Anticardiolipin and antinuclear antibodies in cancer patients - A case control study

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Abstract

Objective

To investigate patients with cancer for the frequency of IgG and IgM aCL and ANA in comparison to a group of age- and sex-matched controls.

Methods

Serum levels of IgG and IgM anticardiolipin antibodies (aCL), antinuclear antibodies (ANA) and anticytoplasmic antibodies were evaluated in 145 cancer patients, including 20 patients with thromboembolic disease (TED) and compared with age- and sex-matched controls.

Results

Higher levels of IgG aCL were found in patients compared to controls ($p < 0.02$). However, there appeared to be no difference in serum aCL levels between TED and the remaining cancer patients. No difference was found in the frequency of antinuclear and anticytoplasmic antibodies between patients and controls and the autoantibody presence in patients was usually not associated with concomitant autoimmune disease.

Conclusion

Apart from increased levels of non-thrombogenic associated IgG aCL, there was no evidence for significantly enhanced B cell autoreactivity in this large collection of cancer patients compared to controls.

Key words
Anticardiolipin antibodies, antinuclear antibodies, cancer, thrombophlebitis.

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Introduction

Trousseau first drew attention to the clinical association between venous thrombosis and malignancy in a series of patients with gastric carcinoma (1). The overall incidence of clinical thromboembolic disease (TED) has been reported to vary between 1% and 11% (2, 3), but is considerably higher in post-mortem studies of cancer patients (4, 5). Historically, pancreatic carcinoma has been associated with the greatest risk of TED, although recent studies suggest a high frequency in patients with carcinoma of the lung (5, 6). Several reports state that chemotherapy, hormone therapy or surgery, increase the risk of TED in cancer patients (7, 8).

Disturbances in coagulation (9) and the presence of anticardiolipin antibodies (aCL) (10) have been observed in patients with cancer. Two recent studies described an association between cancer and aCL positivity in large series of patients with malignancy (11, 12). The association between aCL and TED was first described in patients with systemic lupus erythematosus (SLE), but has subsequently been used to define a primary antiphospholipid antibody syndrome (APS) (13, 14). Recent attention has focused on the role of phospholipid cofactors that may contribute to the generation of aCL and the vascular manifestations of APS (15-17).

Autoantibodies to intracellular antigens have been detected in the majority of the autoimmune diseases, and antinuclear antibodies (ANA) are used as serologic markers for a variety of diseases such as SLE, Sjögren's syndrome and scleroderma (18). An association between ANA and various malignancies has been reported (19), but other studies have shown conflicting results (20).

In the current study we have investigated patients with a range of malignancies for the frequency of IgG and IgM aCL and ANA in comparison to a group of age- and sex-matched controls. Special attention was given to the detection of TED in the cancer population. We have compared aCL levels between patients and controls, between several cancer pathologies and between patients with and without TED. The influence of stage and treatment of the cancer on autoantibody levels was also considered.

Materials and methods

Patients and controls

We studied 145 consecutive cancer patients admitted to the Unit of Hematology/Oncology of the Hospital de Santo Espirito de Angra do Heroísmo, from October 1992 to October 1993. 145 paired controls individually matched for age and sex comprised blood donors, hospital technicians and hospital visitors. TED was diagnosed by phlebography and/or CAT scan in patients with thrombophlebitis; CAT scan was used for the diagnosis of stroke and the one case of pulmonary embolism was diagnosed on clinical grounds and CAT scan. We have also included under the designation of TED the cases diagnosed with superficial thrombophlebitis.

The 145 patients with cancer included 65 males (age range 29 - 86 yrs.) and 80 females (age range 28 - 83 yrs.) with the following primary organ involvement: lung - 11; ear, nose and throat - 7; breast - 34; prostate - 13; stomach - 6; colon - 17, lymphoma -15; gynecological - 17; bladder - 9; others - 16. Patients were considered to have active disease when cancer was detected by clinical examination, blood tests or radiology. They were considered to be in remission if cancer was not detected by the same criteria. All were followed for a minimum of 3 years or until they had a terminal event.

Autoantibody measurement

Blood was collected by venepuncture in both patients and controls, and after clotting was centrifugated at 4000 x g. Serum was stored at -70°C until assayed. aCL beta-2 glycoprotein I (β_2 GPI) dependent and independent IgG and IgM antibodies were detected by ELISA (SELISA, Walker Laboratories, UK). The concentrations were calculated from a 6-point standard curve and referred to as GPL and MPL units (21). Patients with more than 15 GPL or MPL units were considered positive. aCL IgG and IgM antibodies were measured 3 years later in the 9 patients who were still alive and still had high levels of these antibodies using the same methodology.

Screening for ANA was done by indirect immunofluorescence using HEp-2 cells (MarDx Diagnostics, Inc. Germany). The positive results (> 1/40) were con-

firmed using HEp-2 cells (Biodiagnostics, Upton-upon-Severn, UK). Hep-2 cells were incubated for 1 hour with serum diluted 4-fold in phosphate buffered saline (PBS) (from 1:40 to 1:2,560). Washed cells were incubated in fluorescein isothiocyanate-labeled goat anti-human polyvalent conjugate (1:20). Following a second wash, the slides were mounted in glycerol/PBS containing 2.5% DABCO (1,4-diazobicyclo-[2,2,2]-octane) and viewed under a Leitz fluorescence microscope. Ouchterlony double immunodiffusion was carried out on all serum samples that were positive for ANA or for anticytoplasmic antibodies using extracts enriched for all common extractable nuclear antigens (Bradshaw Biologicals, Market Harborough, UK and Biodiagnostics Ltd, Upton-Upon-Severn, UK). Lines of identity were sought with prototype sera of known autoantibody specificities, including sera showing reactivity with topo I, Ro, La, U1RNP, Sm, Jo-1, Pm-Scl, rRNP and Ku.

Statistical methods

Data were entered onto a PC computer and analysed using the SPSS (Statistical Package for Social Sciences) software package. The differences in the serum levels of IgG and IgM aCL antibodies between patients and matched controls were studied using a paired t-test. An estimate of the mean difference, together with a 95% confidence interval, was calculated. In addition, the paired differences were compared in terms of disease status and the presence or absence of treatment using the t-test. Comparisons across the 10 cancer pathologies were performed using the Kruskal-Wallis test. Using the cut-off point of 15.0 units as indicating 'positivity' for IgG and IgM aCL, and greater than 1/40 for ANA and anticytoplasmic antibodies, differences in autoantibody positivity between patients and controls were assessed by the McNemar test. Differences in the proportions of TED patients across disease status and treatment characteristics were analysed using chi-square tests.

Results

aCL in cancer patients

Patients with cancer had significantly higher serum levels of IgG aCL than controls ($p < 0.02$; mean difference = 1.996; 95% CI (0.457; 3.535), Fig. 1). For IgM aCL no significant difference was found between patients and controls ($p > 0.21$; mean difference = -0.873; 95% CI (-2.263; 0.516), Fig. 2). Taking 15 units as the cut-off for a positive IgG aCL result, patients had a higher frequency of positivity ($p < 0.003$) (Fig. 1). On the other hand 'positivity' for IgM aCL was similar in patients and controls ($p = 1.0$). Analysis of the paired differences in serum aCL levels between patients and controls revealed no significant differences between patients in complete remission versus those with active disease (IgG $p > 0.63$; IgM $p > 0.38$) and for those on treatment versus those not on treatment (IgG $p > 0.75$; IgM $p > 0.48$). Furthermore, no significant differences were found across the various cancer pathologies (IgG $p > 0.15$; IgM $p > 0.17$) or between patients who were alive compared with the group who had died in the 3-year period (IgG $p > 0.43$; IgM $p > 0.86$).

Repeated aCL tests

Nine patients with high levels of IgG or IgM aCL were re-tested for aCL years later (Table I). Five of 7 patients with initial elevation of IgG aCL continued

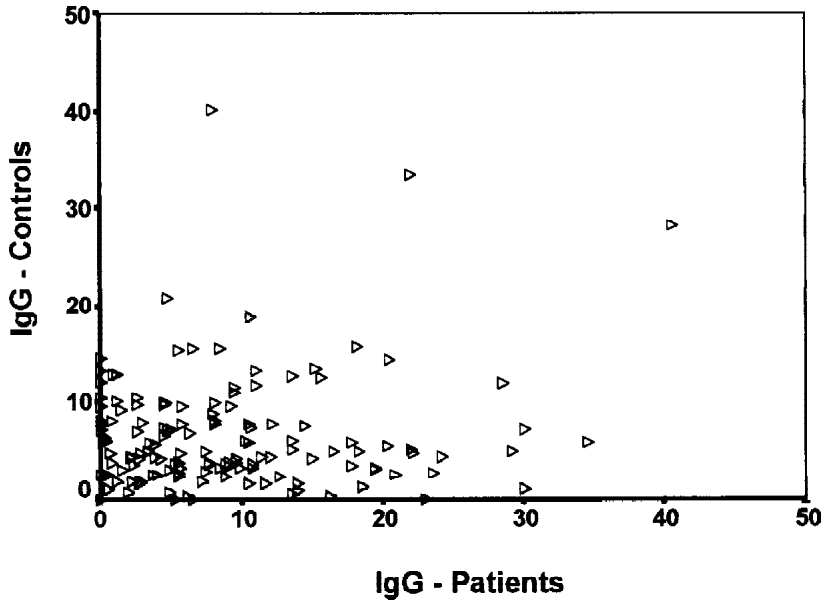

Fig. 1. Levels of IgG in patients and controls.

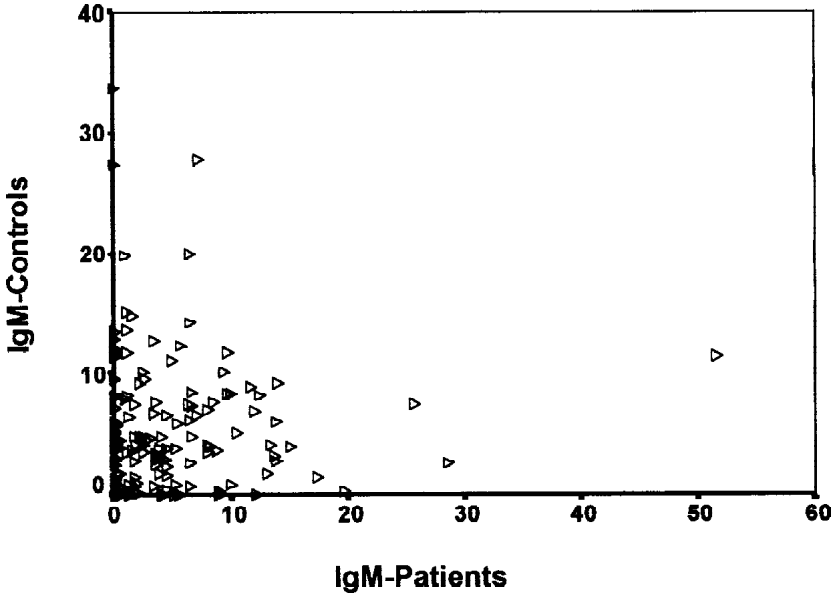

Fig. 2. Levels of IgM in patients and controls.

Table I. Three-year follow-up in cancer patients with elevated levels of aCL.

Pt.	IgG		IgM	
	1996	1999	1996	1999
1	4.5	6.6	25.7	2.2
2	19.4	7.7	51.6	34.5
3	16.3	16.6	15	2.5
4	29.2	30	4	4.4
5	11.6	15.5	17.4	4.3
6	18.1	17.7	6.6	24.5
7	22.1	19.5	0.3	1.2
8	18.6	11	8	7
9	16.5	24.5	0.12	1.6

to have elevated IgG aCL. Only one of 3 patients with initial elevation of IgM aCL was positive for IgM aCL at follow-up, although another one became positive for IgG aCL. Therefore the majority of patients (7/9) had persistently elevated aCL.

Thromboembolic disease

Twenty patients had TED (13.8%) (Table II). All patients with TED had active disease at the time of thromboembolism or thrombophlebitis compared to 77.6% of the remaining patients who had active disease during the time of the study ($p < 0.05$). There was no significant difference in the percentage of patients undergoing chemotherapy treatment between these 2 groups of patients: 16 patients (80%) in the TED group compared to 84 (67.2%) of the remainder ($p > 0.37$). Nineteen patients with TED (95%) died in the 3-year period of the study compared with 70 (56%; $p< 0.003$) in the non-thromboembolic group.

Thrombophlebitis occurred in the lower limbs in 15 patients and in 2 patients was the first manifestation of the cancer; the majority (8) were serious events occurring in the ileo-femoral veins and these patients were admitted and treated with intravenous heparin and oral warfarin. Five patients had calf vein phlebitis and 2 had superficial thrombophlebitis. One patient with breast cancer had a clinical/radiological diagnosis of pulmonary embolus and died shortly after surgery. Four patients had acute cerebrovascular events and 2 of these had high blood pressure. In some of these patients, especially those with pelvic malignancy, anatomic factors such as compression of blood vessels by tumour, may have been at least partially responsible for the lower limb vascular complications.

Thromboembolic disease and aCL

Analysis of the paired differences in serum aCL levels in patients and matched controls showed no significant differences between patients with TED and the remaining cancer patients (IgG $p > 0.67$; IgM $p > 0.16$). There was no significant difference in the percentage of TED in those patients with IgG aCL or IgM aCL or both over the established cut-off (16.7%), compared to those with normal aCL (13%) ($p > 0.82$).

Frequency of ANA and anticytoplasmic antibodies in cancer patients compared to controls

Nine patients had either a positive ANA (n=5) or anticytoplasmic antibodies with a coarse speckled pattern (n=5) (one patient had both) compared to 2 controls who were ANA-positive ($p > 0.06$) (Table III). Two patients with a positive ANA had elevated levels of IgG aCL. One patient with positive anticytoplasmic antibodies (but negative aCL) had TED. One patient with a limited form of breast cancer, treated only with surgery, had anticentromere antibodies (1/2,560 titre). She had a limited form of scleroderma

Table II. Age, gender, thrombotic events and aCL values in 20 patients with TED.

Age	Sex	Type of cancer	IgG aCL	IgM aCL	TED
68	F	Endometrial	4.5	0.12	Ileofemoral
74	M	Colon	3.9	4.5	Ileofemoral
43	M	Lymphoma	1.9	0.0	Ileofemoral
56	F	Renal	7.97	1.3	Superficial
59	F	Breast	30.1	0.0	Ileofemoral
61	M	Prostate	9.1	4.0	Calf
45	M	Pharynx	34.5	0.5	Superficial
68	M	Parotid	15.6	0.5	Calf
64	M	Lung	9.5	1.1	CVA
63	F	Breast	2.2	1.1	Ileofemoral
45	F	Cervix	10.5	1.1	Ileofemoral
73	M	Lymphoma	2.1	0.1	CVA
68	F	Primary unknown	20.3	5.2	CVA
64	F	Ovary	5.5	0.0	Calf
33	F	Cervix	5.2	1.71	Ileofemoral
75	F	Endometrial	2.8	1.1	Ileofemoral
49	F	Endometrial	0.2	0.0	CVA
72	M	Prostate	5.8	0.0	Calf
71	F	Breast	22	1.8	Pulmonary embolus
77	F	Renal	0.8	3.6	Calf

Table III. Clinical features and aCL in ANA and anticytoplasmic antibody cancer patients.

Age	Sex	Type of cancer	ANA pattern	Anti-cyto pattern	IgG aCL	IgM aCL	Other disease
57	M	Lung	1/640 DCS	Negative	0.36	2.2	
55	M	Primary unknown	1/640 H	Negative	20.8	13.8	
65	M	Prostate	Negative	CS	7.7	4.4	
69	M	Prostate	Negative	CS	1.1	1.65	
65	F	Ovary	1/40 FS	1/2560 CS	9.25	11.9	GCA
56	F	Breast	1/2560 ACA	Negative	12.2	6.4	Scleroderma
55	M	Lung	Negative	CS	5.5	0.1	
75	F	Endometrial	Negative	Discrete	2.8	1.1	DVT

Anti-cyto: anticytoplasmic antibodies; DCS: diffuse coarse speckled; H: homogeneous; CS: coarse speckled; FS: fine speckled; ACA: anticentromere; GCA: giant cell arteritis.

involving only the hands and forearms, mild Raynaud's phenomenon and oesophageal disease. Another patient had high titre anticytoplasmic antibodies (1/2,560 titre) and a low positive ANA (1/40). Antineutrophil cytoplasmic antibodies were negative. She had advanced bilateral ovarian cancer with peritoneal invasion. After surgery she was treated with 9 courses of chemotherapy (cisplatin + cyclophosphamide), and went into remission that was maintained throughout the study period. She had a 20-year history of non-erosive intermittent polyarthritis affecting mainly the wrists, ankles, knees, and small joints of both hands. She had been treated with non-steroidal anti-inflammatory drugs. Rheumatoid factor and aCL were negative. Three months after finishing chemotherapy she complained of a severe left temporal headache and further blood tests revealed a high ESR (>100 mm/hr). A temporal artery biopsy showed classical features of giant cell arteritis. She was treated initially with 80 mg prednisolone with rapid improvement. She is now taking 10 mg prednisolone and a recent blood test has shown that anticytoplasmic antibodies are no longer detectable.

Discussion

The association between thromboembolic disease and aCL in autoimmune connective tissue diseases is well established. The first detailed description of large vessel occlusion in SLE was that from Alarcon-Segovia and Osmandson (22). Subsequently an association between large vessel occlusion, gangrene and antiphospholipid antibodies was made (23). Deep venous thrombosis in SLE seems to affect mainly the lower limbs, is frequently multiple and bilateral, and is complicated by pulmonary embolism in 33% of cases (24). Superficial thrombophlebitis may also accompany deep venous thrombosis or may occur independently (25). Several other vascular manifestations have been associated with APS, but cerebral infarcts are one of the most common associations (26). Vascular complications in other autoimmune disorders such as rheumatoid arthritis and scleroderma are less strongly associated with aCL (27, 28). However, aCL of the IgG isotype may be associated with acute giant cell arteritis, especially in those with a history of polymyalgia rheumatica (29).

In keeping with other similar studies, thrombophlebitis was the most common vascular manifestation in our group of cancer patients. Thrombophlebitis did not seem to be related to treatment, but did identify patients with a poorer prognosis. Given the association of antiphospholipid antibodies with macrovascular complications, it was of interest that IgG aCL levels in cancer patients were significantly increased compared to controls. The finding of elevated IgG aCL was not influenced by factors such as the stage or type of cancer or treatment, although our subgroups may have been too small to detect differences. We were not able to establish an association between vascular events in cancer patients and the presence of aCL. Several patients had high levels of aCL antibodies, but no manifestations of vascular disease, and on the other hand several patients with TED had normal levels of aCL antibodies. In both situations it is possible that other mechanisms including anatomical factors and other disturbances in coagulation were involved.

Our results agreed with the findings of Zukerman *et al.* (11) of an increased prevalence of aCL antibodies in cancer patients. However, we were unable to confirm the results of these authors, who reported an increased incidence of thromboembolic disease in anticardiolipin positive patients with malignancy. They studied 241 cancer patients and found that the relationship of TED and aCL antibodies was more evident when aCL levels were over 60 GPL or MPL units, and they were able to find 15 patients with such high levels. Our study involved 145 patients and the absence of statistically significant differences in aCL levels between TED patients and the remaining cancer patients may be related to the small number of patients with thromboembolic phenomena. Also, we were unable to detect high levels (over 60 GPL or MPL units) in our patients or controls. Our results also agreed with the findings of Schved *et al.* who investigated the prevalence and clinical significance of elevated antiphospholipid antibodies in a large series of 1,014 patients admitted to a department of Internal Medicine (12). In the latter study the most frequent disease associated with aCL positivity was cancer. Of 14 patients with aCL and cancer, 9 had advancing malignant disease, 5 were in clinical remission and none of the 14 patients had a history of thrombosis.

Pathogenic aCL antibodies are believed to be β_2-GPI dependant (30). Although we were unable to measure anti-β_2-GPI antibodies directly, the assay we used is claimed by the manufacturers to detect both β_2-GPI dependent and independent aCL. Another shortfall in our study was the lack of resources to perform coagulation studies for the detection of a lupus anticoagulant. It is well known also that thrombotic events are associated with autoimmune anticardiolipin antibodies characterized as 'persistent' antibodies, and that 'transient' antibodies are more frequently related to infectious processes and do not display any prognostic value for thrombosis. We have confirmed in a small group of patients that were still alive, and without thrombotic events, persistently high levels of aCL IgG 3 years later. This finding suggests at least that a subset of malignancy associated aCL antibodies are not thrombogenic.

There has been some evidence to suggest that patients with malignancy may have an increased frequency of other autoantibodies. In a large group of patients referred to a rheumatology clinic because of a positive ANA test, it was found that 2.9% had cancer and no evidence of autoimmune disease (31). In malignant melanoma, the incidence of antinuclear, antinucleolar and anticytoplasmic antibodies was higher than in controls, and antibodies to nucleolar antigens were found more frequently in sera from patients with late disease (32). Changes in the specificity and an increasing titre of ANA may occur when patients with liver cirrhosis and chronic hepatitis develop hepatocellular carcinoma (33). Nucleolar antigens recognised by autoantibodies in cancer patients have been characterised in a group of cancer patients. These autoantigens are also recognised by autoantibodies in sera from patients with autoimmune diseases, suggesting that they do not repre-

sent an autoimmune reaction unique to cancer, but might reflect a breakdown in self tolerance associated with cancer that leads to an antigen-driven immune reaction (34).

The results from our present study suggest a difference in the prevalence of ANA and anticytoplasmic antibodies in cancer patients (6.2%) when compared to the control population (1.2%), but the difference was not statistically significant. Autoimmune disease was diagnosed in only 2 cases and all other patients were asymptomatic, even those few with high ANA titres. Two cases were of interest. One patient with ovarian cancer and high titre anticytoplasmic antibodies who had long-standing polyarthritis developed temporal arteritis, but was consistently negative for both aCL and for anti-neutrophil cytoplasmic antibodies. Vasculitis has been reported frequently in association with neoplasia, although the association is strongest with hematologic malignancies (35). The second case with breast cancer had anticentromere antibodies and a limited form of scleroderma with which this autoantibody is usually associated. A link between breast cancer and scleroderma has been suggested (36).

We conclude that ANA and anticytoplasmic antibodies may be present in some patients with cancer even in the absence of any other known autoimmune disease. However, in our study the frequency of these antibodies was not significantly different from a control population. The presence of more disease specific antibodies such as anticentromere antibodies should alert the clinician to the possibility of an underlying coincident autoimmune condition. Cancer is associated with increased levels of aCL. However, our results are insufficient to draw firm conclusions about the association of these antiphospholipid antibodies and TED in malignancy. Further studies would be of interest because TED is associated with a higher mortality in patients with cancer.

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