Neopterin predicts cardiac dysfunction following cardiac surgery†

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Abstract

OBJECTIVES: Oxidative stress following ischaemia and reperfusion, as well as inflammation, are thought to be important for the development of cardiac dysfunction after cardiac surgery. Our main objective was to investigate whether the inflammatory biomarkers C-reactive protein (CRP), lactoferrin, neopterin and the terminal complement complex (TCC) were associated with cardiac dysfunction after cardiac surgery. Another objective was to assess whether the biomarkers could improve prediction of postoperative cardiac dysfunction compared with clinical variables only.

METHODS: Blood samples and clinical data from 1018 consecutive patients undergoing cardiac surgery from 1 April 2008 to 19 April 2010 at St. Olavs University Hospital, Trondheim, Norway, were collected prospectively. The end-point was postoperative cardiac dysfunction, defined as the need for more than one inotropic agent or an intra-aortic balloon pump occurring after the operation and until the patient was discharged from the department. CRP, lactoferrin, neopterin and TCC were analysed in plasma, and we used logistic regression to evaluate the association of the biomarkers with postoperative cardiac dysfunction. We adjusted for the following clinical variables previously associated with postoperative cardiac dysfunction: urgent operation, operation type, previous cardiac surgery, chronic heart failure, pulmonary hypertension, previous myocardial infarction and haemoglobin. The likelihood ratio test, the integrated discrimination improvement and receiver operating characteristic (ROC) curves were used to assess whether the biomarkers could improve prediction of postoperative cardiac dysfunction compared with clinical variables alone.

RESULTS: Neopterin was the only biomarker significantly associated with postoperative cardiac dysfunction (odds ratio 2.73, 95% confidence interval 1.65–4.51) after adjustment for clinical variables. Neopterin improved risk prediction of cardiac dysfunction following heart surgery compared with clinical variables alone according to the likelihood ratio test (P < 0.0001) and the integrated discrimination improvement (P = 0.02), particularly for patients with intermediate risks.

CONCLUSIONS: Neopterin was associated with cardiac dysfunction following cardiac surgery, and improved the accuracy of risk prediction of postoperative cardiac dysfunction. At present, we do not suggest that neopterin should be measured routinely before heart surgery, but our findings support the hypothesis of the role of oxidative stress and inflammation in development of cardiac dysfunction following heart surgery.

Keywords: Cardiac surgery · Cardiac dysfunction · Biomarker · Inflammation

INTRODUCTION

Cardiac dysfunction after heart surgery is a clinical syndrome characterized by insufficient delivery of blood to the tissues because of reduced cardiac output. A reduction in ventricular function is commonly seen following cardiac surgery, often worsening for several hours postoperatively before recovery [1]. In 5–11% of patients, the cardiac dysfunction is severe enough to require treatment with several inotropic drugs or an intra-aortic balloon pump (IABP) [2–4], and cardiac dysfunction was the most common cause of death after coronary artery bypass grafting in a study from New England, USA [5].

Many factors contribute to the development of cardiac dysfunction following heart surgery. Oxidative stress following ischaemia and reperfusion, and inflammation seem to be crucial [6, 7]. The inflammatory markers C-reactive protein (CRP), lactoferrin, neopterin and the terminal complement complex (TCC) have all been associated with coronary artery disease or the
development of reduced ventricular function following ischaemia [8-10]. An overview of further relevant publications regarding these markers is given in Supplementary material, Table S1. Several models for prediction of cardiac dysfunction following cardiac surgery have been published, but most of them were based on clinical variables alone [2, 4, 5].

To explore some of the inflammatory pathways that might underlie the development of cardiac dysfunction, our main aim was to investigate whether the inflammatory biomarkers CRP, lactoferrin, neopterin and TCC were associated with cardiac dysfunction after cardiac surgery. The secondary aim was to assess whether one or several of the biomarkers could improve the accuracy of risk prediction of cardiac dysfunction after heart surgery compared with a model based on previously published clinical variables [2].

Our hypothesis was that CRP, lactoferrin, neopterin or TCC would be associated with cardiac dysfunction after cardiac surgery, and that one or several of the biomarkers would improve the accuracy of risk prediction compared with clinical variables alone.

MATERIALS AND METHODS

The project was approved by The Regional Research Ethics Committee in Medicine (Project number 4.2007.1528), Trondheim, Norway, on 27 June 2007 (Chairperson Arne Sandvik), and by the Norwegian Data Inspectorate. The present work is part of the Cardiac Surgery Outcome Study (CaSOS).

Data

In this prospective study, all adult patients undergoing cardiac surgery from 1 April 2008 to 19 April 2010 at St. Olavs University Hospital, Trondheim, Norway, were considered eligible for inclusion in the study. Patient characteristics, other diseases and risk factors, blood tests, perioperative data and data on postoperative factors and complications were collected prospectively, quality assured by a senior anaesthesiologist and stored in a local database as part of the department’s quality assurance work.

We collected preoperative peripheral arterial blood samples from consecutive patients. The samples were kept on ice for a maximum of 6 h before they were centrifuged, and stored at −80°C until analysis. Of 1149 eligible patients, 21 did not consent, 32 and 7 were unable to consent due to emergency surgery and language problems, respectively, and 57 had missing blood samples. We also excluded 14 patient samples: 1 had infectious blood, 3 had active endocarditis, 2 underwent off-pump surgery, 1 did not have data on the end-point and 7 samples had an identification error preventing coupling with clinical data. Thus, 1018 patients were normally not considered for elective heart surgery. Data in infection (other than endocarditis) and elevated levels of CRP were normally not considered for elective heart surgery. Data on the end-point and 7 samples had an identification error preventing coupling with clinical data. Thus, 1018 patients were

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Table 1: Variable definitions

<table>
<thead>
<tr>
<th>Variable</th>
<th>Definition</th>
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</thead>
<tbody>
<tr>
<td>Urgent operation</td>
<td>1: standard waiting list; 2: need for operation within 1 week</td>
</tr>
<tr>
<td>Operation type</td>
<td>1: CABG only or repair of atrial septal defect, 2: AVR only, AVR and CABG combined, repair of aneurysm in the ascending aorta or non-ischaeamic mitral valve surgery, 3: miscellaneous procedures such as mitral valve surgery combined with CABG or AVR, AVR combined with procedures other than CABG, operation for dissection of the ascending aorta or rupture of the ventricular septum and other cardiac surgery such as removal of cardiac tumours and pericardectomy</td>
</tr>
<tr>
<td>Previous cardiac surgery</td>
<td>No/yes</td>
</tr>
<tr>
<td>Chronic heart failure</td>
<td>Receiving medication (no/yes)</td>
</tr>
<tr>
<td>Pulmonary hypertension</td>
<td>Systolic PAP &gt;40 mmHg or mean PAP &gt;25 mmHg, echocardiography or catheterization (no/yes)</td>
</tr>
<tr>
<td>Previous myocardial infarction</td>
<td>No/yes</td>
</tr>
<tr>
<td>Preoperative renal dysfunction</td>
<td>Creatinine concentration &gt;140 µmol/l or dialysis (no/yes)</td>
</tr>
<tr>
<td>Preoperative haemoglobin</td>
<td>g/dl (continuous)</td>
</tr>
</tbody>
</table>

CABG: coronary artery bypass grafting; AVR: aortic valve replacement; PAP: pulmonary arterial pressure.

aPreviously published risk factors for cardiac dysfunction after open-heart surgery in our population [2].
bCompared with the previously published definition, we used only two categories instead of three because no patients underwent emergency surgery in our cohort.
cCompared with the previously published definition, we used only three categories instead of four due to only 1 patient needing elective surgery for dissection of the ascending aorta and none undergoing surgery for rupture of the ventricular septum.

probabilities, as differences in the AUC are insensitive measures of improvement in discrimination [15].

General statistics

Descriptive statistics are given as median with 95% confidence intervals for continuous data, and frequencies with percentages for categorical data. For between-group comparisons, we used the Mann–Whitney U-test for continuous data, and Pearson’s χ² test for categorical data. Linear correlation was assessed with Pearson’s correlation coefficient. Statistical analyses were performed using the statistical software R (version 12.2.0; R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria), IBM SPSS (version 18.0; IBM Corporation, Armonk, NY, USA), Minitab 17 (Minitab Ltd, Coventry, UK) and Sigma Plot 13.0 (Systat Software, Inc., San Jose, CA, USA).

RESULTS

Patient characteristics are given in Table 2. In the unadjusted analysis, both CRP and neopterin showed significant associations with cardiac dysfunction following cardiac surgery (Table 3). However, neopterin was the only significant biomarker after adjustment for urgent operation, operation type, previous cardiac surgery, chronic heart failure, pulmonary hypertension, previous myocardial infarction and preoperative haemoglobin concentration (P = 0.0005) (Table 3, Supplementary material, Table S2). Neopterin was correlated with CRP (R = 0.27, P < 0.0005).

Preoperative renal dysfunction was removed from the model because of strong correlation with neopterin (R = 0.37, P < 0.0005). Neither renal dysfunction nor serum creatinine was a significant predictor of cardiac dysfunction in these patients, and the sensitivity analysis showed no difference in odds ratios after removal of renal dysfunction or creatinine from the model. As another sensitivity analysis, we also developed an alternative model including age and sex, but the odds ratios for the biomarkers were essentially unchanged (data not shown).

The likelihood ratio test showed that neopterin improved the model fit (P < 0.0001). When comparing the model containing only clinical variables with the model including neopterin as well, neopterin increased the AUC of the model from 0.817 (0.770–0.863) to 0.833 (0.779–0.874) (P = 0.07) (Fig. 1), and the IDI was 0.014 (P = 0.02), indicating that neopterin increased discrimination.

Figure 1 illustrates that neopterin increased discrimination for a group of patients in particular. These were 380 (37.3%) patients with predicted risks between 2.5 and 6.4%. This corresponded approximately to the 25th percentile, and the 60th percentile, i.e. the patients with intermediate predicted risks, having a few risk factors for cardiac dysfunction.

DISCUSSION

Preoperative neopterin levels were associated with cardiac dysfunction after cardiac surgery, also after adjustment for clinical variables. Moreover, neopterin improved the accuracy of prediction of cardiac dysfunction compared with clinical variables alone.

Cardiac dysfunction after cardiac surgery

Cardiac dysfunction following cardiopulmonary bypass is thought to result from myocardial stunning due to ischaemia and reperfusion [16], and in part also from local and systemic inflammation [7]. It has been proposed that the mechanism involves generation of reactive oxygen species and impaired calcium homeostasis, with damage of the sarcolemma, modification of contractile proteins and reduced calcium sensitivity [6].

Neopterin

Neopterin is released from activated macrophages and monocytes after stimulation with interferon-γ from activated T-lymphocytes [17, 18], and may be seen as a marker of activation of monocytes and the cellular immune system. Neopterin has been associated with left ventricular ejection fraction and cardiac dysfunction in patients with chronic stable angina pectoris [9], and with left ventricular ejection fraction and diastolic left ventricular diameter in patients with critical limb ischaemia [19].

Neopterin has been shown to induce contractile dysfunction in isolated perfused rat hearts [20]. Although the effective concentration in that study was higher than neopterin levels occurring in vivo, it was suggested that long-term influence of lower levels of...
neopterin could lead to cardiac dysfunction in humans [20]. This effect could possibly be mediated through oxidative stress. A previous study has shown that neopterin enhanced the oxidative effect of hydrogen peroxide in vitro [21].

Our findings suggest that neopterin could play a role in the development of cardiac dysfunction. We measured neopterin before cardiac operation, and registered if the patient had cardiac dysfunction postoperatively. It is uncertain whether it was the specific effects of neopterin that enhanced the risk of postoperative cardiac dysfunction, if neopterin acted as a marker of inflammation in general or of activation of macrophages and the cellular immune system, or whether the association was caused by something else. However, it is possible that elevated levels of neopterin before surgery enhanced the effects of oxidative stress resulting from ischaemia and inflammation after aortic cross-clamping and cardiopulmonary bypass during heart surgery.

Other causes of elevated neopterin

Elevated levels of neopterin have been associated with acute infection, autoimmune diseases and malignancy [18], as well as atherosclerosis [22] and left ventricular dysfunction [9]. We excluded patients with active endocarditis, and normally patients with inter-current infections were not eligible for elective cardiac surgery until recovery. We did not have data to identify autoimmune diseases or malignancy, but the total number of affected patients is expected to be low. Atherosclerosis and left ventricular
Neopterin is biologically stable in the circulation and is eliminated by the kidneys [18]. Thus, impaired kidney function could also cause an increase in neopterin concentration. Therefore, we also analysed neopterin with adjustment for serum creatinine as well as the other clinical variables, but this did not change the results.

**Improvement of risk prediction**

Neopterin improved the accuracy of prediction of cardiac dysfunction after cardiac surgery. This was statistically significant according to the likelihood ratio test and the IDI, and almost significant according to the comparison of AUC for the model with and without neopterin (P = 0.07). Differences in the AUC are conservative measures of improvement in discrimination when comparing risk prediction models, yet ROC curves may be useful for describing the discrimination [15, 23]. Figure 1 indicated that neopterin improved risk prediction especially for patients with intermediate risk of postoperative cardiac dysfunction. The added value for high-risk and low-risk patients was less important. For the high-risk patients, it could be that the added effect of several risk factors overshadowed the effect of neopterin. Implementation of new biomarkers in clinical practice should rely on thorough research and evidence of its usefulness, and an evaluation of the benefit compared with the increased expenses. Presently, we therefore do not suggest that neopterin should be measured routinely before heart surgery. However, our findings support the hypothesis of the role of oxidative stress and inflammation in development of cardiac dysfunction following heart surgery.

**C-reactive protein**

CRP was not significant after adjustment for the clinical variables. Previous studies of the association between CRP and several cardiac end-points have shown conflicting results [8–10, 22, 24]. In our study, CRP was somewhat correlated with neopterin, and this could weaken the association between CRP and cardiac dysfunction when CRP and neopterin were analysed in the same model. Moreover, CRP is considered a more general marker of inflammation than neopterin. It is also possible that CRP represents some of the information included in the clinical variables, such as urgent operation, chronic heart failure and previous myocardial infarction, as CRP has been associated with ischaemic heart disease and chronic heart failure in previous studies [10, 24].

CRP circulates as a pentamer, but a recent study showed that the more active monomeric form of CRP, and not the pentameric form, was found in inflamed atherosclerotic plaques and in myocardial infarction lesions in humans [25]. The different results regarding associations of CRP and cardiac end-points could be partly explained by the fact that we are measuring the circulating pentamer, which is more than not necessarily corresponds with the concentration of monomeric, proinflammatory CRP at sites of inflammation.

As patients with signs of intercurrent infection and elevated CRP were not eligible for elective cardiac surgery, the preoperative level of CRP should reflect low-grade inflammation, and for most patients it was not expected to be measurable by routine methods used in clinical practice.

**Strengths and limitations**

Strengths of the present study include the large number of patients and the completeness of data, with few missing observations. However, we cannot exclude that some unknown confounders that we have not adjusted for. Unfortunately, we did not have complete data on left ventricular ejection fraction, and we could therefore not control for the severity of chronic heart failure. Another limitation is that the end-point definition was partly based on clinical judgement, and was therefore less specific than end-points such as mortality or myocardial infarction. The use of data from only one institution may also have introduced a bias.

**CONCLUSION**

Neopterin was associated with cardiac dysfunction following cardiac surgery, and improved the accuracy of risk prediction of cardiac dysfunction after heart surgery, especially in patients with intermediate risk. At present, we do not suggest that neopterin be measured routinely before heart surgery, but our findings support the hypothesis of the role of oxidative stress and inflammation in development of cardiac dysfunction following heart surgery.

**SUPPLEMENTARY MATERIAL**

Supplementary material is available at ICVTS online.

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Conflict of interest: none declared.

REFERENCES


