Prognostic impact of haemostatic derangements in chronic heart failure

Borut Jug1; Nina Vene1; Barbara Gužic Salobir2; Miran Šebeštjen1; Mišo Šabovic1; Irena Keber1

1Department of Vascular Diseases, Clinic of Internal Medicine, University Clinical Center Ljubljana, Slovenia; 2Clinic of Nuclear Medicine, University Clinical Center Ljubljana, Slovenia

Summary
Heart failure is characterised by activation of haemostasis. We sought to explore the prognostic impact of deranged haemostasis in chronic heart failure. In stable, optimally managed outpatients with chronic heart failure, baseline levels of prothrombin fragment F1+2, D-dimer, and tPA and PAI-1 antigens were determined. Clinical follow-up was obtained and the rate of events (heart failure related deaths or hospitalisations) was recorded. We included 195 patients (32.3% female, NYHA class II (66.2%) or III (33.8%), mean age 71 years). During a median follow-up of 693 (interquartile range [IQR] 574–788) days, 63 (30.9%) patients experienced an event; those with an event had higher levels of tPA antigen (median 11.8 [IQR 8.7–14.0] µg/l; p=0.033) and D-dimer (938 [485–1269] vs. 620 [37–1076] µg/l; p=0.018). However, on Cox multivariate analysis, only tPA levels above optimal cut-off value of 10.2 µg/l (but not D-dimer) emerged as an independent predictor of prognosis (HR adjusted 2.695, 95% confidence interval 1.233–5.363; p=0.017). Our findings suggest that elevated tPA antigen levels are an independent prognostic predictor in patients with chronic stable heart failure.

Keywords
Congestive heart failure, prognosis, haemostasis

Introduction
Heart failure is associated with a prothrombotic state (1, 2). However, unlike in atherothrombotic disease, the causes of a procoagulant state in heart failure are more complex; therefore, a modified Wirchow triad - blood stasis in enlarged cardiac chambers, vascular (endothelial) abnormalities and deranged blood coagulation properties - has been proposed as a predisposing mechanism to a procoagulant state in heart failure (3). In fact, increased activity of beta-thromboglobulin, fibrinopeptide A, endothelial procoagulant, von Willebrand factor, fibrinolytic products, D-dimer, tissue plasminogen activator (tPA) and thrombin have all been reported in patients with heart failure and impaired left ventricular systolic function (1, 4–8).

Haemostatic derangements are associated with unfavourable prognosis in a wide range of cardiovascular disorders. Previous studies have reported an association between increased levels of different haemostatic markers and the rate of cardiovascular events in patients with coronary (9–12), cerebrovascular (13–15) and peripheral artery disease (16), and atrial fibrillation (17). In heart failure, haemostatic derangements correlate with disease severity (18). Nevertheless, to date only D-dimer levels have been evaluated and confirmed as an independent predictor of cardiovascular mortality in outpatients with signs and symptoms compatible with heart failure (19).

Most risk-stratification strategies in heart failure rely on prognostic factors that have been identified and validated before the introduction of current management strategies (20, 21). Therefore, management of patients with chronic heart failure calls for a sustained identification of novel and validation of established prognostic factors. In our study, we sought to evaluate and compare the prognostic impact of different haemostatic markers (namely, prothrombin fragment F1+2, fibrin turn-over product D-dimer, and tPA and plasminogen activator inhibitor 1 [PAI-1] antigens) in patients with chronic stable optimally managed heart failure.

Patients and methods

Patients
Patients were recruited from the Heart failure clinic of the University Clinical Center in Ljubljana, Slovenia.
The diagnosis of heart failure (based on signs and symptoms of heart failure or on history of hospitalisation because of heart failure within 12 months prior to inclusion) was confirmed by two independent cardiologists. Patients had either evidence of impaired left ventricular ejection fraction (LVEF, ≤50% as measured by the Simpson biplane method) or preserved left ventricular ejection fraction (LVEF >50%) and either I) an E/Em ratio >15 or alternatively II) an E/Em ratio between 8 and 15 plus one of the following: elevated NT-proBNP levels, increased left ventricular mass or left atrial volume index, or presence of indices of diastolic dysfunction as estimated by transmitted or pulmonary vein flow (E/A ratio, dt, IRT, S/D) (22). We included consecutive real-life patients irrespective of whether the underlying ventricular dysfunction was systolic or isolated diastolic; although landmark heart failure trials focused on patients with impaired LVEF, a growing body of evidence suggests that isolated diastolic dysfunction comprises a wide subgroup of heart failure patients and research is therefore expanding to study patients with isolated diastolic dysfunction as well. Patients were in NYHA functional class II or III, optimally managed according to current guidelines and stable for at least three months prior to inclusion.

We excluded patients with recent (<3 months) myocardial infarction, stroke or thromboembolism, with significant liver (enzymes >3 times the upper reference limit) or renal dysfunction (creatinine level >250 μM), and patients with chronic auto-immune or inflammatory diseases and malignancies. Anticoagulation therapy was an exclusion criterion. Informed consent was obtained from all participants according to a protocol approved by the national Committee for medical ethics and biomedical investigation.

Study design and follow-up
At inclusion, patients underwent thorough clinical examination, echographic assessment, and had their blood drawn. We planned a minimum follow-up of 12 months at regular three-month intervals. If the patient missed a follow-up appointment, telephone contacts with next of kin and/or the general practitioner were carried out and pertinent medical records were examined in order to assess changes in health status.

The predefined primary outcome was a composite of cardiovascular death (pump failure, fatal myocardial infarction or sudden cardiac death) or unplanned hospital admission for management of worsening heart failure. The composite end-point was confirmed by two independent cardiologists blinded for baseline measurements; in case of disagreement, a third cardiologist was consulted for end-point adjudication.

Biochemical analysis
All patients had venous blood samples (EDTA) taken after 30 minutes (min) of rest in supine position from the cubital vein. Samples were centrifuged at 3,000 rpm for 10 min at 0°C and separated immediately afterwards.

Prothrombin fragment F1+2 levels were measured by quantitative sandwich enzyme immunoassay technique (Enzygnost® F1+2 monoclonal, Dade Behring, Marburg, Germany). Intra- and interassay coefficient variations were 3.5% and 10.5%, respectively.

D-dimer levels were measured by quantitative sandwich enzyme immunoassay technique (Asserachrom® D-dimer, Diagnostica Stago, Asnieres, France). Intra- and interassay coefficient variations were 6.4 and 14.4%, respectively.

Tissue plasminogen activator (tPA) and plasminogen activator inhibitor 1 (PAI-1) antigens concentrations were measured by quantitative sandwich enzyme immunoassay technique (Imulyse®, Biopool, Ventura, CA, USA). Intra- and inter assay coefficient variations were 7.0 and 17.7% for tPA, and 5.2 and 8.5% for PAI-1.

Statistical analysis
Sample size was calculated to detect a hazard ratio of 2.0 with the following assumptions: proportional hazards, α=0.80 and β=0.05, median survival time 24 months (taking into account the incidence of heart failure related deaths and hospitalisations in patients with chronic stable heart failure), accrual (recruitment) time 12 months, follow-up time after end of recruitment 12 months (23).

Normal distribution was assessed by the Kolmogorov-Smirnov test. Baseline characteristics were summarized by mean (standard deviation) for normally and by median (inter-quartile range) for non-normally distributed continuous variables, and by frequency (percentage) for categorical variables. Between-group differences were tested by the Student t-test for normally and by the Mann-Whitney U test for non-normally distributed variables, and proportions were compared using chi-square test.

For each haemostatic parameter, optimal cut-offs were determined using a maximum statistic approach (24). The selection interval consisted of the inner 80% (10th to 90th percentile) of each covariate distribution (to avoid having a small number in one of the groups after dichotomisation and thus preventing a substantial loss of statistical power). Candidate cut-offs were selected at each 5th percentile of the selection interval and were entered in a log rank time-to-event analysis. As the type I error might be inflated with the increasing number of cut-off candidates, statistical conservativism was achieved by adjusting p-values with the Bonferroni method.

The estimated hazard (risk) ratios were adjusted for major univariate predictors of morbidity and mortality using a multi-variate Cox proportional hazard regression model. A 2-tailed p-value of less than 0.05 was considered statistically significant.

Results
A total of 195 patients were included. One third of patients were female, median age was 71 years (inter-quartile range [IQR] 62–78). One hundred twenty-nine (66%) patients were in NYHA class II, and 66 (33.8%) were in NYHA class III. The majority (136 patients, 69.7%) had heart failure with impaired LVEF and 59 (30.3%) had heart failure with preserved LVEF. Mean duration of heart failure at inclusion was 17 months, and aetiology was ischaemic in 48%, hypertensive in 17%, ischaemic and hypertensive in 12%, idiopathic dilatational in 14%, other in 9% of patients.

Of the 195 patients with heart failure, 63 patients (32.3%) experienced an event (19 patients died and 44 were hospitalised) after six to 668 days (median 312 days). Additionally, 13 patients...
died because of non-cardiovascular causes. None were lost to follow-up. Median follow-up period was 693 days (range 574–788) days. Cumulative event rate for all patients was 10.2% at six months, 16.9% at 12 months and 31.8% at 24 months.

Patients who experienced an event (heart failure related death or hospitalisation) had increased levels of D-dimer and tPA antigen, they more often had diabetes mellitus and coronary artery disease, lower systolic blood pressure, worse performance on the six-minute walking test, lower LVEF and haemoglobin; they also were more often prescribed a loop diuretic or digoxin, and more often in NYHA class III. On the other hand, levels of PAI-1 and F1+2 did not differ significantly between patients with or without an event (Table 1).

Using the maximum statistic approach, levels of D-dimer >634 µg/l (45th percentile) and levels of tPA antigen >10.2 µg/l (55th percentile) emerged as best cut-off levels to predict heart failure related hospitalisations or deaths (Bonferroni adjusted p=0.006 and p=0.007, respectively). F1+2 and PAI-1 antigen concentrations failed to reach statistical significance in predicting heart failure related events at any tested cut-off level; F1+2 best predicted outcomes at >74 µg/l (35th percentile) and PAI-1 antigen at >25.6 µg/l (60th percentile) with a Bonferroni adjusted p-value of 0.222 and 0.215, respectively.

Presence of diabetes or coronary artery disease, NYHA class III, duration of heart failure >2 years, anaemia (haemoglobin <120 g/l), LVEF, poor performance on the six-minute walking test, log-transformed NT-proBNP levels, fasting plasma glucose, HDL levels below 1.0 mM for men and 1.2 mM for women, triglycerides levels above 1.7 mM, and creatinine levels were also significant univariate predictors of unfavourable outcomes (Table 2).

On Cox multivariate regression model tPA, but not D-dimer emerged as an independent predictor of heart failure related death or hospitalisation (Fig. 1, Table 2).

In a subgroup analysis, a Cox survivorship model for patients with heart failure and preserved LVEF (n=59) was constructed. Due to subgroup size, only a limited number of most significant univariate predictors could be entered the model, namely age, gender, NT-proBNP, and D-dimer and tPA above their respective cut-offs (634 and 10.2 µg/l, respectively). The model failed to reach statistically significant prediction power (chi-square=7.127; p=0.226) as none of the tested covariates retained

### Table 1: Baseline characteristics and differences between patients who did and did not experience a heart failure related adverse event (death or hospitalisation) during a follow-up period of two years.

<table>
<thead>
<tr>
<th>Therapy (%)</th>
<th>All (baseline)</th>
<th>2 years of follow-up</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE inhibitor/ARB</td>
<td>97.4</td>
<td>98.4</td>
<td>96.2</td>
</tr>
<tr>
<td>Beta blocker</td>
<td>90.8</td>
<td>88.9</td>
<td>94.5</td>
</tr>
<tr>
<td>Aldosterone antagonist</td>
<td>46.2</td>
<td>53.1</td>
<td>42.4</td>
</tr>
<tr>
<td>Loop diuretic</td>
<td>58.5</td>
<td>85.9</td>
<td>44.7</td>
</tr>
<tr>
<td>Digoxin</td>
<td>19.0</td>
<td>29.7</td>
<td>13.6</td>
</tr>
</tbody>
</table>

Values are mean ± SD or median (interquartile range) for continuous, and frequency (%) for categoric variables. NT-proBNP, N-terminal pro-brain natriuretic peptide; F1+2, prothrombin fragment F1+2; tPA, tissue plasminogen activator antigen; PAI-1, plasminogen activator inhibitor 1 antigen; 6MWT, 6-minute walking test; MLHFQ, Minnesota living with heart failure questionnaire; LVEF, left ventricular ejection fraction; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker.
its predictory power, including tPA (hazard ratio [HR] 4.134 [0.645–26.509], p=0.134) and D-dimer (HR 2.845 [0.317–25.501], p=0.350).

Discussion

The present study explored and compared prognostic impacts of different haemostatic factors (F1+2, D-dimer, tPA and PAI) in patients with chronic heart failure and showed that tPA is an independent predictor of prognosis after adjustment for usual prognostic factors, in particular for presence of diabetes mellitus, NYHA class, anaemia and NT-proBNP. Previous studies reported a strong association between increased levels of haemostatic factors and cardiovascular events; however, the vast majority of these studies focused on atherosclerotic disease and proposed that haemostatic derangements reflect a procoagulant tendency to (athero)thrombotic events, thus predicting additional vascular risk (9–12, 14, 16). On the other hand, recent evidence

![Figure 1: Survival probability curves according to tissue plasminogen activator (tPA) antigen levels dichotomised by the maximum statistical approach derived optimal cut-off value (A – und adjusted; B – adjusted for other univariate predictors of survival).]
suggest that haemostatic derangements in heart failure result from a cross-talk between the coagulation and fibrinolytic systems, neuroendocrine hyperactivation and inflammatory processes (6, 25–31). Activation of the sympathetic and renin-angiotensin-aldosterone systems provokes a simultaneous increase in molecules of both the coagulation and fibrinolysis pathways within minutes, resulting in net hypercoagulability (32–34). Activation of haemostasis may thus reflect the severity of neurohumoral derangements and the extent of their pharmacological inhibition (the vast majority of patients in our study were on ACE inhibitors/ARB blockers and beta blockers). The prognostic impact of procoagulant factors in heart failure therefore most likely indicates the extent of the (prognostically unfavourable) maladaptive systemic response to the failing ventricle.

Moreover, tPA antigen levels may reflect endothelial dysfunction. tPA antigen (unlike prothrombin fragment F1+2 and fibrin turn-over product D-dimer) is released into the bloodstream from the endothelium and reflects endothelial dysfunction (as opposed to measurement of e.g. tPA activity). Endothelial dysfunction is a hallmark of heart failure severity and a strong pre-

<table>
<thead>
<tr>
<th>Table 2: Univariate and multivariate predictors of heart failure related events (deaths or hospitalisations).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate</strong></td>
</tr>
<tr>
<td><strong>P-value</strong></td>
</tr>
<tr>
<td>tPA &gt;10.2 µg/l*</td>
</tr>
<tr>
<td>D-dimer &gt;634 µg/l*</td>
</tr>
<tr>
<td>F1+2 &gt;74 µg/l*</td>
</tr>
<tr>
<td>PAI-1 &gt;25.6 µg/l*</td>
</tr>
<tr>
<td>Age &gt;75 years</td>
</tr>
<tr>
<td>Gender (male)</td>
</tr>
<tr>
<td>Presence of diabetes mellitus</td>
</tr>
<tr>
<td>Presence of coronary artery disease</td>
</tr>
<tr>
<td>NYHA class III</td>
</tr>
<tr>
<td>Duration of heart failure &gt;2 years</td>
</tr>
<tr>
<td>Hb&gt;120 g/l</td>
</tr>
<tr>
<td>LVEF</td>
</tr>
<tr>
<td>0.35–0.50</td>
</tr>
<tr>
<td>&gt;0.50</td>
</tr>
<tr>
<td>Systolic blood pressure &gt;120 mmHg</td>
</tr>
<tr>
<td>6-minute walking test &gt;310 m</td>
</tr>
<tr>
<td>Log NT-proBNP</td>
</tr>
<tr>
<td>Fasting plasma glucose &gt; 6.1 mM</td>
</tr>
<tr>
<td>Abdominal obesity**</td>
</tr>
<tr>
<td>HDL &lt;1.0 (&lt;1.2 for women) mM</td>
</tr>
<tr>
<td>Triglyceride levels &gt;1.7 mMl</td>
</tr>
<tr>
<td>Creatinine levels (µM)</td>
</tr>
<tr>
<td>Therapy</td>
</tr>
<tr>
<td>ACE inhibitors/ARBs</td>
</tr>
<tr>
<td>Beta blockers</td>
</tr>
<tr>
<td>Antiplaete therapy</td>
</tr>
</tbody>
</table>
| NT-proBNP – N-terminal pro brain natriuretic protein; IL-6 – interleukin-6; hsCRP – high-sensitive C-reactive protein; LVEF – left ventricular ejection fraction; tPA – tissue plasminogen activator antigen, PAI-1 plasminogen activator inhibitor 1 antigen; F1+2 – prothrombin fragment F1+2; HDL – high-density lipoproteins; ACE – angiotensine converting enzyme; ARB – angiotensin receptor blockers. *Cut-offs determined by the maximum statistical approach. **Abdominal obesity was defined as a body mass index >30 kg/m² or waist circumference ≥94 cm in men and ≥80 cm in women.
dictor of its prognosis and may thus result in an increased release of tPA. Taking into account the association of haemostatic derangements and the metabolic syndrome (35, 36), and the high prevalence of insulin resistance in heart failure (37), we included parameters of the metabolic syndrome in our survivorship model; nevertheless, tPA retained its prognostic impact thus suggesting that tPA in heart failure reflects unfavourable haemostatic derangements beyond those associated with insulin resistance. A less plausible explanation – i.e. that undiagnosed coronary thrombosis could also contribute to unfavourable prognosis in heart failure (3) – has recently been challenged; therapeutic targeting of coronary thrombosis has either failed to provide substantial survival benefits (38–40) or has yet to be shown to be safe and effective in patients with heart failure (41).

As opposed to tPA, other haemostatic factors (D-dimer, F1+2 and PAI-1) did not emerge as independent predictors of heart failure related events. Several factors may account for the failure of D-dimer, F1+2 and PAI-1 to predict adverse outcomes in our study. For instance, D-dimer may provide less specific information about the extent of haemostatic derangements in heart failure, since it is not as directly related to neurohumoral derangements as tPA. A previous study exploring the prognostic impact of D-dimer in a community cohort of elderly subjects with signs and symptoms of heart failure showed that D-dimer was an independent predictor of total and cardiovascular mortality (19). However, unlike in our study, only 48% of patients actually had impaired (systolic or diastolic) left ventricular function and less than half patients were optimally managed according to current guidelines (42). In another study, Loh et al. evaluated the prognostic impact of haemostatic derangements in 473 patients with heart failure and LVEF <40% and suggested that only D-dimer was associated with unfavourable prognosis (43). However, the primary end-point was mortality and the study population encompassed a significant proportion (31%) of patients with atrial fibrillation (where D-dimer levels strongly predict unfavourable prognosis regardless of underlying heart failure [17]). D-dimer may thus reflect general age- and comorbidity-related risk rather than cardiovascular prognosis, while our study focused on stable and optimally managed patients with ventricular dysfunction, and primarily explored heart failure related outcomes (death and hospitalisations) which might be better predicted by elevated tPA levels. Similarly, previous prognostic studies of PAI-1 were largely inconclusive (12) mainly because of a strong confounding influence of cardiovascular risk factors (including parameters of insulin resistance) on PAI-1 levels (44–46), which might account for the lack of association between this haemostatic marker and heart failure prognosis in our study. As for F1+2, previous reports showed that thrombin generation usually increases after an acute ischaemic event and persists for up to six months (47); the prognostic role of F1+2 therefore seems to be limited to short-term atherothrombotic risk.

Despite being a strong univariate predictor of prognosis, LVEF failed to independently predict heart failure-related events in our multivariate survivorship model. The lack of association between LVEF and prognosis might be partially explained by the fact that all patients were on optimal pharmacotherapy (which could account for the blunting of the expected prognostic impact of this otherwise important predictor) and by the relatively homogeneous study group. In fact, despite one third of the participants had heart failure with preserved LVEF, more than one half (58.1%) of patients had a LVEF between 0.25 and 0.40; this leptokurtic distribution of LVEF suggests that LVEF as a prognostic marker might fail to discriminate between patients at high and low risk simply because of the relative homogeneity of the studied population. We further explored this unexpected finding with a separate survival analysis in patients with preserved LVEF; the model, however, failed to reach statistical significance, most likely because of the limited subgroup size, as our study was underpowered for such exploration. Further studies are thus called upon to explore the issue of haemostatic derangements and their prognostic impact in patients with heart failure and preserved LVEF.

Despite providing evidence that tPA is a strong predictor of heart failure-related death or hospitalisation, our study has several limitations that need to be taken into account. Firstly, the modest number of participants allowed us to focus on the primary aim of the study – exploring and comparing the prognostic role of haemostatic markers in heart failure. However, the prognostic significance of such derangements could not be properly elucidated in specific subgroups (especially heart failure patients with preserved LVEF). Our study nevertheless provides additional insight in the haemostatic processes and derangements in chronic heart failure, but larger cohorts and studies in specific subgroups should validate clinical applicability of our findings. Secondly, dichotomisation of data using cut-offs might yield to over-optimistic conclusions; however, we minimised the risk of such bias by using a maximum statistical approach, which still allowed us to preserve cut-offs that better than continuous values help estimating a possible clinical usefulness of a prognostic parameter. Finally, we had to exclude some important groups of heart failure patients (e.g. patients with severe renal dysfunction and patients taking anticoagulation therapy) in order to reliably estimate the prognostic impact of haemostatic factors; therefore our conclusions can not be generalised to all heart failure patients as we only included optimally managed ambulatory patients with stable chronic heart failure.

What is known about this topic?
- Heart failure confers a hypercoagulable state.
- Haemostatic factors are prognostic predictors in a variety of cardiovascular diseases.
- Several haemostatic factors are increased in heart failure; however, their prognostic impact has not been established yet.

What does this study add?
- Activation of haemostasis is associated with unfavorable prognosis in chronic heart failure.
- tPA and D-dimer (but not F1+2 or PAI-1) are associated with adverse outcomes in heart failure.
- tPA is an independent predictor of adverse outcomes in chronic heart failure even after adjusting for other prognostic factors.
In conclusion, our findings demonstrate that haemostatic derangements as determined by tPA and D-dimer levels predict heart failure related hospitalizations and deaths. Moreover, tPA levels emerged as an independent predictor of heart failure related events even after allowing for other known prognostic factors, such as presence of diabetes mellitus, coronary artery disease, NT-proBNP, NYHA class and parameters of the metabolic syndrome. Nevertheless, larger cohort studies need to confirm our findings, as well as to address and compare other haemostatic markers of prognosis.

References

4. Lip GYH, Lowe GD, Metcalfe M, et al. Effects of warfarin therapy on plasma fibrinogen, von Willebrand factor, and fibrin D-dimer in left ventricular dysfunc-

5. Pan CH, Conway DS, Chung NA, et al. Interleukin-6, tissue factor and von Willebrand factor in acute 


11. Lowe GD0, Danesh J, Lewington S, et al. Tissue plasminogen activator antigen and coronary heart dis-

15. van der Bom JG, Bots ML, Haverkate F, et al. Activ-

16. Nephew S, Le MK, Ghanji P. Effects of angio-
17. Labnojč P, Newby DE, Pellegrini MP, et al. Po-
tentiation of bradykinin-induced tissue plasminogen 
activator release by angiotensin-converting enzyme in-
22. Massie BM, Collins JF, Ammon SE, et al. Random-
ized Trial of Warfarin, Aspirin, and Clopidogrel in Pa-

tients With Chronic Heart Failure. The Warfarin and Antiplatelet Therapy in Chronic Heart Failure (WATCH) Trial. Circulation 2009;119: Published online before print March 16, 2009, doi: 10.1161/CIRCULATIONAHA.108.801753.

27. Thøgersen AM, Jansson J-H, Hansen K, et al. High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myo-

nolytic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. Circu-

30. Merli PA, Bauer KA, Oltron A, et al. Persistent activation of coagulation mechanism in unstable angi-