Preclinical Carotid Atherosclerosis in Patients With Juvenile-Onset Mixed Connective Tissue Disease

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Background: This study investigated preclinical atherosclerosis in patients with juvenile mixed connective tissue disease (JMCTD), which is a chronic inflammatory disease with a varied phenotype. Mixed connective tissue disease (MCTD) has well known associations with other autoimmune diseases known to have increased risk of cardiovascular disease. However, the cardiovascular risk for patients with the juvenile form remains unclear. *Materials and methods:* Forty-nine patients with JMCTD and 45 age-and sex-matched controls took part in this study. They underwent blood tests, clinical examination, and ultrasound measurement of the carotid arteries. *Results:* We found that patients had significantly higher average carotid intima-media thickness (IMT) as compared to controls (mean 0.57 ± 0.09 versus 0.53 ± 0.06, $P = .03$). IMT also increased with both increasing disease duration (years from diagnosis), and severity as assessed by the physicians global assessment score, after adjustment for age. *Conclusions:* This is the first study to demonstrate increased preclinical atherosclerosis in juvenile MCTD. Our findings suggest that the atherosclerotic burden in this patient group, which was independent of traditional cardiovascular risk factors, might be secondary to the underlying connective tissue disease.

Key Words: Carotid atherosclerosis—connective tissue disease—intima media thickness—carotid ultrasound—stroke

Introduction

Mixed connective tissue disease (MCTD) is a chronic inflammatory, systemic autoimmune disease characterized by high titers of ribonucleoprotein (anti-U1RNP).[1] Up to 23% of patients present with a juvenile form, with a varying constellation of clinical symptoms and findings specific for systemic lupus erythematosus (SLE), systemic sclerosis, and idiopathic inflammatory myositis.[2,3] Patients with inflammatory rheumatic disease including SLE, systemic sclerosis, and inflammatory myositis have an increased risk of premature cardiovascular disease such as stroke and myocardial infarction.[4-8]

Knowledge on the proportional contribution of traditional risk factors, medication for rheumatic disease, and age to this increased risk of cardiovascular disease, is key when treating these patients. However, despite the recognition of increased cardiovascular disease in patients with rheumatic disease, several important questions about the connections between chronic inflammatory disease and premature cardiovascular disease remain unanswered. Firstly, it is uncertain if the known increased cardiovascular risk is conferred by an enhancement of traditional risk factors such as hypertension, diabetes, hyperlipidemia, and smoking,[9-12] or by the disease-specific mechanisms related to the disease itself. More knowledge is needed on the interaction of local vascular and systemic inflammation due to rheumatologic disease with traditional risk factors for cardiovascular disease, and the degree to which these pathways contribute to adverse CV outcomes in this patient population.[13] The majority of reported results on cardiovascular risk in connective tissue disease are from adult patient populations making it difficult to

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Received November 21, 2018; accepted January 25, 2019.

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1052-3057/$ - see front matter

https://doi.org/10.1016/j.jstrokecerebrovasdis.2019.01.027

distinguish these pathways as cardiovascular risk factor are prevalent. Because the impact of traditional cardiovascular risk factors increases with age, a juvenile population is probably better suited to answer this question. Secondly, some of the medications used in treatment of rheumatic disease like corticosteroids, may increase CV risk, whilst others, like methotrexate, may decrease risk.[14] Some patients with longstanding inflammatory disease do not have accentuated atherosclerosis and for these the possible protective effect of medication has been postulated. Thirdly, it is not established if different age-groups have different incremental risk of cardiovascular disease. The assumption that early onset disease, longer disease duration and increased inflammatory burden put juvenile patients at increased risk compared to their adult counterparts is often made. The long-term risk for juvenile patients with mixed connective tissue disease however, is uncertain.[2,3,15,16]

In the adult MCTD population premature atherosclerosis with carotid intima-media thickness (IMT) has been documented and found associated with increasing age, disease duration and to traditional risk factors such as cholesterol levels, systolic blood pressure and diastolic blood pressure.[17] The risk in a juvenile population, however, is unclear. To the best of our knowledge, however, no previous studies have assessed subclinical atherosclerosis in juvenile mixed connective tissue disease. The aim of our study therefore, was to assess subclinical atherosclerosis in a juvenile MCTD population and compare the results with those of a healthy control group.

Because CV events are rare in the young, surrogate markers of atherosclerosis such as carotid IMT are valuable to detect early subclinical atherosclerosis. IMT of the carotid arteries is measured using B-mode ultrasonography (US), a noninvasive and well-recognized method for evaluating generalized atherosclerotic arterial disease.[18,19]

Materials and Methods

Patients and Control Subjects

Data on the patients and controls included in this study were previously published in the nationwide Norwegian study describing long-term outcome and clinical characteristics in a Norwegian juvenile MCTD population.[20]

Different methods were used to identify patients with juvenile MCTD in Norway, as previously described.[20] Inclusion criteria were fulfillment of the Kasukawa or the Alarcon-Segovia and Villareal criteria and a clinical MCTD-diagnosis confirmed by a rheumatologist or a pediatrician, and symptom onset before the age of 18. Controls were randomly selected from the National Population Register after matching for age and sex. Controls with a history of autoimmune disease requiring immunosuppressive medication or lung or heart disease other than mild asthma were excluded. Forty-nine of the identified and examined patients and 49 matched controls agreed to undergo carotid ultrasound and they were all included in the analysis for this current study. As well as carotid ultrasound examination, all patients and controls underwent clinical examination and venous blood sampling.

The study was approved by the Regional Ethics Committee for Medical Research (Id 2012/1721).

Carotid Ultrasound

Patients and controls were examined with color duplex US of the carotid arteries by an experienced ultrasonographer, who was blinded for patients' and controls' clinical and laboratory data.

The examination was carried out in the supine position with the head angled at 45° toward the contralateral side. IMT measurements were synchronized with the QRS-complex on the echocardiogram and made in each carotid artery at the peak of the R wave where the lumen was widest. IMT was defined as the distance between the lumen-adventitia borders of the vessel, identified as a double-line pattern in a longitudinal image. IMT was measured in the carotid arteries on both sides of the neck in 3 segments: (1) in the far wall of the common carotid artery, 1 cm proximal to the bifurcation, over an area of 1 cm and in 3 different projections: lateral, posterior and anterior; (2) in the far wall of the carotid bifurcations over an area of 1 cm in the lateral position; (3) in the far wall of the proximal internal carotid arteries immediately distal to the bifurcation over an area of 1 cm in the lateral projection.

We used the averaged value of the 10 carotid IMT measurements in each subject for further analyses. The carotid US examinations were carried out according to the American Society of Echocardiography guidelines[21] using a Toshiba/Canon medical systems Aplio 500.

Clinical Assessment

All patients and controls underwent a thorough clinical examination at Oslo University Hospital in between March 2013 and June 2015. The examinations included a joint count and Physician global assessment of disease activity (PGA), based on a 10-cm visual analogue scale. There are no validated scores for disease activity or damage in MCTD, thus, we used scores attributable for related diseases: the systemic lupus erythematosus

Disease Activity Index, the Rodnan skin score, the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index and the Juvenile Arthritis Damage Index.

As there are no defined criteria for remission in MCTD, we used the criteria for defining remission Juvenile idiopathic arthritis (JIA),[22] and added the absence of cytopenia, myositis, progressive lung, and oesophageal manifestations, and progressive sclerodactyly (with or without Raynaud phenomena). Clinical remission with

medication was defined as inactive disease with immunosuppressive medication for a minimum of six continuous moths. Clinical remission without medication was defined as inactive disease without immunosuppressive medication for a minimum of 12 months. Active disease was defined as absence of remission.[20]

In patients, medical records were examined, and clinical parameters from time of diagnosis, during the entire disease course and at follow-up examination were recorded. Patients and controls measured height and weight, and body mass index was calculated. Information on physical activity and smoking habits were collected from questionnaires. Fasting blood samples were analysed by routine methods at Oslo University Hospital and included; low density lipoprotein, high density lipoprotein (HDL), triglycerides, apolipoprotein A1, apolipoprotein B, fasting glucose, glycosylated hemoglobin.

Statistical Analysis

Categorical variables were compared with the Mann-Whitney *U* test (two groups) or Kruskal-Wallis comparison test when three groups were compared. Student's *t*-test/paired samples *t*-test was used for comparison of normally distributed data. Patients and controls were compared using paired testing. The χ^2 test was used for analyzing categorical data. Spearman correlation was performed to investigate known risk factors for cardiovascular disease and IMT. All calculations were performed with SPSS for Windows statistical software (Version 21.0; SPSS Inc, Chicago, IL).

Results

Baseline Characteristics of Patients and Controls

Forty-nine patients were included to take part in the study. Four patients were not able to give blood and nine patients were for logistical reasons not examined with carotid ultrasound. All patients with available data were included in the study analysis which resulted in 45 patients with blood for the basic patient characteristics, and 40 patients with ultrasound results who were age-, and sex-matched. Patient characteristics are shown in Table 1. The mean age of diagnosis in the patient group was 14 years, whereas mean duration of disease was 16.8 years.

Cardiovascular risk factors for patients and controls are shown in Table 2. Controls had significantly higher levels of HDL cholesterol compared with patients (1.7 versus 1.3 mmol/l, P <0.01), as well as higher levels of Apolipoprotein A at 1.68 versus 1.38 mmol/L, P <0.01). Patients had higher levels of both triglycerides and erythrocyte sedimentation rate (ESR) compared with controls (1.1 ± 0.7 versus 0.8 ± 0.4 mmol/l, P = 0.05) and (12.1 ± 9.7 versus 5.1 ± 3.7, P = 0.05), respectively.

Table 1. *Patient characteristics*

Characteristics	Patients with MCTD, n = 45
Female gender*	41 (91.0)
Age at inclusion, years	28.0 (10.3)
Age at diagnosis, years	14.3 (4.4)
Disease duration	13.6 (9.1)
PGA at inclusion, cm VAS	1.7 cm (0-5.7)
Current medication at inclusion*	
Steroids per os	14 (31.0)
Azathioprine	5 (11.1)
Methotrexate	13 (28.9)
Hydroxycloroquine	25 (55.5)
Mycophenolate Mofetil	2 (4.44)
Rituximab	1 (2.22)

The values are given as mean ± (SD) or *number (percentage). The values for PGA are given as median (range). JMCTD, juvenile mixed connective tissue disease; PGA, physician global assessment.

IMT in Patients and Controls

The carotid ultrasound findings are shown in Table 2. Patients had significantly higher average carotid IMT as compared to controls (mean 0.57 ± 0.09 versus 0.53 ± 0.06, P <0.01, respectively), Figure 1. No carotid plaques or stenosis were detected in patients or controls.

When analyzing the impact of known, traditional cardiovascular risk factors (body mass index, cigarette smoking, diabetes, hypertension and hyperlipidemia on IMT, we found that these were not significantly associated with increased IMT. In the primary analysis HDL, Triglycerides, ESR and Apolipoprotein A were found to be significantly different between the two groups (Table 2), however, on regression analysis with correction for HDL, APOA and triglycerides, IMT remained statistically significantly different between patients and controls, P = 0.036 CI (0.075-2.069).

IMT in Relation to Disease Duration and Disease Activity

When investigating the impact of disease duration (time from diagnosis to study inclusion) on IMT, we found a statistically significant correlation between increased IMT and longer disease duration, P <0.001, Table 3. Furthermore, patients with longer disease duration (≥10 years) had significantly higher IMT compared with controls; mean IMT 0.60±0.10 versus 0.53±0.08, (P = 0.014, 95% CI (0.014-0.123), found to be persistent also after adjustment for age. Conversely, patients with shorter disease duration (≤10 years) did not have higher IMT compared to controls; mean IMT 0.56±0.04 versus 0.53±0.06, P = 0.21 95% CI (0.01-0.06).

When correlating IMT to disease activity scores used for MCTD we found that IMT increased with increasing

Table 2. *Cardiovascular risk factors in patients with juvenile onset MCTD and controls*

Variables	Patients, n = 40	Controls, n = 40	*P*
BMI, kg/m^2	22,7 ± 3,5	23,4 ± 3,0	0.21
Cigarette smoking, yes° n (%)	7 (14.6)	8 (17.8)	1.00
Diabetes, n (%)	1 (2.0)	0	1.00
Systolic blood pressure, mmHg	111.8 ± 14.7	115.5 ± 14.1	0.24
Diastolic blood pressure, mmHg	64.6 ± 15.1	68.4 ± 10.1	0.13
HDL, mmol/L	1.3 ± 0.3	1.7 ± 0.4	<0.01
LDL, mmol/L	2.5 ± 0.8	2.9 ± 1.0	0.13
Triglycerides, mmol/L	1.1 ± 0.7	0.8 ± 0.4	0.05
ESR mm	12.1 ± 9.7	5.1 ± 3.7	<0.01
HbA1c, %	5.3 ± 0.3	5.3 ± 0.8	0.93
CRP, mg/L*	1.9 ± 2.7	1.2 ± 1.7	0.13
Apolipoprotein A	1.38 ± 0.37	1.68 ± 0.23	<0.01
Apolipoprotein B	0.79 ± 0.23	0.82 ± 0.22	0.50
Mean average IMT, mm	0.57 ± 0.09	0.53 ± 0.06	<0.01

Values are mean ± SD or *median (range). BMI, body mass index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; IMT, intima media thickness; LDL, low-density lipoprotein. Smoke occasionally or every day.

Table 3. *Duration of disease and impact on carotid IMT*

Duration of disease, years	Mean IMT, SD
< 10, n = 16	0.56, ±0.04
≥10, n = 24	0.60, ±0.10

disease activity as measured by PGA. Comparing IMT for patients with PGA under and above the median PGA we found that there was a significantly higher IMT for patients with higher disease activity, Figure 2A. Patients with PGA under median (1.6, n = 25) had mean IMT 0.50 ± 0.02 versus mean 0.62 ± 0.018 for those with disease burden over median (n = 24), P <0.001. Furthermore, patients with PGA above the median had significantly higher IMT as compared with controls, P <0.001, Figure 2B, whereas patients with PGA under median did not.

Six patients were in remission and off medication. Interestingly, these patients had a significantly lower IMT than the rest of the patient group- either patients with active disease, or patients in remission on medication. Mean IMT in patients in remission and off medication 0.51 ± 0.09 versus mean IMT 0.58 ± 0.42, P = 0.030 (r = 0.31).

Discussion

This study investigated the presence of preclinical atherosclerosis in patients with juvenile MCTD. The main findings were as follows: (1) Patients with juvenile MCTD had significantly increased preclinical atherosclerosis quantified by increased IMT compared to healthy age- and sex-matched controls. (2) This atherosclerotic burden increased with longer disease duration as well as with increased disease activity, independently of age and sex. These findings demonstrate a potential significantly increased cardiovascular risk in this patient group. Additionally, we demonstrated this premature atherosclerosis to be present independently of established traditional cardiovascular risk factors, indicating that the accelerated atherosclerosis in this patient group might be secondary to the inflammatory burden associated with the underlying disease itself.

To the best of our knowledge, this is the first study to demonstrate increased preclinical atherosclerosis in juvenile MCTD. The most studied pediatric rheumatic disease is JIA with results regarding premature atherosclerosis being inconsistent where some studies demonstrating increased IMT[23-25] whilst others have not.[15,26,27]

Age and sex are variables strongly related to both the exposure and outcome of interest in this study, with IMT known to significantly increase with advancing age, more in men compared with women, in all carotid segments. To allow for comparison of IMT values we therefore matched 1 control to 1 patient with juvenile MCTD of the same age and sex, which we believe is essential to ensure validity of findings in this type of study. Furthermore, IMT measurements are notoriously operator-dependent demanding a strict ultrasound protocol adhering to consensus recommendations including measurements in more segments of the carotid artery as well as using ECG tracing increases as was done in this study, increasing reliability.[28] The relationship of IMT with cardiovascular disease is continuous and determining a specific threshold IMT value is difficult. Indeed, which IMT values should be considered abnormal is a controversial topic. The ESH/ESC hypertension guidelines however, states carotid IMT >0.9 to be a marker of asymptomatic organ damage for adult patients.[29] Normal reference values of IMT in adults are based on large epidemiological database

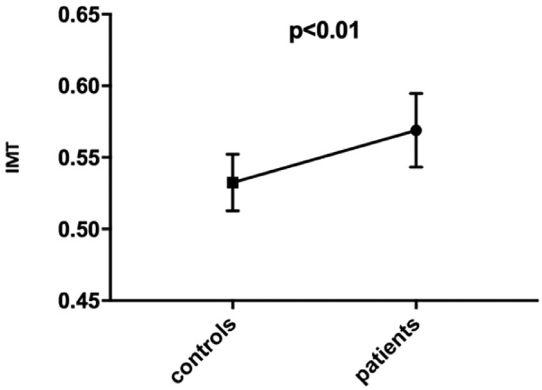

Figure 1. *IMT values in patients and controls. The square and the circle represent the mean IMT and the lines the range of the 95% CI for the mean in controls and patients respectively, 0.53 (0.51-0.56) versus 0.57 (0.54-0.59).*

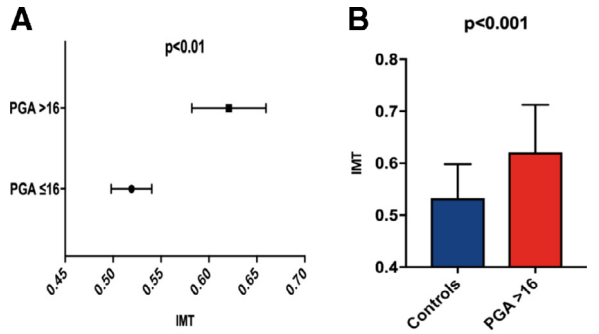

Figure 2. *Comparison of IMT disease score above and below the median PGA. (A) The square and the circle represent the mean IMT and the lines the range of the 95% CI for the mean for patients with PGA under and over the median respectively, (0.52 (0.49-0.54) versus 0.62 (0.58-0.66)). (B) The top of the boxes represent the mean IMT and the line the standard deviation for controls and patients with disease burden above 16 PGA respectively, 0.53 ± 0.07 versus 0.62 ± 0.1. The* P *value is from a Mann Whitney* U *test.*

with data from serial ultrasound measurements of carotid IMT over years. No such data on children are available and normal IMT values in children are therefore uncertain.[30] As a consequence, the given cut −off for children is usually limited to below 30 or below 40 years of age, the value for normal cut-off varies and has suggested to be as low as 0.4 mm for healthy young people below 30 years of age.[31-33] Our finding of increased atherosclerosis in patients with juvenile MCTD is in agreement with recently published findings from Alenghat et al who found that young adults with CTD (aged 18-44) had an even more pronounced prevalence of atherosclerotic CVD relative to their peers.[34] In their cross-sectional analysis including 287,467 patients they found a 1.6-2.2 fold higher prevalence of atherosclerotic cardiovascular disease in middle-aged and older adults with CTD compared to those without CTD, whilst the prevalence in young adults (18-44 years) with connective tissue disease was even higher relative to their healthy counterparts. This disproportionate effect of CTD on younger groups is explained by the lack of other traditional risk factors at this age. In the absence of traditional risk factors, the atherosclerosis −inducing effect of the immune-mediated inflammation becomes evident. Whereas, as the population ages, the atherosclerosis caused by traditional risk factors in both healthy controls and patients blur the differences between the groups. Because of this, a juvenile population is ideal for studying immune-mediated atherosclerosis. In our study the patients and controls had no cardiovascular risk factors, and the increased IMT found in the patient group could therefore be attributed to the chronic inflammation associated with MCTD. Further exploration of levels of immune activity including cytokine profiles could add knowledge on the pathophysiology of inflammatory-driven atherosclerosis in this patient group.

MCTD is a heterogeneous group of patients with varying disease phenotypes. We found preclinical atherosclerosis to increase with increasing Physician global assessment (PGA score), which is commonly used both in clinical practice and research in a number of diseases, including Rheumatoid arthritis (RA) and JIA to assess disease severity.[35-37] The PGA score is also an integrated part of the juvenile arthritis disease activity score.[38] Our group has previously found PGA to correlate well with disease activity demonstrating it could be a useful tool in assessment of disease activity in juvenile MCTD patients.[20] We found higher PGA score indicate higher disease burden to correlate with increased IMT. This is in keeping with the existing knowledge that increased disease burden is associated with increased inflammatory burden, known to cause atherosclerosis. We have not seen this finding demonstrated in previous studies. A direct comparison of atherosclerotic changes however is difficult between studies because of different methods for evaluating IMT and relatively small sample sizes.

Chronic inflammatory diseases such as MCTD, RA and SLE are known to be associated with an increased risk of CVD,[17,39,40] and CVD in adult connective tissue disease has also been associated with an increased prevalence of CVD risk factors such as smoking, diabetes mellitus and hypertension.[41-44] Limiting the findings of many of these prior studies is that they have focused on populations with connective tissue disease with co-existing established CVD with atherosclerotic cardiovascular disease, heart failure as well as cardiac arrhythmias.[6,45] These co-existing diseases are important clinical outcomes associated with certain connective tissue disease, but they may not necessarily respond to systemic inflammation in the same way as atherosclerosis and therefore make interpretation of results difficult. In our study, patients did not have established cardiovascular disease, but we found higher levels of triglycerides and ESR in patients compared to controls, consistent with previous reports of patients with CTD having greater levels of general inflammation than what is found in the general population. Furthermore, HDL and Apolipoprotein were higher in controls compared to patients indicating a possible loss of protection from HDL in the patient group. Further exploration of

these differences in lipid profile among patients and controls might add knowledge to the pathophysiological mechanisms accelerating atherosclerosis in this patient group.

This study has some limitations. Despite the number of patients included in this study being comparable to other published reports on IMT in patients with connective tissue disease, it still remains a small population. The strengths of this study are the long-term follow-up of a well-defined cohort, with paired controls, and the presentation of novel data on preclinical atherosclerosis in JMCDT.

In conclusion, our data demonstrate increased preclinical atherosclerosis in a juvenile population with MCTD, independently of cardiovascular risk factors and age. These findings indicate that this patient group might have increased risk of clinical cardiovascular outcomes and referral for carotid ultrasound should be considered as part of standard patient management, especially for patients with advanced disease. Additionally, further study investigating the mechanisms leading to the increased subclinical atherosclerosis in this patient group is warranted.

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