08013) and written informed consents were all obtained.

Biochemical analysis

Blood samples were taken from an antecubital vein with an evacuated EDTA tube on the morning after patients had fasted overnight. Plasma samples were obtained within 30 min of collection by centrifugation at 3000g for 15 min at 4°C. To avoid repetitive freeze-and-thaw cycles, each sample was divided into seven to nine aliquots, immediately frozen, and stored at –80°C for analysis. Plasma GDF-15 levels were determined by sandwich enzyme-linked immunosorbent assay (ELISA) with a commercially available kit (R&D Co., Minneapolis, MN, USA) according to the manufacturer's instructions. The sample was analysed double-blindly at the Key Laboratory of Molecular Cardiovascular Sciences, Ministry of Education. Plasma NT-proBNP concentrations were measured by electrochemical luminescence methods.

Levels of creatinine, uric acid, white blood cell count and percentage of neutrophils were evaluated at the central chemistry laboratory of Peking University Third Hospital. Creatinine clearance was calculated according to the Cockcroft and Gaultequation (Matz2002).

Echocardiography

Each patient underwent echocardiography lying in the left decubitus position at the time of study entry using a GE-VingMedVechocardiographic machine (Vivid 7) with a 3.3-MHz multiphase array probe. The echocardiographic techniques and calculation of different cardiac dimensions and volumes were performed according to the guidelines of the American Society of Echocardiography (Schiller et al. 1989). Left ventricular ejection fraction (LVEF) was obtained using a modified biplane version of Simpson's method with apical two- and four-chamber views. These studies were carried out by experienced cardiologists. LVMI was calculated according to the Devereux equation (Devereux et al. 1986).

Statistical analysis

The Kolmogorov–Smirnov test was used to test for normal distribution of continuous variables. Variables with two groups were compared with the Student's t-test. One-way ANOVA was used for comparing data for more than two groups; Student–Newman–Keuls method and the Tamhane method were used for homogeneous subsets and non-homogeneous subsets, respectively. Proportions were compared using the χ^2 test. Parametric correlation employed the Pearson correlation coefficient and the Spearman test was used for non-parametric correlation. Multiple linear regression analysis was used to identify factors that were independently associated with GDF-15 level. The cut-off concentration for GDF-15 was selected to yield the highest possible Youden Index score (sensitivity + specificity– 1). The area under the receiver-operatingcharacteristic (ROC) curve for GDF-15 was calculated. All analyses involved use of SPSS 17.0 (SPSS Inc., Chicago, IL, USA). A p-value<0.05 (two-tailed) was considered statistically significant. Data were expressed numerically (as a percentage), as a median (interquartile range) or mean±standard deviation (SD) as appropriate.

Results

Baseline clinical characteristics of participants

Patient characteristics are summarized in Table 1. Stage C patients were more likely have a higher average age, diuretic usage and uric acid level, and a lower creatinine clearance and aspirin usage than the other threegroups, but there were no significant differences among others three groups. The total cholesterol, glycosylated haemoglobin and white blood cells were comparable in these groups.

Plasma levels of GDF-15

As shown in Figure 1, plasma GDF-15was increased gradually from stage A (697.5±324.3 ng l^{-1}), stage B (978.9±278.5 ng l^{-1}) to stage C (1302.3± 324.4 ng l^{-1}) compared with the Contgroup (245.2±101.7ng l^{-1}), and had a significantly positive correlation (r=0.802, p <0.001). The correlation remained (r=0.639, p <0.001) when we excluded the factors that could influence GDF-15 concentrations, including age (r=0.286, p <0.001), diabetic mellitus status (r=0.194, p <0.008), uric acid level (r=0.194, p =0.008), creatine clearance (r=-0.347, p <0.001) and medication (statin, r=0.334, p <0.001) inpartial correlation analysis.

Among echo parameters, plasma GDF-15was most strongly correlated with LVMI (β=0.372, p <0.001) and LVEF (β =-0.391, p <0.01); in multiple regression analyses these adjusted indicators were also significantly correlated with GDF-15: left atrial diameter (r=0.344, p <0.01), left ventricular diastolic end diameter (r=0.299, p <0.01), left atrial pressure (r=0.276, p <0.01).

GDF-15 and NT-proBNP

The concentrations of GDF-15 and NT-proBNP were positively correlated in stage C patients (r=0.392, p =0.016), but were not correlated in either stage A or B patients (p =0.963) (Figure 2A, B).

NT-proBNP was positive (r=0.401, p <0.001), but had a much lower correlation with HF stages. After adjusting for age, creatine clearance and the usage of diuretics, no correlation was found.

The receiver-operating characteristic curves

ROC curves ofGDF-15 and NT-proBNP diagnosing stage BHF are shown in Figure 3A, B. The areas under curve (AUC) for GDF-15 and NT-proBNP were 0.873 and 0.818,

Table 1. Baseline clinical characteristics in different stages of heart failure.

Parameters	Control (n=53)	Stage A (n=49)	Stage B (n=58)	Stage C (n=48)	Total p-value
Clinical characteristics					
Sex (M /F)	34/19	35/14	47/11	33/15	0.321
Age (years)	58.1±11.6	60.7±13.9	62.2±9.7	68.5±11.0[a]	0.005
Smoking	-	51	71 [c]	52	0.007
Hypertension (%)	-	71	53[c]	71	0.012
Hyperlipidaemia (%)	-	49	60[c]	33	0.040
Diabetes mellitus (%)	-	31	29	40	0.491
Medications					
Aspirin(%)	-	88	93	67[b]	0.002
ACEI/ARB(%)	-	71	79	88	0.098
β-Blockers(%)	-	80	91	83	0.242
Statin(%)	-	65	78	73	0.400
Diuretics(%)	-	22	24	63[a]	0.001
Biochemical characteristics					
White blood cells (x 10^9 l^{-1})	5.97 ± 1.59	6.52 ± 1.16	6.68 ± 1.83	6.03 ± 1.43	0.055
Neutrophils (%)	58.1 ± 11.1	58.9 ± 7.5	59.8 ± 8.8	62.5 ± 8.4	0.151
CCr(mlmin^{-1})	72.6 ± 12.8	71.1 ± 21.6	66.4 ± 19.4	51.6 ± 18.9[b]	0.001
UA (μmol l^{-1})	355.1 ± 83.1	350.8 ± 93.2	360.5 ± 73.6	417.5 ± 170.6[a]	0.001
TC (mmol l^{-1})	4.47 ± 0.86	4.47 ± 0.99	4.27 ± 1.15	4.30 ± 1.14	0.739
HbA1C (%)	-	5.9	6.1	6.9	0.466
		(5.5–7.75)	(5.5–7.6)	(6.0–8.3)	
NT-proBNP (pgml^{-1})	-	97.2	463.7	1947[a]	0.001
		(32.2–179.6)	(133.3–1068)	(680.7–4174)	

Continuous variables expressed as mean±deviation (normal distribution) or medians with interquartile range (not normal distribution).
[a]Stage C patients had a higher average age, diuretic usage, uric acid level and NT-proBNP than the other groups. [b]A lower creatinine clearance and aspirin usage were also found in stage C. [c]Stage B patients were more likely to have a history of prior hyperlipidaemia, hypertension and smoking. CCr, creatinine clearance; UA, uric acid; TC, total cholesterol, HbAlC, glycosylated haemoglobin; NT-proBNP, amino-terminal pro-brain natriuretic peptide.

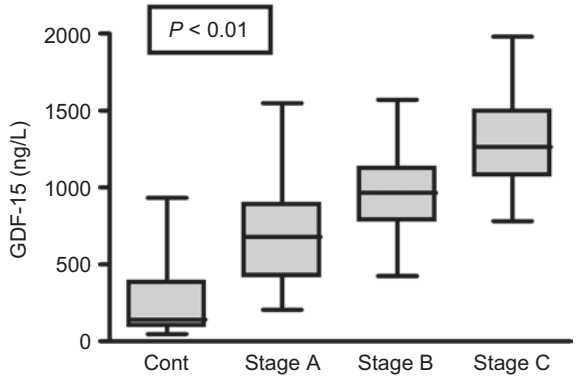

Figure 1. Plasma concentrations of growth-differentiation factor (GDF)-15 are depicted as box plotsat different stages of heart failure. Boxes represent the interquartile range, lines inside boxes represent the median value, whiskers represent 5th and 95th percentiles.

respectively. As shown in Table 2, the odds ratio calculated for structural heart disease in this study was: 32.4 for GDF-15 and 19.286 for NT-proBNP, with the cut-off values at600.1ng l^{-1} and 204.2 $pgml^{-1}$.

Discussion

This study evaluated 208 patients with cardiovascular clinical or echo findings, divided into three groups: (1) those at risk for development of clinical HF (such as coronary heart disease and hypertension); (2) patients with cardiac structural abnormalities resulting from cardiac pathology (such as a previous myocardial infarction and left ventricular hypertrophy); and (3) patients with frankly clinically identifiable HF. Patients were stratified

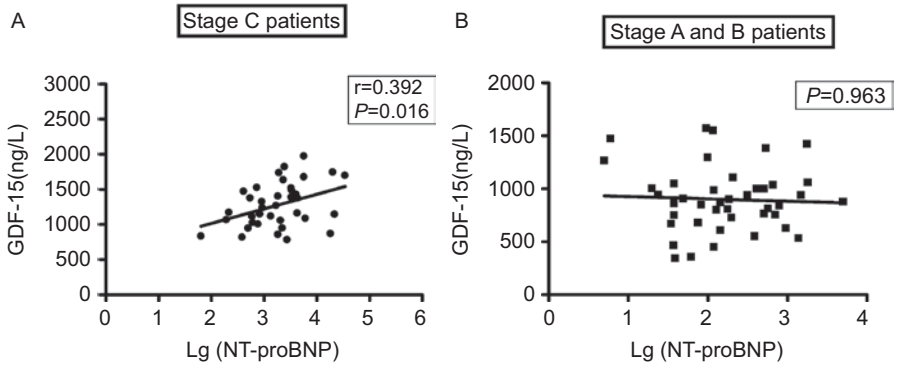

Figure 2. (A, B) Positive correlation between concentrations of growth-differentiation factor (GDF)-15and amino-terminal pro-brain natriuretic peptide (NT-proBNP) in stage C patients(r=0.392, p = 0.016), but no correlation was found in stage A and B patients (p =0.963).

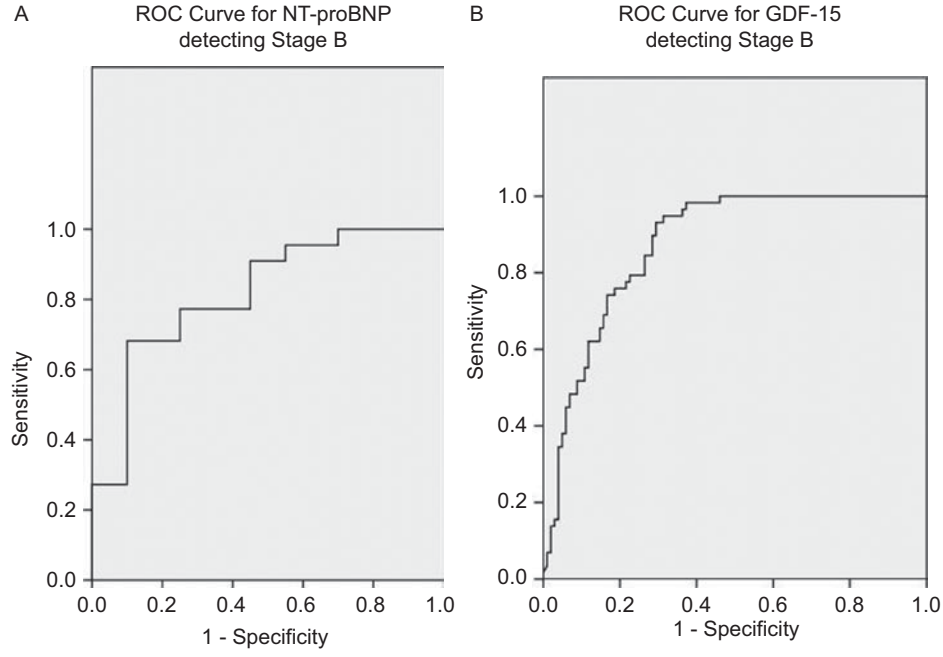

Figure 3. Receiver operating characteristic curves for the ability of growth-differentiation factor(GDF)-15 and amino-terminal pro-brain natriuretic peptide (NT-proBNP) to predict stage B heart failure patients.

Table 2. Diagnostic value of growth-differentiation factor (GDF)-15 and amino-terminal pro-brain natriuretic peptide (NT-proBNP) in detecting stage B patients.

Factors	Cut-off value	AUC	*p*-Value	Sensitivity (%)	Specificity (%)
GDF-15(ng l^{-1})	600.1	0.873	0.000	91.4	70.6
NT-proBNP(pgml^{-1})	204.2	0.818	0.000	68.2	90

AUC, area under the curve.

according to ACC/AHA HF guidelines and the mean GDF-15 level was then analysed in this stratification. In our study, GDF-15 level was positively correlated with the stages of HF. GDF-15, known as macrophage inhibitory cytokine 1(MIC-1) was first cloned on the basis of its increased mRNA expression associated with macrophage activation (Bootcov et al. 1997). GDF-15 first came to attention as a potentially useful clinical marker in heart disease when it was reported that increased circulating levels of GDF-15 were associated with increased risk of future adverse cardiovascular events in elderly women (Brown et al. 2002). Recently, GDF-15 has come under increasing scrutiny as a biomarker for prognosis in patients with clinically evident HF and in relation to disease severity (Kempf et al. 2007a). Stage B HF does not show any specific symptoms that allow its identification during the early stages. Diagnosis is frequently made only after progression to later stages, at which point the dissemination of HF limits effective treatment. Therefore, the development of sensitive and specific methods for early detection has been a priority as a means for improving the treatment of this disease. It was observed that GDF-15 progressively increased proportionally with progression of the stages of HF (r=0.802, p <0.001), and the concentrations of GDF-15 were already elevated at stage B HF patients compared with stage A (without echo-positive observation to find structural heart disease). This suggested that GDF-15 concentrations may have potential clinical value in facilitating the diagnosis of patients who do not have specific current clinical findings or history and maybe progressing silently toward future HF. HF is a progressive condition. The activation of endogenous neurohormonal systems and cardiac remodelling play an important role in the advancement of HF. GDF-15 levels increase gradually according to the stage of HF and also show an association with echo findings. While not normally expressed in cardiac myocytes, GDF-15 does appear to be upregulated in response to oxidative stress, including pressure overload (Xu et al. 2006), HF (Kempf et al. 2007a) and atherosclerosis (Brown et al. 2002). GDF-15-overexpressed mice were shown to be resistant to pressure overload-induced hypertrophy (Xu et al. 2006). These results indicated that GDF-15 was a promising cardioprotective agent. Our results showed that GDF-15 is correlated with echocardiography indicators including reduced LVEF, elevated LVEDD and LAP which are the results of structural abnormality and HF. LVEF and LVMI were most strongly correlated with levels of plasma GDF-15. The reduced LVEF represents impaired left ventricular function and the elevated LVMI illustrates the occurrence of left ventricular remodelling. Therefore the gradual elevation of GDF-15 may be a reflection of heart function deterioration with a cardioprotective compensatory mechanism.

A variety of physiological factors, such as age, sex, exercise, body posture and drugs including diuretics, angiotensin-converting enzyme inhibitors, adrenergic agonists could affect NT-proBNP (Silver et al. 2004). Our finding was that GDF-15 only correlated with age, diabetic mellitus, renal function and statin usage. Maybe due to GDF-15 was given the reported association with inflammatory conditions and NT-proBNP was a neurohormonal marker. In our study, patients without diabetic mellitus or using statins had lower concentrations of GDF-15. It may be partly due to the different status of vascular inflammation. NT-proBNP was influenced by neurohormonal changes. We also showed that plasma concentration of GDF-15 was correlated with NT-proBNP in the clinical HF patients, but among the patients without clinical HF symptoms, there was no correlation. At the same time, a recent study of 455 patients with CHF showed that GDF-15 was a predictor of mortality independent of NT-proBNP (Kempf et al. 2007b). GDF-15 might be a different biomarker from the NT-proBNP that was secreted through other HF pathways. NT-proBNP has been used as a diagnostic and prognostic biomarker, but when it was evaluated prospectively as a screening tool for stage B, the study results were not promising as the majority failed, and the guidelines did not define the use of NT-proBNP as a screening tool. However, among high-risk patients, NT-proBNP did achieve some screening value, which was comparable with other routinely used screening tests such as prostate-specific antigen measurement for prostate cancer (Silver &Pisano2003, de Lemos et al. 2009). In our study, the ROC curve analysis for diagnostic ability showed that GDF-15 was slightly more sensitive than NT-proBNP. Combining more than one biomarker may promote the early detection of cancer (Kumar et al. 2006). Determining the concentrations of GDF-15 and NT-proBNP may reflect the different pathophysiological aspects of HF and the combination might also facility the early detection of stage B to improve the management of HF.

There are some limitations of the current study. First, this is a cross-sectional study, and a standard prospective study would be more convincing to show the GDF-15 concentrations in the different stages of heart failure by long-term follow-up. Second, for the use of GDF-15 as a screening tool for the stage B patients, we need to increase the patient population. Third, group C patients were previously symptomatic and currently asymptomatic when

they were recruited and had CHF; there were no patients with acute heart failure. Finally, the stage C patients were older and had higher uric acid levels and lower creatine clearance, which are common findings in HF patients. Comparable characteristics in the different groups would be better. However, in this study, the correlation remained after adjusting for these factors using partial analysis and the differences between the other groups were also significant without these different clinical characters.

In conclusion, the plasma concentration of GDF-15 increased gradually with progressing of ACC/AHA HF stages. GDF-15 might have potential implications-for detecting stage B HF, and, in combination with NT-proBNP, for monitoring high-risk HF patients.

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Declaration of interest

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References

Baggish AL, van Kimmenade RR, Pinto Y, Richards AM, Lainchbury J, Bayes-GenisA, Santaló M, Ordonez-Llanos J, Januzzi JL.(2010). New York Heart Association class versus amino-terminal pro-B type natriuretic peptide for acute heart failure prognosis. *Biomarkers* 15:307–14.

Bootcov MR, Bauskin AR, Valenzuela SM, et al. (1997). MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily. *Proc Natl Acad Sci U S A* 94:11514–19.

Brown DA, Breit SN, Buring J, et al. (2002). Concentration in plasma of macrophage inhibitory cytokine-1 and risk of cardiovascular events in women: a nested case-control study. *Lancet* 359:2159–63.

deLemos JA, McGuire DK, Khera A, et al. (2009). Screening the population for left ventricular hypertrophy and left ventricular systolic dysfunction using natriuretic peptides: results from the Dallas Heart Study. *Am Heart J* 157:746–53. e2.

Devereux RB, Alonso DR, Lutas EM, et al. (1986). Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol* 57:450–8.

Fox K, Garcia MA, Ardissino D, et al. (2006). Guidelines on the management of stable angina pectoris: executive summary: The Task Force on the Management of Stable Angina Pectoris of the European Society of Cardiology. *Eur Heart J* 27:1341–81.

Hunt SA, Abraham WT, Chin MH, et al. (2005). ACC/AHA 2005 Guideline Update for the Diagnosis and Management of Chronic Heart Failure in the Adult: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure): developed in collaboration with the American College of Chest Physicians and the International Society for Heart and Lung Transplantation: endorsed by the Heart Rhythm Society. *Circulation* 112:e154–235.

Kempf T, Eden M, Strelau J, et al. (2006). The transforming growth factor-beta superfamily member growth-differentiation factor-15 protects the heart from ischemia/reperfusion injury. *Circ Res* 98:351–60.

Kempf T, Horn-Wichmann R, Brabant G, et al. (2007a). Circulating concentrations of growth-differentiation factor 15 in apparently healthy elderly individuals and patients with chronic heart failure as assessed by a new immunoradiometric sandwich assay. *Clin Chem* 53:284–91.

Kempf T, von HS, Peter T, et al. (2007b). Prognostic utility of growth differentiation factor-15 in patients with chronic heart failure. *J Am Coll Cardiol* 50:1054–60.

Kumar S, Mohan A, Guleria R. (2006). Biomarkers in cancer screening, research and detection: present and future: a review. *Biomarkers* 11:385–405.

Lloyd-Jones D, Adams RJ, Brown TM, et al. (2010). Heart disease and stroke statistics–(2010) update: a report from the American Heart Association. *Circulation* 12:e46–e215.

Mancia G, De Backer G, Dominiczak A, et al. (2007). (2007) Guidelines for the management of arterial hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J* 28:1462–536.

Matz R. (2002). Cockcroft–Gault equation and estimation of creatinine clearance. *AmJ Med* 112:684; author reply 684–5.

Pfister R, Schneider CA. (2009). ESC guidelines for the diagnosis and treatment of acute and chronic heart failure (2008): application of natriuretic peptides. *Eur Heart J* 30:382–3; author reply 383.

Schiller NB, Shah PM, Crawford M, et al. 1989. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. *J Am Soc Echocardiogr* 2:358–67.

Silver MA, Maisel A, Yancy CW, et al. (2004). BNP Consensus Panel 2004: a clinical approach for the diagnostic, prognostic, screening, treatment monitoring, and therapeutic roles of natriuretic peptides in cardiovascular diseases. *Congest Heart Fail* 10 (5 Suppl. 3):1–30.

Silver MA, Pisano C. (2003). High incidence of elevated B-type natriuretic peptide levels and risk factors for heart failure in an unselected at-risk population (stage A): implications for heart failure screening programs. *Congest Heart Fail* 9:127–32.

Thygesen K, Alpert JS, White HD. (2007). Universal definition of myocardial infarction. *Eur Heart J* 28:2525–38.

Vasan RS, Benjamin EJ, Larson MG, et al. (2002). Plasma natriuretic peptides for community screening for left ventricular hypertrophy and systolic dysfunction: the Framingham heart study. *JAMA* 288:1252–9.

Whitworth JA. (2003). 2003 World Health Organization (WHO)/ International Society of Hypertension (ISH) statement on management of hypertension. *J Hypertens* 21:1983–92.

Xu J, Kimball TR, Lorenz JN, et al. (2006). GDF15/MIC-1 functions as a protective and antihypertrophic factor released from the myocardium in association with SMAD protein activation. *Circ Res* 98:342–50.