Cystatin C in a composite risk score for mortality in patients with infective endocarditis: A cohort study

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2012-000856
Article Type:	Research
Date Submitted by the Author:	14-Feb-2012
Complete List of Authors:	Bjurman, Christian; Medicine, Clinical and Molecular Medicine Snygg-Martin, Ulrika; Biomedicine, Infectious Diseases Olaison, Lars; Biomedicine, Infectious Diseases Fu, Michael; Medicine, Clinical and Molecular Medicine Hammarsten, Ola; Biomedicine, Clinical chemistry and transfusion medicine
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Infectious diseases
Keywords:	Valvular heart disease < CARDIOLOGY, Cardiology < INTERNAL MEDICINE, INFECTIOUS DISEASES
Note: The following files were submitted by the author for peer review, but cannot be converted to PDF. You must view these files (e.g. movies) online.	
Suppl. Tabl IE HEART	

Cystatin C in a composite risk score for mortality in patients with infective endocarditis: A cohort study

[1]Christian Bjurman [*], MD; [2]Ulrika Snygg-Martin[*], MD, PhD; [2]Lars Olaison, MD, Assoc. Prof; [1]Michael LX Fu, MD, Prof. [3]Ola Hammarsten, MD, Assoc. Prof

[1]Department of Medicine, [2]Department of Infectious diseases, [3]Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska University Hospital, The Sahlgrenska Academy at University of Gothenburg, 413 45 Gothenburg, Sweden

[*]Equally contributed

Address for correspondence:

Ola Hammarsten, MD, PhD, Associate professor, Senior Physician
Department of clinical chemistry and transfusion medicine
Bruna straket 16
Sahlgrenska academy at the University of Gothenburg
SE 413 45 Gothenburg Sweden
Email: ola.hammarsten@clinchem.gu.se
Phone Lab:+46-31-342 1561, 342 9941
Mobile: +46-733-200834
Fax:+46-31-82 84 58

Running title: CysC prognostic in infective endocarditis

Keywords: Infective endocarditis; Cystatin C; five-year survival; 90-day survival; Composite scoring system

Data sharing statement: There is no additional data available.

Contributorship: All authors have contributed to study conception, design and interpretation, as well as drafting and revision of the article. C Bjurman and Ola Hammarsten were also responsible for the data analyses.

All authors have approved the final version of the manuscript submitted.

All presented authors thus fulfil the criteria for authorship.

No else fulfilled the criteria for authorship.

ABSTRACT

Objective: To develop a multi-marker prognostic score for infectious endocarditis (IE).

Design: Retrospective case control

Setting: Secondary care. Single-centre.

Participants: 125 patients with definite IE

Primary outcome measures: 90-day and five-year mortality

Results: Mean age was 62.7 ± 17 years. The 90-day and five-year mortality was 10.4% and 33.6% respectively. CysC levels at admission and over 20% increases in CysC levels during 2 weeks of treatment were prognostic for 90-day and five-year mortality independent of creatinine estimated glomerular filtration rate. In multivariate analyses CysC (odds ratio (OR) 5.42, 95% confidence interval (CI) 1.90–15.5, p = 0.002) and age (OR 1.06, 95% CI 1.02–1.10, p = 0.002) remained prognostic for five-year mortality. NT-proBNP, TNT, CRP and IL-6 were also linked to prognosis. A composite risk scoring system using levels of CysC, NT-proBNP, age and presence of mitral valve insufficiency was able to separate a high and a low risk group.

Conclusions: CysC levels at admission and increase in CysC after two weeks of treatment were independent prognostic markers for both 90-day and five-year mortality in patients with IE. A multi-marker composite risk scoring system including CysC identified a high-risk group.

ARTICLE SUMMARY

Article focus

- Our aim was to develop a multi-marker prognostic score for IE

Key messages

- CysC levels at admission and increase in CysC after two weeks of treatment were independent prognostic markers for both 90-day and five-year mortality in patients with IE.
- A prognostic score including CysC over 1.2 mg/L, NT-ProBNP over 2000 ng/L, presence of any grade of MI and age over 70 years could identify a high and low risk group in IE.
- The prognostic score might be used to improve patient monitoring and assist treatment choices in IE

Strengths and limitations of this study

- We were able to monitor changes in levels of biomarkers during treatment in a large cohort of IE patients since blood samples were collected at admission and after two weeks of treatment.
- One potential weakness was that 36.2% of patients treated for IE during the study period (71/196) were unavailable for biomarker studies since they lacked stored blood samples. The mortality was lower in the study group (125) compared to all IE patients treated for IE (196) during the study period.

INTRODUCTION

Infective endocarditis (IE) is an infection localized to the endocardial surface of the heart. IE mostly involves the heart valves, resulting in local valve destruction and abscess formation as well as development of vegetations with the ability to embolize to various organs. Despite major advances in both diagnostic and therapeutic procedures, neither the incidence [1] nor the mortality of the disease have decreased in the past 30 years, with a current in-hospital mortality of 15–20% and 1-year mortality reaching 40% in developed countries. [2] Several epidemiological studies have identified a number of prognostic factors related to higher mortality including advanced age, *Staphylococcus aureus* etiology, [2-3] cerebral complications, [4] and female sex. [5] In addition, biomarkers of inflammation like erythrocyte sedimentation rate (ESR), [2] hypoalbuminemia, [6] leukocytosis, [6] CRP [7] and procalcitonin [8] can predict poor prognosis but are too non-specific to guide therapy in individual patients. Identification of novel prognostic biomarkers and development of a prognostic score could help to identify IE patients who might benefit from more aggressive therapeutic procedures.

Because IE often influences hemodynamics, biomarkers linked to cardiovascular mortality could have prognostic power in IE. Among these are factors released during cardiovascular stress like NT-proBNP, MR-proANP, copeptin, and troponin T (TnT), which are linked to poor prognosis in heart failure, [9] coronary syndromes, [10] and sepsis. [11] Markers of renal function like creatinine, [12] estimated glomerular filtration rate (eGFR), [13] and cystatin C (CysC) [14] might also be able to predict prognosis in patients with IE similar to their ability to predict cardiovascular mortality. In this study, we analyzed clinical factors and these cardiovascular biomarkers in blood samples collected at admission and after two weeks of therapy among patients with definite IE and examined their ability to predict 90-day and 5-year mortality.

METHODS

In this single-center retrospective cohort study, patients with IE treated between 1999–2005 at the Department of Infectious Diseases, Sahlgrenska University Hospital, University of Gothenburg, Gothenburg, Sweden, were registered between February 1, 1999, and December 3, 2005. During this time, 196 patients were diagnosed with definite IE according to the modified Duke criteria [15] and 125 patients had a blood sample drawn at diagnosis and stored and were therefore included in the study. After two weeks of treatment 120 out of the 125 patients had a second blood sample drawn. Of all 196 IE patients recorded from 1999–2005, 13 patients died (6.6%) within 90 days, and 43 patients died within five years (20.9%). Among the 125 patients with an available admission blood sample, the 90-day mortality was 10.4% and the five-year mortality was 33.6%. Information regarding mortality was collected from the hospital's registry of administrative data. The study was approved by the Ethical Committee at the University of Gothenburg.

All patients were followed for up to five years or until death occurred (mean 1449.1±636.8 days).

Clinical data

Demographic and clinical information regarding age, sex, bacterial etiology, native and prosthetic valve IE, left ventricular ejection fraction (LVEF), comorbidities, and surgery during active IE were obtained from the endocarditis database. The study design included analysis of blood samples at admission and after two weeks of treatment.

Laboratory analyses

Serial blood samples were obtained during the hospital stay and stored until analysis at -

70°C. The first sample was taken at admission and the second sample two weeks later. NT-proBNP was analyzed by Elecsys proBNP assay (Roche Diagnostics, Switzerland). CysC was analyzed in serum using reagents from Dako and the Modular P 2551. Creatinine based eGFR was calculated using "The Modification of Diet in Renal Disease" formula (MDRD). [19] TnT was analyzed by using The Elecsys Troponin T high sensitivity assay (Roche Diagnostics, Switzerland). All other laboratory parameters examined were part of the routine laboratory services provided by the Clinical Chemistry Laboratory, Sahlgrenska University Hospital.

Echocardiography

All patients were examined by transthoracic and transesophageal echocardiography at least once during the study period. Echocardiographic criteria for IE and degrees of valve insufficiency were evaluated. Left ventricular ejection fraction (LVEF) was calculated from long-axis planes (2-, 3-, and 4-chamber views) of the heart.

Statistical analysis

Multiple logistic regression models were used to evaluate possible associations between serum levels of biomarkers (NT-proBNP, TNT, creatinine, and CysC) and clinical variables including age, sex, echocardiographic parameters, and infectious agens and underlying diseases. Univariate comparisons between groups were calculated using conventional t-tests. Mann-Whitney U-tests were used for nonparametric comparisons of medians. Dichotomous variables were analyzed using the chi-square test. Receiver operating characteristic (ROC) curves were used to assess the prognostic properties of biomarkers. The log rank test was used to compare different strata in Kaplan-Meier analyses of survival. Statistical analyses were performed with SPSS version 19. All probabilities were two-tailed, and p values <0.05

were regarded as significant. Odds ratios with confidence intervals were collected from outputs from logistic regression analyses. The coefficient of determination (R^2) was calculated in Microsoft Excel 2007 using Spearman's correlation to assess the strength of the correlation between CysC change and mortality. The add-in Analyse-it was used to compare AUCs between ROC curves.

The study population was chosen to get a Power of over 90% for detecting clinically relevant associations between CysC, NT-proBNP and mortality.

No missing data existed for the variables included in the prognostic score, but for other tested variables, cases sometimes were excluded if data was missing, although no more than two cases in each analysis had to be excluded due to a high degree of data availability.

RESULTS

The mean age among the 125 IE patients was 62.7 ± 16.9 years, 64.8% were male, and Staphylococcus aureus infection was seen in 28.0% of the patients (Supplementary table 1). Prosthetic valve endocarditis was diagnosed in 28 (22.4%) of the patients, and 14 (11.2%) had a pacemaker. Vegetations on pacemaker leads were seen in four of these patients. Most of the patients (91.6%) had LVEF over 40%, and 34.5% underwent heart surgery during antibiotic treatment. Clinical parameters correlating with five-year mortality included age, history of hypertension and mitral valve insufficiency (MI), but not ejection fraction (Supplementary table 1). Aminoglycoside use and duration of aminoglycoside therapy were both positively correlated with five-year survival. No associations were found between the presence of septic emboli and five-year survival, biomarker levels or clinical variables examined in the study (data not shown).

In the univariate analysis CysC levels at admission were associated with both 90-day

(OR 5.7 95% CI 2.2-14.7, p=<0.0001) and five-year mortality (OR 7.11 95% CI 2.6-19.5, p=<0.0001 (Fig. 1). CysC increases over 20% between admission and after two weeks of treatment (Supplementary Fig. 1) were also associated with increased five-year mortality (OR 2.8 95% CI 1.20-6.6, p=<0.017). All eight patients with CysC over 2.1 mg/L died within five years. CysC levels and increase over 20% remained as significant prognostic indicators for five-year mortality with similar ORs when alternative multivariate models, including creatinine-estimated GFR or baseline creatinine were applied (Supplementary table 2).

The area under the receiver operator characteristic curve (AUC) for predicting five-year mortality was 0.70 (95% CI 0.60–0.80, p < 0.001) for CysC levels at admission and 0.74 (95% CI = 0.65–0.83, p < 0.0001) for CysC levels after two weeks of treatment. CysC had a significantly higher AUC compared to creatinine (0.62 (95% CI 0.51-0.73, p = 0.0042 for difference between AUC for CysC and creatinine after two weeks of treatment). Mean creatinine was significantly higher among patients that died within five years (Table 1). In contrast, median creatnine was not significantly different among patients that died or lived after five years.

In the univariate analysis, log NT-proBNP, GFR, creatinine, age, MI, and hypertension were linked to poor prognosis, whereas copeptin and MR-proANP did not reach statistical significance. CRP, IL-6, and TNT at two weeks, but not at admission, were also linked to prognosis (Table 1 and 2). No sex-based differences were present.

The four risk factors with the highest AUC for death within five years that appeared to be independent from each other were used to generate a composite risk score where each factor added one point. The factors included in the score were CysC over 1.2 mg/L, NT-ProBNP over 2000 ng/L, presence of any grade of MI and age over 70 years. Patients with a risk score of 0-2 had a significantly better prognosis (11% (8/71) died within five years) compared to patients with a score of 3-4 (63% (34/54) died within five years, p<0.0001) (Fig.

2). The score's ability to separate patient prognosis was improved when the 34.5% of patients that underwent cardiac surgery were excluded (0-2 points 7% (3/41), 3-4 points 66% (22/33) died within five years, p<0.0001). The composite risk score also predicted death within 90-days (0-2 points 0% (0/71), 3-4 points 24.1% (13/54) died within 90-days, p<0.0001).

Furthermore, dividing the cohort based on a score of 0-2 or 3-4 predicted mortality in patients with left sided IE (OR=14.6, 5.55-38.2 p<0.001), left sided IE that did not undergo surgery (OR=25.7, 6.35-103.7, p<0.001) and staph aureus infected left sided IE that did not undergo surgery (OR 31.5, 2.35-422.3, p=0.009). Lastly, the score was also able to predict mortality if the MI parameter excluded and thus not adding 1 point if present (OR 2.30 (95% CI 1.41-3.72), p=0.001) indicating that the composite score was not reliant on the MI parameter and was able to predict mortality in important subgroups of IE.

DISCUSSION

CysC levels at admission, CysC levels after two weeks, and over 20% increases in CysC levels during two weeks of treatment were prognostic for mortality in patients with definite IE. The combination of CysC levels and three other risk factors generated a powerful risk score.

CysC is a small 13-kDa cysteine protease inhibitor produced at a constant rate by all nucleated human cells tested. CysC is cleared predominantly by renal filtration and is most often used as a replacement for creatinine as a marker of GFR. CysC is regarded as a more accurate marker of kidney function than creatinine (Reviewed in 14). CysC also offers superior ability to diagnose acute kidney injury [16] and declining GFR [17] compared to creatinine. Furthermore, several studies have shown that elevated CysC is a strong risk factor for adverse cardiovascular prognosis in the elderly even when creatinine levels are normal. [18] Increased CysC levels indicate a future risk of developing heart failure, [19] as well as a poor prognosis among patients with already established heart failure, [20] independent of creatinine levels. Therefore, CysC is a marker of kidney function and prognosis that outperforms creatinine in most studies.

The associations between CysC levels and prognosis in our study might reflect that IE impairs kidney function. The ability of CysC to more correctly predict GFR among the elderly and to respond to rapid changes in GFR compared to creatinine could explain the stronger association between CysC levels and IE prognosis compared to creatinine levels.

There are different conceivable explanations for decreased kidney function in IE. One factor could be decreased cardiac output during the acute phase of IE. Although there was no correlation between LVEF and prognosis (Supplementary table 1), levels of the heart failure biomarker, NT-proBNP, and prevalence of MI were significantly higher among patients with poor prognosis. In addition, levels of TnT after two weeks of treatment were associated with prognosis. TnT levels are affected by cardiac stress found among patients with heart failure [21-22] and correlate with prognosis in a number of heart-related conditions. [23] Therefore, the link between CysC levels

and poor prognosis among IE patients could be decreased cardiac output resulting in low kidney perfusion and low GFR.

It is also possible that factors that increase production of CysC, such as increased glucocorticoid levels.[24], and possibly inflammation, [25-27] could explain the correlation between CysC levels and IE prognosis. CysC levels show a stronger association with cardiovascular risk factors compared to creatinine, independent of iohexol clearance. [28] Among patients with chronic renal failure followed for 10 years, CysC levels correlated better with cardiovascular disease mortality compared to creatinine or iothalamate clearance [29] indicating that CysC levels predict prognosis partly independent from GFR. In line with this possibility, we found that CysC levels and change in CysC levels during two weeks of treatment remained prognostic even after multivariate analysis correcting for creatinine levels and creatinine estimated GFR (Supplementary table 2) suggesting that conditions that increase production of CysC may correlate with IE prognosis. One such factor could be persistent inflammation because high CRP or IL-6 levels after two weeks of treatment were also linked to poor prognosis in our study. Moreover, it is also possible that increased glucocorticoid levels due to a general stress response among IE patients could be the link between poor prognosis and CysC levels.

One important aspect of this study was that we could monitor changes in biomarkers during treatment in a large group if IE patients. We found that levels of CRP, IL-6, and TnT after two weeks of treatment, but not admission levels, were significantly associated with five-year survival. This association likely reflects the fact that a rapid response to initial treatment is of great importance for the prognosis. In contrast, levels of the otherwise promising cardiac stress markers MR-proANP and copeptin failed to predict prognosis, even when levels were assessed after two weeks of treatment, indicating that these biomarkers are less valuable among IE patients.

Our results confirmed that MI is a powerful prognostic indicator in IE irrespective of the infected valve. The reason for the significant association between MI of all degrees, and not with

the worst degrees alone (3 and 4), can be explained by the fact that the patients with severe MI often underwent surgery, which is linked to improved survival. [30] This association could, however, not be confirmed in this study (Supplementary table 1).

The multitude of biomarkers and clinical factors included in this study allowed us to explore a composite risk score based on the four strongest and apparently independent risk factors. The composite risk score was able to separate a high and a low risk group with significantly different 90-day and five-year survival. IE has still a poor prognosis despite major advances in diagnostic and therapeutic procedures and we still have limited ability to find the patients that should be considered for more aggressive treatment. In this light, this novel risk score could add decisional information and allow for a multifactorial judgment of patients and consideration for more active intervention like heart valve replacement or surgical removal of vegetations. However, this risk score must be validated in prospective studies before it can be safely implemented in clinical practice.

A potential problem with the current study was that 36.2% of the patients treated for IE during the study period (71/196) lacked stored blood samples. In addition, there was a bias for patients with a worse prognosis in the study group. On the other hand, the study group of 125 IE patients had a five-year prognosis (66.4% five-year survival) closer to the 60% five-year survival reported in most studies [1-2] compared to the complete cohort (79% five-year survival) indicating that the prognosis in our study group were comparable with the outcome in previous studies.

In summary a prognostic score including CysC over 1.2 mg/L, NT-proBNP over 2000 ng/L, presence of any grade of MI and age over 70 years could identify a high and low risk group in IE.

ACKNOWLEDGEMENTS

We are thankful for the expertise and analysis of the laboratory parameters by Bodil Gustafsson, Carina M Gustafsson, and Anne-Sofie Johansson Fällgren.

Potential conflicts of interest: C.B., U.S., L.O., M.F, O.H.: None declared.

COMPETING INTERESTS

No competing interests exist.

FUNDING

This work was supported by the Swedish Cancer Society [O.H.], Swedish Research Council, Swedish Pain Foundation [O.H.], the Sahlgrenska University Hospital Research Foundation [O.H.], Swedish Heart-Lung Foundation [M.F.], Sahlgrenska University Hospital Research Foundation [M.F.] and by government support to the city councils for cost arising (grant ALFGBG-138141) [LO].

REFERENCES

1 Tleyjeh IM, Steckelberg JM, Murad HS, et al. Temporal trends in infective endocarditis: A population-based study in olmsted county, minnesota. Jama 2005;**293**:3022-3028.

2 Murdoch DR, Corey GR, Hoen B, et al. Clinical presentation, etiology, and outcome of infective endocarditis in the 21st century: The international collaboration on endocarditis-prospective cohort study. Arch Intern Med 2009;**169**:463-473.

3 Thuny F, Di Salvo G, Belliard O, et al. Risk of embolism and death in infective endocarditis: Prognostic value of echocardiography: A prospective multicenter study. Circulation 2005;**112**:69-75.

4 Thuny F, Avierinos JF, Tribouilloy C, et al. Impact of cerebrovascular complications on mortality and neurologic outcome during infective endocarditis: A prospective multicentre study. Eur Heart J 2007;**28**:1155-1161.

5 Sambola A, Fernandez-Hidalgo N, Almirante B, et al. Sex differences in native-valve infective endocarditis in a single tertiary-care hospital. Am J Cardiol 2010;**106**:92-98

6 Wallace SM, Walton BI, Kharbanda RK, et al. Mortality from infective endocarditis: Clinical predictors of outcome. Heart 2002;**88**:53-60.

7 Olaison L, Hogevik H, Alestig K. Fever, C-reactive protein, and other acute-phase reactants during treatment of infective endocarditis. Arch Intern Med 1997;**157**:885-92.

8 Kocazeybek B, Kucukoglu S, Oner YA. Procalcitonin and c-reactive protein in infective endocarditis: Correlation with etiology and prognosis. Chemotherapy 2003;**49**:76-84.

9 Palazzuoli A, Gallotta M, Quatrini I, et al. Natriuretic peptides (bnp and nt-probnp): Measurement and relevance in heart failure. Vasc Health Risk Manag 2010;**6**:411-418.

10 Mazzone M, Forte P, Portale G, et al. Brain natriuretic peptide and acute coronary syndrome. Minerva Med 2005;**96**:11-18.

11 Perman SM, Chang AM, Hollander JE, et al. Relationship between b-type natriuretic

peptide and adverse outcome in patients with clinical evidence of sepsis presenting to the emergency department. Acad Emerg Med 2011;**18**:219-222.

12 Rostagno C, Rosso G, Puggelli F, et al. Active infective endocarditis: Clinical characteristics and factors related to hospital mortality. Cardiol J 2010;**17**:566-573.

13 Hillege H, Van Gilst W, de Zeeuw D, et al. Renal function as a predictor of prognosis in chronic heart failure. Heart Fail Monit 2002;**2**:78-84.

14 Lassus J, Harjola VP. Cystatin c: A step forward in assessing kidney function and cardiovascular risk. Heart Fail Rev Published Online First: 23 Mars 2011. doi:10.1007/s10741-011-9242-6.

15 Li JS, Sexton DJ, Mick N, et al. Proposed modifications to the duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis 2000;**30**:633-638.

16 Nejat M, Pickering JW, Walker RJ, et al. Rapid detection of acute kidney injury by plasma cystatin c in the intensive care unit. Nephrol Dial Transplant 2010;**25**:3283-3289.

17 Premaratne E, MacIsaac RJ, Finch S, et al. Serial measurements of cystatin c are more accurate than creatinine-based methods in detecting declining renal function in type 1 diabetes. Diabetes Care 2008;**31**:971-973.

18 Ix JH, Shlipak MG, Chertow GM, et al. Association of cystatin c with mortality, cardiovascular events, and incident heart failure among persons with coronary heart disease: Data from the heart and soul study. Circulation 2007;**115**:173-179.

19 Sarnak MJ, Katz R, Stehman-Breen CO, et al. Cystatin c concentration as a risk factor for heart failure in older adults. Ann Intern Med 2005;**142**:497-505.

20 Alehagen U, Dahlstrom U, Lindahl TL. Cystatin c and nt-probnp, a powerful combination of biomarkers for predicting cardiovascular mortality in elderly patients with heart failure: Results from a 10-year study in primary care. Eur J Heart Fail 2009;**11**:354-360.

21 Peacock WFt, De Marco T, Fonarow GC, et al. Cardiac troponin and outcome in acute

heart failure. N Engl J Med 2008;**358**:2117-2126.

22 deFilippi CR, de Lemos JA, Christenson RH, et al. Association of serial measures of cardiac troponin t using a sensitive assay with incident heart failure and cardiovascular mortality in older adults. JAMA 2010;**304**:2494-2502.

23 Omland T, de Lemos JA, Sabatine MS, et al. A sensitive cardiac troponin t assay in stable coronary artery disease. N Engl J Med 2009;**361**:2538-2547.

24 Bokenkamp A, van Wijk JA, Lentze MJ, et al. Effect of corticosteroid therapy on serum cystatin c and beta2-microglobulin concentrations. Clin Chem 2002;48:1123-1126.

25 Evangelopoulos AA, Vallianou NG, Bountziouka V, et al. Association between serum cystatin c, monocytes and other inflammatory markers. Intern Med J Published Online First: 6 April 2011. doi:10.1111/j.1445-5994.2011.02500.x.

26 Grubb A, Bjork J, Nyman U, et al. Cystatin c, a marker for successful aging and glomerular filtration rate, is not influenced by inflammation. Scand J Clin Lab Invest 2011;**71**:145-149.

27 Stevens LA, Schmid CH, Greene T, et al. Factors other than glomerular filtration rate affect serum cystatin c levels. Kidney Int 2009;**75**:652-660.

28 Mathisen UD, Melsom T, Ingebretsen OC, et al. Estimated gfr associates with cardiovascular risk factors independently of measured gfr. J Am Soc Nephrol 2011;**22**:927-37.

29 Menon V, Shlipak MG, Wang X, et al. Cystatin c as a risk factor for outcomes in chronic kidney disease. Ann Intern Med 2007;**147**:19-27.

30 Lalani T, Cabell CH, Benjamin DK, et al; International Collaboration on Endocarditis-Prospective Cohort Study (ICE-PCS) Investigators. Analysis of the impact of early surgery on in-hospital mortality of native valve endocarditis: use of propensity score and instrumental variable methods to adjust for treatment-selection bias. Circulation 2010;**121**:1005-13.

FIGURE LEGENDS

Figure 1. Kaplan-Meier survival plot by quartiles of admission levels of CysC.

Figure 2: Kaplan-Meier survival plot by risk score for death within five years among patients with IE. Patients with 0-2 points were compared to patients with 3-4 points ($p<0.001$). The presence of these risk factors adds one point each: Any grade of MI, CysC >1. 2mg/L, NT-proBNP >2000 ng/L, Age >70 years.

Supplementary figure 1: Correlation between five-year mortality and percentage change of CysC between admission and two weeks after initiation of treatment ($R^2 = 0.87$). Patients were ordered in six strata based on percentage change of CysC and the mean five-year survival was calculated for each group.

Table 1: Laboratory characteristics of IE patients

Variable	**Total**	**[a]Dead**	**[a]Alive**	**p value**
CRP [mg/L] (at admission)	58.6±57.9	61.1±54.7	57.3±64.3	0.72
CRP [mg/L] (after 2 weeks)	28.0±49.3	45.0±26.7	20.7±78.2	0.013[b]
CRP [mg/L] (peak level)	139.9±94.1	160.8±86.5	130.5±107.3	0.12
IL-6 [ng/L] (at admission)	45.3±112.1	43.5±134.5	46.3±42.2	0.90
IL-6 [ng/L] (after 2 weeks)	24.1±42.5	39.1±25.4	17.9±65.2	0.011[b]
Hb [g/L]	112.2±17.3	109.8±17.5	113.2±16.9	0.48
Creatinine [mol/L]	95.3±66.6	123.2±26.7	81.4±104.1	<0.001[c]
eGFR [mL/min] (MDRD)[d]	81.0±41.2	67.3±44.1	87.0±29.6	0.016[b]
TnT [ng/L] (at admission)	106.9±226.1	156.1±204.7	82.3±259.3	0.083
TnT [ng/L] (after 2 weeks)	75.7±124.2	109.7±104.4	61.1±157.6	0.048[b]
Incremental TNT [%]	40.8%	44.4%	39.3%	0.60

NT-proBNP [ng/L] (at admission)	3874.2±6868.4	8209.8±1962.6	1706.4±10314.3	<0.001[c]
NT-proBNP [ng/L] (after 2 weeks)	2597.2±3363.6	4782.3±2042.7	1660.8±4633.1	<0.001[c]
Incremental NT-proBNP [%]	41.7%	30.6%	46.4%	0.11
CysC [mg/L] (at admission)	1.34±0.67	1.73±0.35	1.15±0.94	<0.001[c]
CysC [mg/L] (after 2 weeks)	1.45±0.81	0.99±0.65	0.66±0.99	<0.001[c]
CysC (>20% increase) [%]	26.7%	41.7%	20.2%	0.015[b]
MR-proANP [pmol/L] (at admission)	327.6±199.3	378.8±227.0	302.0±452.7	0.21
MR-proANP [pmol/L] (after 2 weeks)	290.2±39.6	249.2±219.1	218.8±135.1	0.15
Copeptin [mmol/L] (at admission)	23.3±29.0	30.1±24.3	19.9±59.2	0.17
Copeptin [mmol/L] (after 2 weeks)	24.0±29.0	21.2±31.8	31.7±21.2	0.42

Table 2: Odds ratios[a] for mortality among IE patients

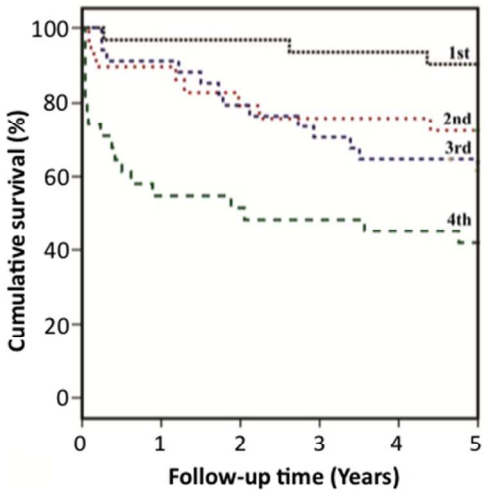
Variable[b]	**OR (all patients)**	**p value**	**OR (no surgery[c])**	**p value**
Age	1.06 (1.03–1.10)	<0.001[d]	1.09 (1.03–1.14)	0.001[d]
MI (all degrees)	2.94 (1.30–6.67)	0.010[e]	5.45 (1.84–16.2)	0.002[d]
Hypertension	3.12 (1.37–7.10)	0.007[e]	2.81 (1.03–7.62)	0.043[e]
CRP [mg/L] [after 2 weeks]	1.01 (1.00–1.03)	0.040[e]	1.00 (0.99–1.01)	0.75
Creatinine [mol/L]	1.02 (1.00–1.02)	0.006[e]	1.02 (1.00–1.04)	0.025[e]
GFR (MDRD)	0.98 (0.97–1.00)	0.012[e]	0.98 (0.96–1.00)	0.041[e]
logNT-proBNP [ng/L] (at admission)	12.2 (4.28–34.9)	<0.001[d]	23.2 (4.68–115.0)	<0.001[d]
logNT-proBNP [ng/L] (after 2 weeks)	5.91 (2.43–14.4)	<0.001[d]	9.44 (2.83–31.5)	<0.001[d]

CysC [mg/L] (at admission)	7.11 (2.59–19.5)	<0.001[d]	37.5 (4.58–308.1)	0.001[d]
CysC [mg/L] (after 2 weeks)	2.55 (1.37–4.76)	0.003[d]	41.7 (5.43–320.6)	<0.001[d]
CysC [mg/L] (>20% increase)	2.82 (1.20–6.59)	0.017[e]	2.60 (0.90–7.50)	0.080
TnT [ng/L] (after 2 weeks)	1.00 (1.00–1.01)	0.059	1.03 (1.01–1.05)	0.008[e]
IL-6 [ng/L] (after 2 weeks)	1.02 (1.00–1.03)	0.044[e]	1.10 (1.04–1.17)	0.002[d]

[a] *From multiple logistic regression,* [b] *Insignificant predictors excluded from the table,*

[c] *Subgroup analysis of patients who did not undergo surgery,* [d] *P <0.005,* [e] *P < 0.05*

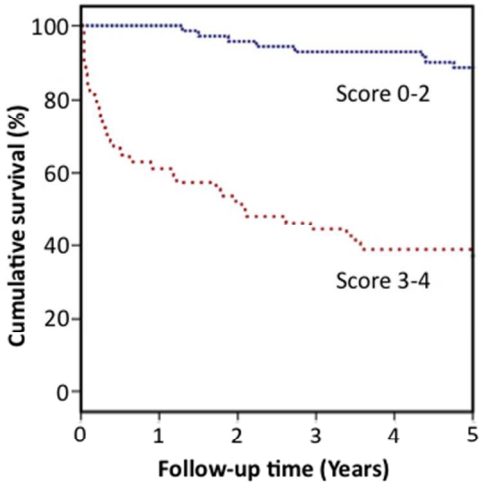
Fig. 1 Bjurman et al.



CysC quartile	CysC level (mg/L)	n	Dead 5 years	p
1	<0.975	31	3	Reference
2	0.975-1.17	29	8	0.066
3	1.17-1.52	34	13	0.008
4	>1.52	31	18	<0.0001

254x190mm (72 x 72 DPI)

Fig. 2 Bjurman et al.



254x190mm (72 x 72 DPI)

Cystatin C in a composite risk score for mortality in patients with infective endocarditis: A cohort study

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2012-000856.R1
Article Type:	Research
Date Submitted by the Author:	24-May-2012
Complete List of Authors:	Bjurman, Christian; Medicine, Clinical and Molecular Medicine Snygg-Martin, Ulrika; Biomedicine, Infectious Diseases Olaison, Lars; Biomedicine, Infectious Diseases Fu, Michael; Medicine, Clinical and Molecular Medicine Hammarsten, Ola; Biomedicine, Clinical chemistry and transfusion medicine
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Infectious diseases
Keywords:	Valvular heart disease < CARDIOLOGY, Cardiology < INTERNAL MEDICINE, INFECTIOUS DISEASES

Cystatin C in a composite risk score for mortality in patients with infective endocarditis: A cohort study

[1]Christian Bjurman [*], MD; [2]Ulrika Snygg-Martin[*], MD, PhD; [2]Lars Olaison, MD, Assoc. Prof; [1]Michael LX Fu, MD, Prof. [3]Ola Hammarsten, MD, Assoc. Prof

[1]Department of Medicine, [2]Department of Infectious diseases, [3]Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska University Hospital, The Sahlgrenska Academy at University of Gothenburg, 413 45 Gothenburg, Sweden

[*]Equally contributed

Address for correspondence:

Ola Hammarsten, MD, PhD, Associate professor, Senior Physician
Department of clinical chemistry and transfusion medicine
Bruna straket 16
Sahlgrenska academy at the University of Gothenburg
SE 413 45 Gothenburg Sweden
Email: ola.hammarsten@clinchem.gu.se
Phone Lab:+46-31-342 1561, 342 9941
Mobile: +46-733-200834
Fax:+46-31-82 84 58

Running title: CysC prognostic in infective endocarditis

Keywords: Infective endocarditis; Cystatin C; five-year survival; 90-day survival; Composite scoring system

Data sharing statement: There is no additional data available.

Contributorship: All authors have contributed to study conception, design and interpretation, as well as drafting and revision of the article. C Bjurman and Ola Hammarsten were also responsible for the data analyses.

All authors have approved the final version of the manuscript submitted.

All presented authors thus fulfil the criteria for authorship.

No else fulfilled the criteria for authorship.

ABSTRACT

Objective: To develop a multi-marker prognostic score for infectious endocarditis (IE).

Design: Retrospective case control

Setting: Secondary care. Single-centre.

Participants: 125 patients with definite IE

Primary outcome measures: 90-day and five-year mortality

Results: Mean age was 62.7 ± 17 years. The 90-day and five-year mortality was 10.4% and 33.6% respectively. CysC levels at admission and over 20% increases in CysC levels during 2 weeks of treatment were prognostic for 90-day and five-year mortality independent of creatinine estimated glomerular filtration rate. In multivariate analyses CysC (odds ratio (OR) 5.42, 95% confidence interval (CI) 1.90–15.5, p = 0.002) and age (OR 1.06, 95% CI 1.02–1.10, p = 0.002) remained prognostic for five-year mortality. NT-proBNP, TNT, CRP and IL-6 were also linked to prognosis. A composite risk scoring system using levels of CysC, NT-proBNP, age and presence of mitral valve insufficiency was able to separate a high and a low risk group.

Conclusions: CysC levels at admission and increase in CysC after two weeks of treatment were independent prognostic markers for both 90-day and five-year mortality in patients with IE. A multi-marker composite risk scoring system including CysC identified a high-risk group.

ARTICLE SUMMARY

Article focus

- Our aim was to develop a multi-marker prognostic score for IE

Key messages

- CysC levels at admission and increase in CysC after two weeks of treatment were independent prognostic markers for both 90-day and five-year mortality in patients with IE.
- A prognostic score including CysC over 1.2 mg/L, NT-ProBNP over 2000 ng/L, presence of any grade of MI and age over 70 years could identify a high and low risk group in IE.
- The prognostic score might be used to improve patient monitoring and assist treatment choices in IE

Strengths and limitations of this study

- We were able to monitor changes in levels of biomarkers during treatment in a large cohort of IE patients since blood samples were collected at admission and after two weeks of treatment.
- One potential weakness was that 36.2% of patients treated for IE during the study period (71/196) were unavailable for biomarker studies since they lacked stored blood samples. The mortality was lower in the study group (125) compared to all IE patients treated for IE (196) during the study period.

INTRODUCTION

Infective endocarditis (IE) is an infection localized to the endocardial surface of the heart. IE mostly involves the heart valves, resulting in local valve destruction and abscess formation as well as development of vegetations with the ability to embolize to various organs. Despite major advances in both diagnostic and therapeutic procedures, neither the incidence [1] nor the mortality of the disease have decreased in the past 30 years, with a current in-hospital mortality of 15–20% and 1-year mortality reaching 40% in developed countries. [2] Several epidemiological studies have identified a number of prognostic factors related to higher mortality including advanced age, *Staphylococcus aureus* etiology, [2-3] cerebral complications, [4] and female sex. [5] In addition, biomarkers of inflammation like erythrocyte sedimentation rate (ESR), [2] hypoalbuminemia, [6] leukocytosis, [6] CRP [7] and procalcitonin [8] can predict poor prognosis but are too non-specific to guide therapy in individual patients. Identification of novel prognostic biomarkers and development of a prognostic score could help to identify IE patients who might benefit from more aggressive therapeutic procedures.

Because IE often influences hemodynamics, biomarkers linked to cardiovascular mortality could have prognostic power in IE. Among these are factors released during cardiovascular stress like NT-proBNP, MR-proANP, copeptin, and troponin T (TnT), which are linked to poor prognosis in heart failure, [9] coronary syndromes, [10] and sepsis. [11] Markers of renal function like creatinine, [12] estimated glomerular filtration rate (eGFR), [13] and cystatin C (CysC) [14] might also be able to predict prognosis in patients with IE similar to their ability to predict cardiovascular mortality. In this study, we analyzed clinical factors and cardiovascular biomarkers in blood samples collected at admission and after two weeks of therapy among patients with definite IE and examined their ability to predict 90-day and 5-year mortality. **Our primary goal was to develop a prognostic score in IE.**

METHODS

In this single-center retrospective cohort study, patients with IE treated between 1999–2005 at the Department of Infectious Diseases, Sahlgrenska University Hospital, University of Gothenburg, Gothenburg, Sweden, were registered between February 1, 1999, and December 3, 2005. During this time, 196 patients were diagnosed with definite IE according to the modified Duke criteria [15] and 125 patients had a blood sample drawn at diagnosis and stored and were therefore included in the study. After two weeks of treatment 120 out of the 125 patients had a second blood sample drawn. Of all 196 IE patients recorded from 1999–2005, 13 patients died (6.6%) within 90 days, and 43 patients died within five years (20.9%). Among the 125 patients with an available admission blood sample, the 90-day mortality was 10.4% and the five-year mortality was 33.6%. Information regarding mortality was collected from the hospital's registry of administrative data. The study was approved by the Ethical Committee at the University of Gothenburg. All patients were followed for up to five years or until death occurred (mean 1449.1±636.8 days).

Clinical data

Demographic and clinical information regarding age, sex, bacterial etiology, native and prosthetic valve IE, left ventricular ejection fraction (LVEF), comorbidities, and surgery during active IE were obtained from the endocarditis database. The study design included analysis of blood samples at admission and after two weeks of treatment. **The indications for surgery in Sweden are presented elsewhere. [31]**

Laboratory analyses

Serial blood samples were obtained during the hospital stay and stored until analysis at -70°C. The first sample was taken at admission and the second sample two weeks later. NT-proBNP was analyzed by Elecsys proBNP assay (Roche Diagnostics, Switzerland). CysC was analyzed in serum using reagents from Dako and the Modular P 2551. Creatinine based eGFR was calculated using "The Modification of Diet in Renal Disease" formula (MDRD). [19] TnT was analyzed by using The Elecsys Troponin T high sensitivity assay (Roche Diagnostics, Switzerland). **All biomarkers were analyzed on frozen serum samples in a single run.** All other laboratory parameters examined were part of the routine laboratory services provided by the Clinical Chemistry Laboratory, Sahlgrenska University Hospital.

Echocardiography

All patients were examined by transthoracic and transesophageal echocardiography at least once during the study period. Echocardiographic criteria for IE and degrees of valve insufficiency were evaluated. Left ventricular ejection fraction (LVEF) was calculated from long-axis planes (2-, 3-, and 4-chamber views) of the heart.

Statistical analysis

Multiple logistic regression models were used to evaluate possible associations between serum levels of biomarkers (NT-proBNP, TNT, creatinine, and CysC) and clinical variables including age, sex, echocardiographic parameters, and infectious agens and underlying diseases. Univariate comparisons between groups were calculated using conventional t-tests. Mann-Whitney U-tests were used for nonparametric comparisons of medians. Dichotomous variables were analyzed using the chi-square test. Receiver operating characteristic (ROC) curves were used to assess the prognostic properties of biomarkers. The log rank test was used to compare different strata in Kaplan-Meier analyses of survival. Statistical analyses

were performed with SPSS version 19. All probabilities were two-tailed, and p values <0.05 were regarded as significant. Odds ratios with confidence intervals were collected from outputs from logistic regression analyses. The coefficient of determination (R^2) was calculated in Microsoft Excel 2007 using Spearman's correlation to assess the strength of the correlation between CysC change and mortality. The add-in Analyse-it was used to compare AUCs between ROC curves.The study population was chosen to get a power of over 90% for detecting clinically relevant associations between CysC, NT-proBNP and mortality **(expected OR for mortality of at least 2 for each SD increase in the independent variable at a study population of 125 in logistic regression)**. No missing data existed for the variables included in the prognostic score, but for other tested variables, cases sometimes were excluded if data was missing, although no more than two cases in each analysis had to be excluded due to a high degree of data availability.

RESULTS

The mean age among the 125 IE patients was 62.7 ± 16.9 years, 64.8% were male, and Staphylococcus aureus infection was seen in 28.0% of the patients (Supplementary table 1). **Vegetations on aorta, mitralis and tricuspidalis were seen in 50.4%, 44.0% and 4.0% respectively**. **Prosthetic valve endocarditis was diagnosed in 28 (22.4%) of the patients, and 14 (11.2%) had a pacemaker. Vegetations on pacemaker leads were seen in four of these patients.** Most of the patients (91.6%) had LVEF over 40%, and 34.5% underwent heart surgery during antibiotic treatment. Clinical parameters correlating with five-year mortality included age, history of hypertension and mitral valve insufficiency (MI), but not ejection fraction (Supplementary table 1). Aminoglycoside use and duration of aminoglycoside therapy were both positively correlated with five-year survival. No associations were found between the presence of septic emboli and five-year survival,

biomarker levels or clinical variables examined in the study (data not shown).

In the univariate analysis CysC levels at admission were associated with both 90-day (OR 5.7 95% CI 2.2-14.7, p=<0.0001) and five-year mortality (OR 7.11 95% CI 2.6-19.5, p=<0.0001 (Fig. 1). CysC increases over 20% between admission and after two weeks of treatment (Supplementary Fig. 1) were also associated with increased five-year mortality (OR 2.8 95% CI 1.20-6.6, p=<0.017). **The relative and absolute changes of all biomarkers were evaluated but no significant associations with prognosis were found except for CysC.**

All eight patients with CysC over 2.1 mg/L died within five years. CysC levels and increase over 20% remained as significant prognostic indicators for five-year mortality with similar ORs when alternative multivariate models, including creatinine-estimated GFR or baseline creatinine were applied (Supplementary table 2).

The area under the receiver operator characteristic curve (AUC) for predicting five-year mortality was 0.70 (95% CI 0.60–0.80, p < 0.001) for CysC levels at admission and 0.74 (95% CI = 0.65–0.83, p < 0.0001) for CysC levels after two weeks of treatment. CysC had a significantly higher AUC compared to creatinine (0.62 (95% CI 0.51-0.73, p = 0.0042 for difference between AUC for CysC and creatinine after two weeks of treatment). Mean creatinine was significantly higher among patients that died within five years (Table 1). In contrast, median creatnine was not significantly different among patients that died or lived after five years.

In the univariate analysis, log NT-proBNP, GFR, creatinine, age, MI, and hypertension were linked to poor prognosis, whereas copeptin and MR-proANP did not reach statistical significance. CRP, IL-6, and TNT at two weeks, but not at admission, were also linked to prognosis (Table 1 and 2). No sex-based differences were present. **There was no association between surgery and prognosis.**

The four risk factors with the highest AUC for death within five years that appeared to be independent from each other were used to generate a composite risk score where each factor added one point. The factors included in the score were CysC over 1.2 mg/L, NT-ProBNP over 2000 ng/L, presence of any grade of MI and age over 70 years. **The AUC of the risk score was 0.74 (95% CI 0.70-0.87, p < 0.001). If substituting CysC for eGFR calculated from creatinine levels, the AUC decreased to 0.67 (95% CI 0.57-0.78).**

Patients with a risk score of 0-2 had a significantly better prognosis (11% (8/71) died within five years) compared to patients with a score of 3-4 (63% (34/54) died within five years, p<0.0001) (Fig. 2). **No significant difference in prognosis could be found between patients differing by 1 point.** The score's ability to separate patient prognosis was improved when the 34.5% of patients that underwent cardiac surgery were excluded (0-2 points 7% (3/41), 3-4 points 66% (22/33) died within five years, p<0.0001). The composite risk score also predicted death within 90-days (0-2 points 0% (0/71), 3-4 points 24.1% (13/54) died within 90-days, p<0.0001).

Furthermore, dividing the cohort based on a score of 0-2 or 3-4 predicted mortality in patients with left sided IE (OR=14.6, 5.55-38.2 p<0.001), left sided IE that did not undergo surgery (OR=25.7, 6.35-103.7, p<0.001) and staph aureus infected left sided IE that did not undergo surgery (OR 31.5, 2.35-422.3, p=0.009). Lastly, the score was also able to predict mortality if the MI parameter was excluded (OR 2.30 (95% CI 1.41-3.72), p=0.001) indicating that the composite score was not reliant on the MI parameter and was able to predict mortality in important subgroups of IE.

DISCUSSION

CysC levels at admission, CysC levels after two weeks, and over 20% increases in CysC levels during two weeks of treatment were prognostic for mortality in patients with definite IE. The combination of CysC levels and three other risk factors generated a powerful risk score.

CysC is a small 13-kDa cysteine protease inhibitor produced at a constant rate by all nucleated human cells tested. CysC is cleared predominantly by renal filtration and is most often used as a replacement for creatinine as a marker of GFR. CysC is regarded as a more accurate marker of kidney function than creatinine (Reviewed in 14). CysC also offers superior ability to diagnose acute kidney injury [16] and declining GFR [17] compared to creatinine. Furthermore, several studies have shown that elevated CysC is a strong risk factor for adverse cardiovascular prognosis in the elderly even when creatinine levels are normal. [18] Increased CysC levels indicate a future risk of developing heart failure, [19] as well as a poor prognosis among patients with already established heart failure, [20] independent of creatinine levels. Therefore, CysC is a marker of kidney function and prognosis that outperforms creatinine in most studies.

The associations between CysC levels and prognosis in our study might reflect that IE itself or nefrotoxic agents used during treatment of IE impairs kidney function. The ability of CysC to more correctly predict GFR among the elderly and to respond to rapid changes in GFR compared to creatinine could explain the stronger association between CysC levels and IE prognosis compared to creatinine levels.

There are different conceivable explanations for decreased kidney function in IE. One factor could be decreased cardiac output during the acute phase of IE. Although there was no correlation between LVEF and prognosis (Supplementary table 1), levels of the heart failure biomarker, NT-proBNP, and prevalence of MI were significantly higher among patients with poor prognosis. In addition, levels of TnT after two weeks of treatment were associated with prognosis. TnT levels are affected by cardiac stress found among patients with heart failure [21-22] and correlate with

prognosis in a number of heart-related conditions. [23] Therefore, the link between CysC levels and poor prognosis among IE patients could be decreased cardiac output resulting in low kidney perfusion and low GFR.

It is also possible that factors that increase production of CysC, such as increased glucocorticoid levels.[24], and possibly inflammation, [25-27] could explain the correlation between CysC levels and IE prognosis. CysC levels show a stronger association with cardiovascular risk factors compared to creatinine, independent of iohexol clearance. [28] Among patients with chronic renal failure followed for 10 years, CysC levels correlated better with cardiovascular disease mortality compared to creatinine or iothalamate clearance [29] indicating that CysC levels predict prognosis partly independent from GFR. In line with this possibility, we found that CysC levels and change in CysC levels during two weeks of treatment remained prognostic even after multivariate analysis correcting for creatinine levels and creatinine estimated GFR (Supplementary table 2) suggesting that conditions that increase production of CysC may correlate with IE prognosis. One such factor could be persistent inflammation because high CRP or IL-6 levels after two weeks of treatment were also linked to poor prognosis in our study. Moreover, it is also possible that increased glucocorticoid levels due to a general stress response among IE patients could be the link between poor prognosis and CysC levels.

One important aspect of this study was that we could monitor changes in biomarkers during treatment in a large group if IE patients. We found that levels of CRP, IL-6, and TnT after two weeks of treatment, but not admission levels, were significantly associated with five-year survival. This association likely reflects the fact that a rapid response to initial treatment is of great importance for the prognosis. In contrast, levels of the otherwise promising cardiac stress markers MR-proANP and copeptin failed to predict prognosis, even when levels were assessed after two weeks of treatment, indicating that these biomarkers are less valuable among IE patients.

Our results confirmed that MI is a powerful prognostic indicator in IE irrespective of the

infected valve. The reason for the significant association between MI of all degrees, and not with the worst degrees alone (3 and 4), can be explained by the fact that the patients with severe MI often underwent surgery, which is linked to improved survival. [30] This association could, however, not be confirmed in this study (Supplementary table 1).

The multitude of biomarkers and clinical factors included in this study allowed us to explore a composite risk score based on the four strongest and apparently independent risk factors. The composite risk score was able to separate a high and a low risk group with significantly different 90-day and five-year survival. IE has still a poor prognosis despite major advances in diagnostic and therapeutic procedures and we still have limited ability to find the patients that should be considered for more aggressive treatment. In this light, this novel risk score could add decisional information and allow for a multifactorial judgment of patients and consideration for more active intervention like heart valve replacement or surgical removal of vegetations.

A potential problem with the current study was that 36.2% of the patients treated for IE during the study period (71/196) lacked stored blood samples. In addition, there was a bias for patients with a worse prognosis in the study group. On the other hand, the study group of 125 IE patients had a five-year prognosis (66.4% five-year survival) closer to the 60% five-year survival reported in most studies [1-2] compared to the complete cohort (79% five-year survival) indicating that the prognosis in our study group were comparable with the outcome in previous studies. **Furthermore data on hemodynamic parameters on admission and during the hospital stay were not recorded. As this was a single center study, our findings must be validated before the risk score can be included in clinical routine.**

In summary a prognostic score including CysC over 1.2 mg/L, NT-proBNP over 2000 ng/L, presence of any grade of MI and age over 70 years could identify a high and low risk group in IE.

ACKNOWLEDGEMENTS

We are thankful for the expertise and analysis of the laboratory parameters by Bodil Gustafsson, Carina M Gustafsson, and Anne-Sofie Johansson Fällgren.

Potential conflicts of interest: C.B., U.S., L.O., M.F, O.H.: None declared.

COMPETING INTERESTS

No competing interests exist.

FUNDING

This work was supported by the Swedish Cancer Society [O.H.], Swedish Research Council, Swedish Pain Foundation [O.H.], the Sahlgrenska University Hospital Research Foundation [O.H.], Swedish Heart-Lung Foundation [M.F.], Sahlgrenska University Hospital Research Foundation [M.F.] and by government support to the city councils for cost arising (grant ALFGBG-138141) [LO].

REFERENCES

1 Tleyjeh IM, Steckelberg JM, Murad HS, et al. Temporal trends in infective endocarditis: A population-based study in olmsted county, minnesota. Jama 2005;**293**:3022-3028.

2 Murdoch DR, Corey GR, Hoen B, et al. Clinical presentation, etiology, and outcome of infective endocarditis in the 21st century: The international collaboration on endocarditis-prospective cohort study. Arch Intern Med 2009;**169**:463-473.

3 Thuny F, Di Salvo G, Belliard O, et al. Risk of embolism and death in infective endocarditis: Prognostic value of echocardiography: A prospective multicenter study. Circulation 2005;**112**:69-75.

4 Thuny F, Avierinos JF, Tribouilloy C, et al. Impact of cerebrovascular complications on mortality and neurologic outcome during infective endocarditis: A prospective multicentre study. Eur Heart J 2007;**28**:1155-1161.

5 Sambola A, Fernandez-Hidalgo N, Almirante B, et al. Sex differences in native-valve infective endocarditis in a single tertiary-care hospital. Am J Cardiol 2010;**106**:92-98

6 Wallace SM, Walton BI, Kharbanda RK, et al. Mortality from infective endocarditis: Clinical predictors of outcome. Heart 2002;**88**:53-60.

7 Olaison L, Hogevik H, Alestig K. Fever, C-reactive protein, and other acute-phase reactants during treatment of infective endocarditis. Arch Intern Med 1997;**157**:885-92.

8 Kocazeybek B, Kucukoglu S, Oner YA. Procalcitonin and c-reactive protein in infective endocarditis: Correlation with etiology and prognosis. Chemotherapy 2003;**49**:76-84.

9 Palazzuoli A, Gallotta M, Quatrini I, et al. Natriuretic peptides (bnp and nt-probnp): Measurement and relevance in heart failure. Vasc Health Risk Manag 2010;**6**:411-418.

10 Mazzone M, Forte P, Portale G, et al. Brain natriuretic peptide and acute coronary syndrome. Minerva Med 2005;**96**:11-18.

11 Perman SM, Chang AM, Hollander JE, et al. Relationship between b-type natriuretic

peptide and adverse outcome in patients with clinical evidence of sepsis presenting to the emergency department. Acad Emerg Med 2011;**18**:219-222.

12 Rostagno C, Rosso G, Puggelli F, et al. Active infective endocarditis: Clinical characteristics and factors related to hospital mortality. Cardiol J 2010;**17**:566-573.

13 Hillege H, Van Gilst W, de Zeeuw D, et al. Renal function as a predictor of prognosis in chronic heart failure. Heart Fail Monit 2002;**2**:78-84.

14 Lassus J, Harjola VP. Cystatin c: A step forward in assessing kidney function and cardiovascular risk. Heart Fail Rev Published Online First: 23 Mars 2011. doi:10.1007/s10741-011-9242-6.

15 Li JS, Sexton DJ, Mick N, et al. Proposed modifications to the duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis 2000;**30**:633-638.

16 Nejat M, Pickering JW, Walker RJ, et al. Rapid detection of acute kidney injury by plasma cystatin c in the intensive care unit. Nephrol Dial Transplant 2010;**25**:3283-3289.

17 Premaratne E, MacIsaac RJ, Finch S, et al. Serial measurements of cystatin c are more accurate than creatinine-based methods in detecting declining renal function in type 1 diabetes. Diabetes Care 2008;**31**:971-973.

18 Ix JH, Shlipak MG, Chertow GM, et al. Association of cystatin c with mortality, cardiovascular events, and incident heart failure among persons with coronary heart disease: Data from the heart and soul study. Circulation 2007;**115**:173-179.

19 Sarnak MJ, Katz R, Stehman-Breen CO, et al. Cystatin c concentration as a risk factor for heart failure in older adults. Ann Intern Med 2005;**142**:497-505.

20 Alehagen U, Dahlstrom U, Lindahl TL. Cystatin c and nt-probnp, a powerful combination of biomarkers for predicting cardiovascular mortality in elderly patients with heart failure: Results from a 10-year study in primary care. Eur J Heart Fail 2009;**11**:354-360.

21 Peacock WFt, De Marco T, Fonarow GC, et al. Cardiac troponin and outcome in acute

heart failure. N Engl J Med 2008;**358**:2117-2126.

22 deFilippi CR, de Lemos JA, Christenson RH, et al. Association of serial measures of cardiac troponin t using a sensitive assay with incident heart failure and cardiovascular mortality in older adults. JAMA 2010;**304**:2494-2502.

23 Omland T, de Lemos JA, Sabatine MS, et al. A sensitive cardiac troponin t assay in stable coronary artery disease. N Engl J Med 2009;**361**:2538-2547.

24 Bokenkamp A, van Wijk JA, Lentze MJ, et al. Effect of corticosteroid therapy on serum cystatin c and beta2-microglobulin concentrations. Clin Chem 2002;48:1123-1126.

25 Evangelopoulos AA, Vallianou NG, Bountziouka V, et al. Association between serum cystatin c, monocytes and other inflammatory markers. Intern Med J Published Online First: 6 April 2011. doi:10.1111/j.1445-5994.2011.02500.x.

26 Grubb A, Bjork J, Nyman U, et al. Cystatin c, a marker for successful aging and glomerular filtration rate, is not influenced by inflammation. Scand J Clin Lab Invest 2011;**71**:145-149.

27 Stevens LA, Schmid CH, Greene T, et al. Factors other than glomerular filtration rate affect serum cystatin c levels. Kidney Int 2009;**75**:652-660.

28 Mathisen UD, Melsom T, Ingebretsen OC, et al. Estimated gfr associates with cardiovascular risk factors independently of measured gfr. J Am Soc Nephrol 2011;**22**:927-37.

29 Menon V, Shlipak MG, Wang X, et al. Cystatin c as a risk factor for outcomes in chronic kidney disease. Ann Intern Med 2007;**147**:19-27.

30 Lalani T, Cabell CH, Benjamin DK, et al; International Collaboration on Endocarditis-Prospective Cohort Study (ICE-PCS) Investigators. Analysis of the impact of early surgery on in-hospital mortality of native valve endocarditis: use of propensity score and instrumental variable methods to adjust for treatment-selection bias. Circulation 2010;**121**:1005-13.

31 Westling K, Aufwerber E, Ekdahl C, et al; Swedish guidelines for diagnosis and treatment of infective endocarditis. Scand J Infect Dis. 2007;**39**:929-46.

FIGURE LEGENDS

Figure 1. Kaplan-Meier survival plot by quartiles of admission levels of CysC.

Figure 2: Kaplan-Meier survival plot by risk score for death within five years among patients with IE. Patients with 0-2 points were compared to patients with 3-4 points (p<0.001). The presence of these risk factors adds one point each: Any grade of MI, CysC >1. 2mg/L, NT-proBNP >2000 ng/L, Age >70 years.

Supplementary figure 1: Correlation between five-year mortality and percentage change of CysC between admission and two weeks after initiation of treatment ($R^2 = 0.87$). Patients were ordered in six strata based on percentage change of CysC and the mean five-year survival was calculated for each group.

Table 1: Laboratory characteristics of IE patients

Variable	**Total**	**[a]Dead**	**[a]Alive**	**p value**
CRP [mg/L] (at admission)	58.6±57.9	61.1±54.7	57.3±64.3	0.72
CRP [mg/L] (after 2 weeks)	28.0±49.3	45.0±26.7	20.7±78.2	0.013[b]
CRP [mg/L] (peak level)	139.9±94.1	160.8±86.5	130.5±107.3	0.12
IL-6 [ng/L] (at admission)	45.3±112.1	43.5±134.5	46.3±42.2	0.90
IL-6 [ng/L] (after 2 weeks)	24.1±42.5	39.1±25.4	17.9±65.2	0.011[b]
Hb [g/L]	112.2±17.3	109.8±17.5	113.2±16.9	0.48
Creatinine [mol/L]	95.3±66.6	123.2±26.7	81.4±104.1	<0.001[c]
eGFR [mL/min] (MDRD)[d]	81.0±41.2	67.3±44.1	87.0±29.6	0.016[b]
TnT [ng/L] (at admission)	106.9±226.1	156.1±204.7	82.3±259.3	0.083
TnT [ng/L] (after 2 weeks)	75.7±124.2	109.7±104.4	61.1±157.6	0.048[b]
Incremental TNT [%]	40.8%	44.4%	39.3%	0.60

NT-proBNP [ng/L] (at admission)	3874.2±6868.4	8209.8±1962.6	1706.4±10314.3	<0.001[c]
NT-proBNP [ng/L] (after 2 weeks)	2597.2±3363.6	4782.3±2042.7	1660.8±4633.1	<0.001[c]
Incremental NT-proBNP [%]	41.7%	30.6%	46.4%	0.11
CysC [mg/L] (at admission)	1.34±0.67	1.73±0.35	1.15±0.94	<0.001[c]
CysC [mg/L] (after 2 weeks)	1.45±0.81	0.99±0.65	0.66±0.99	<0.001[c]
CysC (>20% increase) [%]	26.7%	41.7%	20.2%	0.015[b]
MR-proANP [pmol/L] (at admission)	327.6±199.3	378.8±227.0	302.0±452.7	0.21
MR-proANP [pmol/L] (after 2 weeks)	290.2±39.6	249.2±219.1	218.8±135.1	0.15
Copeptin [mmol/L] (at admission)	23.3±29.0	30.1±24.3	19.9±59.2	0.17
Copeptin [mmol/L] (after 2 weeks)	24.0±29.0	21.2±31.8	31.7±21.2	0.42

Table 2: Odds ratios[a] for mortality among IE patients

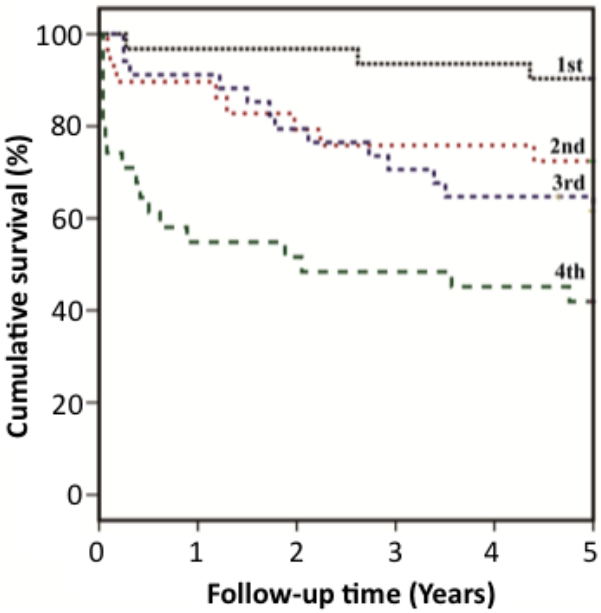
Variable[b]	**OR (all patients)**	**p value**	**OR (no surgery[c])**	**p value**
Age	1.06 (1.03–1.10)	<0.001[d]	1.09 (1.03–1.14)	0.001[d]
MI (all degrees)	2.94 (1.30–6.67)	0.010[e]	5.45 (1.84–16.2)	0.002[d]
Hypertension	3.12 (1.37–7.10)	0.007[e]	2.81 (1.03–7.62)	0.043[e]
CRP [mg/L] [after 2 weeks]	1.01 (1.00–1.03)	0.040[e]	1.00 (0.99–1.01)	0.75
Creatinine [mol/L]	1.02 (1.00–1.02)	0.006[e]	1.02 (1.00–1.04)	0.025[e]
GFR (MDRD)	0.98 (0.97–1.00)	0.012[e]	0.98 (0.96–1.00)	0.041[e]
logNT-proBNP [ng/L] (at admission)	12.2 (4.28–34.9)	<0.001[d]	23.2 (4.68–115.0)	<0.001[d]
logNT-proBNP [ng/L] (after 2 weeks)	5.91 (2.43–14.4)	<0.001[d]	9.44 (2.83–31.5)	<0.001[d]

CysC [mg/L] (at admission)	7.11 (2.59–19.5)	<0.001[d]	37.5 (4.58–308.1)	0.001[d]
CysC [mg/L] (after 2 weeks)	2.55 (1.37–4.76)	0.003[d]	41.7 (5.43–320.6)	<0.001[d]
CysC [mg/L] (>20% increase)	2.82 (1.20–6.59)	0.017[e]	2.60 (0.90–7.50)	0.080
TnT [ng/L] (after 2 weeks)	1.00 (1.00–1.01)	0.059	1.03 (1.01–1.05)	0.008[e]
IL-6 [ng/L] (after 2 weeks)	1.02 (1.00–1.03)	0.044[e]	1.10 (1.04–1.17)	0.002[d]

[a] From multiple logistic regression, [b] Insignificant predictors excluded from the table,

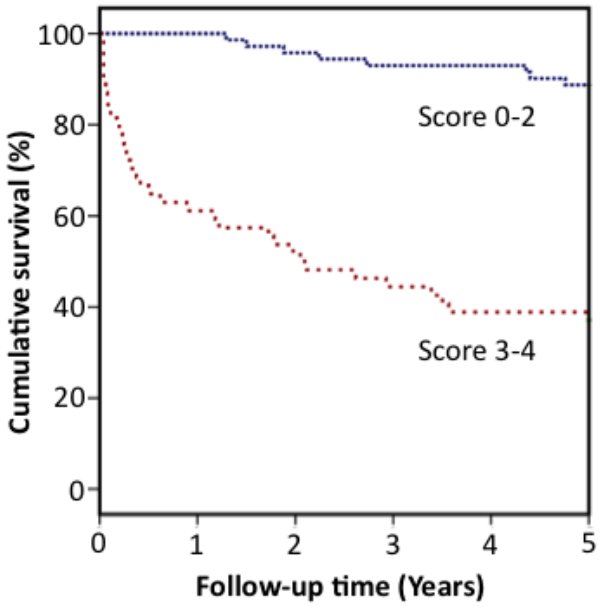
[c] Subgroup analysis of patients who did not undergo surgery, [d] P <0.005, [e] P < 0.05

Fig. 1 Bjurman et al.

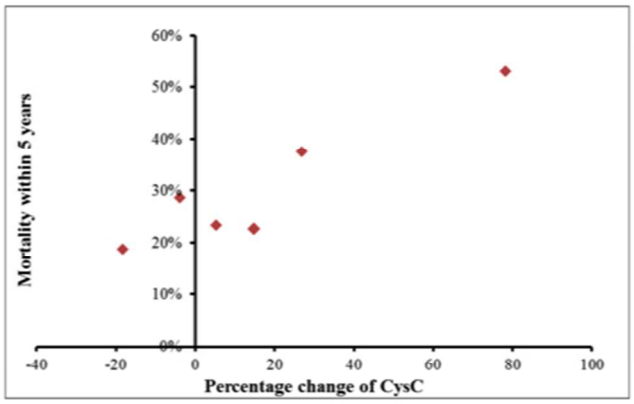


CysC quartile	CysC level (mg/L)	n	Dead 5 years (n)	p
1	<0.98	31	3	Reference
2	0.98-1.17	29	8	0.066
3	1.17-1.52	34	13	0.008
4	>1.52	31	18	<0.0001

Fig. 2 Bjurman et al.



Supplementary Fig. 1 Bjurman et al.



254x190mm (72 x 72 DPI)

Supplementary Table 1: Demographic and clinical characteristics of IE patients

Variable	Total	[a] Dead	[a] Alive	p value
N	125	42	84	
Age [years]	62.7±16.9	71.9±13.6	58.6±16.7	0.0001[b]
Male [%]	64.8%	61.9%	66.3%	0.63
LVEF <0.40	8.0%	11.1%	7.2%	0.49
[c] MI [all degrees]	48.7%	66.7%	41.0%	0.01[b]
[c] MI [>2/4]	14.3%	16.7%	13.3%	0.63
[c] AI [all degrees]	34.5%	33.3%	35.0%	0.87
[c] AI [>2/4]	10.1%	11.1%	9.6%	0.81
Hypertension	40.4%	60.0%	31.6%	0.004[b]
Dialysis	1.8%	2.9%	1.3%	0.56
Cardiac surgery	34.5%	28.6%	37.2%	0.37
NVE	76.8%	71.4%	79.5%	0.14
PVE	23.2%	28.6%	20.5%	0.14
Staphylococcus aureus	29.4%	30.6%	28.9%	0.86

[a] within five years, [b] p <0.05, [c] MI: Mitral Insufficiency, [d] AI: Aortic Insufficiency,

[d] NVE: Native valve endocarditis, [e] PVE: Prosthetic valve endocarditis

Supplementary Table 2: Multivariate models for prediction of five-year mortality (with adjustments for possible confounders)

Variables included in the model[a]	**OR (CysC)**	**p value**
CysC (at admission), MI[b], and age	5.42 (1.90–15.5)	0.002[c]
CysC (at admission), creatinine	9.69 (2.29-41.1)	0.002[c]
	OR (CysC increase >20%)	**p value**
CysC (increase >20%), MI[b], hypertension, and age	4.86 (1.62–14.5)	0.005[c]
CysC (increase >20%), MI[b], eGFR (MDRD), and age	4.07 (1.46–11.4)	0.007[c]
CysC (increase >20%), MI[b], creatinine, hypertension, and age	5.19 (1.63–16.5)	0.005[c]
CysC (increase >20%), MI[b], *Staphylococcus aureus*, hypertension, and age	4.86 (1.59–14.9)	0.006[c]

[a] Models created to adjust cystatin C (at admission or increase >20%) for age, eGFR, baseline creatinine, and/or pathogen (Staphylococcus aureus),[b] MI: Mitral Insufficiency, [c] $p < 0.05$.