

Anti-centromere antibodies as a marker of Raynaud's phenomenon in pediatric rheumatologic diseases

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To examine the possible relationship between anti-centromere antibodies (ACA) and pediatric rheumatologic diseases, we investigated the presence of ACA (using enzyme immunoassay) in the sera of 45 children and adolescents with such diseases and compared the results with a group of 42 age- and gender-matched healthy subjects. ACA were present (≥ 10 U/ml) in three out of five patients (60%) with scleroderma (SCD), in seven out of 16 (43.8%) patients with systemic lupus erythematosus (SLE), in two out of five patients (40%) with mixed connective tissue disease (MCTD), in one out of four patients (25%) with dermatomyositis (DMS), and in two out of 14 patients (14.3%) with juvenile rheumatoid arthritis (JRA). ACA were also detected in a single patient with anti-phospholipid syndrome (APL) who had digital gangrene and hemiparesis, as well as in two healthy subjects. ACA positivity was related to the presence of Raynaud's phenomenon in the studied sample, as 86% of patients suffering from the phenomenon were ACA positive. ACA positivity was associated with older age, high blood pressure and high erythrocyte sedimentation rate (ESR) values, and lower hemoglobin and weight and height percentile values. It was also higher among anti-nuclear antibody-positive subjects. Raynaud's phenomenon and ACA positivity shared almost the same clinical and laboratory associations in the studied patients. Thus, ACA are probably among the markers of Raynaud's phenomenon in pediatric rheumatologic diseases. Their value as predictors of future development of the phenomenon needs further evaluation.

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The sequence of blanching, followed by cyanosis and erythema, occurring spontaneously or in response to cold or physical or emotional stress, was first described by Raynaud, a French medical student, in 1874 (1). Raynaud's phenomenon is relatively rare in children and usually has an underlying cause such as systemic sclerosis (SSC) or scleroderma (SCD), systemic lupus erythematosus (SLE) or mixed connective tissue disease (MCTD), or rare conditions such as cryoglobulinemia. Some individuals with no apparent clinical disease have laboratory abnormalities such as anti-nuclear antibody (ANA) or capillary abnormalities in the nail beds; such individuals have a high probability of developing rheumatic diseases (2). Anti-centromere antibodies (ACA) are auto-

antibodies that react with protein antigens located in the kinetochore region of chromosomes (3) and are present in 70–90% of adult patients with limited cutaneous SCD or the CREST (Calcinosis, Raynaud's phenomenon, Oesophageal involvement, Sclerosis of the skin and Telangiectasias) syndrome (4). As a result of structural changes in the small blood vessels, patients with SSC are prone to develop digital ulcers and infarctions that may necessitate amputation of digits (5,6). Cases of digital gangrene and ACA without SCD have been reported in adults (7–9). The situation in children is less clear. Therefore, it was of interest to search for a possible relationship between ACA and pediatric rheumatologic diseases in relation to various manifestations and parameters of activity.

Subjects and methods

Subjects

The study comprised 45 children and adolescents with some rheumatologic connective tissue diseases who were enrolled consecutively following attendance at the Pediatric Allergy and Immunology Unit of Ain Shams University Children's Hospital, Cairo. The study group comprised 31 females and 14 males, mean age 10.7 ± 3.4 years (range 6–18 years). A group of 42 age- and gender-matched healthy subjects were included for comparison (29 girls and 13 boys; mean age 11.1 ± 3.7 years, range 6–18 years). They were enrolled from children and adolescents presenting for minor medical, dental or surgical procedures after clinical exclusion of present, past or a family history of collagen vascular diseases or any chronic illness.

The patients were diagnosed according to the criteria of the American College of Rheumatology (2,10,11) and stratified into the following groups:

1. SLE: 16 children and adolescents, 9–18 years old (one male, 15 females).
2. Juvenile rheumatoid arthritis (JRA): 14 children, 6–13 years old (eight boys and six girls). Eight patients presented with polyarticular JRA, three had oligoarticular JRA, and three had systemic-onset JRA.
3. SCD: five children and adolescents, 6–14 years old (three boys and two girls). Three had limited cutaneous sclerosis with Raynaud's phenomenon, one presented with morphea, and one had arthralgia with the linear sclerodermal manifestations.
4. Dermatomyositis (DMS): four female children, 8–10 years old.
5. MCTD: five subjects, 8–17 years old (three girls and two boys).
6. One 6.5-year-old female child with primary anti-phospholipid syndrome and digital gangrene was included. She did not fulfil any of the criteria for diagnosis of rheumatic diseases of children. She was diagnosed on clinical grounds aided by the presence of ACA (2).

Methods

This was a cross-sectional study. The patients were evaluated clinically and by laboratory analyses, as described below.

Clinical evaluation. Clinical evaluation was carried out to determine the presence of Raynaud's

phenomenon and skin and joint manifestations. Renal involvement was assessed as well as the systolic and diastolic blood pressure values in comparison to normal reference values (12). The weights and heights in relation to normal percentiles (13) were recorded.

Laboratory investigations. The hemoglobin (Hb) concentration (Coulter MicroDiff 18, USA) was included as well as the first-hour erythrocyte sedimentation rate (ESR) (Werstergren method). The complement components C_3 and C_4 were measured by turbidimetry (Turbiquant C_3 and C_4 ; Behring Diagnostics GmbH, Marburg, Germany). The presence of rheumatoid factor (RF) was estimated by latex agglutination (Avitex-RF; Omega Diagnostics, Alloa, Scotland, UK). ANA and anti-DNA antibodies were examined by indirect immunofluorescent microscopy (Sanofi Diagnostics-Pasteur, Chaska, MN, USA). Lastly, the ACA were measured by immunometric enzyme immunoassay (EIA) (14) for the quantitative determination of immunoglobulin G (IgG) auto-antibodies directed against centromere B (anticentromer B; ORGentec Diagnostika GmbH, Kupferbergstrasse, Mainz, Germany). According to the manufacturer, normal values are below 10 U/ml, hence ACA concentrations ≥ 10 U/ml were considered as elevated.

Control subjects

The weight, height, and blood pressure of the control subjects were evaluated in relation to the same percentile values described above, and their sera were tested for ACA in the same laboratory as the patients' samples.

Statistical analysis

The results were analyzed via a standard computer program (statview) using the chi-square test, Student's *t*-test, and Mann–Whitney (*Z*) test, after Welch correction for unequal variance. The Kruskal–Wallis test (*H*) was used to compare ACA concentrations in patient groups, and the correlation coefficient (*r*) test was used to compare various numerical data of the patients and controls. The numerical data are presented as mean $\pm$ standard deviation (mean $\pm$ SD).

Results

ACA were detected in 16 out of the 45 (35.6%) patients with various rheumatologic disorders in comparison to two out of the 42 healthy subjects (4.8%). The difference was statistically highly

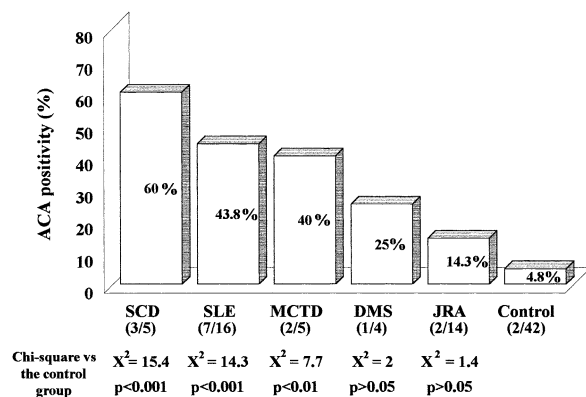


Fig. 1. Anti-centromere antibody (ACA) positivity in the studied sample. DMA, dermatomyositis; JRA, juvenile rheumatoid arthritis; MCTD, mixed connective tissue disease; SCD, scleroderma; SLE, systemic lupus erythematosus.

significant (chi-square=12.6, $p < 0.001$). Children and adolescents with the diagnoses of SCD, SLE, and MCTD had significantly higher ACA positivity rates while those with DMS and JRA did not vary statistically from the control value (Fig. 1). The mean concentrations of ACA in the individual patient groups were statistically comparable ($H = 6.1$, $p > 0.05$). The single patient

with primary anti-phospholipid syndrome and digital gangrene had a high ACA level (30 U/ml).

ACA positivity was considered to be linked to the presence of Raynaud's phenomenon, as 12 out of the 16 ACA-positive patients suffered from its manifestations (Table 1). On the other hand, 12 out of the 14 patients with Raynaud's phenomenon had high serum levels of ACA. Furthermore, the mean concentration of ACA in patients with Raynaud's phenomenon was 16.1 ± 12.5 U/ml while that in patients not suffering from the phenomenon was 5.7 ± 2.9 U/ml; the difference was statistically significant ($Z = 3.9$, $p < 0.001$).

ACA-positive patients had higher rates of ANA positivity and lower Hb concentrations than the ACA-negative patients. ACA positivity was also found to be linked to ESR values. A higher mean ESR (70.6 ± 20.5 mm/h) was found in ACA-positive patients than in those who were ACA negative (35.3 ± 13.3 mm/h) ($t = 6.2$, $p < 0.001$). The ESR values could be positively correlated to the ACA serum concentrations. The ACA level was also positively correlated with the age of patients ($r = 0.36$, $p < 0.05$), systolic blood pressure ($r = 0.38$, $p < 0.05$), and diastolic blood

Table 1. Relation of anticentromere antibody (ACA) positivity to some clinical and laboratory data of the patients

Patients' data	ACA positive (n=16)	ACA negative (n=29)	Statistical analysis
Gender			
Males	4	10	$\chi^2 = 0.5$
Females	12	19	$p > 0.05$
Skin involvement			
Present	11	15	$\chi^2 = 1.2$
Absent	5	14	$p > 0.05$
Arthritis			
Present	11	17	$\chi^2 = 0.5$
Absent	5	12	$p > 0.05$
Nephropathy			
Present	6	7	$\chi^2 = 0.9$
Absent	10	22	$p > 0.05$
Raynaud's phenomenon			
Present	12	2	$\chi^2 = 22.3$
Absent	4	27	$p < 0.001^*$
Rheumatoid factor			
Positive	1	1	$\chi^2 = 0.2$
Negative	15	28	$p > 0.05$
C ₃			
Decreased	7	9	$\chi^2 = 0.7$
Normal	9	20	$p > 0.05$
C ₄			
Decreased	3	3	$\chi^2 = 0.7$
Normal	13	26	$p > 0.05$
ANA			
Positive	12	11	$\chi^2 = 5.7$
Negative	4	18	$p < 0.05^*$
Anti-DNA			
Positive	5	6	$\chi^2 = 0.6$
Negative	11	23	$p > 0.05$

*Significant.

ACA, anti-centromere antibodies; ANA, anti-nuclear antibodies; C₃, C₄, complement components 3 and 4; anti-DNA, anti-deoxyribonucleic acid antibodies.

pressure ($r = 0.34$, $p < 0.05$) while it was negatively correlated to weight ($r = -0.44$, $p < 0.01$) and height percentiles for age ($r = -0.32$, $p < 0.01$).

The presence of Raynaud's phenomenon was linked to almost the same factors that were associated with ACA positivity, such as older age, higher blood pressure and ESR values, and lower growth percentiles. Gender did not influence the ACA positivity and neither did the presence of cutaneous manifestations, arthritis or nephropathy. RF positivity, anti-DNA positivity, and serum complement levels showed no correlation with ACA positivity or concentration.

In the control group, the ACA concentrations, although normal in most subjects (< 10 U/ml), were positively correlated with age ($r = 0.68$, $p < 0.001$) and systolic ($r = 0.67$, $p < 0.001$) and diastolic ($r = 0.45$, $p < 0.01$) blood pressure. The blood pressure values of the control subjects were within the normal percentiles for age and were significantly lower than the patients' values ($p < 0.05$).

Discussion

ACA were present (≥ 10 U/ml) in 16 (35.6%) out of 45 children and adolescents with various forms of rheumatic connective tissue diseases as compared to only two (4.8%) of the healthy subjects. The highest rate of positivity (60%) was found in the group of five patients with SCD: three of these patients had Raynaud's phenomenon together with the cutaneous features and ACA positivity, one had linear SCD with arthralgia, and one had morphea – both of the latter patients were ACA negative. None of the five children in this group had any clinical or investigational evidence of internal organ involvement and it is well known that SSC is uncommon in children (1,2).

ACA were also prevalent in the SLE group, being detected in 43.8% of the subjects, a value that was comparable to that of the SCD group. This may reflect the high frequency of Raynaud's phenomenon in these children, being detected in six patients (37.5%). It is reported that 20% of adult SLE patients suffer from Raynaud's phenomenon owing to small vessel occlusion by proliferative endarteritis (15). In children, a figure of 13% was reported by the American College of Rheumatology (2). However, of all causes of Raynaud's manifestations in a large series, SLE accounted for 60% of cases in children compared to 3% in adults, while idiopathic Raynaud's disease was the cause in only 5% of cases in childhood compared to 70% in adults (1).

Two patients in the group of MCTD (40%) showed an intermittent course of Raynaud's phenomenon and were ACA positive. Among MCTD characteristics, arthritis and Raynaud's phenomenon are the most common manifestations encountered in children (1). Moreover, Raynaud's phenomenon is considered as one of the commonest early presenting symptoms of MCTD in adults (3).

Two out of the four children with DMS in our series had Raynaud's phenomenon, of which only one was ACA positive. In adults, Raynaud's phenomenon occurs in $\approx 30\%$ of patients with dermatomyositis or polymyositis (15). The frequency in children is reported to be less as DMS is thought to represent only 3% of the causes of Raynaud's phenomenon in childhood (1). The difference in ACA positivity between our DMS and healthy subjects was insignificant.

Within the group of JRA, only one girl suffered from obvious episodes of Raynaud's phenomenon and was ACA positive, while a teenage boy had an ACA level > 10 U/ml and had never suffered from any manifestations suggestive of the phenomenon. The ACA positivity in the JRA group was also comparable to the control value. Although Raynaud's phenomenon frequently develops in adult patients with RA (15), JRA is thought to account for only 1% of the causes of Raynaud's phenomenon in children (1). ACA were detected in one out of 10 adults with RA in the study conducted by Onozuka et al. (16).

A girl with the diagnosis of primary antiphospholipid syndrome was included in the study. She presented at the age of 6 years with fever, pallor, arthralgia, and digital gangrene that necessitated amputation of the right index finger. She gave a frank history of Raynaud's phenomenon and Livido reticularis. The latter was formerly thought to be a cutaneous manifestation of vasculitis in SLE patients. It was subsequently considered as a thrombotic process rather than a vasculitic one and is not specific for SLE; it may occur even in normal children – in a reversible form – upon exposure to cold (17). The girl's ESR was 100 mm/h and the blood count showed anemia (Hb 7.5 g/dl) and neutrophilia. The ANA, anti-DNA, serum C_3 and C_4 levels, and renal function tests were normal, but her anti-cardiolipin IgG and IgM antibodies were elevated. Doppler examination of upper limb vessels revealed complete occlusion of the right radial artery. She later developed hemiparesis owing to infarction in the left frontal region and improved partially on corticosteroids and anti-coagulants. Her ACA level was high (30 U/ml), which is probably related to her Raynaud's phenomenon

and digital gangrene. Picillo et al. (9) reported a case of a 22-year-old female with digital gangrene and ulcers in the extremities with no SCD, showing ACA as the main finding. Takahashi et al. (14) stated that the presence of ACA may cause the same pathological presentation of vascular damage as seen in SSC. They suggested that ACA may be directly toxic to the endothelial cells.

From the foregoing results, ACA positivity was found to be linked to the presence of Raynaud's phenomenon whatever its cause. With few exceptions, most children and adolescents who were ACA positive experienced the phenomenon in an intermittent or continuous form. Two healthy adolescents, a 13-year-old girl with SLE and a 14-year-old boy with polyarticular JRA, who was also ANA positive, had an ACA ≥ 10 U/ml and never complained of Raynaud's phenomenon. When the two ACA-positive healthy subjects were re-investigated, no other serologic markers of rheumatic diseases were found. However, one of the patients, a 15-year-old boy, said that his elder sister (23 years old) used to complain of what he described as cold intolerance in her limbs. The presence of positive ACA levels in the others could not be explained. It is possible that ACA positivity may precede the appearance of Raynaud's manifestations in the way that it sometimes precedes digital ischemic manifestations in SSC (18). Apart from connective tissue diseases, ACA acquisition was reported in primary biliary cirrhosis and autoimmune hepatitis and rarely in chronic hepatitis C infection (16). Malignancy, especially malignant lymphomas, also may be accompanied with ACA formation (19,20). ACA positivity was rarely reported in primary Raynaud's disease (1,8).

The relatively high frequency of Raynaud's phenomenon and, hence, ACA positivity in our series may be of somewhat limited significance because of the small size and uneven distribution of the sample. However, the finding deserves attention as it may need to be considered in management strategies. Raynaud's phenomenon may be caused by the disease itself or by some medications, such as the adrenergic blockers used to control the blood pressure in these children. The positive correlations with the systolic and diastolic blood pressure values support this observation. Other causes of Raynaud's phenomenon, such as pulmonary hypertension, atherosclerosis, carpal tunnel syndrome, intervertebral disk disease, cryoglobulinemia or vibration injury owing to piano playing or typing (15), were not encountered in our series.

ACA positivity was significantly related to the presence of ANA in this study. This may reflect the high ACA positivity in the SLE patients, who are essentially ANA positive. In fact, the centromere is a nuclear component and its antibody stains with discrete speckles when analysed by immunofluorescence (20). Chikanza et al. (21) reported that children with primary Raynaud's syndrome who are ANA positive have a higher probability of developing livido reticularis or some features of scleroderma. Adult patients with ACA were reported to develop limited cutaneous SSC or SLE subsequently (8).

Thus, ACA were found to be closely associated with the presence of Raynaud's phenomenon in children and adolescents with various rheumatologic disorders. Their predictive and prognostic values in these diseases were not evaluated and further studies are needed to investigate their pathogenetic role in the causation of Raynaud's phenomenon or digital gangrene as a complication.

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