Seropositivity for cytomegalovirus in patients with end-stage renal disease is strongly associated with atherosclerotic disease

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Abstract

Background. Infection with cytomegalovirus (CMV) is considered a risk factor for progression of atherosclerotic disease. Patients with end-stage renal disease (ESRD) display signs of frequent CMV re-activation, which may be caused by the uraemia-associated defect in cellular immunity. The possible contribution of CMV seropositivity to the hugely increased risk for cardiovascular disease in patients with ESRD is not clear.

Methods. In a retrospective study we analysed the clinical data of patients with ESRD that were evaluated for renal transplantation from January 2002 to March 2006. Classical cardiovascular risk factors and CMV seropositivity were related to the prevalence of atherosclerotic disease.

Results. A total of 408 patients were evaluated with a median age of 52 years (range 18–81 years). Multivariate logistic regression identified age (odds ratio; OR 2.7 per decade), smoking (OR 2.2), hypertension (OR 1.9), C-reactive protein (CRP) (OR 2.6) and CMV seropositivity (OR 2.7) as independent variables that were significantly associated with a positive medical history of atherosclerotic disease. The average titre for anti-CMV immunoglobulin G was higher in patients with atherosclerotic disease (100 AU/ml vs 71 AU/ml, P < 0.05). CMV seropositivity was independently associated with an elevated CRP. In addition, patients with the combination of a high CRP and CMV seropositivity showed the highest prevalence of atherosclerotic disease.

Conclusion. CMV seropositivity is significantly associated with atherosclerotic disease in ESRD patients. Our data suggest that the risk for progressive atherosclerosis is specifically increased in patients with an inflammatory response to CMV.

Keywords: atherosclerotic disease; C-reactive protein; cytomegalovirus; end-stage renal disease

Introduction

Patients with end-stage renal disease (ESRD) carry a greatly increased risk for cardiovascular disease [1]. In recent years, it has become evident that the risk for cardiovascular morbidity and mortality is already increased when the renal function is only moderately impaired [1,2]. Traditional risk factors like smoking, hypertension and hypercholesterolaemia can be identified but do not explain the full magnitude of the increment in risk [3]. A correlation exists between increased markers of inflammation and cardiovascular mortality in individuals with or without impaired renal function [2,3]. With the loss of renal function, these inflammatory parameters increase and strongly correlate with the concomitant increase of risk for cardiovascular disease [2]. Thus far, the causal relationship between markers of inflammation (e.g. C-reactive protein (CRP), interleukin-6 and cardiovascular disease remains obscure. For instance, CRP may reflect the inflammatory status of the atherosclerotic plaques, but inflammation by itself, as it occurs after infection, may foster the progression of atherosclerosis as well [4,5].

Animal models have confirmed that infection with cytomegalovirus (CMV) is capable of accelerating arteriosclerosis [6]. Seropositivity for CMV in humans has also been related to an increased risk for cardiovascular disease in several studies [7–10]. Subclinical CMV re-activation seems more prevalent in ESRD patients, and rarely, even CMV disease has been observed [11,12]. This may be caused by the uraemia-associated immune deficiency in these patients, which negatively affects the cellular immune responses [13,14]. As frequent CMV re-activation may worsen the burden of cardiovascular disease, CMV seropositivity may therefore, be particularly detrimental for ESRD patients.
To date, there is a paucity of data on CMV infection and cardiovascular disease in patients with progressive chronic kidney disease and such a relation could not be found for haemodialysis patients [15]. In this study, we retrospectively analysed the relationship between CMV seropositivity, inflammation and cardiovascular disease in ESRD patients that were evaluated before undergoing renal transplantation.

**Patients and methods**

**Patient population**

Patient data collected in the period between January 2002 and June 2006 were evaluated. Patients with ESRD, defined as a glomerular filtration rate of 15 ml/min or less with or without renal replacement therapy, were referred to our outpatient clinic of renal transplantation by their treating nephrologists. The referring area consists of the southeast part of the Netherlands and contains ~3 million inhabitants. First and second generation immigrants from outside Western Europe were classified as being from non-Western European origin.

**Clinical evaluation**

All patients referred to our outpatient clinic were screened for the presence of symptomatic coronary artery disease. Myocardial infarction (MI) reported in the medical history was confirmed if the medical record review demonstrated symptoms consistent with MI and the presence of either diagnostic ECG changes or cardiac enzymes. Evidence for cardiac ischaemia at the time of pre-operative evaluation was obtained by either graded exercise on a bicycle, or a dobutamine stress echocardiography was performed. These procedures were followed by coronary angiography when changes on the ECG were consistent with cardiac ischaemia. Signs of coronary atherosclerotic disease on coronary angiography were noted as either being absent or present.

Symptomatic carotid artery disease was considered to be present when an atherosclerotic diseased carotid artery had been documented by angiography or ultrasonography, in most cases after a cerebrovascular accident. A cerebrovascular accident because of atherosclerosis was considered to be present if confirmed by a computed tomography scan of the brain.

Atherosclerotic disease of the large peripheral arteries, including the aorta, was documented as being present if confirmed by angiography or computed tomography. The latter were performed because of symptoms of claudication intermittents, abnormalities noted on physical examination that indicated peripheral arterial stenosis or occlusion, or aneurysmal enlargement of the aorta.

**Risk factors**

Risk factors considered in this study included age, male sex, cigarette smoking, diabetes, hyperlipidaemia, hypertension, levels of CRP and seropositivity status to CMV. A history of past and current smoking was obtained. A patient who stopped smoking >10 years ago was considered not to have smoking as a risk factor. A patient was considered to have diabetes if he or she was taking insulin or oral hypoglycaemic agents or had previously received such treatment and was currently using dietary modification to control the condition. A patient was considered to have hyperlipidaemia if he or she had a serum cholesterol value of >6.5 mmol/l and/or was receiving antihyperlipidaemic treatment. A patient was considered to have hypertension if he or she had received the diagnosis, or was being treated with antihypertensive medications at the time of evaluation.

**CMV serology and CRP level**

Serum immunoglobulin G (IgG) antibodies to CMV were measured with an enzyme immuno assay (Biomerieux, VIDAS, Lyon, France) and expressed as arbitrary units/ml (AU/ml). Following the manufacturers guidelines, a test result exceeding 6 AU/ml was considered positive for the presence of CMV-specific IgG antibodies. The CRP level was measured with a fluorescence polarization immunoassay (TDxFLEX analyzer; Abbott Labs); 95% of healthy individuals had a CRP level of ≤8 mg/l.

**Statistical analysis**

Categorical data were analysed by the $\chi^2$ test. All statistical tests were two-sided. All covariates were examined in relation to the presence of atherosclerotic disease in a multivariate logistic regression analysis with forward and backward modelling. Covariates that were considered were age, sex, smoking, diabetes, hypercholesterolaemia, ethnicity, hypertension and a seropositive status for CMV. To analyse the association with underlying renal disease, the patients were grouped into two categories; hypertensive nephropathy and primary renal disease. The CRP levels were log-transformed before adding them as a covariate in the model. The upper level of normal serum CRP concentration (≤8 mg/l) was used to stratify patients into groups of high ($n = 60$) and non-high ($n = 120$) CRP levels. Differences between groups were analysed with the student’s $t$-test when the variable was normally distributed and otherwise by the Mann–Whitney test. The SPSS software version 10.1 was used for all statistical tests.

**Results**

**Clinical characteristics**

The medical records of 408 patients with ESRD were studied and the clinical characteristics are shown in Table 1. The percentage of CMV-seropositive patients was 68.7%, which is in the range of the reported prevalence of CMV seropositivity in the Dutch population [16]. Both age and ethnicity are known to influence the prevalence of CMV seropositivity, which was also observed in our group of patients (Figure 1). In the patients of non-Western European origin the prevalence for CMV seropositivity was 84% as compared with 66% in the other patients ($P < 0.01$). After the third decade, the seroprevalence of CMV infection stabilized for both groups.
Classical risk factors and CMV seropositivity and the prevalence of atherosclerotic disease

Age, smoking, hypertension and CMV seropositivity were statistically and independently associated with the presence of atherosclerotic disease by multivariate analysis (Table 2). All other clinical characteristics, as shown in Table 1, were not statistically significantly associated with atherosclerotic disease. The prevalence of atherosclerotic disease was 28% in CMV-seropositive patients vs 13% in CMV-seronegative patients (P-value <0.01). In each decade, the CMV seropositive patients had a higher prevalence of atherosclerotic disease (Figure 2).

The patients were grouped according to the underlying kidney disease into two categories; hypertensive nephropathy and primary renal disease. Only within the last category an association between CMV seropositivity and prevalence of atherosclerotic disease was observed with a high OR of 7.9 (P = 0.006).

Within the group of CMV-seropositive patients, the average titre of anti-CMV IgG was significantly higher (mean 100 AU/ml vs 71 AU/ml, P = 0.04) in patients with atherosclerotic disease (Figure 3).

CMV seropositivity and CRP concentration

CRP levels were available for analysis in 180 patients. Logistic regression modelling within this group of patients identified age (OR 2.5, P < 0.001), smoking (OR 3.2, P = 0.03), hypertension (OR 2.4, P = 0.05) and CRP levels (OR 3.1, P = 0.02) as independent covariables for the presence of atherosclerotic disease.

Table 1. Clinical characteristics of patients with end-stage renal disease before renal transplantation

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>408</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median and range in years)</td>
<td>52 (18–81)</td>
</tr>
<tr>
<td>Male</td>
<td>59.0%</td>
</tr>
<tr>
<td>Non-Western European origin</td>
<td>30.0%</td>
</tr>
<tr>
<td>Previous renal transplant</td>
<td>20.8%</td>
</tr>
<tr>
<td>Years after starting dialysis treatment</td>
<td>3 (0–29)</td>
</tr>
<tr>
<td>Not receiving dialysis treatment</td>
<td>15.2%</td>
</tr>
<tr>
<td>History of atherosclerotic disease</td>
<td>23.2%</td>
</tr>
<tr>
<td>– Cardiovascular</td>
<td>13.4%</td>
</tr>
<tr>
<td>– Cerebrovascular</td>
<td>6.5%</td>
</tr>
<tr>
<td>– Aorta/peripheral arteries</td>
<td>7.2%</td>
</tr>
<tr>
<td>Underlying kidney disease</td>
<td></td>
</tr>
<tr>
<td>– Hypertensive nephropathy</td>
<td>19.1%</td>
</tr>
<tr>
<td>– Primary glomerulopathy</td>
<td>30.5%</td>
</tr>
<tr>
<td>– Diabetic nephropathy</td>
<td>8.2%</td>
</tr>
<tr>
<td>– Polycystic kidney disease</td>
<td>13.6%</td>
</tr>
<tr>
<td>– Other</td>
<td>12.3%</td>
</tr>
<tr>
<td>– Unknown</td>
<td>16.1%</td>
</tr>
</tbody>
</table>

Prevalence of risk factors for atherosclerosis

| Hypertension | 48.3% |
| Hyperlipidaemia | 15.9% |
| Smoking | 41.4% |
| Diabetes mellitus | 15.3% |

Table 2. Odds ratio of risk factors and a positive history of atherosclerotic disease in patients with end-stage renal disease (n = 408)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Significance</th>
<th>Odds ratio</th>
<th>95.0% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age^a</td>
<td>0.000</td>
<td>2.6</td>
<td>1.9</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.343</td>
<td>1.3</td>
<td>0.7</td>
</tr>
<tr>
<td>DM^b</td>
<td>0.236</td>
<td>1.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.017</td>
<td>2.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Ethnicity^c</td>
<td>0.277</td>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.037</td>
<td>1.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>0.561</td>
<td>0.8</td>
<td>0.3</td>
</tr>
<tr>
<td>CMV^d</td>
<td>0.011</td>
<td>2.7</td>
<td>1.2</td>
</tr>
</tbody>
</table>

^a per 10 years, ^b diabetes mellitus, ^c non-Western European, ^d cytomegalovirus seropositivity.

All odds ratios shown were calculated after multivariate adjustment.

Fig. 1. The percentage of CMV-seropositive patients with end-stage renal disease in relation to age (shown per decade) and ethnic origin (n = 408). The patients are divided as being from a non-Western European origin (filled bars) vs patients from a Western European origin (open bars).

Fig. 2. The prevalence of atherosclerotic disease in patients with end-stage renal disease per decade (n = 408). The filled bars represent the cytomegalovirus-seronegative patients and the open bars the CMV-seropositive patients. The numbers below the x-axis represent the number of patients in each category.
CMV seropositivity showed an OR of 2.7 but with a P-value of 0.11.

In CMV-seropositive patients, the median CRP level was significantly increased compared with CMV-seronegative patients (median 3 mg/l vs 6 mg/l, P = 0.04). Multivariate logistic regression showed that both age (OR per decade 1.4, range 1.0–1.8, P = 0.02) and CMV seropositivity (OR 2.5, range 1.0–6.35, P = 0.05) were independently associated with a high level of CRP (>8 mg/l). These findings indicated a relation between CMV seropositivity and levels of CRP. Therefore, patients were stratified according to CRP level (above 8 mg/l or not) and the presence of CMV seropositivity (Figure 4). The data show that the prevalence of atherosclerotic disease is particularly high (54%) in the group of patients with CMV seropositivity and a high CRP, compared with 13% in CMV-seronegative patients without a high level of CRP. Similar results were obtained when the median CRP level (above 5 mg/l or not) was used to stratify patients (data not shown). The interaction between CMV and CRP remained present after adjusting for the covariables age, smoking and hypertension.

**Discussion**

In this study we have shown a strong association between CMV seropositivity and the prevalence of atherosclerotic disease in patients with ESRD. The only other published report on this subject by Wolf et al. [15], did not show such an association in haemodialysis patients. The high prevalence of CMV seropositivity (100%) in their cases, the overall longer duration of dialysis treatment and the lack of stratification for underlying kidney disease, may account for the lack of association.

In our study, the prevalence of atherosclerotic disease was greatly increased in the CMV seropositive ESRD patients with a high serum CRP level. This finding is remarkably similar to the results of several studies in patients with normal renal function [8–10] and the association between CMV seropositivity, a high CRP and atherosclerotic disease seems even stronger in ESRD patients. Also, prospective studies showed that CMV-seropositive patients, with proven cardiovascular disease and an elevated serum CRP or interleukin-6 concentration, were at a specifically high risk for a lethal cardiovascular event [9,10].

However, the association between CMV seropositivity and atherosclerosis is not unequivocal in epidemiological studies. The relation seems most consistent when CMV seropositivity is considered as a risk factor for secondary atherosclerotic events [7]. Also, in animal models in which CMV is shown to be pro-atherogenic, aggravation of pre-existing atherosclerosis and not a primary CMV-induced atherosclerosis is studied [6].

Loss of renal function is an independent risk factor for atherosclerosis and patients with ESRD may be considered as atherosclerosis prone [1–3]. Also, in this study, the prevalence of atherosclerotic disease among patients with ESRD was as high as 23% and well above 40% in patients 60 years and older. The prevalence is probably even underestimated, as subclinical atherosclerosis was not recorded. Therefore, CMV seropositivity is likely to play a role as an atherosclerosis-promoting factor in an already diseased vascular system, such as the arteries of patients with ESRD.

Animal studies have indicated that the cellular immune response and not CMV infection per se is responsible for the pro-atherogenic activity of CMV [6]. Serological studies support the concept of frequent CMV re-activation in ESRD patients and rarely even CMV disease has been reported [11,12]. In addition, we recently showed that the relative and total numbers of a subset of circulating T cells, known to be specific for
CMV, increases as the loss of renal function progresses and cellular immunity decreases [14]. The CMV-specific T cells (both CD4 and CD8-positive T cells) are potent effector cells, easily secreting cytokines upon stimulating and displaying a highly cytotoxic potential [17]. In a series of studies, it was shown that this type of CD4-positive T cells is related to instable atherosclerotic plaques [18,19]. In a mouse model carrying a human carotid artery plaque, these cells were shown to invade and increase inflammation in the atherosclerotic plaques, in the absence of CMV infection [19]. Therefore, one may hypothesize that frequent subclinical CMV re-activation in ESRD patients will generate an increasing number of circulating T cells with the potential of increasing the inflammation of atherosclerotic plaques. This hypothesis obviates the need for CMV antigen within the atherosclerotic plaque, which has not been convincingly documented thus far [20]. In this study, the group of CMV-seropositive patients with atherosclerotic disease had a significant higher titre of CMV antibodies, confirming earlier data on the presence of subclinical atherosclerosis in the carotid artery in relation to CMV seropositivity [7]. This observation supports the concept that frequent CMV re-activation may indeed play a part in the pathogenesis of atherosclerotic disease in patients with ESRD.

Limitations

Admittedly, there are inherent limitations of a retrospective study design and a possible unrecognized bias in our results might exist. However, several observations support the concept of a true association between CMV seropositivity and atherosclerosis. First, the prevalence of CMV and the relation with age within our patient group are consistent with published data of the Dutch population and therefore, do not indicate a major selection bias [16]. Second, several known traditional risk factors like age, smoking and hypertension were found to be associated with the presence of atherosclerotic disease, indicating an adequate data collection. In this respect, the observed strong association between CRP levels and atherosclerotic disease is also in line with previous published studies [2,3] and confirms the firmness of our data. We could not identify hypercholesterolaemia, sex and diabetes mellitus as risk factors for atherosclerosis. Possible explanations could be that dyslipidaemia in ESRD patients is not that strongly associated with atherosclerotic disease [1] and the number of patients with diabetes mellitus was relatively low.

The results of this study and others indicate that only when CMV seropositivity causes an inflammatory response, is the risk for progressive atherosclerotic disease increased. Areas of uncertainty include whether the elevated CRP level is triggered by an increased systemic inflammation, either CMV induced or not, or by increased inflammation of the atherosclerotic plaques. Also, it is not known if the CMV-specific T-cell responses differ in quantity or quality between patients with or without an elevated CRP. To date, our concept of frequent CMV re-activation in ESRD patients is hypothetical although supported by the above-mentioned immunological data on CMV IgG titres and CMV-specific T cells. Direct evidence, e.g. by detection of CMV antigens or DNA, is as yet not available.

In conclusion, we have identified CMV seropositivity as an independent variable associated with the presence of atherosclerotic disease in patients with ESRD. Particularly, patients with CMV seropositivity and an inflammatory response show a very high prevalence of atherosclerotic disease.

Conflict of interest statement. None declared.

References


17. van Leeuwen EM, Remmerswaal EB, Vossen MT et al. Emergence of a CD4<sup>+</sup>CD28<sup>-</sup> granzyme B<sup>+</sup>, cytomegalovirus-specific T cell subset after recovery of primary cytomegalovirus infection. *J Immunol* 2004; 173: 1834–1841


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