

# Anti-vimentin antibodies are an independent predictor of transplant-associated coronary artery disease following cardiac transplantation

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## Introduction

Transplant-associated coronary artery disease (TxCAD), a rapidly progressing obliterative vascular disease developing in transplanted heart, is the major complication after the first year of cardiac transplantation<sup>1</sup>. A similar vasculopathy occurs following kidney transplantation where it is designated chronic rejection<sup>2</sup>. We have recently developed an enzyme-linked immunoassay for detection of anti-vimentin antibodies<sup>3</sup>. The aim of this study was to investigate whether anti-vimentin antibodies are also associated with development of TxCAD<sup>4</sup>.

## Methods

**Patient study group.** One hundred and nine patients who received orthotopic cardiac allografts at Harefield Hospital between 1987 and 1993 were studied. Their clinical details are described in table I<sup>4</sup>. Serum samples were collected pre-transplant and at 3, 6, 9, 12, 18, 24, 36, 48 and 60 months post-transplant. In total 880 samples were assayed, 109 pre-transplant and 771 post-transplant. Serum samples from 20 healthy subjects were used as negative control.

**Angiography.** Coronary angiograms were reviewed for TxCAD (> 25% stenosis of one or more coronary arteries in two successive annual angiograms). All patients underwent angiography annually, 1-5 years following transplantation.

**Acute rejection.** During the first post-transplant year all patients were monitored by surveillance endomyocardial biopsy. Biopsy

fragments were graded according to the histological criteria of the International Society of Heart and Lung Transplantation<sup>5</sup>. Indications of rejection in two or more subsequent biopsies spanning more than 3 weeks were considered as persistent rejection.

**ELISA for anti-vimentin antibodies.** Recombinant human vimentin (Cymbus Biotechnology Ltd, Chandlers Ford, Hants, UK) was used and the assay was performed as previously described. Results are given as mean titre unit  $\pm$  SE for IgM anti-vimentin antibodies. Normal sera (from 20 samples) gave a mean titre unit of  $53 \pm 32.1$ .

**Statistical analysis.** Difference in means were assessed using various tests as detailed in table I. A two-sided p value of < 0.05 was considered statistically significant. Kaplan-Meier was used to assess relationship between vimentin titre and time to develop TxCAD. Multivariate analysis was carried out using a logistic regression model and the Cox proportional hazards model for survival (time till occurrence of TxCAD).

## Results

**Correlation between anti-vimentin antibodies and transplant-associated coronary artery disease.** Thirty-eight patients out of 109 developed TxCAD at 5 years giving an incidence of 34.9%. The mean titre of anti-vimentin antibodies in patients prior to transplantation ( $59.0 \pm 11.4$ ) was not different to normal sera ( $53 \pm 32.1$ ). There was no significant difference between the pre-transplant titre units of anti-

**Table I.** Patient characteristics, mean values  $\pm$  SE and univariate analysis of association of risk factors with transplant-associated coronary artery disease (TxCAD).

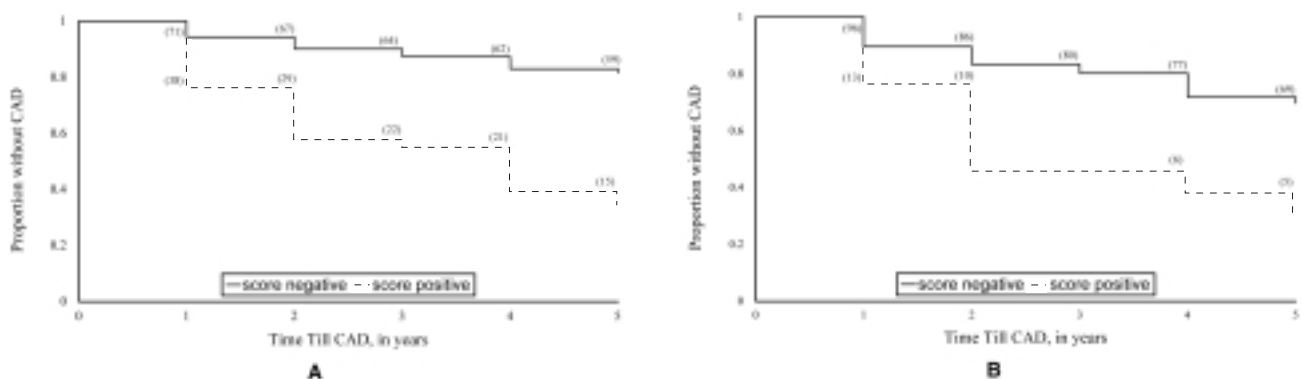
|                                     | TxCAD<br>(n=38) | Non-CAD<br>(n=71) | p      | Test used         |
|-------------------------------------|-----------------|-------------------|--------|-------------------|
| Recipient age (n=106)               | 47.8 $\pm$ 1.9  | 44.2 (1.6)        | 0.14   | 2-sample t-test   |
| Donor age (n=102)                   | 30.4 $\pm$ 1.8  | 26.6 (1.2)        | 0.084  | 2-sample t-test   |
| Recipient sex (n=109)               | M 35/F 3        | M 58/F 13         | 0.17   | Fisher exact test |
| Donor sex (n=107)                   | M 21/F 17       | M 41/F 28         | 0.69   | Fisher exact test |
| Diagnosis (n=109)                   | IHD 28/CM 10    | IHD 37/CM 34      | 0.04   | Fisher exact test |
| No. rejections in year 1 (n=107)    | 1.89 $\pm$ 0.20 | 1.41 $\pm$ 0.16   | 0.020  | Mann-Whitney      |
| Persistent rejection                | 17              | 9                 | 0.0002 | Fisher exact test |
| Not persistent rejection            | 19              | 62                |        |                   |
| HLA-A mismatch (n=99)               |                 |                   | 0.033  | $\chi^2$ test     |
| 0                                   | 7               | 3                 |        |                   |
| 1                                   | 11              | 31                |        |                   |
| 2                                   | 17              | 30                |        |                   |
| HLA-B mismatch (n=99)               |                 |                   | 0.89   | $\chi^2$ test     |
| 0                                   | 2               | 3                 |        |                   |
| 1                                   | 11              | 23                |        |                   |
| 2                                   | 22              | 38                |        |                   |
| HLA-DR mismatch (n=80)              |                 |                   | 0.57   | $\chi^2$ test     |
| 0                                   | 4               | 4                 |        |                   |
| 1                                   | 13              | 26                |        |                   |
| 2                                   | 10              | 23                |        |                   |
| Lipoprotein(a) (n=39) (mg/dl)       | 52.4 $\pm$ 11   | 47.8 $\pm$ 8      | 0.82   | Mann-Whitney      |
| Median                              | 48.8            | 37.3              |        |                   |
| Vimentin titre over 5 years (n=109) | 217 $\pm$ 25    | 112 $\pm$ 13      | 0.0001 | Mann-Whitney      |
| Median                              | 188             | 90                |        |                   |

CM = cardiomyopathy; HLA = human leukocyte antigen; IHD = ischaemic heart disease. From Jurcevic et al.<sup>4</sup>, with permission.

vimentin antibodies in patients who later developed TxCAD (73.0  $\pm$  2.7) and those who remained disease free at 5 years (51.4  $\pm$  9.7,  $p = 0.88$ ). The majority (107 out of 109) of patients increased their anti-vimentin antibody titres after transplantation. The average titre unit obtained over 5 years was significantly higher in the TxCAD positive group (217  $\pm$  25) than in the non-CAD group (112  $\pm$  13,  $p < 0.0001$ ). Assuming that chronic rejection is an ongoing process and in order to devise a predictive test that can be performed in the first one or 2 years after transplantation, we analysed the data from

the first 2 years only. The average titre for year 1 (188  $\pm$  30 for TxCAD vs 101  $\pm$  16 for non-CAD) and the average (combined) titre for years 1 and 2 (202  $\pm$  23 for TxCAD vs 105  $\pm$  14 non-CAD) were significantly higher in the TxCAD group than those who remained disease free at 5 years ( $p = 0.0038$  and  $p < 0.0001$ , respectively).

**Multivariate analysis for occurrence of transplant-associated coronary artery disease.** In this series of patients, number of rejection episodes, persistent rejection



**Figure 1.** A: Kaplan-Meier actuarial survival to demonstrate time to development of transplant-associated coronary artery disease (CAD) in patients with 1-year mean anti-vimentin titre  $\geq$  270 or persistent rejection (dotted line) compared to patients who are negative for this test (solid line). B: Kaplan-Meier actuarial survival to demonstrate time to development of transplant-associated CAD in patients with 1-year mean anti-vimentin titre  $\geq$  270 (dotted line) compared to patients who are negative for this test (solid line). Numbers of patients at risk at each time point are given in parenthesis. From Jurcevic et al.<sup>4</sup>, with permission.

tion, diagnosis (ischaemic heart disease) and number of matches at the human leukocyte antigen-A locus correlated significantly with TxCAD (Table I). Multivariate logistic regression demonstrated that persistent rejection, and both the 1-year mean titre and 2-year mean titres were independent predictors of TxCAD.

**Time till occurrence of transplant-associated coronary artery disease: Kaplan-Meier survival curve.**

Figure 1A shows the estimated probability of surviving without TxCAD for up to 5 years after transplantation. It is clear that patients with titre  $\geq 270$  or persistent rejection tend to have shorter times till occurrence of TxCAD. Patients with 1-year high antibody titre without persistent rejection (Fig. 1B) also show a shorter time to disease development.

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