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# C-reactive protein in the monitoring of acute rejection after heart transplantation

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**Abstract** Histological examination of endomyocardial biopsy (EMB) is the main technique for rejection surveillance after heart transplantation. This technique is elaborate, inconvenient for the patient, and not without complications. We prospectively analyzed whether the measurement of C-reactive protein (CRP), an acute phase protein that quickly rises when there is inflammation, can serve as a marker for immunological quiescence and as an indicator for withholding EMB. During a 6-month period, CRP was measured in all patients referred for EMB as part of the routine follow-up after heart transplantation. Acute rejection in patients with a follow-up of more than 1 year was rare (1/76). In the majority of cases, EMB was taken within the 1-year post-transplantation (170/246 = 69 %). In 71/170 biopsies (42 %), CRP was ≤ 1; in the other 99/170 (58 %), CRP was ≥ 2. When CRP was ≤ 1, acute rejection was diagnosed in 12/70 cases (17 %). In contrast, acute rejection was found in 28/99 cases (28 %) with CRP ≥ 2 ($P = 0.1$). Although CRP is elevated more often in the presence of acute rejection, its sensitivity does not allow CRP to replace the routine performance of EMB for monitoring rejection after heart transplantation. We did, however, find a prognostic significance with regard to the effect of rejection treatment: in all acute rejections with a CRP ≤ 3 ($n = 11$), steroids were effective.

**Key words** Heart transplantation, monitoring, C-reactive protein · Monitoring protein, heart transplantation, C-reactive protein · C-reactive protein, acute rejection, heart

## Introduction

Histological examination of endomyocardial biopsies (EMB) is the gold standard for rejection surveillance following heart transplantation. Increasing numbers of transplant recipients and their good survival rates result in vast numbers of EMB. This constitutes a considerable burden for the transplant program. Moreover, the invasive biopsy procedure is inconvenient for the patient and not without complications. Other methods to detect and monitor acute rejection after heart transplantation are being sought. An alternative strategy is to establish a marker for immunological quiescence and, therefore, for the absence of rejection. C-reactive protein (CRP), an acute phase protein produced in the liver that quickly rises when there is any inflammation, could be such a marker [4]. Daily measurements of CRP are known to parallel the degree of inflammation. Although immuno-

suppressive therapy has a depressing effect on the CRP response [16], changes in CRP concentrations have been found to correlate with acute rejection in renal transplant patients [6, 16].

We conducted a prospective study in heart transplant recipients to see whether measurement of CRP could help identify patients with a low probability of acute rejection, for whom routine EMB would be unnecessary. A second question we tried to answer in this study was whether CRP measurement could predict the outcome of antirejection treatment. Currently, it is not possible to predict whether an acute rejection will respond to steroid treatment. This may lead to a delay in adequate treatment if the rejection proves to be steroid-resistant. On the other hand, overtreatment is a likely consequence if all rejection episodes are primarily treated with ATG or OKT3. Biopsies of severe rejections show more inflammation and edema, and such rejections might be related to higher CRP levels than less severe rejection episodes.

## Patients and methods

From 30 October 1995 until 30 April 1996, we measured CRP (Array Protein System, Beckman, USA) in peripheral blood (ng/ml) from all heart transplant patients referred for EMB. Right ventricular endomyocardial biopsies (four samples per procedure) were obtained after gaining venous access via the internal jugular vein. A 9 Fr bioptome was used to obtain myocardial tissue samples.

In our heart transplant program, EMB are routinely performed weekly during the 1st 6 weeks, biweekly during the next 2 months, and monthly for the next 4 months after transplantation. Thereafter, the interval between EMB is gradually increased to twice yearly after 1 year. Biopsies are repeated more frequently after a rejection episode. All patients were on maintenance immunosuppression with cyclosporin and prednisone. In some patients, azathioprine had been added because of either recurrent rejections or cyclosporin nephrotoxicity.

Biopsies were graded according to the guidelines of the International Society for Heart and Lung Transplantation [7]. Biopsies graded 3A or higher were regarded as representing rejection episodes necessitating antirejection treatment. Acute rejections were treated with 1 gram methylprednisolone (Solu-Medrol) intravenously on 3 consecutive days. After treatment, control biopsies were taken 4–7 days after the last dose. Refractory or frequently recurring acute rejections were treated with rabbit ATG (RIVM, Bilthoven, The Netherlands) or OKT3 (Janssen-Cilag, Tilburg, The Netherlands).

This study was approved by the medical ethics committee of the University Hospital Rotterdam and all patients gave their informed consent for the CRP measurements. For the determination of levels of statistical significance, two-sided *P*-values were calculated using Fisher's exact test.

## Results

Within the 6-month study period, 267 EMB procedures were performed in 134 patients. In 2 EMB procedures, insufficient amounts of tissue were obtained, and in 19 other procedures CRP was not measured. The number of EMB procedures per patient ranged from 1 to 9. The results of 246 EMB-CRP combinations were available for analysis: 170 in patients with a follow-up of less than 1 year and 76 in patients with an EMB taken after a follow-up of more than 1 year after transplantation.

In EMB taken after the 1st year, the incidence of acute rejection was only 1/76 (1.3%). Within the 1st year, an acute rejection was diagnosed in 40/170 EMB (24%).

The results of these 170 cases are shown in Table 1. In 71/170 cases (42%), the CRP was ≤ 1; in the other 99/170 (58%), CRP was ≥ 2. In the biopsies with a CRP ≤ 1, 12/71 acute rejections (17%) were found. In the CRP ≥ 2 group, EMB showed 28/99 acute rejections (28%). In the cases with CRP ≥ 2 and acute rejection, CRP ranged from 2 to 36 (mean 9). After rejection treatment, subsequent CRP values were below the CRP rejection level in 65% of cases.

In Table 2, the result of steroid treatment for the rejection episode is shown. Clearly, in cases with a CRP ≤ 3, steroid treatment was effective in all (11/11) cases.

**Table 1** C-reactive protein (CRP) as a marker for the absence of acute rejection * $P = 0.1$

| | No rejection | Acute rejection | |
|---|---|---|---|
| CRP ≤ 1 | 59 | 12* | 71 |
| CRP ≥ 2 | 71 | 28 | 99 |
| | 130 | 40 | 170 |

**Table 2** The prognostic significance of C-reactive protein (CRP) for the effect of high-dose steroids as rejection treatment

| | Steroid-responsive | Steroid-resistant | |
|---|---|---|---|
| CRP ≤ 3 | 11 | 0 | 11 |
| CRP ≥ 4 | 8 | 7 | 15 |
| | 19 | 7 | 26 |

## Discussion

In the Rotterdam Heart Transplant Program, 307 heart transplantations have been performed since 1984. The number of EMB taken yearly has increased annually. In 1995, a total of 660 EMB procedures were performed. In centers where more heart transplants are being performed each year, the number of EMB procedures are even higher. This results in a considerable workload for cardiologists and pathologists. Moreover, the procedure is highly inconvenient for the patient and is associated with a number of complications [2]. To avoid EMB, alternative methods have been proposed for monitoring

acute rejection [1, 3, 8–10, 12, 18, 19]. However, the sensitivity and specificity of these techniques have not allowed widespread use and omission of EMB in the routine follow-up. Some groups advocate omitting routine EMB after 6 [14] – 12 [20] months post-transplantation.

Instead of looking for an alternative to EMB in the monitoring of heart transplant patients, we looked for a marker of immunological quiescence with high sensitivity. This would enable us to skip EMB safely. C-reactive protein (CRP) is an acute phase protein that quickly rises during inflammation [11]. Using CRP, it is not possible to distinguish the cause of inflammation, i.e. infection or rejection [5, 17]. Our hypothesis was that by measuring CRP it would be possible to select a large population of patients in whom the likelihood of acute rejection is very low. However, the sensitivity of this method does not appear to be high enough to allow us to stop taking EMB. Relying on CRP alone would have resulted in missing 12/40 acute rejections (30%). Although false-negative results may be obtained even with EMB [13], the false-negative rate of our CRP method was found to be too high. Whether there will ever be a noninvasive marker for rejection with sufficient sensitivity and specificity is doubtful. Replacing EMB procedures with those that determine of (semi-) continuous, noninvasive markers would overburden laboratory technicians and rejections could still be missed because of inappropriate timing with respect to sampling. Steinhoff et al. published data on urinary CRP concentrations, simultaneously measured with myeloperoxidase and α2-macroglobulin, after kidney transplantation [15]. Continuous monitoring of all three parameters would lead to high costs.

A major reduction in the number of EMB can be achieved by omitting routine EMB in patients who are more than 1 year post-transplantation. In our series, this would mean that 76/246 of the patients (31%) would not have had an EMB and that only one acute rejection would have been missed. Some centers have already adopted this approach [14, 16]. EMB, in the long-term management phase, should only be performed for special indications or on the basis of clinical symptoms such as cardiac arrhythmia, unexplained fatigue, or heart failure. We have now also adopted this strategy.

Our hypothesis that CRP levels might predict the outcome of high-dose steroids as antirejection treatment proved to be true. In all acute rejection episodes with a CRP $\leq 3$, this rejection proved to be steroid-responsive, whereas acute rejections with a CRP $\geq 4$ were steroid-responsive in only 8/15 cases (53%). A similar prognostic value for CRP was found in acute rejection after kidney transplantation [6]. Apart from having an impact on the choice of treatment, this may also have implications for control biopsies taken after antirejection treatment. A high likelihood of successful treatment reduces the need to perform early control biopsies.

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