Anti-Cardiolipin Antibodies and Overall Survival in a Large Cohort: Preliminary Report

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Background: Anti-cardiolipin antibodies have been associated with both arterial and venous thrombosis, but their overall impact on all-cause or vascular mortality is unknown. In this study, we evaluated the influence of anti-cardiolipin antibodies on all-cause and vascular mortality.

Methods: All individuals who fulfilled the inclusion criteria (completeness of data, no admission from an intensive care unit, unique identification with name and date of birth) and whose anti-cardiolipin antibodies were measured between October 2002 and February 2004 were included in this study (n = 4756; 64% female; median age, 46 years). Death/survival and cause of death were obtained from the Austrian Death Registry. The median observation period was 1.5 years, and the study comprised 7189 person-years.

Results: During the study period, 184 patients (3.9%) died. There were no associations between either anti-cardiolipin IgM or IgG antibodies and both vascular death and noncancer mortality as outcome variables in a Cox regression analysis adjusted for age and sex. In contrast, the risk of cancer-related mortality was increased 2.6-fold.

Conclusions: Anti-cardiolipin antibodies are associated with cancer mortality, likely as an epiphenomenon of malignancy, but they are not predictive of vascular mortality or noncancer mortality. Hence, although a clear association between anti-cardiolipin antibodies and (mostly nonfatal) vascular events has been described in the literature, our data indicate that this finding is not necessarily associated with an increase in vascular mortality.

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Anti-cardiolipin antibodies are a heterogeneous family of autoantibodies directed against protein–phospholipid complexes (1, 2). Most anti-cardiolipin antibody immunoassays require β2-glycoprotein I to react with anti-cardiolipin (3). The presence of these antibodies is 1 of 2 laboratory diagnostic criteria for antiphospholipid syndromes characterized by venous and arterial thrombosis, recurrent abortion, and thrombocytopenia (2). Anti-cardiolipin antibodies are observed in a large proportion of patients with systemic lupus erythematosus (4) and other autoimmune diseases, but also in patients without any apparent autoimmune diseases (5, 6). Anti-cardiolipin antibodies have also been identified as independent risk factors for venous (5, 7) and arterial thrombosis leading to ischemic stroke (8) and myocardial infarction (6) in individuals with and without underlying autoimmune diseases (9).

Most anti-cardiolipin studies have been retrospective analyses. However, increased anti-cardiolipin antibodies are not uncommon in healthy individuals (10, 11) without clinical implications. As a consequence, in some prospective cohort studies, the association between anti-cardiolipin antibodies and thrombosis could not be confirmed (12, 13).

To date, the impact of positive anti-cardiolipin antibodies on survival and vascular mortality has not been assessed in a large patient sample. Such an assessment could yield an objectively defined endpoint that would allow more accurate risk estimates, whereas previous studies had very heterogeneous endpoints with mostly limited sample size. To evaluate the relevance of anti-cardiolipin antibodies as a potential predictor for overall and vascular mortality, we performed a database query of
our laboratory computer system and analyzed overall survival, noncancer mortality, and all-cause vascular mortality in a large-scale cohort.

**Patients and Methods**

**Patients**
All first-ever admissions to the Clinical Institute for Medical and Chemical Laboratory Diagnostics for determination of anti-cardiolipin antibodies between October 2002 and February 2004 were included in our study. Inclusion criteria were valid anti-cardiolipin antibody IgG and IgM determinations and complete patient data required for successful record linkage, including sex, name, and date of birth. Exclusion criteria were incomplete patient data and admission from an intensive care unit. Record linkage was performed via database query of the Austrian Death Registry, which gave date of death (if it occurred between October 2002 and December 2004) and cause of death encoded according to the International Code of Diseases, Ver. 10 (ICD10).4 The Austrian Death Registry comprises all deaths within Austria and the deaths of Austrian citizens in foreign countries if reported to Austrian officials. According to Austrian laws, an autopsy must be performed if the final cause of death is not evident from the patient’s history; the overall autopsy frequency in our study was 30%. For statistical analysis only, coded data were used containing no personal information except age in years and sex. The study was approved by the local ethics committee.

**Anti-Cardiolipin Antibody Measurements**
Measurements of anti-cardiolipin IgM and IgG antibodies were performed in patent serum within routine laboratory analyses, using Varelisa Cardiolipin IgG antibodies and Varelisa Cardiolipin IgM antibodies (both from Pharmacia Diagnostics) according to the manufacturer’s instructions. Both assays are based on the principle of ELISA with cardiolipin-coated polystyrol plates and β2-glycoprotein as a cofactor for anti-cardiolipin antibodies added within the sample buffer. The measuring range was 1–100 kilounits/L. The assays showed a total (inter- and intraassay) variability between 6.6% (IgM Varelisa) and 9.8% (IgG Varelisa). Both assays had reference intervals of <10 kilounits/L for negative anti-cardiolipin antibodies, 10–15 kilounits/L for equivocal, and >15 kilounits/L for positive anti-cardiolipin antibodies.

**Determination of Outcome Variables and Statistical Analysis**
The main outcome variable was all-cause mortality, defined as death occurring after anti-cardiolipin antibody measurement and before December 31, 2004, regardless of diagnosis. Noncancer mortality was defined as death occurring for causes other than neoplasia (defined as ICD10 groups C00 to D99); all-cause vascular mortality was considered present in ICD10 groups I00 to I99, including the arterial and venous system. Observation time was calculated in years from anti-cardiolipin antibody measurement to death or until the end of the observation period (December 31, 2004) in case of survivors. Age was calculated in years at time of anti-cardiolipin antibody measurement.

To facilitate analysis, individuals with anti-cardiolipin antibody concentrations above the reference limit (>15 kilounits/L) were grouped together and compared with individuals with anti-cardiolipin antibody concentrations <15 kilounits/L, who served as the reference category. The rationale for this categorization is the previously reported association of anti-cardiolipin antibodies with venous or arterial thrombosis; thus, individuals with anti-cardiolipin antibodies below the reference limit should have a lower risk for thrombosis. The influence of increased anti-cardiolipin antibody concentrations on all-cause, cancer, noncancer, and vascular mortality was assessed in a multivariate Cox regression adjusting for sex and age as possible confounders. To evaluate the effect of positive IgM or IgG autoantibodies, relative risks and 95% confidence intervals (95% CIs) were calculated as hazard ratios (HRs) derived from the Cox proportional-hazards regression model. Multivariable models were fitted by use of the available clinical covariates. The assumptions underlying the proportional-hazards model (proportional hazards, lack of interaction, and linearity of continuous variables) were tested and found valid unless otherwise indicated.

We performed regression analysis according to standard recommendations. A two-sided P value <0.05 was considered statistically significant. Unless otherwise stated, all continuous variables are given as the median and interquartile range and categorical variables as the number and percentage.

**Results**
A total of 4756 patients were included in our study population. Patient characteristics and prevalence of the antibodies are reported in Table 1. The median observation period was 1.5 years, giving a total of 7189 person-years studied (Table 1). Women were overrepresented in our sample (64%); however, increased anti-cardiolipin antibodies were equally frequent in women (6.9%) and men (6.0%; P = 0.31). We obtained similar results when we compared women older and younger than 46 years, indicating no relevant association with menopausal status. Within the observation period, a total of 184 (3.9%) deaths were recorded.

In approximately one third of the patients who died during the study, neoplasia was the cause of death (n = 60; 1.3%), and in another one third, death was attributable to any kind of vascular disease (n = 57; 1.2%). IgM anti-cardiolipin antibodies were present in concentrations

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4 Nonstandard abbreviations: ICD10, International Code of Diseases, Ver. 10; 95% CI, 95% confidence interval; and HR, hazard ratio.
Table 1. Characteristics of the study population (n = 4756).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M/F (%)</td>
<td>1721/3035 (36.2/63.8)</td>
</tr>
<tr>
<td>Median (IQR)a age, years</td>
<td>46 (33–61)</td>
</tr>
<tr>
<td>Median (IQR) observation period, years</td>
<td>1.5 (1.2–1.9)</td>
</tr>
<tr>
<td>All-cause mortality, n (%)</td>
<td>184 (3.9)</td>
</tr>
<tr>
<td>Noncancer mortality, n (%)</td>
<td>124 (2.6)</td>
</tr>
<tr>
<td>Cancer-related mortality, n (%)</td>
<td>60 (1.3)</td>
</tr>
<tr>
<td>Vascular mortality, n (%)</td>
<td>57 (1.2)</td>
</tr>
</tbody>
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Patients with increased anti-cardiolipin antibodies, n (%)
- Increased anti-cardiolipin IgM: 199 (4.2)
- Increased anti-cardiolipin IgG: 178 (3.7)
- Any anti-cardiolipin antibody increased: 340 (7.1)

*a IQR, interquartile range.

above the upper reference limit in 199 (4.2%) patients, and 178 (3.7%) had increased IgG anti-cardiolipin antibodies, giving a total of 340 individuals (7.1%) with either increased IgG or IgM anti-cardiolipin antibodies, or both.

In a multivariate Cox regression adjusted for sex and age as possible confounders, only IgM anti-cardiolipin antibody concentrations were significantly associated with all-cause mortality [P = 0.03; HR = 1.84 (95% CI 1.06–3.18); Fig. 1]. In contrast, neither IgG anti-cardiolipin antibody nor (any) anti-cardiolipin antibody concentrations were significantly associated with all-cause mortality (Fig 1). Anti-cardiolipin antibodies were significantly associated with cancer mortality [HR = 2.57 (95% CI, 1.03–6.42) for IgG anti-cardiolipin antibodies and HR = 2.63 (95% CI, 1.13–6.14) for IgM anti-cardiolipin antibodies] in a Cox regression adjusted for sex and age. This effect was not age dependent. There was no significant association of any increased anti-cardiolipin antibodies with noncancer or vascular mortality (Figs. 1 and 2).

Results remained essentially unchanged after we categorized patient data according to clearly negative anti-cardiolipin antibody results [<10 kilounits/L; n = 4144 (87.1%)], equivocal results [10–15 kilounits/L; n = 340 (7.1%)], and positive anti-cardiolipin antibody [>15 kilounits/L; n = 340 (7.1%); see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol52/issue6/]. Interestingly, death rates in individuals with equivocal results for either IgG or IgM anti-cardiolipin antibodies (4%) were similar to those in antibody-negative patients (3.7%), whereas death rates for patients with increased anti-cardiolipin antibodies were slightly higher (5.3%; P = 0.36). Results remained essentially unchanged after stratification into short-term (death within 6 months) and long-term (survival >6 months) follow-up groups.

**Discussion**

Anti-cardiolipin antibodies have been associated with both arterial and venous thrombosis. In our study, we evaluated the prognostic value of anti-cardiolipin antibodies in regard to survival in a large population. Both increased anti-cardiolipin IgG and IgM were associated with a higher risk of cancer mortality. This finding seems plausible because anti-cardiolipin antibodies are frequently observed in individuals with malignant tumors (14, 15). Because the median observation period was only 1.5 years, it is likely that these cases already had clinically manifest malignancies at the time of anti-cardiolipin antibody measurements. Thus, increased anti-cardiolipin antibody concentrations seem to occur as an epiphenomenon of malignancy.

On the other hand, we were unable to identify any association between anti-cardiolipin antibodies and vascular death or noncancer mortality, including all causes of death except neoplasia. These findings are in contrast to previous studies establishing anti-cardiolipin antibodies as risk factors for both arterial and venous thrombosis (16). However, because treatment of patients with venous thrombosis is highly effective and overall mortality is low in this patient group (17), the impact on mortality is most probably much smaller.

Another reason for the lack of association of anti-cardiolipin antibodies with vascular mortality might be the limited number of cases (n = 57) in our study. However, this number should be sufficient to detect at least a 3-fold increase in vascular mortality with a statistical power of 80%. Many studies that found a positive association between anti-cardiolipin antibodies and arterial or venous thrombosis were performed retrospectively in selected patient groups and could not be confirmed in prospective studies, especially in patients without lupus anticoagulants (12, 13). Unfortunately, data on the presence of lupus anticoagulant were not available for our patient cohort.

Anti-cardiolipin antibodies can be detected in up to 7% of the healthy population (10, 11, 18) and might be present in increased concentrations in selected patient groups, such as those suffering from chronic viral infections, including HIV or hepatitis. In addition, Delgado...
Alves et al. (19) reported that anti-cardiolipin antibodies frequently interact with other plasma lipids, including HDL-cholesterol and apolipoprotein A1. Thus, measurement of anti-cardiolipin antibodies might be biased in patients with increased plasma lipids. Unfortunately, HDL concentrations were not available in our patients, and we can only speculate whether anti-cardiolipin antibody concentrations correlate with plasma lipids, although this finding undoubtedly would be of clinical relevance, especially when assessing cardiovascular risk profiles.

In contrast to anti-cardiolipin antibodies, lupus anticoagulant is rarely present in asymptomatic individuals (18); thus, our findings cannot be applied to other markers of antiphospholipid syndromes, such as lupus anticoagulant.

Interestingly, in our study, individuals with equivocal results showed an overall mortality similar to that for patients with negative anti-cardiolipin antibodies, indicating that the risk for these individuals might be comparable to the risk for antibody-negative patients.

**STRENGTHS AND LIMITATIONS OF THIS STUDY**

In our study, we evaluated the association of anti-cardiolipin antibodies with mortality in a large population. The chosen record linkage approach of linking laboratory data with the death registry offers a unique opportunity to evaluate the outcome of risk markers in a large population (n = 4756), yielding an observation period of 7189 person-years. Because Austrian laws stipulate that all deaths must be recorded in the central death registry, this approach allows an almost complete follow-up of all patients. The only losses might occur as a result of spelling errors in names, leading to faulty record linkage, or missing persons, who are not recorded as deaths until 50 years after the date of disappearance. Overall, we estimate that these losses affected less than 1% of the study population and are negligible for statistical analysis. In contrast to clinical diagnoses, which are subject to examiner bias and usually vary because of different diagnostic criteria, death is usually reliably recorded and misdiagnoses rarely occur. Because of the high autopsy frequency (~31%) and the Austrian legal situation, we estimate that cause-of-death diagnoses were recorded correctly in >90% of the cases.

Our study had several limitations. Patients admitted for determination of anti-cardiolipin antibodies are not a representative sample of the healthy Austrian population and might be preselected to worse outcome. Thus, the prevalence of increased anti-cardiolipin antibodies within the Austrian population might be lower than that reported in our study (Table 1). In addition, anti-cardiolipin assays are still poorly standardized.

In our study, we used a commercial assay with highly reproducible results. However, we are well aware that reproducibility among assays from different manufacturers is still far from completely satisfactory (20, 21). Thus, we cannot exclude the possibility that different anti-cardiolipin assays might yield different results regarding risk prediction. We are well aware that endpoints other than vascular mortality associated with increased anti-cardiolipin antibodies (e.g., pulmonary embolism, non-fatal stroke, or myocardial infarction) were not covered in our study. Venous thrombosis, if properly treated, leads only infrequently to death through pulmonary embolism (17).

Another limitation of our study is the moderate length of the median observation period (1.5 years). Although to our knowledge this is the largest study evaluating anti-cardiolipin antibodies and survival, we cannot exclude an effect of anti-cardiolipin antibodies on long-term survival.
In conclusion, in our study anti-cardiolipin antibodies were associated with cancer mortality, likely as an epiphenomenon of malignancy. However, they were not predictive of vascular mortality or noncancer mortality. Although there is agreement within the literature that anti-cardiolipin antibodies are associated with nonfatal arterial and venous thrombosis, our data indicate that they were not major predictors of vascular mortality in a large hospital-based cohort of patients.

This work is part of the AKH Biobank Project, with the aim of identifying new biomarkers within the Austrian population. We would also like to thank Peter Bayer (Statistik Austria, Vienna) for providing data from the Austrian Death Registry.

References