行政院國家科學委員會補助專題研究計畫成果報告

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※ Soluble Inflammatory Markers in Coronary Sinus and Peripheral Blood of ※

※ Heart Transplant Recipients ※

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執行期間： 90 年 08 月 1 日 至 91 年 07 月 31 日

計畫主持人：許榮彬

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執行單位：臺大醫院心臟外科

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壹、 摘要

Cardiac allograft rejection is a focal inflammation and soluble markers are released into coronary sinus (CS). We investigated whether plasma soluble markers in CS is better to predict the clinical status of transplant recipients than in peripheral blood (PB). Between February 1998 and January 2001, 51 patients admitted for endomyocardial biopsy were included. The clinical events of the transplant recipient were recorded as: early post-transplant, long-term uneventful status, infection, acute rejection and transplant coronary artery disease. The plasma levels of interleukin-2 (IL-2), tumor necrosis factor- . (TNF-), ICAM-1, P-selectin, high-sensitive C-reactive protein (CRP) and troponin-I of CS and PB were determined. There were 71 blood samples. In patients within one month after heart transplant, there was a higher level of P-selectin, ICAM-1, CRP and troponin-I in CS and PB. In patients with infection, there was a higher level of all inflammatory markers except IL-2 in CS and PB. Patients with a long-term uneventful status had a lower level of CRP in PB but not in CS. In patients with infection, there was a higher level of all soluble markers except IL-2 in CS and PB. Patients with a long-term uneventful status had a lower level of CRP in PB but not in CS. Patients with acute rejection had a higher level of IL-2 in PB but not in CS. Patients with transplant coronary artery disease had a higher level of TNF- in PB but not in CS. Soluble markers in CS failed to predict the occurrence of acute or chronic rejections.

貳、源由與目的

Acute and chronic rejection are the two major causes of morbidity and mortality following heart transplantation (1). The diagnosis of acute and chronic allograft rejection relies mostly on the pathological examination of endomyocardial biopsy specimens and annual surveillance coronary angiography. However, this approach is invasive and is associated with significant complications. Recently, most heart transplant centers have investigated the uses of noninvasive methods to diagnose allograft rejection. The easiest way for early detection of rejection is to find the

serological markers in the blood of the recipients. Given the pivotal role of inflammation in organ rejection, previous studies (2-11) have focused on whether plasma levels of inflammatory markers and tissue injury can predict individuals at an increased risk of rejection.

As rejection is a focal inflammation, these soluble markers are generated in the cardiac allograft, released into the cardiac veins and finally drained to coronary sinus (CS). Fyfe et al (12) and Wildhirt et al (13-15) have reported the levels of nitric oxide metabolites and cytokines of CS in heart transplant recipients, but their clinical correlation were not appropriately assessed. Here, we investigated whether plasma soluble markers in CS is better to predict clinical status of transplant recipients than in peripheral blood (PB).

參、結果與討論

Patients.

The patients in this cross-sectional study were parts of the transplant program in our hospital. Between February 1998 and January 2001, 51 patients admitted for endomyocardial biopsy were included in this study. The median age at transplantation of this group was 52 years (range, 18 to 69), and 80 % were males. All patients received triple-drug immunosuppressive therapy according to our heart transplantation protocol (16).

Endomyocardial biopsy was performed weekly in the first month, biweekly in the second month, monthly in the 6 months and yearly six months after transplantation. Rejection was defined as a clinical event leading to specific immunosuppressive intervention. The scale of the International Society for Heart Transplantation is used for grading of rejection. This scale ranges from grade 0, which denotes no sign of acute rejection through 1A, 1B, 2, 3A, 3B to grade 4 which denotes diffuse aggressive inflammation, myocyte necrosis and damage, edema, hemorrhage, and vasculitis (17). Humoral rejection was diagnosed by the immunofluorescent staining of immunoglobulins and complement.

For those patients surviving for more than 6 months after transplantation, coronary angiography was performed annually for surveillance of transplant coronary artery disease. Side-by-side comparisons were done. The diagnosis of transplant coronary artery disease was made from the evidence of any coronary artery irregularity or diffuse narrowing in proximal and distal vessels.

Clinical data.

Clinical events including acute rejection, infection, transplant coronary artery disease and death were recorded. The recipient characteristics including age, sex, body weight, smoking, hypertension, diabetes, cholesterol level, triglyceride and medications were recorded. The clinical events of the transplant recipient were recorded as: 1) early post-transplant; 2) infection; 3) long-term uneventful status; 4) acute rejection and 5) transplant coronary artery disease. Early post-transplant was defined as within one month after transplantation. Infection was defined as clinical evidence of local or systemic infection with or without leukocytosis. Acute rejection was defined as a clinical event leading to specific immunosuppressive intervention and the rejection grade was ≥ 2 by endomyocardial biopsy. Long-term uneventful status was defined as no clinical significant complications, including rejection and infection, after transplantation..

Blood samples and plasma determination.

Under the guidance of fluoroscopy, we cannulated CS through right internal jugular approach. The correct position in CS was verified by analysis of the blood oxygen saturation. Study blood samples were collected directly from CS and PB. Ten millimeters of blood were collected. The samples are collected in vacuum tubes, kept in ice slush, and centrifugated. The plasma samples are then immediately frozen at -70^0C, in 500-ul aliquots, and stored until analysis. The plasma levels of interleukin-2 (IL-2), tumor necrosis factor- . (TNF- ., intercellular adhesion molecule-1 (ICAM-1) and P-selectin were determined by the commercially available enzyme-linked immunosorbent assays (ELISAs) (R&D Systems USA, Minneapolis, MN). Tropinin-I was also determined by ELISAs. High sensitive C-reactive protein (CRP) was determined by immunoturbidometry.

Statistical analysis.

The measured plasma levels of troponin-I, CRP, IL-2, TNF- , ICAM-1 and P-selectin of CS and PB were compared between patients with and without each clinical event of the transplant recipient. The plasma levels of these soluble markers were compared between CS and PB among patients without early post-transplant and infection. Whitney-Mann test and Spearman correlation were used for independent two-sample comparison. A p-value ≤ 0.05 was considered statistically significant.

Results

Patient data.

There were 71 blood samples from 51 patients of heart transplantation. The post-transplantation period ranged from one week to 5 years.

Among the 71 clinical events, there were 19 early post-transplant, 16 acute rejection, 9 transplant coronary artery disease, 10 infection, and 32 long-term uneventful status.

Soluble markers and clinical events.

The plasma levels of soluble markers between patients with and without early post-transplant were shown in Table 1. In early post-transplant patients, there was a higher level of P-selectin, ICAM-1, CRP and troponin-I in both CS and PB. In patients with infection, as shown in Table 2, there was a higher level of all soluble markers except IL-2 in both CS and PB.

In patients with long-term uneventful status, as shown in Table 3, there was a lower level of CRP in PB but not in CS. In patients with acute rejection, as shown in Table 4, there was a higher level of IL-2 in PB but not in CS. In patients with transplant coronary artery disease, as shown in Table 5, there was a higher level of TNF- in PB but not in CS. Among the 48 specimen in patients without early post-transplant and infection, patients with acute rejection had a higher level of IL-2, TNF- , and CRP in PB (Table 6) but not in CS.

Soluble markers between CS and PB (Table 7).

Among the 48 specimen in patients without early post-transplant and infection, the plasma levels of P-selectin, IL-2, and TNF- . were higher in CS than in PB, but the level of ICAM-1 was higher in PB than in CS.

Discussion

Inflammatory processes play a pivotal role in the pathogenesis of transplantation rejection and mediate many of the stages of rejection from acute cellular rejection to chronic rejection (transplant coronary artery disease). Noninvasive methods to assess immune activation would be helpful in optimizing therapy after heart transplantation to reduce rejection and complications caused by excessive immunosuppression. Elevated plasma levels of several soluble markers of the inflammatory cascade and tissue injury have been shown to predict future risk of rejection and graft loss (2-11). These markers included troponin-I (2), CRP (3-5), cytokines (TNF- . and ILs) (6), and adhesion molecules (7-11). But the results were conflicting with limited clinical utility. The plasma levels of these soluble markers released from the transplant hearts were mixed with the blood from peripheral circulation. Therefore, the measured plasma levels represented the sums of CS and PB. Rejection is a focal inflammation and soluble markers are released into CS. It prompted us to investigate whether plasma soluble markers in CS is better to predict the clinical event of transplant recipients than in PB. Wildhirt et al. (13-15) reported a series of studies of CS expression of nitric

oxide metabolites, TNF- , IL-2 and IL-6 in transplant recipients. Patients with acute rejection or infection were excluded. The primary end point of these studies was microvascular endothelial dysfunction, but not clinical event. Fyfe et al. in 1993 (12) reported that elevated CS levels of TNF- and IL-6 in transplant recipients. But they found no relation between the CS or vena cava cytokine concentration or profile and severity of rejection. Since then, there was no extensive study of CS expression of soluble markers in transplant recipients.

Troponin-I. Previous studies (2,18) have yielded conflicting data regarding whether a relationship exists between elevated cardiac troponin-I levels and acute allograft rejection in patients who have received heart transplants. In a large prospective study (2), all heart transplant recipients had elevated troponin-I levels during the first month after transplantation. Troponin-I levels remained persistently elevated during the first 12 months in one-half of patients. Patients with persistent elevation of serum troponin-I had significantly increased risk for subsequent development of chronic rejection. However, markers of tissue injury were inadequate predictors of acute rejection in cardiac allografts (18). We had a similar result. Patients in early post-transplant period had an elevated titer of troponin-I both in CS and PB. But the levels of tropinin-I, either in CS or in PB, was not high in patients with rejetcion.

CRP. Elevated levels of plasma CRP were associated with decreased graft survival in cardiac transplant recipients (4). Although CRP was elevated more often in the presence of acute rejection, its sensitivity didn't allow CRP to replace routine endomyocardial biopsy for monitoring rejection (5). In our study, the level of CRP was high in CS and PB in patients with early post-transplant and infection, but it failed to predict the occurrence of acute rejection and transplant coronary artery disease. But CRP seemed to have a predicting role in long-term uneventful status.

Cytokines and adhesion molecules. The cardiac allograft is a major source of cytokines and adhesion molecules after heart transplantation (8). Pathological studies (19,20) suggested that immune events resulting in surface changes of coronary endothelial cells and production of cytokines play a role in the pathogenesis of acute rejection and may contribute to the long-term complication of transplant coronary artery disease. Cytokines are of great importance in the mechanisms of transplant rejection and this, in some cases, is reflected in the serum. However, this is not sufficiently consistent to be of diagnostic value (21). Measurement of serum cytokine levels in heart transplant recipients does not appear to correlate with findings on endomyocardial biopsy (22). We had a similar result. In patients with early post-transplant and infection, there was a higher

level of all inflammatory markers except IL-2 in CS and PB. The depressed level of IL-2 was due to immunosuppression modification because most of immunosuppressive agents (cyclosporine and tacrolimus) were aimed to suppress IL-2 expression.

Positivity for circulating ICAM-1 in heart transplant recipients has been claimed to predict the development of coronary artery disease and risk of graft failure (7). However, more recent studies showed that increased circulating ICAM-1 levels did not correlate with endomyocardial biopsy scores (23,24). When the measured serum levels were correlated with the clinical status of the transplant recipient, soluble adhesion molecules only weakly discriminated between rejection and infection and the clinical utility of ICAM-1 in non-invasive monitoring is, therefore, limited (25).

P-selectin level increased progressively with increasing rejection grade (26). In heart transplant recipients, P-selectin levels are highly predictive of organ rejection. Significant prolongation of graft survival was observed in mice treated with anti-P-selectin monoclonal antibodies. The enhanced endothelial mRNA expression of P-selectin was observed in the rejecting cardiac allografts (27). These results suggest that P-selectin is critically involved in the early development of acute rejection. In our study, patients with acute rejection had a higher level of IL-2, TNF- ..and CRP in PB but not in CS. Soluble markers in CS failed to predict the occurrence of acute or chronic rejections.

On interesting finding of our study was that, as shown in Table 7, the plasma levels of P-selectin, IL-2, and TNF- . were higher in CS than in PB, but the level of ICAM-1 was higher in PB than in CS. It indicated that ICAM-1 was mostly released from the systemic circulation rather than from the cardiac allograft. Its clinical significance needed further investigation.

In conclusion, in patients with heart transplantation, soluble markers in CS is not better to predict the occurrence of acute or chronic rejections than in PB.

References

1. Hosenpud JD. Bennett LE. Keck BM. Boucek MM. Novick RJ. The Registry of the International Society for Heart and Lung Transplantation: seventeenth official report-2000. J Heart Lung Transplant 2000: 19: 909.
2. Labarrere CA, Nelson DR, Cox CJ, Pitts D, Kirlin P, Halbrook H. Cardiac-Specific Troponin I Levels and Risk of Coronary Artery Disease and Graft Failure Following Heart Transplantation.

JAMA 2000: 284: 457.

3. Pethig K, Heublein B, Kutschka I, Haverich A. Systemic inflammatory response in cardiac allograft vasculopathy: high-sensitive C-reactive protein is associated with progressive luminal obstruction. Circulation 2000: 102(Suppl): III233.
4. Eisenberg MS, Chen HJ, Warshofsky MK, Sciacca RR, Wasserman HS, Schwartz A, Rabbani LE. Elevated levels of plasma C-reactive protein are associated with decreased graft survival in cardiac transplant recipients. Circulation 2000: 102: 2100.
5. van Gelder T, Balk AH, Zondervan PE, Maat AW, Mochtar B, van der Meer P, Weimar W. C-reactive protein in the monitoring of acute rejection after heart transplantation. Transpl Int 1998: 11: 361.
6. Gullestad L. Simonsen S. Ueland T. Holm T. Aass H. Andreassen AK. Madsen S. Geiran O. Froland SS. Aukrust P. Possible role of proinflammatory cytokines in heart allograft coronary artery disease. Am J Cardiol 1999: 84: 999.
7. Ohtani H, Strauss HW, Southern JF, Tamatani T, Miyasaka M, Sekiguchi M, Isobe M. Intercellular adhesion molecule-1 induction: a sensitive and quantitative marker for cardiac allograft rejection. J Am Coll Cardiol 1995: 26: 793.
8. Labarrere CA, Nelson DR, Faulk WP. Endothelial Activation and Development of Coronary Artery Disease in Transplanted Human Hearts. JAMA 1997: 278: 1169.
9. Ballantyne CM. Mainolfi EA. Young JB. Windsor NT. Cocanougher B. Lawrence EC. Pollack MS. Entman ML. Rothlein R. Relationship of increased levels of circulating intercellular adhesion molecule 1 after heart transplantation to rejection: human leukocyte antigen mismatch and survival. J Heart Lung Transplant 1994: 13: 597.
10. Andreassen AK, Nordoy I, Simonsen S, Ueland T, Muller F, Froland SS, Gullestad L, Aukrust P. Levels of circulating adhesion molecules in congestive heart failure and after heart transplantation. Am J Cardiol 1998: 81: 604.
11. Labarrere CA. Nelson DR. Park JW. Pathologic markers of allograft arteriopathy: insight into the pathophysiology of cardiac allograft chronic rejection. Curr Opin Cardiol 2001: 16: 110.
12. Fyfe A. Daly P. Galligan L. Pirc L. Feindel C. Cardella C. Coronary sinus sampling of cytokines after heart transplantation: evidence for macrophage activation and interleukin-4 production within the graft. J Am Coll Cardiol 1993: 21: 171.
13. Wildhirt SM. Weis M. Schulze C. Conrad N. Rieder G. Enders G. Hoepp C. von Scheidt W.

Reichart B. An association between microvascular endothelial dysfunction, transcardiac nitric oxide production and pro-inflammatory cytokines after heart transplantation in humans. Transplant International. 2000: 13 (Suppl 1): S228.

14. Weis M. Wildhirt SM. Schulze C. Pehlivanli S. Fraunberger P. Meiser BM. von Scheidt W. Modulation of coronary vasomotor tone by cytokines in cardiac transplant recipients. Transplantation 1999: 68: 1263.
15. Wildhirt SM, Weis M, Schulze C, Conrad N, Pehlivanli S, Rieder G, Enders G, von Scheidt W, Reichart B. Expression of endomyocardial nitric oxide synthase and coronary endothelial function in human cardiac allografts. Circulation 2001: 104(Suppl): I336.
16. Hsu RB, Chu SH, Wang SS, Ko WJ, Chou NK, Lee CM, Chen MF, Lee YT. Low incidence of transplant coronary artery disease in Chinese heart recipients. J Am Coll Cardiol 1999: 33: 1573.
17. Billingham ME. Cary NR. Hammond ME. Kemnitz J. Marboe C. McCallister HA. Snovar DC. Winters GL. Zerbe A. A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection: heart rejection study group. J Heart Lung Transplant 1990: 9: 587.
18. Wang CW. Steinhubl SR. Castellani WJ. Van Lente F. Miller DP. James KB. Young JB. Inability of serum myocyte death markers to predict acute cardiac allograft rejection. Transplant 1996: 62: 1938.
19. Salom RN, Maguire JA, Hancock WW. Endothelial activation and cytokine expression in human acute cardiac allograft rejection. Pathology 1998: 30: 24.
20. Van Hoffen E. Van Wichen D. Stuij I. De Jonge N. Klopping C. Lahpor J. Van Den Tweel J. Gmelig-Meyling F. De Weger R. In situ expression of cytokines in human heart allografts. Am J Path 1996: 149: 1991.
21. Grant SC, Lamb WR, Hutchinson IV, Brenchley PE. Serum soluble adhesion molecules and cytokines in cardiac allograft rejection. The Manchester Transplant Cytokine Group. Transplant Immunol 1994: 2: 321.
22. Jordan SC. Czer L. Toyoda M. Galfayan K. Doan D. Fishbein M. Blanche C. Trento A. Serum cytokine levels in heart allograft recipients: correlation with findings on endomyocardial biopsy. J Heart Lung Transplant 1993: 12: 333.
23. Campana E, Parlapiano C, Borgia MC, Papalia U, Laurenti A, Pantone P, Giovanniello T,

Marangi M, Sanguigni S. Are elevated levels of soluble ICAM-1 a marker of chronic graft disease in heart transplant recipients? Atherosclerosis. 2000: 148: 293.

24. Tanio JW. Basu CB. Albelda SM. Eisen HJ. Differential expression of the cell adhesion molecules ICAM-1, VCAM-1, and E-selectin in normal and posttransplantation myocardium. Cell adhesion molecule expression in human cardiac allografts. Circulation 1994: 89: 1760.
25. Weigel G, Grimm M, Griesmacher A, Seebacher G, Sichrovsky T, Wolner E, Laufer G, Muller MM. Adhesion molecule behavior during rejection and infection episodes after heart transplantation. Clin Chem Lab Med 2000: 38: 403.
26. Segal JB, Kasper EK, Rohde C, Bray PF, Baldwin WM 3rd, Resar JR, Hruban RH, Kickler TS. Coagulation markers predicting cardiac transplant rejection. Transplantation 2001: 72: 233.
27. Yamazaki S, Isobe M, Suzuki J, Tojo S, Horie S, Okubo Y, Sekiguchi M. Role of selectin-dependent adhesion in cardiac allograft rejection. J Heart Lung Transplant 1998: 17: 1007.

Table 1. Comparison of plasma levels of soluble markers between patients with early post-transplant (n=19) and without early post-transplant (n=52)

Markers	With		Without		P-value
	Median	Range	Median	Range	
Coronary sinus					
P-selectin (ng/ml)	304	87-854	264	36-778	0.2321
ICAM-1 (ng/ml)	282	9-847	19	6-962	0.0072*
TNF- . (pg/ml)	2.03	1.16-8.22	2.15	1.00-13.61	0.3877
IL-2 (pg/ml)	1.19	0.84-2.02	1.30	0.6-5.1	0.6308
CRP (pg/ml)	2.14	0.23-21.63	0.16	0.01-16.65	<0.0001*
Troponin-I (ng/ml)	3.3	0.01-17.7	0.06	0.01-6.1	<0.0001*
Peripheral blood					
P-selectin (ng/ml)	116	31-389	81	17-437	0.0201*
ICAM-1 (ng/ml)	457	185-1082	261	81-1081	0.0012*
TNF- . (pg/ml)	1.67	0.81-11.48	1.90	0.78-274.21	0.6262
IL-2 (pg/ml)	0.78	0.59-2.1	0.87	0.6-5.92	0.1704
CRP (pg/ml)	2.35	0.16-23.77	0.17	0.001-16.28	<0.0001*
Troponin-I (ng/ml)	1.6	0.01-76.5	0.1	0.01-3.6	<0.0001*

ICAM=intercellular adhesion molecule; TNF=tumor necrosis factor; IL=interleukin; CRP=C-reactive protein.

* P< 0.05 by Whitney-Mann test.

Table 2. Comparison of plasma levels of soluble markers between patients with infection (n=10) and without infection (n=61)

Markers	With		Without		P-value
	Median	Range	Median	Range	
Coronary sinus					
P-selectin (ng/ml)	327	92-854	263	36-791	0.2610
ICAM-1 (ng/ml)	355	11-847	25	6-962	0.0158*
TNF- . (pg/ml)	3.88	2.03-8.22	1.87	1.0-13.61	0.0026*
IL-2 (pg/ml)	1.16	0.89-3.77	1.26	0.6-5.1	0.7472
CRP (pg/ml)	7.78	1.85-16.65	0.23	0.01-21.63	<0.0001*
Troponin-I (ng/ml)	2.05	0.01-9.0	0.10	0.01-17.7	0.0047*
Peripheral blood					
P-selectin (ng/ml)	117	78-257	85	17-437	0.0492*
ICAM-1 (ng/ml)	650	254-1082	269	81-935	0.0002*
TNF- . (pg/ml)	3.19	1.4-28.09	1.65	0.78-274.21	0.0036*
IL-2 (pg/ml)	0.84	0.59-4.71	0.84	0.6-5.92	0.7472
CRP (pg/ml)	11.93	2.05-23.77	0.19	0.001-13.78	<0.0001*
Troponin-I (ng/ml)	1.4	0.01-8.2	0.1	0.01-76.5	0.0048*

ICAM=intercellular adhesion molecule; TNF=tumor necrosis factor; IL=interleukin; CRP=C-reactive protein.

* P< 0.05 by Whitney-Mann test.

Table 3. Comparison of plasma levels of soluble markers between patients with long-term uneventful status (n=32) and without long-term uneventful status (n=39)

Markers	With		Without		P-value
	Median	Range	Median	Range	
Coronary sinus					
P-selectin (ng/ml)	330	58-854	259	36-791	0.3433
ICAM-1 (ng/ml)	34	6-847	35	7-962	0.9172
TNF- . (pg/ml)	2.11	1.18-7.56	2.04	1.0-13.61	0.8578
IL-2 (pg/ml)	1.25	0.6-5.1	1.25	0.71-4.86	0.7638
CRP (pg/ml)	0.16	0.01-9.32	0.30	0.03-21.63	0.0679
Troponin-I (ng/ml)	0.1	0.01-17.7	0.2	0.01-9.0	0.7297
Peripheral blood					
P-selectin (ng/ml)	95	17-437	85	25-359	0.9539
ICAM-1 (ng/ml)	298	116-703	282	81-1082	0.4219
TNF- . (pg/ml)	1.67	0.78-26.44	1.98	0.91-274.21	0.3288
IL-2 (pg/ml)	0.83	0.6-5.92	0.86	0.59-5.74	0.4771
CRP (pg/ml)	0.17	0.001-21.38	0.29	0.02-23.77	0.0273*
Troponin-I (ng/ml)	0.1	0.01-76.5	0.2	0.01-8.2	0.1318

ICAM=intercellular adhesion molecule; TNF=tumor necrosis factor; IL=interleukin; CRP=C-reactive protein.

*P< 0.05 by Whitney-Mann test.

Table 4. Comparison of plasma levels of soluble markers between patients with acute rejection (n=16) and without acute rejection (n=55)

Markers	With		Without		P-value
	Median	Range	Median	Range	
Coronary sinus					
P-selectin (ng/ml)	231	62-630	304	36-854	0.0603
ICAM-1 (ng/ml)	17	7-414	142	6-962	0.0632
TNF- . (pg/ml)	2.25	1.22-7.25	2.08	1.0-13.61	0.5726
IL-2 (pg/ml)	1.46	0.8-4.86	1.19	0.6-5.1	0.2104
CRP (pg/ml)	0.26	0.01-3.61	0.28	0.03-21.63	0.4957
Troponin-I (ng/ml)	0.2	0.01-6.1	0.1	0.01-17.7	0.8315
Peripheral blood					
P-selectin (ng/ml)	80	25-174	91	17-437	0.3388
ICAM-1 (ng/ml)	274	81-775	301	116-1082	0.5222
TNF- . (pg/ml)	2.34	0.99-26.44	1.68	0.78-274.21	0.2769
IL-2 (pg/ml)	1.24	0.67-5.74	0.83	0.59-5.92	0.0409*
CRP (pg/ml)	0.24	0.001-0.855	0.23	0.001-23.77	0.7726
Troponin-I (ng/ml)	0.15	0.01-2.3	0.20	0.01-76.5	0.4479

ICAM=intercellular adhesion molecule; TNF=tumor necrosis factor; IL=interleukin; CRP=C-reactive protein.

*P< 0.05 by Whitney-Mann test.

Table 5. Comparison of plasma levels of soluble markers between patients with transplant coronary artery disease (n=9) and without transplant coronary artery disease (n=62)

Markers	With		Without		P-value
	Median	Range	Median	Range	
Coronary sinus					
P-selectin (ng/ml)	264	140-469	277	36-854	0.9106
ICAM-1 (ng/ml)	27	9-674	41	6-962	0.8492
TNF- . (pg/ml)	2.21	1.31-13.61	2.09	1.0-8.22	0.2997
IL-2 (pg/ml)	1.08	0.74-3.77	1.27	0.6-5.1	0.5921
CRP (pg/ml)	0.29	0.07-12.80	0.27	0.01-21.63	0.5743
Troponin-I (ng/ml)	0.1	0.01-1.2	0.1	0.01-17.7	0.2432
Peripheral blood					
P-selectin (ng/ml)	83	31-257	88	17-467	0.5113
ICAM-1 (ng/ml)	470	163-656	281	81-1082	0.2650
TNF- . (pg/ml)	3.97	1.27-274.21	1.67	0.78-28.09	0.0498*
IL-2 (pg/ml)	0.82	0.66-4.71	0.85	0.59-5.92	0.7425
CRP (pg/ml)	0.28	0.04-15.19	0.22	0.001-23.77	0.5861
Troponin-I (ng/ml)	0.01	0.01-1.2	0.2	0.01-76.5	0.1255

ICAM=intercellular adhesion molecule; TNF=tumor necrosis factor; IL=interleukin; CRP=C-reactive protein.

*P< 0.05 by Whitney-Mann test.

Table 6. Comparison of plasma levels of soluble markers between non-early post-transplant, non-infection patients with acute rejection (n=14) and without acute rejection (n=34)

Markers	With		Without		P-value
	Median	Range	Median	Range	
Peripheral blood					
TNF- . (pg/ml)	2.58	1.25-26.44	1.60	0.78-274.21	0.0339*
IL-2 (pg/ml)	1.27	0.67-5.74	0.83	0.6-5.92	0.0458*
CRP (pg/ml)	0.23	0.001-0.855	0.21	0.001-0.635	0.0412*

ICAM=intercellular adhesion molecule; TNF=tumor necrosis factor; IL=interleukin; CRP=C-reactive protein.

*P< 0.05 by Whitney-Mann test.

Table 7. Comparison of plasma levels of soluble markers between coronary sinus (CS) and peripheral blood (PB) in 48 patients with non-early post-transplant and non-infection.

Markers	CS		PB		P-value
	Median	Range	Median	Range	
P-selectin (ng/ml)	264	36-778	80	17-437	<0.0001*
ICAM-1 (ng/ml)	19	6-962	250	81-935	<0.0001*
TNF- . (pg/ml)	2.08	1.0-13.61	1.67	0.78-274.21	0.0007*
IL-2 (pg/ml)	1.29	0.6-5.1	0.86	0.6-5.92	0.0293*
CRP (pg/ml)	0.16	0.01-2.303	0.12	0.001-0.855	0.0595
Troponin-I (ng/ml)	0.01	0.01-6.1	0.01	0.001-3.6	0.9999

ICAM=intercellular adhesion molecule; TNF=tumor necrosis factor; IL=interleukin; CRP=C-reactive protein.

*P< 0.05 by Whitney-Mann test.