Cardiometabolic risk and subclinical vascular damage assessment in idiopathic inflammatory myopathies: a challenge for the clinician

S. Barsotti[1,2], C. Saponaro[3], M. Gaggini[3], R. Talarico[1], E. Bianchini[3], N. Di Lascio[3,4], C. Ferrari[1], E. Buzzigoli[3], M. Mosca[1], A. Gastaldelli[3,4], R. Neri[1], M.A. Morales[3]

[1]Rheumatology Unit, University of Pisa; [2]Department of Medical Biotechnologies, University of Siena; [3]Institute of Clinical Physiology CNR, Pisa; [4]Institute of Life Science, Scuola Superiore Sant'Anna, Pisa, Italy.

Abstract

Objective

A high prevalence of cardiovascular disease (CVD), not fully explained by the prevalence of traditional risk factors only, is reported in patients with idiopathic inflammatory myopathies (IIMs). Thus, we investigated if novel markers of CVD risk, like carotid diameter and advanced glycated end products, can better predict increased CVD risk in IIM patients.

Methods

We studied 43 consecutive patients diagnosed with IIM. All the patients underwent a clinical and laboratory evaluation of cardiovascular risk factors and characterisation of myositis disease activity. Non-invasive instrumental examinations performed included the measurement of carotid parameters (intima-media thickness, IMT and mean arterial diameter, mAD) by ultrasonic techniques, advanced glycation end-product accumulation in the skin by autofluorescence (AF) and body composition by bioelectrical impedance analysis. The parameters were compared to those measured in 29 controls, with similar mean age, BMI, blood pressure and smoking habits.

Results

IIM patients showed normal carotid IMT and distensibility, but higher carotid mAD ($p=0.012$), higher skin AF ($p<0.001$), lower fat free mass ($p=0.036$) and increased waist circumference compared to controls. A significant correlation was observed among AF and mAD ($rho=0.317$ $p<0.05$), carotid distension ($rho=0.391$ $p=0.036$) and IMT ($rho=0.627$ $p<0.001$).

Conclusion

Abnormalities of the studied parameters suggest a higher risk of CV disease in IIM patients independent of disease activity. In this population, a thorough assessment of CV risk is recommended also in absence of overt CV disease during the clinical evaluation.

Key words

idiopathic inflammatory myopathies, intima-media thickness, ultrasound, advanced glycation end products, polymyositis, dermatomyositis, cardiovascular risk

Simone Barsotti, MD
Chiara Saponaro, PhD
Melania Gaggini, PhD
Rosaria Talarico, MD, PhD
Elisabetta Bianchini, PhD
Nicole Di Lascio, PhD
Claudia Ferrari, MD
Emma Buzzigoli, PhD
Marta Mosca, MD, PhD
Amalia Gastaldelli, PhD
Rossella Neri, MD, PhD
Maria Aurora Morales, MD, PhD

Please address correspondence to:
Dr Simone Barsotti,
Reumatologia,
Università di Pisa,
Via Roma 67,
56126 Pisa, Italy.
E-mail: simone.barsotti.pisa@gmail.com

Received on November 22, 2018; accepted in revised form on February 18, 2019.

Competing interests: E. Bianchini is co-founder of Quipu srl (Italy).

The other co-authors have declared no competing interests.

Background

Idiopathic inflammatory myopathies (IIMs) are rare autoimmune diseases in which a systemic inflammatory process usually affects both skeletal muscles and internal organs, in particular, the lungs and oesophagus. The most common subtypes of IIMs are polymyositis (PM) and dermatomyositis (DM), which share similar clinical symptoms, despite a different pathogenesis; furthermore, DM patients present a characteristic skin rash (1).

Several studies have shown that inflammation increases the risk of cardiovascular disease (CVD) *per se* (2, 3). In patients with IIM, a higher incidence of stroke and myocardial infarction has been reported, when compared to the general population (4-8).

Although the traditional cardiovascular risk factors may contribute to the pathogenesis of CVD in IIM patients (9), signs of accelerated atherosclerosis, which cannot be fully explained by the prevalence of traditional risk factors, such as smoke, cholesterol, systemic hypertension and diabetes, have been documented in different cohorts (6, 10-13). Additionally, metabolic syndrome (MS) is more prevalent in IIM as compared to healthy subjects (14, 15) and it is also associated to an increased risk of CVD (16).

In asymptomatic populations, assessment of the risk of CVD should rely on non-invasive, low cost techniques; great emphasis has been placed on the predictive role of ultrasonic-derived vascular parameters at carotid artery level such as intima-media thickness (IMT), artery diameter and distensibility (17). On the other hand, skin autofluorescence (AF), apart from being totally non-invasive, allows us to assess the accumulation of advanced glycation end-products (AGEs) which are an index of abnormal glucose metabolism (18). Clinical studies suggest that AGEs predict long-term vascular complications (19-21). Finally, alterations in body composition and the increase in fat mass and fat distribution are known to be related to presence of cardiovascular disease (22, 23).

The main objective of the present study was to assess CV risk and potential subclinical CV involvement in IIM, through the analysis of parameters derived from the ultrasonographic assessment of carotid arteries, skin autofluorescence and body composition as compared to a control group. We also evaluated their possible association to traditional cardiovascular risk factors and autoimmune disease activity parameters.

Materials and methods

Patients

In a case-control study in which we prospectively enrolled 43 consecutive in- and out-patients with a previous diagnosis of IIM according to the Bohan and Peter criteria (24, 25) referred to our Centre from January 2015 to December 2016 (35 definite IIM, 8 probable). Eighteen patients were affected by DM and 25 by PM. The mean disease duration was 8.8±7 years.

Patients with a known history of CVD and impaired renal function as defined by reduced estimated creatinine clearance (Cockroft-Gault formula) were excluded from the study.

Serum samples were tested for myositis specific and myositis associated autoantibodies (MSA and MAA) by line blot (Myositis profile 3, Euroimmun, Lubeck) for the following specificity: Mi-2, Ku, PM-Scl100, PM-Scl75, Jo-1, SRP, PL-7, PL-12, EJ, OJ, Ro-52, MDA5, Tif1gamma. Among MSA, positivity was found in 16 patients: 9 Jo-1, 2 Pl-7, 1 Pl-12, 2 Tif1g, and 1 anti-SRP. MAA were positive in 9 patients: 8 Ro-52, 1 PM/Scl75.

Twenty-nine subjects without overt CVD, autoimmune and/or metabolic diseases were recruited as controls (CT).

The study was approved by the local ethics committee and conducted according to the Helsinki declaration; written informed consent was obtained from all the subjects enrolled in the study.

Methods

At the time of enrolment all patients underwent clinical and laboratory evaluations, which included:

Disease activity according to the International Myositis Assessment and Clinical Studies group (IMACS) which included: physician's and patient's global activity (PGA) (26), manual

muscle testing 8 (MMT8) (27), health quality assessment – HAQ (28), serum muscle enzymes: creatine kinase (CK), aldolase, alanine aminotransferase (ALT), aspartate aminotransferase (AST). Patients were classified as "clinically active" if physician VAS>3 and MMT8<72 or CK or aldolase blood levels were higher than twice the upper limit for normality.

Previous and ongoing treatment of the disease: corticosteroids cumulative dose, immunosuppressants, intravenous immunoglobulins.

Blood analyses by routine techniques for fasting glucose, glycated haemoglobin (HbA1c), total cholesterol, triglyceride, low- and high-density lipoproteins (LDL and HDL), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR).

In both the patients and the CT we searched for traditional CV risk factors: smoking habit, presence of diabetes mellitus (according to the American Diabetes Association (29), arterial hypertension (values ≥140 mmHg systolic and/or ≥90 mmHg diastolic blood pressure (30) or use of antihypertensive drugs), hypercholesterolaemia, family history of CVD (first-degree relatives suffered before 60 years of age), MS according to the American Heart Association (31).

In 14 patients we also evaluated fasting insulin concentrations and insulin resistance as HOMA index (HOMA-IR) (fasting glucose*fasting insulin/22.5) (32). HOMA-IR was classified as normal if <2.60, "borderline high" if ≥2.60 <3.80, and as "high" if HOMA-IR ≥3.80 (32). Blood pressure was measured by an automatic sphygmomanometer and an average of three consecutive measures obtained in supine resting condition was considered.

Carotid ultrasound analysis

For each subject, ultrasound B-mode image sequences of common carotid arteries were acquired by high-resolution B-mode ultrasound equipment (10-MHz linear-array probe, 25 frame/sec, MyLabAlpha-ESAOTE™ , Genoa, Italy) and analysed by an automatic system (Carotid Studio, Quipu, Italy) for the measurement of IMT and mean arterial diameter (mAD) (33). In addition, pulse pressure (PP) was estimated by the sphygmomanomer at brachial level, and the distensibility coefficient derived.

Briefly, carotid longitudinal scans were performed by a trained operator. The systolic and diastolic carotid diameters were automatically measured, 1-2 cm beneath the bifurcation. Carotid diameter was calculated as the distance between media-adventitia interfaces. For each frame the arterial interfaces were automatically detected using a contour tracking algorithm that can process B-mode ultrasound sequences of the longitudinal section of the vessel and arterial diameter calculated.

Cross-sectional distensibility coefficient was estimated through the variations in arterial cross-sectional area and blood pressure during systole and computed as: $\Delta A/(PP*A)$, where A is the diastolic lumen area, ΔA is the stroke change in lumen area, and PP is the pulse pressure. The lumen area and stroke change in the lumen area were calculated from diameter and distension values, assuming the cross section of the artery to be circular.

Common carotid IMT was automatically and simultaneously measured in the far wall of the common carotid artery, 1 cm proximal to the carotid bulb, as the distance between the lumen-intima and media-adventitia interfaces.

Assessment of skin AGE accumulation

Skin AGE accumulation was assessed by means of skin AF, following the principles of the AGE reader (AGE Reader™ , DiagnOptics technologies, The Netherlands) (34). Skin AF was assessed using a fluorescent lamp which illuminates the skin of the forearm. Measurements were performed at a skin site without lesions, to prevent influence of skin disease, especially in DM patients. The spectrum at the skin with lamp on was subtracted from the spectrum with the lamp off and the AF values were automatically calculated by the software.

Determination of body composition

We measured waist circumference (WC) and body weight and calculated waist to height ratio (WHR) and body mass index (BMI) as routine measurements of obesity and fat distribution. Body composition was also evaluated by the bioelectrical impedance analysis (BIA - Tanita, The Netherlands) after at least 3 hours of fasting. Briefly, BIA uses a constant high frequency current source applied by electrodes positioned under the feet of the subjects (35). The current passes freely through the fluids contained in muscle tissue and in blood, but encounters difficulty when passing through fat tissue, which contains little water. By measuring bioelectrical impedance in the body along with gender, age, height and weight, BIA can quickly and reliably calculate body composition as fat percentage (FAT%), the body mass fat (FATM - expressed in kg), the free fat body mass (FFM - expressed in kg).

Statistical analysis

All variables were expressed as median±interquartile range (IQR). The difference between patients and CT was calculated using the parametric test (ANOVA) when normally distributed or using the Mann-Whitney and Kruskal-Wallis non-parametric tests when variables were not normally distributed (StatView and JMP, SAS Institute Inc). Correlations between variables were calculated using the Spearman correlation coefficient. A multivariate analysis was used to assess possible differences in the evaluated parameters due to underlying treatment, adjusted for age and duration of the disease.

Results

Characteristics of patients and controls

Anthropometric and clinical characteristics of patients and CT are reported in Table I; they were comparable for age and gender, personal and familiar history of CV diseases, BMI, presence of diabetes mellitus, smoking habits.

Patients with IIM presented a slight increase of total cholesterol levels with normal HDL, LDL and triglycerides values and a slight increase of HbA1c with normal fasting glucose values (Table II). A significant difference was observed for lipid lowering drugs use, since 2 patients were treated with ezetimibe and 1 with pravastatin while

one control only was treated with simvastatin. Moreover, although baseline systolic and diastolic blood pressures were similar in both groups, 10 IIM patients were under antihypertensive treatment compared to 4 CT.

Twenty-nine IIM patients were treated with immunosuppressive (IS) drugs: 11 cyclosporine A, 8 methotrexate, 7 mycofenolate mophetil, 2 azathioprine, and 1 cyclophosphamide, 4 rituximab. Twelve patients were receiving intravenous immunoglobulins. All IIM patients were on steroid therapy with an approximate mean cumulative dose of 13.3±26.5 grams of methylprednisolone (minimum 0.5, maximum 138 grams). At the moment of the enrolment, patients were treated with a mean daily dose of 4.37±6.37 mg of methylprednisolone.

Disease activity parameters in the IIM cohort are reported in Table III. According to the definition specified in the methods section, 14 patients were classifiable as "clinically active".

Carotid ultrasonography parameters

Arterial parameters in the study population and CT are reported in Table IV. Patients and CT were similar as far as IMT, while a significant statistical difference was found in mean arterial diameter (7.46±0.2 in patients *vs*. 6.80±0.15 in CT p=0.012). A trend towards a lower distensibility coefficient was observed in IIM, although not statistically significant (p=0.08).

In both patients and CT, mAD was not related to sex and age at the evaluation and no correlations were found among carotid parameters and disease activity parameters in IIM patients.

Although carotid parameters were not different in patients with diabetes and those without diabetes, mAD was correlated to HbA1c serum levels (rho=0.62 p<0.003) and fasting glucose levels (rho=0.48 p=0.028). A higher mAD was identified in patients with compared to those without MS (8.35±1.83 *vs*. 7.1±1.4 p=0.011), while no differences were identified as far as others carotid parameters. In IIM, both WC and WHR correlated with mAD (rho=0.756 p<0.001 and rho=0.673 p<0.001 respectively) and IMT (rho=0.388 p=0.046 and rho=0.428 p=0.026) – corrected for gender.

No correlations were found among carotid values and other traditional CV and metabolic risk factors. No difference was observed according to disease activity parameters.

Skin AF in patients and controls

The analysis of the AGEs accumulation in the skin, as evaluated by AF, showed that patients with IIM presented higher AF values than CT (2.70±0.6 *vs*. 2.10±0.7 p<0.001) – Table IV.

AF was related to WHR (rho=0.375 p=0.014) and fasting insulin (rho=0.315 p=0.007) but not with WC.

A significant correlation was observed among AF and mAD (rho=0.317 p<0.05), carotid distension (rho=0.391 p=0.036) and IMT (rho=0.627 p<0.001) in IIM patients only (Fig. 1). No difference was found between AF levels and traditional cardiovascular risk factors, metabolic and disease activity parameters.

Body composition and metabolic status

Compared to CT, patients with IIM showed a lower FFM (p=0.036) while they were not different for FAT% and FATM (Table IV). Although it is known that the female population has higher FAT% compared to males, these results

Table I. Clinical characteristics of the cohort in patients and controls (CT).

	Patients (n=43)	CT (n=29)	p-value
Age (years)	57 ± 2	51 ± 2	NS
Female/male ratio	31:12	15:14	NS
Mean disease duration (years)	8.8 ± 7	NA	NA
History of CV disease	0	0	NS
Family history of CV disease	18 (41.9%)	9 (31.0%)	NS
BMI (kg/m^2)	24.4 ± 0.61	24.8 ± 0.67	NS
Waist circumference (cm)	88 ± 22.3	79 ± 19	p=0.026
Waist height ratio	0.53 ± 0.12	0.48 ± 0.11	p=0.003
Body weight (kg)	61.2 ± 22	71.5 ± 18	NS
Systemic hypertension	10 (23.2%)	4 (13.8 %)	NS
Blood pressure			
systolic	130.9 ± 2.2	126.6 ± 2.3	NS
diastolic	81.7 ± 1.5	83.8 ± 1.4	NS
Hypercholesterolaemia	3 (7%)	1 (3%)	NS
Smoking habits	7 (16%)	5 (17%)	NS
Diabetes mellitus	4 (9.3%)	2 (6.9 %)	NS
Metabolic syndrome	10 (23.3%)	0 (0%)	p<0.001
Steroid dose evaluation, mean (gr)	4.3 ± 0.75	0	NA
Cumulative steroid dose, mean (gr)	~ 13.3 ± 26.5	~ 0	NA

Values are expressed in mean ± standard deviation NA: not available; NS: not statistical significant.

Table II. Laboratory parameters in IIM patients. Values are expressed in median ± interquartile range (IQR).

Metabolic parameter	Values ± IQR	Normal value (range)	Patient out of normal value (%)
HbA1c (mmol/mol)	38.0 ± 6.0	20-39	8 (18.6)
Total cholesterol (mg/dl)	219.5 ± 64.3	<200	22 (51.2)
HDL (mg/dl)	61.0 ± 34.5	>45	7 (16.3)
LDL (mg/dl)	131.9 ± 47.0	<130	13 (30.2)
Triglycerides (mg/dl)	114.0 ± 103.5	<150	11 (25.6)
Fasting glucose (mg/dl)	85.0 ± 15.8	(74-109)	4 (9.3)
Fasting insulin (mU/l)	25.1 ± 4.2	<25	11
Creatinine(mg/dl)	0.72 ± 0.21	<0.8	7 (16.3)
CRP (mg/dl)	0.21 ± 0.51	<0.5	6 (14.0)
ESR (mm/h)	24.0 ± 27.8	<30	15 (38.9)

HbA1c: glycated haemoglobin; HDL: high density lipoproteins; LDL: low density lipoproteins; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

Table III. Disease activity parameters according to IMACS criteria in IIM patients. Values are expressed in median ± interquartile range (IQR)

IMACS core set	Values ± IQR	Normal value (range)	Patient out of normal value (%)
MMT8	74.0 ± 14.0	80 (0-80)	31 (71.1)
HAQ	0.5 ± 1.0	0 (0-3)	32 (74.4)
Physician's VAS (cm)	2.1 ± 3.0	0 (0-10)	37 (86.0)
Patient's VAS (cm)	3.9 ± 4.0	0 (0-10)	35 (81.4)
Serum parameters			
CK (U/L)	143.5 ± 180.25	<190	11 (25.6)
Aldolase (U/L)	8.3 ± 3.5	<7.5	19 (44.2)
ALT (U/L)	24.5 ± 17.5	<41	7 (16.3)
AST (U/L)	23.0 ± 23.4	<40	8 (18.6)

MMT8: manual muscle test 8; HAQ: health quality assessment; VAS: visual analogue scale; CK creatine-kinase; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

Table IV. Carotid, AGEs and body composition parameters in the IIM patients and controls (CT). Data are expressed as median ± interquartile range.

Carotid parameters	Patients	CT	*p*-value
mAD, mm	7.46 ± 0.2	6.80 ± 0.90	0.012
Carotid distensibility, Kpa^{-1} 10^{-3}	24.8 ± 10.8	26.9 ± 16.7	N.S.
IMT, MM	0.61 ± 0.13	0.59 ± 0.22	N.S.
AGEs			
Skin Autoflorescence	2.70 ± 0.6	2.10 ± 0.7	$p<0.001$
BIA			
FAT%	27.1 ± 11.2	24.3± 9.0	N.S.
FAT, Kg	19.1 ± 9.1	17.1 ± 10.2	N.S.
FFM, Kg	44.2 ± 14.7	52.0 ± 1709	0.036

AGEs: advanced glycation end-products; BIA: bioelectrical impedance analysis; mAD: mean arterial diameter; IMT: intima-media thickness; FAT%: percentage of body fat; FATM: fat mass; FFM: free fat mass.

were confirmed also when the female population only both among patients and controls was analysed.

Correlations between mAD and FFM were reported in both IIM patients and CT (rho=0.485 p=0.006 and rho=0.480 p=0.011, respectively). In IIM patients a correlation between mAD and FATM was also reported (rho=0.57 p<0.001).

Patients with MS had higher values of FATM (29.2±7.1 *vs*. 16.2±8.6 p<0.001), FAT% (40.4±10.3 *vs*. 25.5±12.4 p=0.006) and lower FFM (46.3±9.0 *vs*. 41.6±12.3 p=0.039) when compared to those without MS. HOMA-IR was calculated in 14 patients: respectively 3 and 2 patients had a normal or borderline HOMA-IR and 9 patients had high values, suggesting the presence of clear correlates of peripheral insulin resistance in the IIM population. Patients with HOMA-IR out of the normal range (≥2.6) had higher FFM compared to those with normal HOMA-IR (44.6 *vs*. 35.4 p=0.007). As far as skin AF, relations were observed with FAT% (rho=0.417 p=0.008) and FATM (rho=0.343 p=0.032) in IIM patients only.

No difference was found among parameters of body composition and traditional cardiovascular risk factors and disease activity parameters.

Relation with autoantibodies, disease duration and treatment

The presence of specific autoantibodies subsets, *i.e*. the antisynthetase autoantibodies, MSA and MAA, were not related to the carotid ultrasonographic derived parameters, AF or indices of body composition.

To assess the possible influence of disease duration on the different parameters under study, the enrolled population was divided in two groups according to a disease duration greater or lower than 5 years. No differences between the two groups could be observed for all the analysed parameters.

When the role of underlying treatments (corticosteroids cumulative dose and immunosuppressants) was considered, no significant differences were identified for the parameters included in this study by a multiparametric approach.

Discussion

In the present study non-invasive techniques were employed to identify patients with IIM at higher risk of CVD beyond traditional risk factors like smoke, diabetes, arterial hypertension, hypercholesterolaemia and family history of CVD. Accelerated atherosclerosis has been reported in several systemic autoimmune diseases (36), but the first study on subclinical atherosclerosis in IIM patients was published by Vincze and colleagues in 2014 (10), reporting an increased arterial stiffness and decreased flow-mediated vasodilatation in the brachial artery, hypothesising a high risk for these patients to develop CVD.

A statistically significant difference in carotid mAD between IIM patients and CT was found, and mAD was higher in patients with IIM and MS. IMT still remains a main CV risk factor in IIM patients (10, 11), but a larger carotid diameter may also predict CVD independently of IMT (37, 38), as the chronic inflammatory process may play an adjunctive role in the maladaptive remodelling process in these patients (39). Our findings are similar to those reported for rheumatoid arthritis (RA) (40, 41). Although in this study we used an automatic analysis of carotid artery parameters that allowed a more reproducible evaluation of the carotids, also the measurements obtained by conventional ultrasounds may give reliable results provided that the images are acquired by a skilled technician.

We also investigated AGEs accumulation in the skin. AGEs are produced from non-enzymatic glycation of proteins (42) and their formation may be increased in conditions of reduced renal clearance (43) or oxidative stress (44, 45), as expected in patients with chronic inflammation. AGEs can predict long-

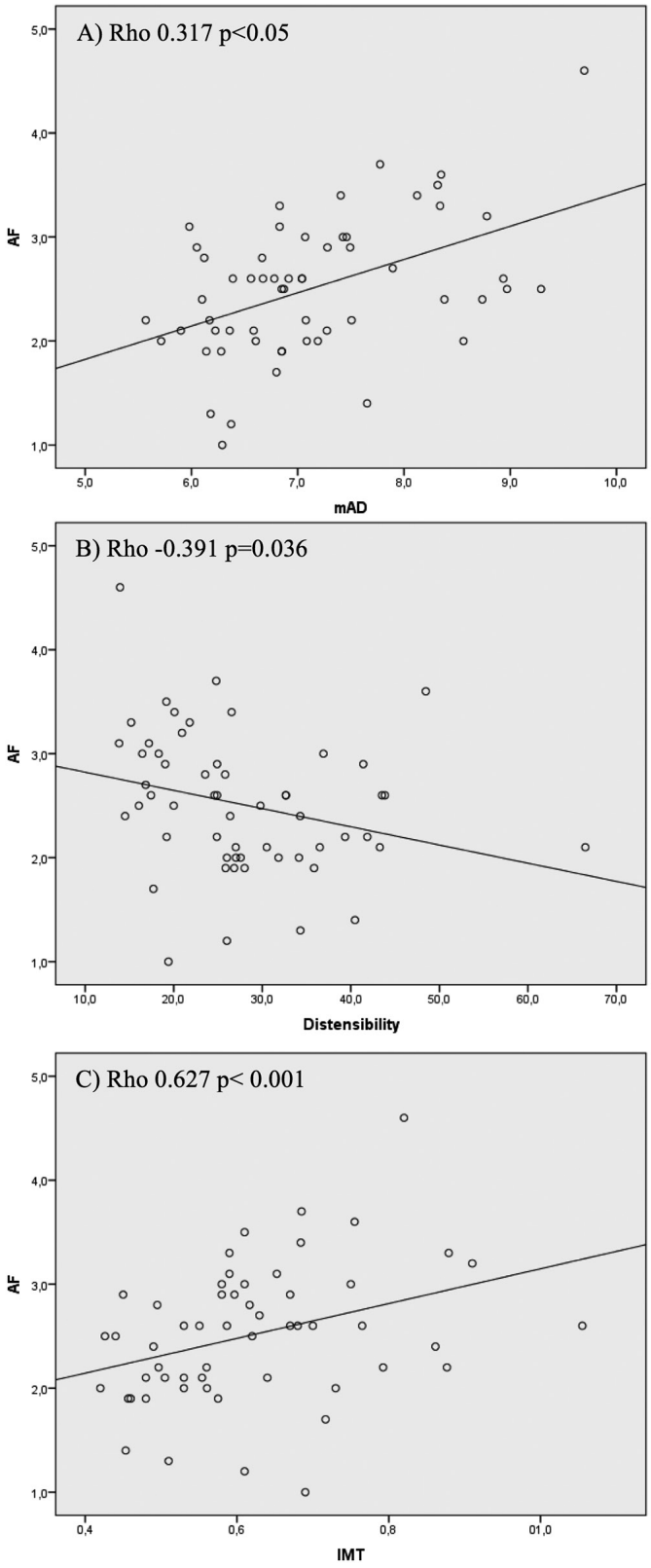

Fig. 1. Scatter plots of autofluorescence in IIM patients for: **A**: mean arterial diameter; **B**: carotid distension; **C**: intima-media thickness. Statistical analysis was performed using Spearman's correlation. mAD: mean arterial diameter, IMT: intima-media thickness, AF: autofluorescence.

term vascular complications (19-21) and the accumulation of AGEs may contribute to the progression of atherosclerosis, leading to the reduction of elasticity and increasing thickness of the vascular wall (46). Traditional measurements of AGEs accumulation require invasive sampling but the results obtained using an AF reader are strictly related to the AGEs obtained by skin biopsies (47) and are associated to increased risk of CVD (48-50). To our knowledge no study has been performed on AGE skin accumulation in IIM, while few data are available about other systemic autoimmune diseases such as systemic sclerosis (SSc) (51), RA (52, 53) and systemic lupus erythematosus (SLE) (46, 54). As already reported in SLE, we did not found a correlation between skin AF and CRP or HbA1c (46) probably due to the pivotal role of the inflammatory process in AGEs production and accumulation in patients with autoimmune disease. The reported association between AF and mAD suggests that in IIM patients, skin AF may be related with subclinical carotid abnormalities and linked to the presence of subclinical cardiovascular involvement. Previously, Dadoniene *et al*. reported an association between AGEs accumulation and subclinical cardiovascular involvement in SSc patients (55); in SLE and RA patients, a positive correlation between skin AF and IMT was also demonstrated (46, 52). These observations indicate a contribution of AGEs to the accelerated atherosclerosis in patients with chronic inflammatory diseases, including IIM patients.

Body composition assessment, in particular an increase FATM, has been proposed as an important tool in the prediction of cardiovascular and total mortality in patients with CVD (22). In IIM patients we reported a lower FFM as compared to CT. Muscular inflammation, which may cause muscular atrophy and fatty infiltrates, as often observed by muscular MRI in this population (56, 57), could be responsible of the reduced FFM. Although without statistical significance, IIM patients showed higher FAT% and FATM compared to CT with similar BMI. Body fat has been correlated to higher cardiometabolic risk in RA patients (58); in our study FAT% was related to AF and FATM with both mAD and AF, suggesting an interaction between the different parameters included in our study and strengthening the role of a combined multiparametric approach in IIM for a

more comprehensive assessment of CV risk and subclinical CV involvement.

Patients with MS had alterations of all the parameters measured by bioimpedance analysis. Recent studies associated the high prevalence of MS and basal insulinaemia in IIM, as well as the increase in pro-inflammatory cytokines, which are able to impair insulin signalling (59). In our cohort, insulin concentrations were available only in a small subgroup, but indicated increased insulin resistance associated with increased AF.

It is well known that corticosteroids chronic treatment may increase cardiometabolic risk factors, since they may cause hyperglycaemia, systemic hypertension and abnormalities in lipid metabolism. However, we could not report any correlation between the steroid and immunosuppressive treatments and the parameters included in the study. This could be explained by a possible role of this treatments in reducing the inflammatory component of the atherosclerotic process (60).

The main strength of our study lies in the evaluation of different aspects of subclinical CV involvement, which relies on the combined acquisition of clinical, laboratory and instrumental data during the same outpatient visit, thus reducing the variability due to temporally spaced analysis. The main limitations of this study are represented by the lack of a control group of untreated myositis patients, which could have provided data not influenced by current treatment and the unavailability of biochemical assessment of controls at the enrolment. Despite growing literature data in rheumatic and metabolic diseases, skin AF is not yet a validated biomarker of CV involvement. The use – for technical reasons – of brachial pressure instead of local carotid pulse pressure for the determination of carotid parameters might impair the measurement of carotid distensibility. However, the possible discrepancy in the results should not be significant since patients and CT are middle aged, and the pulse pressure amplification between carotid and brachial pressure is less evident than in younger subjects.

In summary, this multiparametric clinical approach allowed to define an increased cardiometabolic risk in IIM patients as compared to CT. Our results suggest that patients with IIM are more likely to develop CVD as compared to CT even in absence of traditional CV risk factors. However, as the presence of MS is associated to alterations in body composition and carotid parameters, we recommend that all IIM patients should be assessed for the presence of MS since the diagnosis.

We can conclude that the techniques used in our study, all non-invasive, repeatable and providing on-line information, may be proposed as a screening tool for the assessment of CVD risk in IIM patients during routine clinical visits.

References

1. DALAKAS MC, HOHLFELD R: Polymyositis and dermatomyositis. *Lancet* 2003; 362: 971-82.
2. SITI HN, KAMISAH Y, KAMSIAH J: The role of oxidative stress, antioxidants and vascular inflammation in cardiovascular disease (a review). *Vascul Pharmacol* 2015; 71: 40-56.
3. GOLIA E, LIMONGELLI G, NATALE F *et al*.: Inflammation and Cardiovascular Disease: From Pathogenesis to Therapeutic Target. *Curr Atheroscler Rep* 2014; 16: 435.
4. VAN GELDER H, CHARLES-SCHOEMAN C: The heart in inflammatory myopathies. *Rheum Dis Clin North Am* 2014; 40: 1-10.
5. LUNDBERG IE, FORBESS CJ: Mortality in idiopathic inflammatory myopathies. *Clin Exp Rheumatol* 2008; 26: S109-14.
6. DIEDERICHSEN LP: Cardiovascular involvement in myositis. *Curr Opin Rheumatol* 2017; 29: 598-603.
7. BARSOTTI S, BRUNI C, COMETI L *et al*.: One year in review 2017: Idiopathic inflammatory myopathies. *Clin Exp Rheumatol* 2017; 35: 875-84.
8. MARASCO E, CIOFFI E, COMETI L *et al*.: One year in review 2018: idiopathic inflammatory myopathies. *Clin Exp Rheumatol* 2018; 36: 937-47.
9. DIEDERICHSEN LP, SIMONSEN JA, DIEDERICHSEN AC *et al*.: Cardiac abnormalities in adult patients with polymyositis or dermatomyositis as assessed by noninvasive modalities. *Arthritis Care Res* (Hoboken) 2016; 68: 1012-20.
10. VINCZE M, DÉR H, KEREKES G *et al*.: Decreased flow-mediated dilatation with increased arterial stiffness and thickness as early signs of atherosclerosis in polymyositis and dermatomyositis patients. *Clin Rheumatol* 2014; 33: 1635-41.
11. SOLTÉSZ P, DÉR H, KEREKES G *et al*.: A comparative study of arterial stiffness, flow-mediated vasodilation of the brachial artery, and the thickness of the carotid artery intima-media in patients with systemic autoimmune diseases. *Clin Rheumatol* 2009; 28: 655-62.
12. RIEMEKASTEN G, OPITZ C, AUDRING H *et al*.: Beware of the heart: the multiple picture of cardiac involvement in myositis. *Rheumatology* (Oxford) 1999; 38: 1153-57.
13. GRUNDTMAN C, LUNDBERG IE: Vascular involvement in the pathogenesis of idiopathic inflammatory myopathies. *Autoimmunity* 2009; 42: 615-26.
14. ARAUJO PAO, SILVA MG, BORBA EF, SHINJO SK: High prevalence of metabolic syndrome in antisynthetase syndrome. *Clin Exp Rheumatol* 2018; 36: 241-47.
15. DE SOUZA FHC, SHINJO SK: The high prevalence of metabolic syndrome in polymyositis. *Clin Exp Rheumatol* 2014; 32: 82-7.
16. DE MORAES MT, DE SOUZA FHC, DE BARROS TBM, SHINJO SK: Analysis of metabolic syndrome in adult dermatomyositis with a focus on cardiovascular disease. *Arthritis Care Res* (Hoboken) 2013; 65: 793-99.
17. LAURENT S, COCKCROFT J, VAN BORTEL L *et al*.: Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J* 2006; 27: 2588-605.
18. VISTOLI G, DE MADDIS D, CIPAK A, ZARKOVIC N, CARINI M, ALDINI G: Advanced glycoxidation and lipoxidation end products (AGEs and ALEs): an overview of their mechanisms of formation. *Free Radic Res* 2013; 47: 3-27.
19. MEERWALDT R, LUTGERS HL, LINKS TP *et al*.: Skin autofluorescence is a strong predictor of cardiac mortality in diabetes. *Diabetes Care* 2007; 30: 107-12.
20. GENUTH S, SUN W, CLEARY P *et al*.: Glycation and carboxymethyllysine levels in skin collagen predict the risk of future 10-year progression of diabetic retinopathy and nephropathy in the diabetes control and complications trial and epidemiology of diabetes interventions and complications participants with type 1 diabetes. *Diabetes* 2005; 54: 3103-11.
21. KILHOVD BK, JUUTILAINEN A, LEHTO S *et al*.: High serum levels of advanced glycation end products predict increased coronary heart disease mortality in nondiabetic women but not in nondiabetic men: a population-based 18-year follow-up study. *Arterioscler Thromb Vasc Biol* 2005; 25: 815-20.
22. SRIKANTHAN P, HORWICH TB, TSENG CH: Relation of muscle mass and fat mass to cardiovascular disease mortality. *Am J Cardiol* 2016; 117: 1355-60.
23. MORELLI M, GAGGINI M, DANIELE G, MARRACCINI P, SICARI R, GASTALDELLI A: Ectopic fat: the true culprit linking obesity and cardiovascular disease? *Thromb Haemost* 2013; 110: 651-60.
24. BOHAN A, PETER JB: Polymyositis and dermatomyositis (second of two parts). *N Engl J Med* 1975; 292: 403-7.
25. BOHAN A, PETER JB: Polymyositis and dermatomyositis (first of two parts). *N Engl J Med* 1975; 292: 344-47.
26. RIDER LG, FELDMAN BM, PEREZ MD *et al*.: Development of validated disease activity and damage indices for the juvenile idiopathic inflammatory myopathies: I. Physician, parent, and patient global assessments. Juvenile

Dermatomyositis Disease Activity Collaborative Study Group. *Arthritis Rheum* 1997; 40: 1976-83.
27. RIDER LG, KOZIOL D, GIANNINI EH *et al*.: Validation of manual muscle testing and a subset of eight muscles for adult and juvenile idiopathic inflammatory myopathies. *Arthritis Care Res* (Hoboken) 2010; 62: 465-72.
28. ALEXANDERSON H, DEL GRANDE M, BINGHAM CO *et al*.: Patient-reported Outcomes and Adult Patients' Disease Experience in the Idiopathic Inflammatory Myopathies. Report from the OMERACT 11 Myositis Special Interest Group. *J Rheumatol* 2014; 41: 581-92.
29. Classification and diagnosis of diabetes. *Diabetes Care* 2017; 40 (Suppl. 1): S11-S24.
30. MANCIA G, FAGARD R, NARKIEWICZ K *et al*.: 2013 ESH/ESC guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J* 2013; 34: 2159-19.
31. GRUNDY SM: Definition of Metabolic Syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association Conference on Scientific Issues Related to Definition. *Circulation* 2004;109: 433-38.
32. MATTHEWS DR, HOSKER JP, RUDENSKI AS, NAYLOR BA, TREACHER DF, TURNER RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28: 412-9.
33. BIANCHINI E, BOZEC E, GEMIGNANI V *et al*.: Assessment of carotid stiffness and intima-media thickness from ultrasound data: comparison between two methods. *J Ultrasound Med Off J Am Inst Ultrasound Med* 2010; 29: 1169-75.
34. MULDER DJ, WATER T VAN DE, LUTGERS HL *et al*.: Skin autofluorescence, a novel marker for glycemic and oxidative stress-derived advanced glycation endproducts: an overview of current clinical studies, evidence, and limitations. *Diabetes Technol Ther* 2006; 8: 523-35.
35. KYLE UG, BOSAEUS I, DE LORENZO AD *et al*.: Bioelectrical impedance analysis – part II: utilization in clinical practice. *Clin Nutr* 2004; 23: 1430-53.
36. SOLTÉSZ P, KEREKES G, DÉR H *et al*.: Comparative assessment of vascular function in autoimmune rheumatic diseases: considerations of prevention and treatment. *Autoimmun Rev*. 2011; 10: 416-25.
37. EIGENBRODT ML, BURSAC Z, TRACY RE, MEHTA JL, ROSE KM, COUPER DJ: B-mode ultrasound common carotid artery intima-media thickness and external diameter: cross-sectional and longitudinal associations with carotid atherosclerosis in a large population sample. *Cardiovasc Ultrasound* 2008; 6: 10.
38. TERRY JG, TANG R, ESPELAND MA *et al*.: Carotid arterial structure in patients with documented coronary artery disease and disease-free control subjects. *Circulation* 2003; 107: 1146-51.
39. JENSEN-URSTAD K, JENSEN-URSTAD M, JOHANSSON J: Carotid artery diameter correlates with risk factors for cardiovascular disease in a population of. *Stroke* 1999; 39: 1572-77.
40. VAN SIJL AM, VAN DEN HURK K, PETERS MJL *et al*.: Different type of carotid arterial wall remodeling in rheumatoid arthritis compared with healthy subjects: a case-control study. *J Rheumatol* 2012; 39: 2261-66.
41. SCHOTT LL, KAO AH, CUNNINGHAM A *et al*.: Do carotid artery diameters manifest early evidence of atherosclerosis in women with rheumatoid arthritis? *J Womens Health (Larchmt)* 2009; 18: 21-29.
42. SCHLEICHER ED, WAGNER E, NERLICH AG: Increased accumulation of the glycoxidation product N(epsilon)-(carboxymethyl)lysine in human tissues in diabetes and aging. *J Clin Invest* 1997; 99: 457-68.
43. MIYATA T, WADA Y, CAI Z *et al*.: Implication of an increased oxidative stress in the formation of advanced glycation end products in patients with end-stage renal failure. *Kidney Int* 1997; 51: 1170-81.
44. MONNIER VM, SELL DR, ABDUL-KARIM FW, EMANCIPATOR SN: Collagen browning and cross-linking are increased in chronic experimental hyperglycemia. Relevance to diabetes and aging. *Diabetes* 1988; 37: 867-72.
45. NISHIKAWA T, EDELSTEIN D, DU XL *et al*.: Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 2000; 404: 787-90.
46. DE LEEUW K, GRAAFF R, DE VRIES R *et al*.: Accumulation of advanced glycation endproducts in patients with systemic lupus erythematosus. *Rheumatology* (Oxford) 2007; 46: 1551-56.
47. MEERWALDT R, GRAAFF R, OOMEN PHN *et al*.: Simple non-invasive assessment of advanced glycation endproduct accumulation. *Diabetologia* 2004; 47: 1324-30.
48. NOORDZIJ MJ, LEFRANDT JD, LOEFFEN EAH *et al*.: Skin autofluorescence is increased in patients with carotid artery stenosis and peripheral artery disease. *Int J Cardiovasc Imaging* 2012; 28: 431-38.
49. DE VOS LC, NOORDZIJ MJ, MULDER DJ *et al*.: Skin autofluorescence as a measure of advanced glycation end products deposition is elevated in peripheral artery disease. *Arterioscler Thromb Vasc Biol* 2013; 33: 131-38.
50. DE VOS LC, MULDER DJ, SMIT AJ *et al*.: Skin autofluorescence is associated with 5-year mortality and cardiovascular events in patients with peripheral artery disease. *Arterioscler Thromb Vasc Biol* 2014; 34: 933-38.
51. HETTEMA ME, BOOTSMA H, GRAAFF R, DE VRIES R, KALLENBERG CGM, SMIT AJ: Skin autofluorescence, as marker of accumulation of advanced glycation endproducts and of cumulative metabolic stress, is not increased in patients with systemic sclerosis. *Int J Rheumatol*. 2011;2011:417813.
52. DE GROOT L, HINKEMA H, WESTRA J *et al*.: Advanced glycation endproducts are increased in rheumatoid arthritis patients with controlled disease. *Arthritis Res Ther* 2011; 13: R205.
53. MATSUMOTO T, TSURUMOTO T, BABA H *et al*.: Measurement of advanced glycation endproducts in skin of patients with rheumatoid arthritis, osteoarthritis, and dialysis-related spondyloarthropathy using non-invasive methods. *Rheumatol Int* 2007; 28: 157-60.
54. NIENHUIS HL, DE LEEUW K, BIJZET J *et al*.: Skin autofluorescence is increased in systemic lupus erythematosus but is not reflected by elevated plasma levels of advanced glycation endproducts. *Rheumatology* 2008; 47: 1554-58.
55. DADONIENE J, CYPIENE A, RYLISKYTE L, RUGIENE R, RYLIŠKIENE K, LAUCEVIČIUS A: Skin autofluorescence in systemic sclerosis is related to the disease and vascular damage: a cross-sectional analytic study of comparative groups. *Dis Markers* 2015; 2015: 837470.
56. KUBÍNOVÁ K, MANN H, VENCOVSKÝ J: MRI scoring methods used in evaluation of muscle involvement in patients with idiopathic inflammatory myopathies. *Curr Opin Rheumatol* 2017; 29: 623-31.
57. BARTELS EM, SØRENSEN ER, HARRISON AP: Multi-frequency bioimpedance in human muscle assessment. *Physiol Rep*. 2015; 3: e12354.
58. LICHTASH CT, CUI J, GUO X *et al*.: Body adiposity index versus body mass index and other anthropometric traits as correlates of cardiometabolic risk factors. *PLoS One* 2013; 8: e65954.
59. DE OLIVEIRA DS, SILVA MG, SHINJO SK: Insulin resistance is increased in adult patients with dermatomyositis. *Medicalexpress* 2018; 5: mo18003.
60. TUTTOLOMONDO A, DI RAIMONDO D, PECORARO R, ARNAO V, PINTO A, LICATA G: Atherosclerosis as an inflammatory disease. *Curr Pharm Des* 2012; 18: 4266-88.