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ORIGINAL RESEARCH ARTICLE

Circulating growth differentiation factor-15 as a novel biomarker in heart transplant

Nithi Tokavanich[1], Supanee Sinphurmsukskul[2], Narisorn Kongruttanachok[3], Kanokwan Thammanatsakul[2], Supaporn Sritangsirikul[2], Aekarach Ariyachaipanich[1,2], Pat Ongcharit[4,5], Sarawut Siwamogsatham[1,6], Smonporn Boonyaratavej[1,4] and Sarinya Puwanant[1,2,4]*

[1]*Division of Cardiovascular Medicine, Department of Medicine, Faculty of Medicine, Chulalongkorn University, 1873 Rama IV Rd, Pathumwan, Bangkok, 10330, Thailand;* [2]*Excellent Center for Organ Transplantation, King Chulalongkorn Memorial Hospital, the Thai Red Cross Society, Bangkok, Thailand;* [3]*Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand;* [4]*Cardiac Center, King Chulalongkorn Memorial Hospital, the Thai Red Cross Society, Bangkok, Thailand;* [5]*Division of Cardiothoracic Surgery, Department of Surgery, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand;* [6]*Chula Clinical Research Center, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*

Abstract

Aims This study aimed to examine (i) whether circulating growth differentiation factor-15 (GDF-15) is associated with acute cellular cardiac allograft rejection (ACR); (ii) a longitudinal trend of GDF-15 after heart transplantation; and (iii) the prognostic value of GDF-15 in predicting a composite outcome of severe primary graft dysfunction (PGD) and 30 day mortality post-transplant.

Methods and results Serum samples were collected before heart transplantation and at every endomyocardial biopsy (EMB) post-heart transplantation in *de novo* transplant patients. A total of 60 post-transplant serum samples were matched to the corresponding EMBs. Seven (12%) were considered International Society for Heart Lung Transplantation Grade 1R ACR, and one (2%) was identified as Grade 2R ACR. GDF-15 levels in patients with ACR were not different from those in the non-rejection group (6230 vs. 6125 pg/mL, P = 0.27). GDF-15 concentration gradually decreased from 8757 pg/mL pre-transplant to 5203 pg/mL at 4 weeks post-transplant. The composite adverse outcome of PGD and 30 day mortality was significantly associated with increased post-operative GDF-15 (odds ratio: 40; 95% confidence interval: 2.01–794.27; P = 0.005) and high inotrope score post-transplant (odds ratio: 18; 95% confidence interval: 1.22–250.35; P = 0.01).

Conclusions Circulating GDF-15 concentration was markedly elevated in patients with end-stage heart failure and decreased after heart transplantation. GDF-15 was significantly associated with post-transplant PGD and mortality. A lack of association between ACR and GDF-15 did not support routine use of GDF-15 as a biomarker to detect ACR. However, GDF-15 may be potentially useful to determine heart transplant recipients at high risk for adverse post-transplant outcomes. We suggest that GDF-15 levels in recipient serum can provide risk stratification for severe PGD including death during post-operative period. This novel biomarker may serve to inform and guide timely interventions against severe PGD and adverse outcomes during the first 4 weeks after transplantation. Further studies to support the utility of GDF-15 in heart transplantation are required.

Keywords Growth differentiation factor-15; Heart transplant; Primary graft dysfunction; Biomarker; Outcomes

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**Correspondence to: Sarinya Puwanant, MD, FASE, Division of Cardiovascular Medicine, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bhumisiri Mangkhalanusorn Building, M Floor, Zone C, 1873 Rama IV Rd, Pathumwan, Bangkok 10330, Thailand. Email: spuwanant@gmail.com*

Introduction

Growth differentiation factor-15 (GDF-15) is a novel biomarker responsible for tissue injury, inflammation, and cellular stress.[1–5] The expression of the GDF-15 is enhanced in inflammatory conditions, malignant tissue, chronic kidney disease, aging, organ dysfunction, and metabolic diseases including obesity and diabetes mellitus.[4,6–12] A growing body of evidence has demonstrated that GDF-15 is a promising prognostic biomarker in patients with heart failure and various cardiovascular diseases.[4,13–16] Elevated GDF-15 levels have been found predictive of cardiovascular events in

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women with atherosclerosis.[17] Circulating levels of GDF-15 in patients with heart failure or ventricular dysfunction have been found to increase with heart failure severity.[18] As myocardial inflammation is a hallmark feature of acute cellular cardiac allograft rejection (ACR), the use of a biomarker associated with tissue inflammation has been proposed. Previous studies failed to indicate the clinical use of biomarkers including natriuretic peptide, soluble ST2, procalcitonin, interferon-α, interleukin, and troponin, to detect ACR or to predict post-transplant outcomes.[19,20] Whether GDF-15 is associated with ACR or clinical outcomes after heart transplantation has not yet been studied. This study aimed to fill this gap by examining three areas: (i) whether GDF-15 is associated with ACR, (ii) a longitudinal trend of the levels of GDF-15 after heart transplantation, and (iii) the prognostic value of GDF-15 in predicting a composite outcome composed of severe primary graft dysfunction (PGD) and 30 day mortality post-transplant.

Methods

Patients

The study population enrolled *de novo* transplant recipients undergoing heart transplantation from February 2019 to February 2020. The study protocol was approved by the Chulalongkorn University Institutional Review Board. All patients provided written informed consent.

Serum sample collection and growth differentiation factor-15 measurements

Serum samples were collected before heart transplantation and at every endomyocardial biopsy (EMB) at Weeks 1, 2, 3, and 4 post-transplant. Serum samples were taken from the vascular access prior to administration of anaesthesia in patients undergoing heart transplantation and prior to EMBs in transplant recipients. The samples were processed immediately and stored at −70°C. GDF-15 concentrations were analysed by electrochemiluminescence immunoassay using Elecsys® assay (Roche Diagnostics, Germany), measuring 400–50 000 pg/mL (ng/L). All analyses were conducted in once. The assay has an intra-assay coefficient of variation of 0.99% at 1410 pg/mL and an inter-assay coefficient of variation of 1.2% at 1410 pg/mL and 0.97% at 6800 pg/mL.

Endomyocardial biopsies

Using a standard protocol, EMBs were obtained at multiple times post-transplant (Weeks 1, 2, 3, and 4). Pathological analysis for ACR was performed in accordance with the International Society for Heart Lung Transplantation 2005 working formulation: (i) Grade 0R: no rejection; (ii) Grade 1R: an infiltrate without or with only one focus of myocyte damage; (iii) Grade 2R: an infiltrate with multifocal myocyte damage; and (iv) Grade 3R: diffuse myocyte damage with or without oedema, haemorrhage, or vasculitis.[21] Post-transplant serum samples were matched with EMBs.

Clinical outcomes

Patients were clinically managed by the standard of post-heart transplant care. The primary endpoint included a composite outcome composed of severe PGD and 30 day mortality post-transplant. Severe PGD-left ventricle (LV) was defined as post-transplant LV ejection fraction (EF) $\leq$ 40% or haemodynamics with right atrial pressure (RAP) $>$ 15 mmHg, pulmonary capillary wedge pressure (PCWP) $>$ 20 mmHg, and cardiac index $<$ 2.0 L/min/m^2 requiring mechanical circulatory support (MCS), excluding intra-aortic balloon pump, as previously described in the International Society for Heart Lung Transplantation report from a consensus conference on PGD after cardiac transplantation.[22]

Statistical analyses

Continuous variables are presented as median and range or mean and standard deviation. Categorical variables are presented as frequency and percentage. Comparisons of continuous variables were tested using a Student's *t*-test for normally distributed continuous data and a Wilcoxon rank-sum test for non-normally distributed continuous data. Comparisons of categorical variables between patients with and without ACR or adverse outcomes were tested using a χ^2 test or Fisher's exact test, where appropriate. Univariate logistic regression was employed to identify the predictors of the composite events. Due to the small number of composite outcomes, a multivariate analysis was not performed. The original sample size was estimated based on the prevalence of ACR in our centre.[23] *P*-values of $<$0.05 were considered significant.

Results

Characteristics of transplant patients and collection of samples

The baseline characteristics of the patients enrolled in this study are shown in *Table 1*. During the study period, serum samples were obtained and matched to their EMBs. A total of 60 serum samples for GDF-15 measurements were collected from 15 *de novo* transplant recipients who

underwent clinically indicated or routine EMBs during the study period. These serum samples were matched to the EMBs and included in the study.

Post-transplant growth differentiation factor-15 and acute cellular cardiac allograft rejection

Growth differentiation factor-15 was detected in all post-transplant samples at a mean concentration of 6218 pg/mL. Of 60 EMBs, seven (12%) were considered Grade 1R ACR, and one (2%) was identified as Grade 2R ACR. The GDF-15 concentration in the ACR group was not different from the non-ACR group (6230 vs. 6125 pg/mL, $P = 0.27$).

Longitudinal trends of growth differentiation factor-15 after explanting the failing hearts

In pre-operative serum samples, the mean GDF-15 concentration prior to heart transplant was high at 8757 pg/mL. GDF-15 concentration gradually decreased over time with each subsequent biopsy. GDF-15 levels decreased from the first week (mean 7238 pg/mL) to the nadir (mean 5203 pg/mL) at 4 weeks post-transplant (*Figure 1A*).

Prognostic power of growth differentiation factor-15 in post-transplant adverse outcomes

At 30 days post-transplant, six of the 15 *de novo* heart transplant recipients (40%) met the composite of severe PGD ($n = 5$) and 30 day mortality ($n = 1$). Post-transplant GDF-15 concentrations were significantly higher in patients who had severe PGD or died at 30 days post-transplant than those without events [pre-transplant Week 0 (13 032 vs. 2344 pg/mL; $P = 0.08$), post-transplant Week 1 (14 301 vs. 2530 pg/mL; $P = 0.025$), Week 2 (15 538 vs. 1651 pg/mL; $P = 0.034$), Week 3 (10 394 vs. 1778 pg/mL; $P = 0.025$), Week 4 (9914 vs. 2062; $P = 0.045$), and overall post-transplant (12 537 vs. 2005; $P = 0.025$)] (*Figure 1B*). The cut-off for post-transplant GDF-15 at 4208 pg/mL had a sensitivity of 83% and a specificity of 100% for predicting the composite adverse outcome. This equates to an area under the receiver operating characteristic curve of 0.85 [95% confidence interval (CI) 0.66–1.0]. Factors associated with a composite of severe PGD or death at 30 days post-transplant included post-transplant high inotrope score (odds ratio: 18; 95% CI: 1.22–250.35; $P = 0.01$). Increased GDF-15 post-transplant was associated with an increase in a composite adverse outcome (odds ratio: 40; 95% CI: 2.01–794.27; $P = 0.005$).

Discussion

This study examined changes in GDF-15 levels among patients with end-stage heart failure and post-heart transplantation. Key findings of our study are as follows: (i) GDF-15 concentration was not associated with ACR; (ii) GDF-15 levels decreased over time in the first month after heart transplantation; and (iii) high levels of post-transplant GDF-15 were significantly associated with adverse outcomes (severe PGD or 30 day mortality).

Growth differentiation factor-15 and acute cellular cardiac allograft rejection

Although GDF-15 is a biomarker of the inflammatory process, the association between GDF-15 and ACR remains speculative as the incidence of significant (≥2R) ACR was lower than anticipated rejection rates in this study.[25,26] GDF-15 activation may involve other pathways of inflammation including myocyte wall stress, cell injury, or apoptosis. These results do not provide any support for the clinical use of GDF-15 as a biomarker to detect ACR. The results of previous studies examining the relationship of biomarkers and ACR have been conflicting. Patel *et al*. studied the role of high-sensitivity cardiac troponin I to screen for ACR in 98 heart transplant recipients.[27] The authors found that using a cut-off 15 ng/L produced a sensitivity of 94% and a specificity of 60% in detecting Grade 2R ACR or higher. The authors concluded that a high-sensitivity troponin I may play an important role to rule out ACR, although additional studies are needed to validate this finding.[27] Other studies have failed to demonstrate the usefulness of biomarkers, including high-sensitivity troponin T assay, B-type natriuretic peptide, soluble ST2, and troponin T and I in the clinical detection of ACR.[28–31] Agbor-Enoh *et al*. recently demonstrated that a novel non-invasive genomic blood test or per cent donor-derived cell-free DNA can detect ACR with a high area under the curve of 0.92 and a negative predictive value of 99% in 171 cardiac transplant recipients.[32] Their findings highlight the promising path for clinical utility of ACR detection and may minimize a need for EMB.

Growth differentiation factor-15 after heart transplantation

Cardiac transplant replaces the dysfunctional failing heart and restores normal haemodynamics. Findings from our study support cardiac haemodynamic restoration to be associated with a decrease in GDF-15 concentration. This association is compatible with the knowledge that surgical stress, myocardial injury, and the inflammatory response begin to decrease after transplant.[33] Comparing different

Table 1 Baseline clinical and biochemical characteristics

	De novo transplant recipients (n = 15)	Patients with composite events (n = 6)	Patients with no composite events (n = 9)	P-value[a]
Recipient characteristics				
Age (years)	39 ± 15	38 ± 17	41 ± 15	0.813
Male, *n* (%)	14 (93)	5 (83)	9 (100)	0.205
Pre-transplant ischaemic aetiology, *n* (%)	4 (27)	2 (33)	2 (22)	0.634
Diabetes, *n* (%)	1 (7)	0	1 (11)	0.398
Stroke, *n* (%)	1 (7)	1 (11)	0	0.205
Pre-transplant anaemia, *n* (%)	6 (40)	2 (33)	4 (44)	0.667
Pre-transplant hepatopathy, *n* (%)	8 (53)	5 (83)	3 (33)	0.060
Pre-transplant systolic blood pressure (mmHg)	95 ± 12	91 ± 8	100 ± 14	0.260
Pre-transplant haemodynamics				
PVR (WU)	3.4 ± 1.8	3.5 ± 2.5	3.3 ± 1.4	0.723
PASP (mmHg)	48 ± 14	51 ± 12	47 ± 15	0.516
PADP (mmHg)	23 ± 9	26 ± 10	22 ± 8	0.409
PCWP (mmHg)	23 ± 7	26 ± 6	21 ± 7	0.133
CVP (mmHg)	12 ± 6	14 ± 3	11 ± 7	0.138
Cardiac index (L/min/m^2)	2.28 ± 0.61	2.16 ± 0.55	2.34 ± 0.66	0.463
Pulmonary arterial oxygen saturation (%)	66.1 ± 8.1	62.8 ± 8.0	68.0 ± 8.0	0.205
Pre-transplant BUN (mg/dL)	30 ± 13	39 ± 9	24 ± 12	0.034*
Pre-transplant Cr (mg/dL)	1.2 ± 0.4	1.42 ± 0.53	1.03 ± 0.28	0.195
Pre-transplant serum sodium (mEq/L)	135 ± 5	136 ± 6	134 ± 4	0.592
Pre-transplant LVEF (%)	22 ± 8	25 ± 7	20 ± 9	0.110
Pre-transplant mechanical circulatory support, *n* (%)	2 (13)	2 (33)	0	0.143
Pre-transplant INTERMACS	4.3 ± 1.2	4.1 ± 1.5	4.3 ± 1.0	0.900
Waiting time on waiting list (days)	66 ± 87	50 ± 63	77 ± 101	0.593
Donor characteristics				
Age (years)	27 ± 10	27 ± 10	28 ± 10	0.859
Male, *n* (%)	15 (100)	6 (100)	9 (100)	N/A
Operative characteristics				
Ischaemic time (min)	235 ± 51	251 ± 30	224 ± 60	0.479
Post-operative conditions				
Maximal inotrope score[b]	106 ± 68	165 ± 62	67 ± 37	0.009*
Post-transplant Cr (mg/dL)	1.35 ± 0.93	1.89 ± 1.21	0.99 ± 0.49	0.113
Serum lactate (mmol/L)	2.4 ± 2.7	3.6 ± 3.7	1.7 ± 1.6	0.342
Immunosuppressive therapy at hospital discharge				
FK 506	12 (80)	4 (67)	8 (89)	0.292
Cyclosporin	3 (20)	2 (33)	1 (11)	0.292
MMF	15 (100)	6 (100)	9 (100)	N/A
Everolimus	1 (6)	1 (17)	0	0.205
Prednisolone	15 (100)	6 (100)	9 (100)	N/A

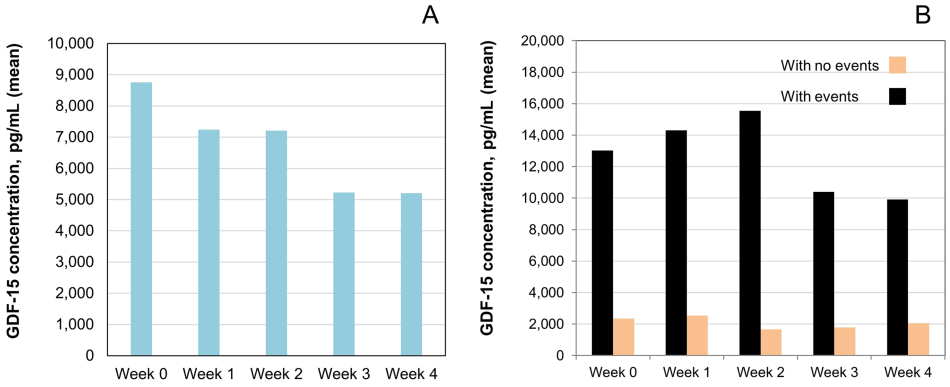
BUN, blood urea nitrogen; Cr, creatinine; CVP, central venous pressure; FK 506, Tacrolimus; LVEF, left ventricular ejection fraction; MMF, mycophenolate mofetil; N/A, not applicable; PADP, pulmonary artery diastolic pressure; PASP, pulmonary artery systolic pressure; PCWP, pulmonary artery capillary wedge pressure; PVR, pulmonary vascular resistance.
Data are presented as *n* (%) or mean ± standard deviation.
[a]*P*-value was comparing the data between patients with and without composite events.
[b]Inotrope score: dopamine (×1) + dobutamine (×1) + amrinone (×1) + milrinone (×15) + epinephrine (×100) + norepinephrine (×100) with each drug dosed in mcg/kg/min. Adapted from Iyer *et al*.[24]
*$p < 0.05$

biomarkers, Kimball *et al*.[34] have demonstrated that interleukin-6 decreased to a normal range within 3 weeks after heart transplantation. A recent study by Nykänen *et al*.[35] showed that serum N-terminal pro-B-type natriuretic peptide levels also decreased after transplantation. Similarly, Agbor-Enoh *et al*. demonstrated that donor-derived cell-free DNA levels decayed after surgery by Day 28. Boilson *et al*.[36] also found evidence that migration of recipient cells into donor myocardium resulted in decreased inflammatory responses, reduced oedema, and LV dysfunction over time after transplantation. These findings are consistent with our observation of a decrease in GDF-15 during the first 4 weeks after heart transplantation.

Growth differentiation factor-15 and post-transplant adverse outcomes

We observed that a much higher post-transplant GDF-15 concentration was found in patients with adverse post-transplant outcomes, including severe PGD and 30 day mortality. Our findings are in stark contrast with the study by Starling *et al*.[20] that analysed immune biomarkers and heart transplant outcomes in a cohort of 200 primary heart transplant recipients at 12 US transplant centres. They concluded that there was no relationship between immune biomarkers and a composite index of death, graft loss, ACR, and cardiac allograft vasculopathy at 6–8 weeks

Figure 1 (A) Growth differentiation factor-15 (GDF-15) concentrations before and after heart transplantation. (B) GDF-15 concentrations stratified by the presence or absence of composite of severe primary graft dysfunction, significant acute cellular rejection, or 30 day post-transplant mortality.



GDF-15 levels (pg/nl)	Week 0	Week 1	Week 2	Week 3	Week 4
Mean	**8,757**	**7,238**	**7,206**	**5,225**	**5,203**
SD	8,984	9,225	8,248	7,975	6,597

	GDF-15 levels (pg/nl)	Week 0	Week 1	Week 2	Week 3	Week 4
Events	**Mean**	**13,032**	**14,301**	**15,538**	**10,394**	**9,914**
	SD	9,425	11,506	18,771	11,147	8,772
No Events	**Mean**	**2,344**	**2,530**	**1,651**	**1,778**	**2,062**
	SD	1,649	1,956	369	472	581

GDF-15: Growth differentiation factor-15; SD: standard deviation
Events included severe primary graft dysfunction post-transplant and 30-day mortality

post-transplant. Their study did not include GDF-15. Elevated post-transplant GDF-15 in severe PGD may involve a similar pathophysiology as described in heart failure, including pressure overloading, oxidative stress, myocyte injury, and/or end-organ dysfunction. Donor characteristics, catecholamine associated with brain lesions, inotropic drugs, and cardiac preservation may also play important roles in the pathophysiology of PGD.[22] Elevated tumour necrosis factor-α, interleukin-6, neutrophils, procalcitonin, troponin assay, and natriuretic peptides in cardiac donors have been described as potential predictors of PGD. [19,37–40] However, little is known about the roles of these biomarkers obtained in cardiac transplant recipient serum samples in association with PGD. The predictive value of GDF-15 may be useful in determining transplant recipients at high risk of early cardiac allograft dysfunction and/or end-organ dysfunction. As GDF-15 levels gradually declined post-transplant, increases in GDF-15 levels occurred among recipients experiencing severe adverse outcomes. Repeated GDF-15 determinations during the first 4 weeks can play a potential role in rapidly identifying patients at risk. Those patients should have intensive peri-operative and post-operative monitoring and timely intervention, including initiation of MCS therapy for PGD when indicated. While the previous studies have shown that pre-operative classical biomarkers in donor serum can predict PGD, the advantage of GDF-15 is that GDF-15 levels in recipient serum can provide risk stratification for severe PGD including death during the post-operative Weeks 1 to 4 period. The risk stratification characteristics of this novel biomarker can serve to inform and guide timely therapeutic interventions against severe PGD and adverse clinical outcomes. Additionally, a combination of biomarkers may be more effective than individual biomarker in identifying heart transplant recipients at risk of developing severe PGD as different biomarkers are involved in different mechanisms that may develop into severe PGD. Further studies to establish the clinical utility of GDF-15 or the best set of biomarkers in heart transplantation are required.

Study limitations

The small sample in our study reduced the ability to identify additional factors at play especially moderate or small differences, which could be important. Adverse outcomes occurred in only six recipients, which is generally too small to draw firm conclusions, and follow-up was limited to 4 weeks post-transplant. Another limitation of our study was the lower than anticipated rate of Grade ≥2R ACR leading to reduced ability to find statistically significant differences between ACR and GDF-15. Our study also enrolled only one female, which may influence levels of GDF-15. Further studies with a broader gender recruitment, a larger number of serum samples in *de novo* recipients, and a longer follow-up period in post-heart transplant are required.

Conclusions

This paper presents findings from the first study to examine the role of GDF-15 in heart transplantation. Our results did not find an association between GDF-15 and ACR. GDF-15 levels declined precipitously during each of the 4 weeks post-transplant. Elevated post-transplant GDF-15

concentrations were found strongly associated with a 40 times odds increase in severe PGD including death. Although our findings do not support the clinical use of GDF-15 as a biomarker to detect ACR, GDF-15 may be potentially useful to identify heart transplant recipients at increased risk of adverse outcomes. We suggest that GDF-15 can provide risk stratification for severe PGD including death in recipients during post-operative period. This novel biomarker may serve to inform and guide timely interventions against severer PGD and adverse clinical outcomes during the first 4 weeks after transplantation. Our findings highlight the need for further studies to explore the value of GDF-15 in improving heart transplantation outcomes.

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Conflict of interest

None declared (for all co-authors).

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