bjh research paper

Haemostatic and inflammatory biomarkers in advanced chronic heart failure: role of oral anticoagulants and successful heart transplantation

Massimo Cugno,[1,2] Daniela Mari,[1,2] Pier Luigi Meroni,[1,3] Edoardo Gronda,[4] Francesco Vicari,[1] Maria Frigerio,[5] Raffaella Coppola,[1,2] Bianca Bottasso,[1,2] Maria Orietta Borghi[1,3] and Luisa Gregorini[2,6]

[1]Department of Internal Medicine, University of Milan, [2]IRCCS Ospedale Maggiore, Milan, [3]Istituto Auxologico, IRCCS, [4]Istituto Clinico Humanitas, [5]De Gasperis Cardiac Department, Niguarda-Ca' Granda Hospital, and [6]Experimental Surgery and Transplantation Institute, University of Milan, Milan, Italy

Summary

Advanced chronic heart failure (CHF) is associated with abnormal haemostasis and inflammation, but it is not known how these abnormalities are related, whether they are modified by oral anticoagulants (OAT), or if they persist after successful heart transplantation. We studied 25 patients with CHF (New York Heart Association class IV, 10 of whom underwent heart transplantation) and 25 age- and sex-matched healthy controls by measuring their plasma levels of prothrombin fragment 1 + 2 (F1 + 2), thrombin–antithrombin (TAT) complexes, tissue plasminogen activator (t-PA), plasminogen activator inhibitor-1 (PAI-1), D-dimer, factor VII (FVII), fibrinogen, von Willebrand factor (VWF), tumour necrosis factor (TNF), soluble TNF receptor II (sTNFRII), interleukin 6 (IL-6), soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), endothelial-selectin (E-selectin) and thrombomodulin. CHF patients had higher plasma levels of TAT, D-dimer, t-PA, fibrinogen, VWF, TNF, IL-6, sTNFRII, sVCAM-1 ($P = 0{\cdot}0001$), sICAM-1 ($P = 0{\cdot}003$) and thrombomodulin ($P = 0{\cdot}007$) than controls. There were significant correlations ($r = 0{\cdot}414$–$0{\cdot}595$) between coagulation, fibrinolysis, endothelial dysfunction and inflammation parameters, which were lower in those patients treated with OATs. Heart transplantation led to reductions in fibrinogen ($P = 0{\cdot}001$), VWF ($P = 0{\cdot}05$), D-dimer ($P = 0{\cdot}05$) and IL-6 levels ($P = 0{\cdot}05$), but all the parameters remained significantly higher ($P = 0{\cdot}01$–$0{\cdot}0001$) than in the controls. Advanced CHF is associated with coagulation activation, endothelial dysfunction and increased proinflammatory cytokine levels. Most of these abnormalities parallel each other, tend to normalize in patients treated with OATs and, although reduced, persist in patients undergoing successful heart transplantation, despite the absence of clinical signs of CHF.

Keywords: coagulation, inflammation, heart failure, oral anticoagulants, heart transplantation.

Received 13 February 2004; accepted for publication 19 March 2004

Correspondence: Massimo Cugno, Department of Internal Medicine, University of Milan and IRCCS Maggiore Hospital, Via Pace 15, 20122 Milano, Italy. E-mail: massimo.cugno@unimi.it

Neuroendocrine mechanisms (i.e. activation of the renin-angiotensin and sympathetic nervous systems) are major contributors to the haemodynamic abnormalities that lead to symptoms in chronic congestive heart failure (CHF) and structural changes in left ventricle remodelling (Francis *et al*, 1984; Pfeffer & Pfeffer, 1988; Cohn, 2001), but there is a growing body of evidence indicating that cytokine-mediated immunological responses also play a role in the pathogenesis of heart failure (Finkel *et al*, 1992; Bozkurt *et al*, 1998). Since the first report of high serum tumour necrosis factor-α (TNF α) levels in CHF patients (Levine *et al*, 1990), a number of studies have shown that other proinflammatory cytokines and chemokines are involved in cardiac depression and the progression of heart failure (Aukrust *et al*, 1998; Kapadia *et al*, 1998;

 doi:10.1111/j.1365-2141.2004.04977.x

Sharma *et al*, 2000), and new lines of evidence suggest that cytokines can be considered treatment targets (Baumgarten *et al*, 2000).

Chronic heart failure is also associated with endothelial and blood coagulation abnormalities that may lead to progressive heart disease, and the increased risk of stroke and venous thromboembolism (Lip & Gibbs, 1999; Davis *et al*, 2000) in patients with severe CHF (Diet & Erdmann, 2000). It has been shown that haemostatic abnormalities are correlated with neuroendocrine activation in CHF (Sbarouni *et al*, 1994) but their relationship with cytokines and inflammation is unclear, although the modulatory role of cytokines on haemostasis is well known (Kerr *et al*, 2001).

Proinflammatory cytokines are also related to endothelial dysfunction in heart transplant recipients (Aukrust *et al*, 2000) and contribute to the development of transplant coronary artery disease (Gullestad *et al*, 1999). Heart transplant recipients also show prothrombotic changes that tend to be more evident in patients with accelerated coronary sclerosis (Hunt *et al*, 1993).

The aim of this study was to investigate the haemostatic, endothelial and inflammatory abnormalities in patients with advanced heart failure in order to determine their possible relationships, and to see whether they are modified by oral anticoagulants (OATs) or persist after successful heart transplantation.

Methods

Patients

The study was performed according to the ethical guidelines of the Declaration of Helsinki, and all of the subjects gave their informed consent before participating.

We studied 25 male patients aged 22–65 years (mean ± SD, 54 ± 10) with advanced CHF [New York Heart Association (NYHA) class IV] who were consecutively admitted to the University Department of Cardiology during the course of 1 year. The patient's clinical, haemodynamic and biochemical characteristics are shown in Table I. All of the patients were treated with diuretics, angiotensin-converting enzyme (ACE)-inhibitors, beta-blockers and anti-aldosterone; 11 also received OAT treatment designed to maintain a prothrombin time international normalized ratio (INR) of between 2 and 3. Although the patients receiving and not receiving OAT treatment were not identical, there was no significant clinical difference between the groups in terms of the aetiology of CHF, left ventricular ejection fraction, blood pressure or routine laboratory parameters.

After 4 ± 3 months, 10 of the 25 patients underwent transplantation, nine of whom (age 22–62 years; mean 51 ± 14) were re-evaluated after a period of 24 ± 7 months. Prior to heart transplantation, these nine patients had clinical conditions and laboratory tests similar to those observed in the untransplanted patients and 44% of the patients in both groups were treated with OATs. The ischaemic period of the cardiac grafts ranged from 120 to 320 min. At the time of the re-evaluation, all of the transplant patients were clinically and haemodynamically stable (NYHA class I) and had shown no signs of rejection or infection in the previous 6 months; none of them was receiving OATs. Five were treated with ciclosporin, prednisolone and azathioprine (one of whom discontinued prednisolone because of major side effects 6 months before the date of blood collection); the remaining four received only ciclosporin and prednisolone.

Table I. Clinical, haemodynamic and laboratory parameters in 25 patients with advanced heart failure.

Patients (*n*)	25
Age (years, mean ± SD)	54 ± 11
Males (*n*)	25
NYHA Class IV advanced (*n*)	25
Aetiology	
Ischaemic (*n*)	11
Idiopathic (*n*)	13
Valvular (*n*)	1
Left ventricular ejection fraction (% mean ± SD)	21 ± 5
Systolic blood pressure (mmHg, mean ± SD)	101 ± 8
Diastolic blood pressure (mmHg, mean ± SD)	65 ± 7
Treatment	
Furosemide (mg/d)	50–500
Potassium canrenoate (mg/d)	25–100
Enalapril (mg/d)	10–20
Laboratory	
Serum creatinine (μmol/l, mean ± SD)	148 ± 68
Serum cholinesterase(U/L, mean ± SD)	7721 ± 2182
Serum bilirubin (μmol/l, mean ± SD)	21 ± 9·0
Serum sodium (mmol/l, mean ± SD)	131 ± 7
Serum potassium (mmol/l, mean ± SD)	4·23 ± 0·47
Haemoglobin (g/dl, mean ± SD)	12·9 ± 2·0

Controls

The control group consisted of 25 age-matched male healthy subjects.

Blood sampling

After an overnight fast, 23 ml of blood were collected in the morning into polypropylene tubes containing different anticoagulant mixtures: (i) sodium citrate 3·8% (1 ml of anticoagulant/9 ml of blood) for the measurement of tissue plasminogen activator (t-PA), D-dimer fragment, prothrombin fragment 1 + 2 (F1 + 2), thrombin–antithrombin (TAT) complexes, fibrinogen, von Willebrand factor (VWF) antigen, factor VII (FVII), activated factor XII (FXIIa) and thrombomodulin; (ii) ethylenediaminetetraacetic acid sodium salt (EDTA) 200 mmol/l (0·250 ml of anticoagulant/5 ml of blood) to determine the levels of interleukin-6 (IL-6), TNFα,

soluble TNF receptor II (sTNFRII), soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1) and soluble endothelial selectin (sE-selectin); (iii) prechilled 0·5 mol/l citrated buffer pH 4·3 (Stabilyte, Biopool, Umea, Sweden) (0·5 ml of anticoagulant/4·5 ml of blood) for the plasminogen activator inhibitor-1 (PAI1) assay and (iv) a cocktail containing protease inhibitors (100 mmol/l trisodium citrate, 67 mmol/l citric acid and 2% dextrose, pH 4·5, 100 mmol/l benzamidine, 400 μg/ml hexadimethrine bromide, 2 mg/ml soybean trypsin inhibitor, 263 μmol/l leupeptin and 20 mmol/l aminoethylbenzenesulphonylfluoride) (0·5 ml of anticoagulant/4·5 ml of blood) for the evaluation of cleaved high-molecular-weight kininogen (HK). Plasma aliquots of 0·4 ml were frozen and stored at −80°C until testing when they were thawed for 10 min in a water bath at 37°C.

Biomarkers

Fibrinogen was measured by means of a commercial coagulometric method (Diagnostica Stago, Asnières, France).

TAT complexes were measured using a sandwich enzyme-linked immunosorbent assay (ELISA; Enzygnost TAT micro, Behring Diagnostics GmbH, Marburg, Germany) with intra- and inter-assay coefficients of variation (CVs) of 2·5% and 5%.

F1 + 2 was assessed using a sandwich ELISA (Enzygnost F1 + 2; Behring Diagnostics GmbH) with intra- and inter-assay CVs of 5% and 8%.

FVII antigen was measured using a commercial ELISA (Asserachrom VII:Ag, Diagnostica Stago) according to the manufacturer's instructions. The intra- and inter-assay CVs were 5·2% and 6·9%.

FXIIa was measured using a sandwich ELISA (Asserachrom FXIIa, Diagnostica Stago) with intra- and inter-assay CVs of <7%.

Cleaved high-molecular-weight kininogen (HK) was assessed by means of sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting analysis: native HK appeared as a band of Mr 130 000, and cleaved HK as two bands of Mr 107 000 and 98 000.

t-PA antigen was measured using a commercial ELISA (Imunolyse t-PA; Biopool, Umea, Sweden) according to the manufacturer's instructions. The intra- and inter-assay CVs were 6·5% and 8%.

Active PAI-1 was measured using a commercial bioimmunoassay (Chromolize PAI-1; Biopool) with intra- and inter-assay CVs of 2·4% and 4·5%.

D-dimer was measured using a commercial ELISA (Enzygnost D-dimer; Behring Diagnostics GmbH) according to manufacturer's instructions. The intra- and inter-assay CVs were 10% and 15%.

VWF antigen was measured in citrated plasma by means of a sandwich ELISA that uses two monoclonal antibodies against different VWF epitopes (11B6·18 and 7G10·8) (Mannucci & Coppola, 1999). The intra- and inter-assay CVs were <8%.

Thrombomodulin was measured in plasma using a commercial sandwich ELISA (Asserachrom Thrombomodulin, Diagnostica Stago) with intra- and inter-assay CVs of <10%.

TNF−α was measured in plasma using a direct solid-phase immunoassay (Enzyme Amplified Sensitivity Immunoassay; EASIA, Biosource, Flerus, Belgium) with intra- and inter-assay CVs of 8% and 10%.

sTNFRII was measured using a sandwich ELISA (Quantikine Human sTNFRII Immunoassay; R&D Systems Inc., Minneapolis, MN, USA) with intra- and inter-assay CVs of 2·5% and 5·1%.

IL-6 was measured in plasma using a sandwich ELISA (Quantikine Human IL-6 Immunoassay, R & D Systems Inc.) with intra- and inter-assay CVs of 4·2% and 6·4%.

sICAM-1 was measured in plasma using a sandwich ELISA (Parameter Human sICAM-1 Immunoassay; R&D Systems Inc.) with intra- and inter-assay CVs of 4·8% and 10·1%.

sVCAM-1 was measured in plasma using a sandwich ELISA (Parameter Human sVCAM-1 Immunoassay; R&D Systems Inc.) with intra- and inter-assay CVs of 5·9% and 10·2%.

sE-selectin was measured in plasma using a sandwich ELISA (Parameter Human sE-selectin Immunoassay; R&D Systems Inc.) with intra- and inter-assay CVs of 5·0% and 8·8%.

Statistical analysis

The descriptive statistics are reported as median values (range) because of the skewed distribution of the investigated variables. Inter-group differences were evaluated by means of the Wilcoxon Mann–Whitney non-parametric test, and correlations by means of Spearman's test. A *P*-value of <0·05 was considered significant.

Results

Coagulation and fibrinolysis parameters

The coagulation and fibrinolysis abnormalities of the CHF patients are shown in Table II. They exhibited significantly higher plasma levels of fibrinogen ($P = 0{\cdot}0001$), TAT ($P = 0{\cdot}001$), t-PA ($P = 0{\cdot}0001$) and D-dimer ($P = 0{\cdot}0001$) than the controls.

Of the coagulation parameters, prothrombin fragment F1 + 2 directly correlated with TAT (0·644, $P = 0{\cdot}001$) and FVII antigen (0·607, $P = 0{\cdot}001$). There was no difference in any of the coagulation and fibrinolysis parameters between the patients with ischaemic disease and those with idiopathic cardiomyopathy.

When the CHF patients were divided on the basis of anticoagulant treatment, lower levels of TAT ($P = 0{\cdot}01$), F1 + 2 ($P = 0{\cdot}0001$) and FVII (0·0001) were observed in the 11 treated patients (Table III), who also had higher levels of PAI activity ($P = 0{\cdot}01$).

The nine patients undergoing successful heart transplantation had significantly reduced plasma fibrinogen ($P = 0{\cdot}0001$)

Table II. Plasma coagulation, fibrinolysis and endothelial parameters in 25 healthy subjects (controls) and 25 patients with advanced CHF, including nine patients evaluated after successful heart transplantation.

	Controls ($n = 25$)	Total CHF patients ($n = 25$)	Transplant patients ($n = 9$)
Fibrinogen (g/l)	3·0 (1·96–3·8)	5·13*** (2·74–7·48)	3·55*††† (2·35–4·87)
TAT (ng/ml)	2·4 (1·3–4·9)	3·8** (1·6–16·8)	4·5*** (2·9–22·2)
F1 + 2 (ng/ml)	1·2 (0·9–1·9)	1·0 (0·3–3·7)	1·1 (0·3–3·1)
FVII (ng/ml)	100 (60–139)	74* (16–138)	145**†† (80–215)
FXIIa (ng/ml)	2·40 (0·90–5·00)	2·50 (1·19–4·50)	5·50***††† (2·50–8·10)
Cleaved HK (% of total HK)	18 (1–32)	18 (8–32)	16 (12–21)
PAI (ng/ml)	14·8 (2·9–29·4)	7·6 (0·1–80·7)	18·7 (2·6–47·3)
t-PA (ng/ml)	6·9 (2·7–13·5)	16·0*** (7·7–36·5)	15·1*** (7·4–21·8)
D-dimer (ng/ml)	15 (7–41)	74*** (12–815)	41***† (32–102)
VWF:Ag (% of normal)	109 (50–204)	264*** (85–720)	175*† (109–360)
Thrombomodulin (ng/ml)	17·3 (0·7–29·3)	22·7* (13·4–55·4)	58·5***††† (23·7–83·6)

Median values (range in parentheses).
Significance *versus* controls ***$P = 0·0001$; **$P = 0·001$; *$P = 0·01$.
Significance *versus* CHF patients †††$P = 0·0001$; ††$P = 0·001$; †$P = 0·01$.
VWF:Ag, von Willebrand factor antigen; CHF, chronic heart failure; TAT, thrombin–antithrombin complex; PAI, plasminogen activator inhibitor.

Table III. Plasma levels of the coagulation and inflammation parameters that were significantly different between the 11 CHF patients treated with OAT and the 14 CHF patients who were not.

	CHF patients not receiving OAT ($n = 14$)	CHF patients receiving OAT ($n = 11$)
TAT (ng/mL)	4·9 (2·6–16·8)	2·3* (1·6–9·2)
F1 + 2 (ng/ml)	1·6 (0·9–1·9)	0·6** (0·3–3·7)
FVII (ng/ml)	101 (21–138)	29** (16–74)
PAI (ng/ml)	5·2 (1·1–44·0)	10·5* (0·1–80·7)
sTNFRII (pg/ml)	5748 (3530–8374)	4374* (2975–7681)
sVCAM (ng/ml)	1360 (613–4608)	882* (401–2042)

Median values (range in parentheses).
Statistical significance **$P = 0·0001$; *$P = 0·01$.
CHF, chronic heart failure; OAT, oral anticoagulant therapy; sVCAM, soluble vascular cell adhesion molecule; sTNFRII, soluble tumour necrosis factor II; TAT, thrombin–antithrombin complex; PAI, plasminogen activator inhibitor.

and D-dimer levels ($P = 0·01$), and significantly increased FVII antigen and FXIIa levels ($P = 0·0001$) (Table II). These differences were also significant when their pre- and post-transplant values were compared (the most important differences are reported in Fig 1). The levels of TAT complexes remained higher than those of the normal controls (Table II). In these nine patients before heart transplantation, coagulation and fibrinolysis parameters were not different from those of untransplanted patients.

Markers of endothelial dysfunction

The CHF patients had higher plasma VWF ($P = 0·0001$), thrombomodulin ($P = 0·01$) and t- PA ($P = 0·0001$) levels than the normal controls, and lower plasma sE-selectin levels ($P = 0·001$) (Tables II and IV). The markers of endothelial dysfunction were not significantly different between the ischaemic and idiopathic cardiomyopathy patients.

Plasma VWF antigen levels tended to correlated directly with plasma thrombomodulin ($r = 0·428$, $P = 0·03$) and t-PA antigen levels ($r = 0·477$, $P = 0·01$).

The nine patients studied after heart transplantation showed a slight reduction in plasma VWF levels ($P = 0·01$), although these remained higher than in the controls ($P = 0·01$), and a further increase in thrombomodulin levels ($P = 0·0001$) (Table II). The differences were also significant when the pre- and post-transplant values were compared (the most important differences are reported in Fig 1). Plasma t-PA levels remained high, but those of sE-selectin normalized (Tables II and IV). In these patients before heart transplantation, markers of endothelial dysfunction were not different from those of untransplanted patients.

Inflammatory cytokines and adhesion molecules

The CHF patients had higher plasma levels of TNF ($P = 0·0001$), sTNFRII ($P = 0·0001$), IL-6 ($P = 0·0001$), sICAM ($P = 0·001$) and sVCAM ($P = 0·0001$) than the normal controls (Table IV). There was no significant difference in inflammation parameters between the patients with ischaemic disease and those with idiopathic cardiomyopathy.

The patients receiving anticoagulant treatment had lower levels of sTNFRII ($P = 0·01$) and sVCAM ($P = 0·01$) than those who were not (Table III).

The plasma levels of sTNFRII directly correlated with those of TNF ($r = 0·422$, $P = 0·03$), IL-6 ($r = 0·481$, $P = 0·01$), sICAM ($r = 0·473$, $P = 0·02$) and sVCAM ($r = 0·797$, $P = 0·0001$), and there was a correlation between IL-6 and sICAM levels ($r = 0·619$, $P = 0·001$).

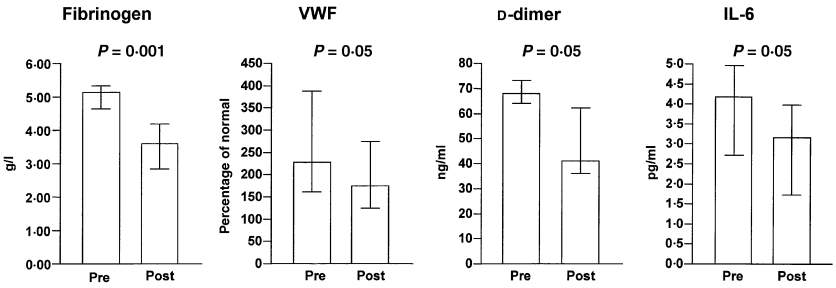

Fig 1. Plasma levels of fibrinogen, von Willebrand Factor (VWF), D-dimer and interleukin-6 (IL-6) in nine patients with advanced chronic heart failure before heart transplantation (pre) and in the same patients after successful heart transplantation (post). Values are expressed as median and interquartile range (*P*-values refer to the Wilcoxon Mann–Whitney non-parametric test).

Table IV. Plasma levels of inflammatory cytokines and adhesion molecules in 25 healthy subjects (controls) and 25 patients with advanced CHF, including nine patients after successful heart transplantation.

	Controls ($n = 25$)	Total CHF patients ($n = 25$)	Transplant patients ($n = 9$)
TNF (pg/ml)	17·8 (12·7–25·7)	31·1*** (19·6–93·1)	22·5* (15·9–41·3)
sTNFRII (pg/ml)	2555 (1762–3557)	5257*** (2975–8374)	6238*** (3478–12062)
IL-6 (pg/ml)	0·12 (0·00–4·60)	7·24*** (0·76–232·16)	3·16***† (1·35–6·79)
sICAM (ng/ml)	348 (210–605)	486** (167–2432)	305† (285–480)
sVCAM (ng/ml)	551 (226–898)	1027*** (401–4608)	849*** (660–1198)
sE-selectin (ng/ml)	45·0 (4·0–150·0)	26·0** (16·0–81·0)	45·6 (19·2–70·8)

Median values (range in parentheses).
Significance *versus* controls ***$P = 0·0001$; **$P = 0·001$; *$P = 0·01$.
Significance *versus* CHF patients †$P = 0·01$.
CHF, chronic heart failure; TNF, tumour necrosis factor; IL-6, interleukin 6; sVCAM, soluble vascular cell adhesion molecule; sICAM, soluble intercellular adhesion molecule; sTNFRII, soluble tumour necrosis factor II; E-selectin, endothelial-selectin.

In the nine patients studied after heart transplantation, there was a significant reduction ($P = 0·01$) in the plasma levels of IL-6 (which, however, remained higher than normal) and sICAM (which normalized) (Table IV). These differences were also significant when the pre- and post-transplant values were compared (the most important differences are reported in Fig 1). In these patients before heart transplantation, plasma levels of inflammatory cytokines and adhesion molecules were not different from those of untransplanted patients.

Correlations between coagulation, fibrinolysis and endothelial function parameters, inflammatory cytokines and adhesion molecules

There were statistically significant correlations between the coagulation and fibrinolysis parameters: fibrinogen directly correlated with tPA antigen ($r = 0·512$, $P = 0·009$); and PAI inversely correlated with FVII antigen ($r = -0·572$, $P = 0·003$).

The coagulation and fibrinolysis parameters correlated with the endothelial markers: VWF directly correlated with fibrinogen ($r = 0·511$, $P = 0·009$), TAT complexes ($r = 0·566$, $P = 0·003$) and t-PA antigen ($r = 0·477$, $P = 0·01$), and sE-selectin directly correlated with cleaved HK ($r = 0·531$, $P = 0·006$) and D-dimer ($r = 0·431$, $P = 0·03$).

In relation to the coagulation/fibrinolysis and inflammation parameters, IL-6 levels directly correlated with fibrinogen ($r = 0·541$, $P = 0·005$) and t-PA antigen ($r = 0·482$, $P = 0·01$).

There were direct correlations between the markers of endothelial dysfunction and cytokine and adhesion molecule levels: VWF correlated with sTNFRII ($r = 0·486$, $P = 0·01$), IL-6 ($r = 0·465$, $P = 0·01$) and sICAM ($r = 0·524$, $P = 0·007$), and thrombomodulin with sTNFRII ($r = 0·456$, $P = 0·02$) and sICAM ($r = 0·462$, $P = 0·02$).

No correlation was found between the patients' age or prognosis at 18 months or cardiac graft ischaemic time and the investigated coagulation, endothelial function and inflammation parameters.

Discussion

We found that, regardless of its aetiology, advanced CHF was associated with the activation of coagulation and fibrinolysis, endothelial dysfunction and increased proinflammatory cytokine and adhesion molecule levels. Most of these abnormalities paralleled each other, were less in patients treated with OAT therapy and, although reduced, persisted after successful heart transplantation even in the absence of any clinical signs of CHF.

Analysis of the coagulation parameters in our patients indicated that CHF leads to a hypercoagulable state, with high levels of thrombin formation markers, such as TAT complexes. The activation of the coagulation system in CHF has been

previously reported (Yamamoto *et al*, 1995; Davis *et al*, 2000), and suggested to be important in disease progression and thromboembolic complications (Davis *et al*, 2000). On the basis of the results of recently published clinical trials, the expected incidence of thromboembolic events is approximately 2% a year (Isnard & Komajda, 2001), but it is higher in patients with severe CHF, who have an annual stroke risk of 4% (Brown & Cleland, 1998). Our patients had high levels of fibrinogen, which has been identified as a major independent risk factor for cardiovascular disease (Juhan-Vague, 1996); they also had high levels of D-dimer, a marker of fibrinolysis (probably secondary to coagulation activation) that has been found to be associated with the risk of myocardial infarction (Cushman *et al*, 1999).

There is still no consensus concerning the usefulness of antithrombotic therapy in CHF (except in the presence of atrial fibrillation) because of the relatively high (2·3–6·8%) risk of major haemorrhages during anticoagulant treatment (Koniaris & Goldhaber, 1998; Isnard & Komajda, 2001). However, there is a need to extend the indications for treatment in CHF patients (Sirajuddin *et al*, 2002). Eleven of our patients received OATs, and had lower levels of coagulation activation markers than those who did not (Table III). The very low F1 + 2 levels in the patients taking anticoagulant treatment explain why they were not increased in the CHF patients as a whole (Table II). The association between OAT treatment and lower levels of the markers of thrombin generation has been previously described (Bruhn *et al*, 1992), and may be related to a favourable effect induced by the reduction in hypercoagulability, although no clear correlation between plasma F1 + 2 levels and the degree of anticoagulation has yet been demonstrated. FVII levels were also very low in our patients receiving anticoagulant treatment, and this may be protective as high levels have been associated with increased cardiovascular mortality (Juhan-Vague, 1996). We found that the changes in coagulation after heart transplantation remained (although to a lesser degree), and these have been correlated with accelerated coronary sclerosis and cardiac transplant rejection (Hunt *et al*, 1993; Segal *et al*, 2001).

The plasma levels of inflammatory cytokines, adhesion molecules and markers of endothelial dysfunction in our CHF patients were higher than in the normal controls, and remained high after heart transplantation, albeit to a lesser extent. High plasma levels of VWF, which have been associated with a hypercoagulable state in CHF patients (Gibbs *et al*, 2001), were observed also in our transplanted patients in agreement with a previous study (Hunt *et al*, 1993); these patients had normal levels of sE-selectin as previously reported (Andreassen *et al*, 1998). High serum levels of proinflammatory cytokines have been related to the pathogenesis of allograft rejection, although a systematic relationship between cytokine levels and histological rejection has been excluded (Grant *et al*, 1996). Persistent intracardiac TNF expression may contribute to the development of cardiac allograft hypertrophy (Stetson *et al*, 2001). However, given the potential immune stimulation caused by a transplanted heart and the immune suppression caused by therapy, the differences in the pre- and post-transplantation levels of inflammation markers in our patients should be interpreted cautiously.

The activation of the immune system in patients with CHF is well known, but its origin remains elusive. Two hypotheses have been proposed on the basis of experimental and clinical data: one hypothesized that bowel wall oedema leads to bacterial translocation, with subsequent endotoxin release and immune activation (Anker *et al*, 1997), and the second hypothesized that the heart is the main source of cytokines, as shown by the fact that TNF α is produced by a failing, but not a normal, myocardium (Habib *et al*, 1996; Torre-Amione *et al*, 1996). However, a single source of cytokine production (gut or heart) does not seem to be sufficient to explain the multiple organ involvement and systemic inflammation of CHF, which have also been related to systemic hypoxia (Hasper *et al*, 1998). Whatever the cause of the immune system stimulation, an intermediate mechanism may be the activation of the coagulation system: our patients treated with OATs, which efficiently reduced the markers of hypercoagulability, showed reduced levels of sensitive inflammation markers, such as sTNFRII and sVCAM (Table III). A link between the activation of the coagulation and immune systems in CHF was also supported by our observation of direct correlations between the markers of both systems. It has been demonstrated that thrombin can activate macrophage adhesion and induce the production of IL-6 and monocyte chemoattractant protein-1 in an *in vivo* animal model (Szaba & Smiley, 2002), and it is known that immune system activation may promote coagulation as there is evidence that IL-6 induces the increased expression of tissue factor, fibrinogen, FVIII and VWF, and activates endothelial cells (Kerr *et al*, 2001).

In conclusion, the novel findings of this study are that: (i) a thrombophilic state is closely associated with markers of endothelial dysfunction and systemic inflammation in advanced CHF; (ii) after a successful heart transplantation, these abnormalities still persist (although to a lesser extent) even in the absence of clinical signs of CHF; and (iii) OAT treatment tends to normalize not only the alterations in coagulation, but also the parameters of inflammation. Our findings suggest that OAT treatment may be beneficial in patients with advanced CHF, although a larger and randomized study is necessary to verify whether or not the benefits of prolonged OAT treatment outweigh its side effects.

References

Andreassen, A.K., Nordoy, I., Simonsen, S., Ueland, T., Muller, F., Froland, S.S., Gullestad, L. & Aukrust, P. (1998) Levels of circulating adhesion molecules in congestive heart failure and after heart transplantation. *American Journal of Cardiology*, **81,** 604–608.

Anker, S.D., Egerer, K., Volk, H.-D., Kox, W.J., Poole-Wilson, P.A. & Coats, A.J.S. (1997) Elevated soluble CD14 receptors and altered

cytokines in chronic heart failure. *American Journal of Cardiology*, **79,** 1426–1430.

Aukrust, P., Ueland, T., Muller, F., Andreassen, A.K., Nordoy, I., Aas, H., Kjekshus, J., Simonsen, S., Froland, S.S. & Gullestad, L. (1998) Elevated circulating levels of C-C chemokines in patients with congestive heart failure. *Circulation*, **97,** 1136–1143.

Aukrust, H.T., Andreassen, A.K., Ueland, T., Brosstad, F., Froland, S.S., Simonsen, S. & Gullestad, L. (2000) Peripheral endothelial dysfunction in heart transplant recipients: possible role of proinflammatory cytokines. *Clinical Transplantation*, **14,** 218–225.

Baumgarten, G., Knuefermann, P. & Mann, D.L. (2000) Cytokines as emerging targets in the treatment of heart failure. *Trends in Cardiovascular Medicine*, **10,** 216–223.

Bozkurt, B., Kribbs, S., Clubb, Jr, F.J., Michael, L.H., Didenko, V.V., Hornsby, P.J., Seta, Y., Oral, H., Spinale, F.G. & Mann, D.L. (1998) Pathophysiologically relevant concentrations of tumor necrosis factor-alpha promote progressive left ventricular dysfunction and remodeling in rats. *Circulation*, **97,** 1382–1391.

Brown, A. & Cleland, J.G.F. (1998) Influence of concomitant disease on patterns of hospitalisation in patients with heart failure discharged from Scottish hospitals in 1995. *European Heart Journal*, **19,** 1063–1069.

Bruhn, H.D., Liebsch, J. & Wagner, C. (1992) Documentation of hypocoagulability by measurement of prothrombin fragment F 1 + 2 when introducing oral anticoagulant therapy. *Thrombosis Research*, **68,** 317–319.

Cohn, J.N. (2001) Left ventricle and arteries: structure, function, hormones, and disease. *Hypertension*, **37,** 346–349.

Cushman, M., Lemaitre, R.N., Kuller, L.H., Psaty, B.M., Macy, E.M., Sharrett, A.R. & Tracy, R.P. (1999) Fibrinolytic activation markers predict myocardial infarction in the elderly. The Cardiovascular Health Study. *Arteriosclerosis Thrombosis and Vascular Biology*, **19,** 493–498.

Davis, C.J., Gurbel, P.A., Gattis, W.A., Fuzaylov, S.Y., Nair, G.V., O'Connor, C.M. & Serebruany, V.L. (2000) Hemostatic abnormalities in patients with congestive heart failure: diagnostic significance and clinical challenge. *International Journal of Cardiology*, **75,** 15–21.

Diet, F. & Erdmann, E. (2000) Thromboembolism in heart failure: who should be treated? *European Journal of Heart Failure*, **2,** 355–363.

Finkel, M.S., Oddis, C.V., Jacob, T.D., Watkins, S.C., Hattler, B.G. & Simmons, R.L. (1992) Negative inotropic effects of cytokines on the heart mediated by nitric oxide. *Science*, **257,** 387–389.

Francis, G.S., Goldsmith, S.R., Levine, T.B., Olivari, M.T. & Cohn, J.N. (1984) The neurohumoral axis in congestive heart failure. *Annals of Internal Medicine*, **101,** 370–377.

Gibbs, C.R., Blann, A.D., Watson, R.D. & Lip, G.Y. (2001) Abnormalities of hemorheological, endothelial, and platelet function in patients with chronic heart failure in sinus rhythm: effects of angiotensin-converting enzyme inhibitor and beta-blocker therapy. *Circulation*, **103,** 1746–1751.

Grant, S.C., Lamb, W.R., Brooks, N.H., Brenchley, P.E. & Hutchinson, I.V. (1996) Serum cytokines in human heart transplant recipients. Is there a relationship to rejection? *Transplantation*, **62,** 480–491.

Gullestad, L., Simonsen, S., Ueland, T., Holm, T., Aass, H., Adreassen, A.K., Madsen, S., Geiran, O., Froland, S.S. & Aukrustb, P. (1999) Possible role of proinflammatory cytokines in heart allograft coronary artery disease. *American Journal of Cardiology*, **84,** 999–1003.

Habib, F.M., Springall, D.R., Davies, G.J., Oakley, C.M., Yacoub, M.H. & Polak, J.M. (1996) Tumor necrosis factor and inducible nitric oxide synthase in dilated cardiomyopathy. *Lancet*, **347,** 1151–1155.

Hasper, D., Hummel, M., Kleber, F.X., Reindl, I. & Volk, H-D. (1998) Systemic inflammation in patients with heart failure. *European Heart Journal*, **19,** 761–765.

Hunt, B., Segal, H. & Yacoub, M. (1993) Hemostatic changes in heart transplant recipients and their relationship to accelerated coronary sclerosis. *Transplantation*, **55,** 309–315.

Isnard, R. & Komajda, M. (2001) Thromboembolism in heart failure, old ideas and new challenges. *European Journal of Heart Failure*, **3,** 265–269.

Juhan-Vague, I. (1996) Haemostatic parameters and vascular risk. *Atherosclerosis*, **124,** S49–S55.

Kapadia, S., Dibbs, Z., Kurrelmeyer, K., Karla, D., Seta, Y., Wang, F., Bozkurt, B., Oral, H., Sivasubramanian, N. & Mann, D.L. (1998) The role of cytokines in the failing human heart. *Cardiology Clinics*, **16,** 645–656.

Kerr, R., Stirling, D. & Ludlam, C.A. (2001) Interleukin 6 and haemostasis. *British Journal of Haematology*, **115,** 3–12.

Koniaris, L.S. & Goldhaber, S.Z. (1998) Anticoagulation in dilated cardiomyopathy. *Journal of the American College of Cardiology*, **31,** 745–748.

Levine, B., Kalman, J., Mayer, L., Fillit, H.M. & Packer, M. (1990) Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. *New England Journal of Medicine*, **323,** 236–241.

Lip, G.Y.H. & Gibbs, C.R. (1999) Does heart failure confer a hypercoagulable state? Virchow's triad revisited. *Journal of the American College of Cardiology*, **33,** 1424–1426.

Mannucci, P.M. & Coppola, R. (1999) Von Willebrand Factor. In: *Laboratory Techniques in Thrombosis. A Manual* (ed. by J. Jespersen, R.M. Bertina & F. Haverkate), pp. 115–119. Kluwer Academic Publishers, Boston.

Pfeffer, J.M. & Pfeffer, M.A. (1988) Angiotensin converting enzyme inhibition and ventricular remodeling in heart failure. *American Journal of Medicine*, **84,** 37–44.

Sbarouni, E., Bradshaw, A., Andreotti, F., Tuddenham, E., Oakley, C.M. & Cleland, G.F. (1994) Relationship between hemostatic abnormalities and neuroendocrine activity in heart failure. *American Heart Journal*, **127,** 607–612.

Segal, J.B., Kasper, E.K., Rohde, C., Bray, P.F., Baldwin III, W.M., Resar, J.R., Hruban, R.H. & Kickler, T.S. (2001) Coagulation markers predicting cardiac transplant rejection. *Transplantation*, **72,** 233–237.

Sharma, R., Coats, A.J. & Anker, S.D. (2000) The role of inflammatory mediators in chronic heart failure: cytokines, nitric oxide, and endothelin-1. *International Journal of Cardiology*, **72,** 175–186.

Sirajuddin, R.A., Miller, A.B. & Geraci, S.A. (2002) Anticoagulation in patients with dilated cardiomyopathy and sinus rhythm: a critical literature review. *Journal of Cardiac Failure*, **8,** 48–53.

Stetson, S.J., Perez-Verdia, A., Mazur, W., Farmer, J.A., Koerner, M.M., Weilbaecher, D.G., Entman, M.L., Quinones, M.A., Noon, G.P. & Torre-Amione, G. (2001) Cardiac hypertrophy after transplantation is associated with persistent expression of tumor necrosis factor-alpha. *Circulation*, **104,** 676–681.

Szaba, F.M. & Smiley, S.T. (2002) Roles for thrombin and fibrin(ogen) in cytokine/chemokine production and macrophage adhesion in vivo. *Blood*, **99,** 1053–1059.

Torre-Amione, G., Kapadia, S., Lee, J., Durand, J.B., Bies, R.D., Young, J.B. & Mann, D.L. (1996) Tumor necrosis factor-alpha and tumor necrosis factor receptors in the failing human heart. *Circulation*, **93,** 704–711.

Yamamoto, K., Ikeda, U., Furuhashi, K., Irokawa, M., Nakayama, T. & Shimada, K. (1995) The coagulation system is activated in idiopathic cardiomyopathy. *Journal of the American College of Cardiology*, **25,** 1634–1640.