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Original article

Abnormal paraoxonase-1 (PON1) enzyme activity in idiopathic inflammatory myopathies

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Abstract

Objectives. Patients with idiopathic inflammatory myopathies (IIM) have severe vascular involvement, which contributes to disease morbidity and mortality. Paraoxonase-1 (PON1) is a high-density lipoprotein (HDL) associated protein that protects the vascular endothelium from oxidative injury and damage. The current work assessed the functional and genetic determinants of PON1 activity in IIM patients.

Methods. A total of 184 IIM patients and 112 healthy controls (HC) were included. PON1 enzyme activity was assessed by paraoxonase, arylesterase and lactonase assays, and the Q192R PON1 single nucleotide polymorphism (SNP) was analysed. Multivariate regression models examined associations of PON1 activity with IIM diagnosis and myositis disease outcomes.

Results. The arylesterase and lactonase activities of PON1 were significantly lower in IIM patients compared with HC. Higher myositis disease activity, the presence of severe IIM-associated interstitial lung disease (ILD), and the presence of MDA5 or anti-synthetase antibodies were significantly associated with lower PON1 activity. The PON1 Q192R polymorphism was strongly linked to the paraoxonase activity of PON1 in IIM, and patients with the PON1 QQ genotype had better IIM disease outcomes compared with patients with the QR or RR genotypes.

Conclusions. The arylesterase and lactonase activities of PON1 are significantly impaired in IIM patients compared with HC, and inversely associate with IIM disease activity and the presence of severe ILD. The PON1 QQ genotype associates with more favourable disease outcomes in IIM patients. Large prospective studies are needed to further evaluate the role of PON1 and PON1 genetic polymorphisms in the development and propagation of IIM and IIM-ILD.

Key words: idiopathic inflammatory myopathy, dermatomyositis, polymyositis, inclusion body myositis, paraoxonase1, PON1, paraoxonase, arylesterase, lactonase

CLINICAL SCIENCE

Rheumatology key messages

- IIM patients have suppressed arylesterase and lactonase activities of the HDL-associated antioxidant enzyme, PON1, compared to controls.
- Lower arylesterase and lactonase activities associate with higher IIM disease activity and presence of severe ILD.
- The QQ genotype of the PON1 Q192R polymorphism associated with more favorable IIM disease outcomes.

Introduction

Idiopathic inflammatory myopathies (IIM) are systemic autoinflammatory diseases of the muscle, associated with high morbidity and mortality [1–3]. Activation and damage to the microvasculature in IIM are strongly implicated in IIM disease pathogenesis, with microvascular damage identified in connective tissue of the muscle, skin and lung, particularly in patients with DM [4, 5]. Mechanisms for increased vascular damage in IIM are incompletely understood.

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Paraoxonase-1 (PON1) is a high-density lipoprotein (HDL)-associated enzyme that promotes the antioxidant, anti-inflammatory function of HDL, and protects the vascular endothelium from damage due to oxidized phospholipids, which accumulate under conditions of oxidative stress [6, 7]. PON1 hydrolyzes a wide range of substrates, and a comprehensive assessment using multiple substrates is important in understanding the functional properties of PON1. Several pharmaceuticals such as statins and aspirin may modulate the activity of PON1 [8–10], and work has also demonstrated that PON1 can affect glucocorticoid metabolism through hydrolysis of glucocorticoid γ-lactones and cyclic carbonates [11]. Genetic polymorphisms, such as the Q192R variant in the coding region of PON1, contribute to variations in PON1 activity [12, 13]. No work to date has comprehensively evaluated the functional and genetic determinants of PON1 activity in patients with IIM.

Methods

Study population

Myositis patients and healthy controls (HC) were recruited from the UCLA medical centre. All myositis patients met EULAR)/ACR Classification Criteria for adult IIM for at least 'probable' IIM [14] and subclasses including DM, PM and inclusion body myositis (IBM) were verified by chart review. Antisynthetase syndrome (ASS) was defined as patients with autoantibodies against an aminoacyl transfer RNA (tRNA) synthetase and characteristic clinical features such as interstitial lung disease (ILD), non-erosive arthritis, Raynaud's phenomenon and fever. All subjects gave written informed consent for the study approved by the Human Research Subject Protection Committee at UCLA (IRB# 10–001833).

Clinical assessments

Laboratory studies including creatine phosphokinase (CPK) levels, inflammatory markers [high-sensitivity CRP (hsCRP) and westergren ESR], and fasting lipid profiles [total cholesterol, low-density lipoprotein-cholesterol (LDL-C), HDL-cholesterol (HDL-C), triglycerides] were performed by the UCLA clinical laboratory using standard methods. Additional blood was collected in heparinized tubes (Becton Dickinson, Mississauga, ON, Canada), and plasma stored at -80°C for PON1 activity assessments.

Myositis autoantibodies (autoAb) were available for 114/184 IIM patients analysed in the current study (100 specimens analysed at the Oklahoma Medical Research Foundation and 14 specimens analysed in other clinical labs using standardized protocols).

Cardiovascular (CV) risk and health information including the presence of concomitant malignancy was obtained by questionnaire and chart review. Statin intensity was defined by the American Heart Association Task Force on Practice Guidelines [15]. Disease activity and damage were assessed using physician global scales by visual analogue scale (VAS) and 5-point Likert scale [16]. ILD was defined by radiographic findings consistent with ILD on high-resolution chest CT (HRCT) per a radiologist read showing at least one of the following: (i) reticulation and fibrosis; (ii) traction bronchiectasis; (iii) honeycombing; or (iv) ground glass opacification [17]. All HRCT and pulmonary function test (PFT) results closest to blood collection date were included in the analysis. Patients with IIM-associated ILD (IIM-ILD) were divided by severity based on the diffusion capacity (DLCO $\geq$40% predicted as mild to moderate; DLCO $<$40% predicted as severe) and separately by forced vital capacity (FVC $\geq$50% predicted as mild to moderate, FVC $<$50% predicted as severe), with cut points supported by clinical trials in idiopathic pulmonary fibrosis [18]. DLCO rather than FVC cutoffs were selected *a priori* to determine severity of ILD as FVC is frequently impacted by respiratory muscle weakness in patients with IIM.

Determination of PON1 activity and genotype

PON1 activity was quantified using three different substrates (paraoxon, dihydrocoumarin and phenylacetate) to assess its paraoxonase, lactonase and arylesterase activities respectively as described previously [19]. The PON1 Q192R polymorphism was determined in IIM patients and controls as described previously [20].

Statistical analysis

A student's *t test* or Wilcoxon rank-sum test was used to compare continuous variables, and a $\chi 2$ test or fisher's exact test was used to compare categorical variables between groups. Patients with IBM were analysed separately with an age-matched control group, as patients with IBM were significantly older than patients with DM or PM.

Multivariate forward stepwise linear regression analysis was performed to examine the association of IIM diagnosis with PON1 activity after accounting for other variables previously linked to PON1 activity. PON1 activity was included as the outcome variable in the linear regression analysis and the IIM/control variable was included as one of the predictors. Other variables in the model included age, sex, traditional lipid levels, statin use [21–24], BMI, diabetes, HTN and inflammatory markers. Additional traditional CV risk factors considered in the stepwise regression model included history of coronary artery disease (CAD), family history of premature CAD and smoking status. The final stepwise model was selected to minimize the Bayes Information Criterion (BIC) [25].

Further analysis of the IIM cohort alone (excluding HC) was performed using univariate linear regression analysis to identify clinical and laboratory characteristics of IIM patients associated with PON1 activity. Additional multivariate stepwise linear regression analyses were performed to evaluate predictors of PON1 activity as

well as disease activity and damage in the IIM cohort, using a similar approach as described above. All statistical analyses were performed using JMP Pro 14.0 (SAS Institute Inc., Cary, NC, USA). All *P*-values are two sided, with significance level at <0.05. Logarithm transformation was applied for skewed variables (hsCRP, CPK) when included in linear models. In all linear models, a single representative variable was selected in cases where variables were correlated ($r \geq 0.4$) to avoid collinearity, and categorical variables with a small number of observations in an individual category were excluded due to them producing unstable regression coefficients.

Results

Demographics and clinical characteristics of IIM patients and healthy controls (HC)

A total of 184 patients with IIM and 112 HC were included in the analysis. DM/PM patients ($n = 169$) were compared with the entire HC group ($n = 112$) (Table 1), while IBM ($n = 15$) patients were compared with an age-matched subgroup of HC ($n = 15$) (Supplementary Table S1, available at *Rheumatology* online). No significant differences between IIM patients and HC were observed in age, sex, race or ethnicity (Table 1 and Supplementary Table S1, available at *Rheumatology* online). Patients with DM/PM had significantly higher levels of systemic inflammation compared with HC (Table 1). Several traditional CV risk factors were also increased in the IIM group compared with HC including higher mean BMI (DM/PM/IBM), diabetes (DM/PM), hypertension (IBM), and higher levels of total cholesterol, LDL cholesterol levels (DM/PM) and triglycerides (DM/PM/IBM) (Table 1 and Supplementary Table S1, available at *Rheumatology* online).

IIM patients were predominantly female (71%), Caucasian (76%), and had chronic disease (mean disease duration >4 years). The majority of patients had DM (67%) with mild-moderate disease activity [physician global activity VAS 39 (19), CPK 608 (1485) U/l, mean (S.D.), and 35% with elevated CPK] and mild-moderate disease damage [physician global damage VAS 34 (23), mean (S.D.)]. IBM patients had lower disease activity by Likert scale ($P < 0.01$), and higher damage scores by VAS compared with DM/PM patients ($P = 0.03$).

A total of 114 patients had myositis autoAb testing performed, and 103 patients (91%) had at least one myositis-associated or specific autoAb. The most common autoAb were antisynthetase ab (21 patients: 16 Jo1, 2 PL-7, 1 PL-12, 1 EJ, 1 KS) and anti-TIF1-γ ab (18 patients; Table 1).

Fifty-five (32%) patients had IIM-ILD and these patients had significantly lower FVC compared with patients without ILD [68 (22) *vs* 92 (15)% predicted, mean (S.D.), $P < 0.001$] and DLCO [57 (23) *vs* 87 (15)% predicted, mean (S.D.), $P < 0.001$]. Among patients with ILD, 12 (22%) had severe ILD with DLCO $\leq 40\%$ predicted.

IIM patients have lower arylesterase and lactonase activities of the PON1 enzyme compared with HC

The activity of PON1 was significantly lower in IIM patients compared with HC as measured by both arylesterase and lactonase assays (Table 1 and Supplementary Table S1, available at *Rheumatology* online). IIM diagnosis remained strongly associated with lower PON1 activity measured by both arylesterase and lactonase activity assays after multivariate adjustment for other variables associated with PON1 activity (Supplementary Table S2, available at *Rheumatology* online). No difference in paraoxonase activity was observed between IIM and HC groups.

Clinical characteristics of IIM patients associate with PON1 activity

Univariate linear regression analysis was performed to determine factors associated with plasma PON1 activity in the IIM patient cohort ($n = 184$, Table 2). Older age, male sex, aspirin use, high dose prednisone (>40 mg/day), and higher ESR and hsCRP associated with lower PON1 activity as measured by different PON1 activity assays, whereas higher cholesterol levels associated with higher PON1 activity (Table 2). PON1 activity did not associate with IIM type, IIM disease duration or other medication use (Table 2).

Association of IIM disease activity with arylesterase and lactonase activities of the PON1 enzyme

Myositis disease activity (physician global activity VAS, CPK) and damage (physician global damage VAS) were significantly associated with PON1 activity measured by both arylesterase and lactonase assays in univariate analyses (Table 2). Higher myositis disease activity and damage were associated with lower arylesterase and lactonase activities of PON1, whereas higher levels of arylesterase and lactonase activity associated with lower IIM disease activity and damage (Table 2). The association of IIM disease activity with arylesterase and lactonase activities of PON1 remained strong after multivariate adjustment for other clinical factors affecting PON1 enzyme function (Table 3). The physician global damage assessment did not remain significantly associated with PON1 activity after multivariate adjustment (Table 3).

Severe IIM-associated ILD (IIM-ILD) and ILD-associated myositis autoAb correlate with lower PON1 activity

Additional analyses investigated associations of PON1 activity with ILD in IIM patients. IIM patients with severe ILD (DLCO $\leq 40\%$) had significantly lower lactonase activity compared with patients with mild-moderate ILD (DLCO >40%) (Fig. 1). Similar trends were noted in the arylesterase activity of PON1, which was lowest in

Table 1 Demographic and clinical characteristics of DM/PM/ASS patients compared with healthy controls

	DM/PM group				HC ($n = 112$)
	DM ($n = 112$)	PM ($n = 36$)	ASS ($n = 21$)	DM/PM/ASS ($n = 169$)	
Age, years	48 (14)	53 (13)	48 (14)	49 (14)	47 (16)
Sex, female	85 (75)	27 (75)	14 (67)	126 (75)	87 (78)
Race, caucasian	93 (84)	21 (58)	13 (62)	126 (75)	61 (54)
Ethnicity, hispanic	24 (21)	5 (14)	4 (19)	33 (20)	19 (17)
Lipid levels, mg/dl					
Total cholesterol	204 (47)	226 (60)	212 (42)	210 (50)[a]	196 (41)
LDL-C	117 (39)	132 (50)	134 (40)	122 (42)[a]	112 (34)
HDL-C	60 (21)	59 (25)	55 (16)	59 (21)	61 (19)
Triglycerides	154 (95)	181 (134)	197 (168)	165 (116)[a]	114 (66)
BMI, kg/m^2	27 (6)	28 (6)	30 (8)	28 (6)[a]	26 (6)
CVD risk factors[b]					
History of CAD	2 (2)	2 (4)	0 (0)	4 (2)	1 (2)
Hypertension	28 (25)	14 (39)	1 (5)	43 (25)	18 (16)
Diabetes	9 (8)	12 (33)	1 (5)	22 (13)[a]	3 (5)
Current smoker	3 (3)	0 (0)	0 (0)	3 (2)	2 (2)
Past smoker	24 (22)	5 (14)	4 (19)	33 (20)	18 (16)
Family history of premature MI	3 (3)	4 (11)	0 (0)	7 (4)	5 (4)
Cholesterol lowering medication use	14 (13)	3 (8)	1 (5)	18 (11)	14 (13)
Aspirin	11 (10)	8 (22)	2 (10)	21 (12)	14 (13)
ESR, mm/h, median (IQR)	20 (8–42)	29 (13–47)	34 (15–59)	23 (11–46)[a]	11 (5–21)
hsCRP, mg/l, median (IQR)	1.6 (0.8–6.6)	2.7 (0.9–6.0)	4.9 (1.3–12.6)	2.0 (0.8–7.1)[a]	1.3 (0.4–3.1)
PON1 activity					
Arylesterase (U/ml)	167.9 (49.1)	175.6 (38.2)	142.6 (45.9)	166.3 (47.8)[a]	274.7 (67.5)
Lactonase (U/ml)	16.5 (6.8)	15.8 (4.9)	14.0 (6.1)	16.1 (6.3)[a]	29.7 (9.9)
Paraoxonase (U/ml)	540.2 (369.8)	613.2 (409.0)	539.6 (290.9)	555.9 (369.3)	523.6 (300.6)
PON1 genotype[a,c]					
QQ	39 (36)	13 (41)	6 (30)	59 (35)	30 (58)
QR	54 (50)	13 (41)	11 (55)	79 (47)	16 (31)
RR	14 (13)	6 (19)	3 (15)	23 (14)	6 (12)
IIM disease characteristics					
Disease duration, years, median(IQR)	1.5 (0.4–5.3)	1.2 (0.2–5.2)	1.2 (0.2–5.4)	1.2 (0.2–5.2)	
ILD, yes	30 (28)	9 (26)	16 (76)	55 (33)	
FVC, % predicted	86 (23)	80 (18)	70 (19)	68 (22)	
DLCO,% predicted	79 (22)	73 (24)	56 (23)	57 (23)	
Severe ILD, *n* (%ILD)[d]	5 (16)	2 (22)	5 (31)	12 (22)	
Malignancy, yes[e]	5 (4)	0 (0)	0 (0)	5 (3)	
Ab subtype[f]					
Antisynthetase ab	0 (0)	0 (0)	21 (100)	21 (19)	
MDA5 ab	9 (7)	0(0)	0 (0)	9 (8)	
SRP ab	2 (2)	8 (17)	0 (0)	10 (9)	
HMGCR ab	0 (0)	3 (7)	0 (0)	3 (3)	
TIF1-γ	18 (15)	0 (0)	0 (0)	18 (16)	
Mi2	6 (5)	0 (0)	0 (0)	6 (5)	
MJ	8 (7)	0 (0)	0 (0)	8 (7)	
Other MSA/MAA	13 (10)	2 (4)	0 (0)	15 (14)	
Unidentified ab	10 (8)	1 (2)	0 (0)	11 (10)	
No autoAb	9 (7)	1 (2)	0 (0)	10 (9)	
Physician global activity VAS (0–100 mm)	40 (19)	34 (19)	49 (19)	40 (19)	
Physician global activity Likert (0–4), median (IQR)	2 (1–2)	1.5 (1–2)	2 (1–3)	2 (1–2)	
Physician global damage VAS (0–100 mm)	29 (22)	31 (20)	53 (18)	33 (23)	

(continued)

TABLE 1 Continued

	DM/PM group				HC ($n = 112$)
	DM ($n = 112$)	PM ($n = 36$)	ASS ($n = 21$)	DM/PM/ASS ($n = 169$)	
Physician global damage Likert (0–4), median (IQR)	1 (1–2)	2 (1–2)	2 (2–3)	1 (1–2)	
CPK, U/l, median(IQR)	86 (53–161)	276 (157–1228)	328 (104–1353)	108 (62–356)	
HAQ	0.94 (0.77)	0.93 (0.70)	1.00 (0.81)	0.96 (0.73)	

Values are mean (S.D.) or *n* (%) unless specified otherwise. [a]$P < 0.05$ for comparison between DM/PM group *vs* HC group using a student's *t test* [summary reported as mean (S.D.)] or Wilcoxon rank-sum test (summary statistic reported as median IQR) to compare continuous variables, and a $\chi 2$ test or fisher's exact test to compare categorical variables. [b]CV risk factors missing in 16 controls, family history of premature MI defined as first degree relative male with history of MI before age 55, females before age 65. [c]PON1 genotype testing available in 159 IIM patients and 52 HC. [d]Severe ILD defined as DLCO $\leq$ 40%predicted. [e]Patients with cancer associated myositis or concomitant malignancy. [f]Ab testing available in 111 DM/PM patients (also available in three IBM patients). Other MSA/MAA (myositis-specific or myositis-associated antibodies): anti-PM-Scl, -Ku, -U1/U2/U3RNP, -SUMO-1 activating enzyme (SAE), -Ro; Unidentified ab: unable to be definitively identified by immunoprecipitation; No autoAb: no detectable autoAbs. ASS: antisynthetase syndrome; CAD: coronary artery disease; CPK: creatine phosphokinase; CVD: cardiovascular disease; DLCO: diffusing capacity of the lungs for carbon monoxide; FVC: forced vital capacity; HAQ: health assessment questionnaire; HC: healthy controls; hsCRP: high sensitivity CRP; MI: myocardial infarction; MSA/MAA: myositis specific antibodies/myositis associated antibodies; VAS: visual analogue scale.

patients with severe ILD compared with patients with mild ILD or no ILD (Fig. 1). Among patients with ILD, arylesterase and lactonase activity were also positively correlated with DLCO (Pearson correlation coefficient $r = 0.3$, $P = 0.04$ for arylesterase; $r = 0.4$, $P = 0.007$ for lactonase); higher PON1 activity associated with less severe disease (higher DLCO). No associations of paraoxonase activity of PON1 with ILD were noted. Similar trends were noted when severe ILD was defined as FVC <50% predicted (Supplementary Fig. 1, available at *Rheumatology* online).

PON1 activity was also examined between myositis autoantibody subgroups. Patients with antibodies associated with ILD (anti-MDA5 and antisynthetase antibodies) had the lowest PON1 activity of all antibody groups as measured by both arylesterase and lactonase assays (Table 4). The presence of an antisynthetase ab (compared with no autoAb) was also strongly associated with lower arylesterase activity in univariate analysis (Table 2), and this association remained significant in multivariate analysis (Table 3).

The paraoxonase activity of PON1 associates closely with the PON1 Q192R genotype in IIM patients

Paraoxonase activity significantly associated with the Q192R polymorphism in IIM patients. Paraoxonase activity was lowest in the QQ genotype group and highest in RR genotype group (QQ<QR<RR, $P < 0.001$ for all comparisons, Table 5). Similar trends were also observed in the HC cohort although only 52/112 patients had DNA available for analysis (data not shown). Lactonase activity showed trends for highest activity in patients with the QQ genotype as compared with QR or RR genotype, but did not reach statistical significance (Table 5).

The PON1 Q192R polymorphism associates with IIM disease characteristics

Because the PON1 Q192R polymorphism is a major determinant of the enzyme activity of PON1, and has been associated with both vascular and pulmonary outcomes in the general population [12, 26], we examined its association with disease outcomes in IIM patients. Clinical characteristics of IIM patients were first examined between PON1 QQ, QR and RR genotypes (Table 5). Demographics and traditional CV risk factors were similar between the groups except for white race and HDL-C levels, which were lowest in the RR genotype group.

Overall, patients with the QQ genotype had better disease outcomes compared with patients with QR or RR genotypes, with the lowest disease activity scores, damage scores and markers of inflammation (Table 5). QQ genotype group had no patients with severe ILD, and the incidence of ILD was also lowest in the QQ genotype group (24%), which was significantly lower compared with patients with RR genotype (61%, $P = 0.01$). Mean DLCO was the highest in the QQ genotype group and was significantly higher than in the RR genotype group. Similar trends were seen with FVC (QQ>QR>RR; Table 5).

Multivariate regression analysis was performed to determine whether the PON1 Q192R polymorphism was predictive of disease outcomes in IIM patients after controlling for other IIM patient characteristics. In linear regression models controlling for other significant predictors of disease activity in univariate analysis (Supplementary Table S3, available at *Rheumatology*

TABLE 2 Univariate regression analysis of variables associated with PON1 activity in IIM patients ($n = 184$)

Variable	Arylesterase	Lactonase	Paraoxonase
PON1 Q192R genotype, QQ (*vs* QR/RR)	4.1 (−10.3, 18.4)	1.0 (−0.9, 2.9)	**−378.7 (−475.5, −281.8)**[a]
Age, 10 years	−8.0 (−12.5, −3.6)[a]	−0.7 (−1.3, −0.1)[a]	−36.1 (−71.5, −0.6)[a]
Sex, female	17.7 (2.6, 32.8)[a]	1.3 (−0.7, 3.3)	147.7 (30.8, 264.6)[a]
Race, white	1.7 (−14.4, 17.8)	1.0 (−1.2, 3.1)	−127.9 (−249.5, −6.3)[a]
IIM type, DM	2.7 (−11.9, 17.4)	0.9 (−1.0, 2.8)	−10.4 (−124.7, 103.9)
PM	13.5 (−3.7, 30.8)	−0.1 (−2.4, 2.2)	88.1 (−46.0, 222.3)
ASS	−26.4 (−49.1, −3.6)[a]	−2.0 (−5.1, 1.0)	30.3 (−151.0, 211.5)
Disease duration, years	0.2 (−0.8, 1.2)	0.03 (−0.1, 0.2)	3.3 (−4.7,11.3)
ILD, present	−7.8 (−23.2, 7.6)	−1.5 (−3.5, 0.5)	9.3 (−21.8, 40.4)
Malignancy	−30.1 (−71.4, 12.9)	0.3 (−5.3, 5.8)	−38.0 (−366.3, 290.3)
Ab subgroups, positive (*vs* no autoAb)[b]			
Antisynthetase ab	−26.4 (−49.1, −3.6)[a]	−2.0 (−5.1, 1.0)	30.3 (−151.0, 211.5)
MDA5 ab	−20.4 (−53.1, 12.2)	−1.4 (−5.7, 2.9)	51.0 (−203.9, 305.9)
SRP or HMGCR ab	−8.2 (−35.1, 18.7)	−1.5 (−5.0, 2.1)	115.1 (−94.9, 325.1)
TIF1-γ	−0.2 (−23.4, 23.1)	2.2 (−0.9, 5.3)	153.7 (−27.6, 334.9)
Other MSA/MAA[c]	−1.7 (−22.1, 18.7)	0.4 (−2.3, 3.1)	−25.2 (−184.4, 134.0)
Unidentified ab	10.1 (−18.7, 38.8)	0.5 (−3.3, 4.3)	98.8 (−144.4, 341.9)
No autoAb (REF)	–	–	–
Physician global activity VAS, 0–10 cm	−4.7 (−8.2, −1.1)[a]	−0.5 (−1.0, −0.03)[a]	−8.4 (−36.0, 19.3)
Physician global damage VAS, 0–10 cm	−3.6 (−6.7, −0.5)[a]	−0.5 (−0.9, −0.1)[a]	−8.7 (−32.2, 14.8)
CPK, log U/l[d]	−6.5 (−11.7, −1.4)[a]	−0.7 (−1.4, −0.1)[a]	6.3 (−33.7, 46.2)
ESR, 10 mm/h	−2.6 (−5.3, 0.1)	−0.5 (−0.9, −0.2)[a]	−21.3 (−42.3, −0.3)[a]
hsCRP, log mg/l[d]	−3.6 (−8.3, 1.0)	−0.7 (−1.3, −0.1)[a]	−15.6 (−53.4, 22.1)
Medications			
MTX	2.0 (−13.9, 17.9)	−1.4 (−3.4, 0.7)	−65.0 (−190.5, 60.5)
TNF inhibitor	5.1 (−37.3, 47.4)	−0.2 (−5.8, 5.4)	−47.4 (−377.4, 282.6)
LEF	11.9 (−35.2, 59.1)	3.5 (−2.7, 9.7)	−36.3 (−403.1, 330.6)
AZA	5.4 (−15.3, 26.1)	0.6 (−2.2, 3.4)	−15.0 (−184.2, 154.1)
IVIG	−0.9 (−17.9, 16.1)	0.5 (−1.8, 2.7)	−85.5 (−217.4, 46.4)
Mycophenolate	−0.6 (−17.0, 15.9)	1.1 (−1.1, 3.3)	16.7 (−111.2, 144.5)
Rituximab	3.7 (−23.1, 30.6)	2.5 (−1.0, 6.1)	−65.8 (−274.5, 142.9)
CYC	1.4 (−30.4, 33.1)	−0.6 (−4.8, 3.6)	−74.0 (−322.4, 174.3)
Prednisone	7.5 (−7.5, 22.5)	0.4 (−1.6, 2.4)	−0.3 (−118.3, 117.7)
Prednisone (mg/day)	0.1 (−0.2, 0.5)	0.02 (−0.02, 0.07)	−2.5 (−5.1, 0.1)
Prednisone high dose (>40 mg/day)	0.7 (−17.03, 18.41)	0.3 (−2.03,2.65)	−150.4 (−286.7, −14.0)[a]
Statin	−10.4 (−32.4, 11.6)	−1.6 (−4.5, 1.4)	−6.1 (−237.0, 114.5)
Moderate-high intensity statin[e]	−12.5 (−35.1, 10.1)	−2.0 (−4.9, 1.0)	−81.7 (−261.7, 97.7)
Aspirin	−29.3 (−48.4, −10.3)[a]	−3.4 (−5.9, −0.9)[a]	−134.6 (−284.3, 15.1)
Lipid panel, 10 mg/dl			
Total cholesterol	2.4 (1.1, 3.8)[a]	0.4 (0.2, 0.6)[a]	−0.3 (−1.4, 0.8)
LDL-C	1.4 (−0.3, 3.1)	0.2 (0.02, 0.5)[a]	−9.2 (−22.3, 4.1)
HDL-C	4.5 (1.1, 7.8)[a]	0.8 (0.4, 1.3)[a]	14.6 (−11.9, 41.2)
Triglyceride	0.4 (−0.2, 1.0)	0.05 (−0.03, 0.1)	−0.5 (−5.2, 4.1)
CVD risk factors			
CAD	0.8 (−46.6, 48.0)	−3.9 (−10.1, 2.3)	313.3 (−49.8, 676.4)
Hypertension	−9.2 (−24.5, 6.0)	−1.1 (−3.2, 0.9)	−52.1 (−171.5, 67.3)
Diabetes	−9.6 (−29.8, 10.4)	−1.2 (−3.9, 1.4)	75.5 (−82.8, 233.7)
Family history of premature MI	7.4 (−25.9, 40.7)	1.6 (−2.8, 6.1)	22.4 (−234.9, 279.7)
Smoking history, ever smoker (*vs* never)	−15.2 (−32.0, 1.5)	−1.3 (−3.5, 0.9)	−55.5 (−187.6, 76.5)
BMI, kg/m^2	0.2 (−1.0, 1.4)	0.1 (−0.1 0.s)	−2.9 (−11.9, 6.1)

Values reported are regression coefficient β (95% CI). Regression coefficient is presented per unit increase unless specified for continuous variables, and for category assessed compared with referent group for categorical variables. [a]$P < 0.05$. [b]Ab testing results available in 114 patients. [c]Anti-Mi2, -MJ were included in other MSA/MAA group given small numbers. [d]Log transformed in regression model for skewness. [e]According to ACC/AHA practice guidelines. Ab: antibodies; ASS: antisynthetase syndrome; CAD: coronary artery disease; CVD: cardiovascular disease; ILD: interstitial lung disease; MI: myocardial infarction; MSA/MAA: myositis specific antibodies/myositis associated antibodies; VAS: visual analogue scale.

TABLE 3 Multivariate linear regression analysis in IIM cohort of variables associated with arylesterase and lactonase activity in IIM patients ($n = 184$)

Variables	Arylesterase			Lactonase		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
Physician global activity VAS (0–100 mm), 10 mm	−5.5 (−8.9, −2.0)[a]	—	—	−0.6 (−1.0, −0.02)[a]	—	—
Physician global damage VAS (0–100 mm), 10 mm	—	−0.8 (−3.9, 2.3)	—	—	−0.3 (−0.7,0.1)	—
CPK, logU/l	—	—	−5.2 (−10.1, −0.2)[a]	—	—	−0.8 (−1.4, −0.1)[a]
Age, 10 years	−8.1 (−12.7, −3.5)[a]	−7.6 (−12.4, −2.7)[a]	−7.6 (−12.3, −2.9)[a]	−0.4 (−1.1,0.2)	−0.2 (−1.0,0.4)	−0.2 (−0.9, 0.4)
Sex, female	3.8 (−10.3, 17.9)	3.5 (−11.4, 18.4)	7.8 (−7.5, 23.1)	−0.6 (−2.5,1.3)	−0.7 (−2.7, 1.3)	−0.5 (−2.6, 1.6)
Total cholesterol, 10 mg/dl	2.7 (1.4, 4.0)[a]	2.6 (1.2, 4.0)[a]	2.4 (1.0, 3.8)[a]	0.4 (0.2, 0.6)[a]	0.4 (0.2,0.6)[a]	0.3 (0.2, 0.6)[a]
HDL-C, 10 mg/dl	1.2 (−2.1, 4.4)	2.1 (−1.2, 5.4)	2.0 (−1.3, 5.4)	0.4 (−0.1, 0.8)	0.4 (−0.0, 0.1)	0.3 (−0.1, 0.8)
Antisynthetase ab, yes	−21.1 (−41.0, −1.3)[a]	−23.7 (−45.2, −2.2)[a]	−21.7 (−41.6, −0.9)[a]	—	—	—
hsCRP, log mg/l	—	—	—	−0.4 (−1.1, 0.3)	−0.4 (−1.1, 0.3)	−0.6 (−1.2, 0.1)
Statin use, yes	13.6 (−8.5, 35.6)	10.1 (−12.8, 32.9)	4.3 (−18.9, 27.5)	1.2 (−1.8, 4.2)	0.8 (−2.2, 3.9)	−0.2 (−3.2, 2.9)
Aspirin use, yes	−27.9 (−48.4, −7.2)[a]	−25.1 (−46.8, −3.4)[a]	−22.4 (−44.2, −0.7)[a]	−3.8 (−6.7, −1.0)[a]	−3.7 (−6.6, −0.7)[a]	−3.6 (−6.5, −0.6)[a]

Values reported are regression coefficient β (95% CI). [a]$P < 0.05$. Model 1 with disease activity measured as VAS, Model 2 with disease damage measured as VAS, Model 3 with disease activity measured as CPK. Hs-CRP and CPK was transformed to the log scale due to skewness. LDL-C was not included in MV model as highly correlated with total cholesterol ($r = 0.8$, $P < 0.001$), ESR correlated with hsCRP ($r = 0.5$, $P < 0.001$, respectively). CPK: creatine phosphokinase; hsCRP: high sensitivity CRP; VAS: visual analogue scale.

online), the PON1 QQ genotype remained a significant predictor of lower disease activity measured by physician global scores (Supplementary Table S4, available at *Rheumatology* online). As expected, higher arylesterase or lactonase activity of the PON1 enzyme also remained associated with lower disease activity in these models (Supplementary Table S4, available at *Rheumatology* online). The PON1Q192R polymorphism did not remain significantly associated with disease damage or DLCO after multivariate adjustment (data not shown).

Discussion

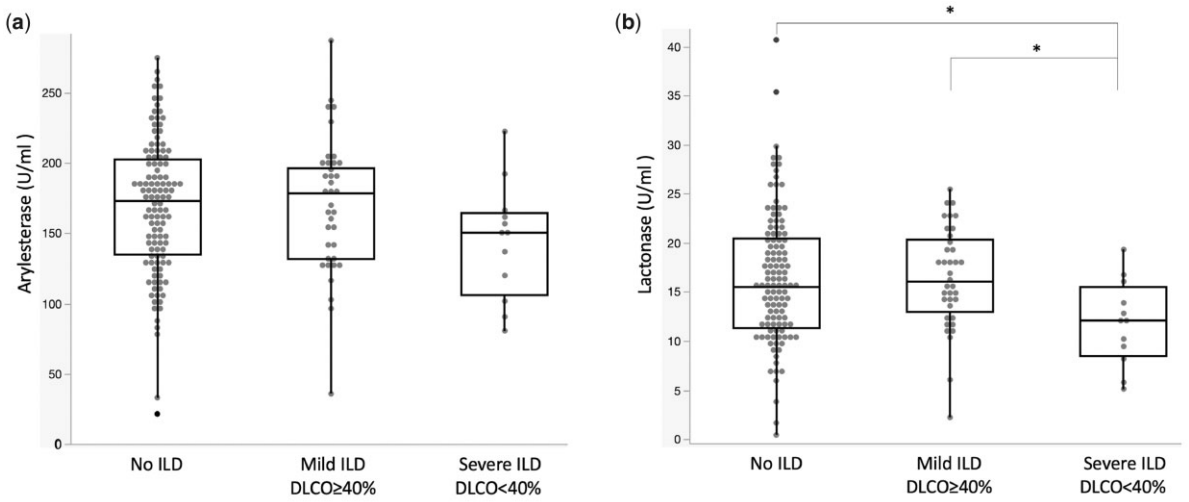
The current study is the first work to comprehensively evaluate the biochemical and genetic determinants of PON1 activity in a large cohort of patients with IIM. We recently reported that the antioxidant function of HDL in IIM patients is impaired compared with healthy controls [27]. Because the function of HDL is directly affected by enzymatic changes within the HDL particle, in the current study we assessed the activity of PON1, which is a major HDL-associated enzyme.

PON1 is largely responsible for the protective, antioxidant function of HDL by neutralizing inflammatory bioactive lipids such as oxidized phospholipids, which directly activate inflammatory signalling pathways in the vascular endothelium [28, 29]. Because microvascular inflammation and damage are strongly implicated in the disease pathogenesis of IIM, particularly in patients with DM [4, 5], understanding the role of PON1 may be important in IIM patients. In addition, PON1 has been implicated directly in the immune response, with impact on macrophage differentiation [30], suppression of macrophage pro-inflammatory responses [31], and regulation of T-cell development [32].

In the current work, PON1 activity was significantly impaired in IIM patients compared with controls as assessed by both arylesterase and lactonase assays, and this finding remained strong after multivariate adjustment for differences between groups. Our work is consistent with data in other inflammatory diseases including RA [33, 34], SLE [35] and psoriatic arthritis [36], which have reported lower PON1 activity compared with controls. Our study also identified several clinical characteristics of patients previously associated with PON1 activity in non-IIM populations, including older age, male sex and higher inflammatory markers [9, 10, 21, 22, 37, 38].

We also studied the associations of several therapeutic agents previously linked to modulation of PON1 levels and activity. Statin and aspirin have been associated with higher PON1 activity in several clinical studies [8, 10]. There were very few patients using these agents in our IIM cohort, and we did not see an association of PON1 activity with statin use or intensity. Interestingly, aspirin use was associated with significantly lower arylesterase and lactonase activities of PON1 in both univariate and multivariate models. This observation was

FIG. 1 PON1 activity by arylesterase and lactonase assays in patients with no ILD ($n = 116$), mild-moderate ILD ($n = 37$) and severe ILD ($n = 12$)



Boxplots indicate median and quartiles. * indicates P value <0.05 by pairwise Wilcoxon test.

TABLE 4 PON1 activity in myositis autoantibody subgroups

Ab subgroup	*n*	Arylesterase (U/ml)	Lactonase (U/ml)	Paraoxonase (U/ml)
Antisynthetase ab	21	142.6 (45.8)	13.9 (6.0)	539.5 (290.9)
MDA5 ab	9	148.5 (52.1)	14.6 (7.4)	560.4 (289.0)
SRP or HMGCR ab	13	172.2 (41.1)	15.1 (5.3)	665.9 (395.6)
TIF1-γ	18	169.7 (52.8)	18.5 (6.7)	698.3 (540.6)
Other MSA/MAA[a]	30	162.7 (50.9)	16.0 (5.7)	471.0 (349.1)
Unidentified ab	12	173.8 (60.9)	17.0 (9.3)	487.7 (347.6)
No autoAb	11	164.0 (46.7)	18.0 (8.9)	651.8 (530.0)

Reported mean (S.D.). [a]Other MSA/MAA (myositis specific or myositis associated antibodies): anti-Mi2, -MJ, -PM-Scl, -Ku, -U1RNP, -U2RNP, -SUMO-1 activating enzyme (SAE), -Ro. unidentified ab: undefined autoAb (unable to be definitively identified by immunoprecipitation); no autoAb: no detectable autoAbs.

unexpected, and given the small numbers of IIM patients taking aspirin in our cohort, warrants further investigation. High-dose prednisone was associated with both higher patient disease activity as well as lower paraoxonase activity of the PON1 enzyme.

The current work is unique compared with previous studies in rheumatic diseases because of its comprehensive assessment of all three enzyme activities of PON1 as well as the assessment of the PONQ192R polymorphism, which may significantly influence PON1 activity. This comprehensive assessment of PON1 is important in understanding its biologically relevant functions, as the PON1 enzyme may hydrolyze a wide range of substrates *in vivo* [39].

Myositis disease activity was strongly associated with both lactonase and arylesterase activities of the PON1 enzyme. Higher lactonase and arylesterase activity of PON1 associated with lower disease activity whereas suppressed enzyme activities were noted in patients with poor disease control. The paraoxonase activity of PON1 did not associate with IIM disease activity, and interestingly, work by Husni and colleagues described a similar disease activity association of the arylesterase activity, but not paraoxonase activity, in patients with psoriatic arthritis [36]. Previous work has suggested that the activity of PON1 may be impaired in the setting of active inflammation, in part by accumulation of inflammatory bioactive lipids in the HDL particle, which inhibit enzyme function [20, 40]. Further work is warranted to determine whether PON1 contributes to disease control in IIM by metabolism of biologically active substrates, or is merely impaired in the setting of inflammation from active IIM. We recently demonstrated that overexpression of the human PON1 transgene reduced inflammatory arthritis in a mouse model of RA in association with suppression of pro-inflammatory oxidized fatty acids [19].

ILD is a common complication of IIM [3, 41], which associates with high morbidity and mortality. In the

Table 5 Demographic and clinical characteristics of IIM patients by PON1 Q192R genotype

	QQ ($n = 67$)	QR ($n = 83$)	RR ($n = 24$)
PON1 activity			
Arylesterase (U/ml)	167.3 (51.8)	519.9 (46.2)	174.7 (41.7)
Lactonase (U/ml)	16.6 (6.5)	15.6 (6.7)	15.6 (4.5)
Paraoxonase (U/ml)	301.9 (208.3)	605.7 (292.0)[a]	915.3 (385.7)[a]
Age, years	53 (16)	4 9 (15)	50 (13)
Sex, female	45 (67)	58 (70)	20 (83)
Race, white	59 (88)	60 (72)	13 (54)[†]
Ethnicity, hispanic	9 (13)	19 (23)	5 (21)
Lipid levels, mg/dl			
Total cholesterol	206 (48)	210 (54)	199 (48)
LDL-C	116 (37)	125 (46)	110 (38)
HDL-C	61 (21)	57 (20)	51 (15)[a]
Triglycerides	160 (106)	171 (118)	197 (164)
BMI, kg/m^2	28.1 (6.4)	27.5 (6.5)	28.7 (5.7)
CVD risk factors			
History of CAD	1 (1)	1 (1)	2 (8)
Hypertension	22 (33)	20 (24)	5 (21)
Diabetes	7 (10)	13 (16)	3 (13)
Ever smoker	18 (27)	13 (16)	6 (26)
Family history of premature MI	1 (1)	3 (4)	2 (8)
Cholesterol lowering medication use	8 (12)	10 (12)	1 (4)
ESR, mm/h, median (IQR)	20 (9–42)	23 (9–47)	23 (13–41)
hsCRP, mg/l, median (IQR)	1.7 (0.6–5.0)	2.0 (0.9–6.9)	4.1 (1.3–12.7)
IIM type			
DM	42 (62)	61 (73)	14 (58)
PM	16 (24)	17 (20)	9 (38)
IBM	9 (13)	5 (6)	1 (4)
Disease duration, years, median (IQR)	1.4 (0.4–5.3)	0.6 (0.1–2.7)[a]	1.9 (0.4–6.6)
Physician global activity VAS (0–100 mm)	32 (18)	44 (19)[a]	38 (20)
Physician global activity Likert (0–4), median (IQR)	1 (1–2)	2 (1–2)[a]	2 (1–2)
Physician global damage VAS (0–100 mm)	31 (21)	34 (25)	38 (19)
Physician global damage Likert (0–4), median (IQR)	1 (1–2)	1 (1–2)	2 (1–2)[a]
CPK, U/l, median (IQR)	107 (52–332)	122 (66–370)	168 (68–556)
FVC, %predicted	87 (20)	80 (23)	76 (22)
DLCO,% predicted	80 (17)	73 (26)	64 (26)[a]
ILD, yes	14 (24)	24 (30)	14 (61)[b]
Severe ILD[c], *n* (% ILD)	00	7 (29)	4 (29)[b]
Medications			
MTX	17 (25)	23 (28)	5 (21)
TNF inhibitor	2 (2)	3 (4)	00
LEF	1 (1)	2 (2)	00
AZA	13 (19)	5 (6)	5 (21)[b]
IVIG	16 (24)	17 (20)	3 (13)
Mycophenolate	12 (18)	19 (23)	6 (25)
Rituximab	6 (9)	6 (7)	1 (4)
CYC	1 (1)	7 (8)	00[b]
Prednisone	47 (70)	55 (66)	17 (71)
Prednisone high dose (>40 mg/day)	8 (12)	21 (26)	4 (17)
Prednisone (mg/day)	15 (21)	19 (21)	13 (16)
Statin	8 (12)	10 (12)	1 (4)
Moderate-high intensity statin	8 (12)	9 (11)	1 (4)
Aspirin	13 (19)	10 (12)	2 (8)

Values are mean (S.D.) or *n* (%) unless specified. [a]$P < 0.05$ compared with QQ genotype. [b]$P < 0.05$ by $\chi 2$ test or fishers exact test. [c]Severe ILD defined as patients with ILD and DLCO $\leq 40\%$ predicted. CAD: coronary artery disease; CPK: creatine phosphokinase; CVD: cardiovascular disease; DLCO: diffusing capacity of the lungs for carbon monoxide; FVC: forced vital capacity; hsCRP: high sensitivity CRP; ILD: interstitial lung disease; MI: myocardial infarction; VAS: visual analogue scale.

current work, patients with severe IIM-ILD had significantly lower plasma PON1 activity measured by its lactonase activity compared with IIM patients with mild IIM-ILD or no ILD, and showed similar trends for arylesterase activity. Furthermore, patients with ILD-associated antibodies had the lowest arylesterase and lactonase activities of any antibody subgroup. In contrast, higher PON1 activity by both assays correlated with better lung function in patients with ILD measured by a higher DLCO. This data is consistent with our previous work in which we showed impairment in HDL function and higher levels of oxidized fatty acids in HDL of patients with IIM-ILD compared with IIM patients without ILD and controls [27]. A major role of PON1 is to metabolize inflammatory oxidized lipids, which can accumulate in HDL, suggesting that PON1 may be an important determinant of HDL function in IIM patients. Of note, endothelial activation and increased oxidative stress leading to vascular damage have been suggested to play a direct role in IIM-ILD. Funauchi *et al.* compared IIM patients with interstitial pneumonitis to IIM patients without lung disease, and found higher circulating markers of endothelial damage [42]. Further investigation of PON1 as a mechanism for vascular damage and disease in IIM-ILD may be warranted.

The PON1 Q192R polymorphism involves a mutation from glutamine (Q wild type) to arginine (R variant) at amino acid position 192 of the protein sequence, and strongly influences the paraoxonase activity of the PON1 enzyme in patients without IIM [43]. The current work is the first study to report a strong, significant association of the PON1 RR genotype as compared with the QQ genotype with higher paraoxonase activity in IIM patients. Few studies in any patient group have examined associations of the PON1 Q192R polymorphism with lactonase and arylesterase activities of the PON1 enzyme. In the current work, we noted a trend for higher lactonase activity in QQ genotype patients as compared with patients with the QR or RR genotype. Zhou and colleagues studied a larger group of 347 women with gestational diabetes and reported that the PON1 Q allele associates with significantly higher lactonase activity compared with the R allele [44]. In our recent work studying 1969 patients with RA, we also reported that the QQ genotype had a highly significant association with higher lactonase and arylesterase activities of PON1 compared with the RR genotype [45].

The PON1 QQ genotype strongly associated with better disease outcomes in patients with IIM in the current study, including a significant association of the QQ genotype with lower IIM disease activity in multivariate analysis. We hypothesize that this finding may relate to the association of the Q allele with higher lactonase activity of the PON1 enzyme as described above. However, further confirmation in larger IIM patient groups and longitudinal analyses is warranted and is ongoing.

There are limitations to the present study. First, while our current findings support the hypothesis that PON1 may play a role in IIM and IIM-ILD, observations from a single time point are not enough to determine causality. Additional work including prospective studies evaluating the effect of the PON1 Q192R polymorphism and longitudinal PON1 activity over time on disease outcomes is underway. Second, although the current cohort of 184 IIM patients represents a relatively large cohort for a rare disease, further work in multicentre cohorts with larger numbers of clearly defined specific IIM disease subsets such as myositis autoantibody subgroups is warranted. In the meantime, while the data in myositis antibody subgroups is hypothesis generating, our current results should be interpreted with caution. Also, the majority of patients in the current study had DM, and limited numbers of patients with IBM were evaluated. Third, while physician global scores and CPK are widely used outcome measures, future analysis may include more comprehensive validated IIM disease-specific measures such as manual muscle testing, skin disease activity and damage scores and patient reported outcomes. A more extensive review of HRCT scans to quantify the extent of ILD may further enhance our findings. Fourth, although plasma PON1 activity is widely used in assessing PON1's impact on the vascular endothelium [12], direct assessment of PON1 in target organs such as the skeletal muscle and lung may be worthwhile.

In summary, we report the first study to comprehensively assess the biochemical and genetic determinants of PON1 activity in a large cohort of IIM patients. We demonstrate that the arylesterase and lactonase activities of the enzyme are significantly lower in IIM patients relative to population controls and have an inverse association with IIM disease activity. The PON1 Q192R polymorphism showed a strong association with paraoxonase activity in IIM, and the PON1 QQ genotype associated with more favourable disease outcomes. Patients with severe IIM-ILD had decreased arylesterase and lactonase activities of the PON1 enzyme, and the association between low PON1 activity and higher severity of ILD was more apparent in non QQ genotype patients. Large prospective studies may be warranted to further evaluate the role of PON1 and PON1 genetic polymorphisms in the development and propagation of IIM and IIM-ILD.

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Data availability statement

The pertinent data underlying this article are available in the article and in its online supplementary material. Additional data underlying this article will be shared on reasonable request to the corresponding author.

Supplementary data

Supplementary data are available at *Rheumatology* online.

References

1 Dalakas MC. Polymyositis, dermatomyositis and inclusion-body myositis. N Engl J Med 1991;325:1487–98.

2 Diederichsen LP, Diederichsen AC, Simonsen JA *et al.* Traditional cardiovascular risk factors and coronary artery calcification in adults with polymyositis and dermatomyositis: a Danish multicenter study. Arthritis Care Res 2015;67:848–54.

3 Dobloug GC, Svensson J, Lundberg IE, Holmqvist M. Mortality in idiopathic inflammatory myopathy: results from a Swedish nationwide population-based cohort study. Ann Rheum Dis 2018;77:40–7.

4 Grundtman C, Lundberg IE. Pathogenesis of idiopathic inflammatory myopathies. Curr Rheumatol Rep 2006;8:188–95.

5 Emslie-Smith AM, Engel AG. Microvascular changes in early and advanced dermatomyositis: a quantitative study. Ann Neurol 1990;27:343–56.

6 Navab M, Hama SY, Anantharamaiah GM *et al.* Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: steps 2 and 3. J Lipid Res 2000;41:1495–508.

7 Navab M, Hama SY, Cooke CJ *et al.* Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: step 1. J Lipid Res 2000;41:1481–94.

8 Ferretti G, Bacchetti T, Sahebkar A. Effect of statin therapy on paraoxonase-1 status: a systematic review and meta-analysis of 25 clinical trials. Prog Lipid Res 2015;60:50–73.

9 Santanam N, Parthasarathy S. Aspirin is a substrate for paraoxonase-like activity: implications in atherosclerosis. Atherosclerosis 2007;191:272–5.

10 Blatter-Garin MC, Kalix B, De Pree S, James RW. Aspirin use is associated with higher serum concentrations of the anti-oxidant enzyme, paraoxonase-1. Diabetologia 2003;46:593–4.

11 Biggadike K, Angell RM, Burgess CM *et al.* Selective plasma hydrolysis of glucocorticoid gamma-lactones and cyclic carbonates by the enzyme paraoxonase: an ideal plasma inactivation mechanism. J Med Chem 2000;43:19–21.

12 Bhattacharyya T, Nicholls SJ, Topol EJ *et al.* Relationship of paraoxonase 1 (PON1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. JAMA 2008;299:1265–76.

13 Wang M, Lang X, Zou L, Huang S, Xu Z. Four genetic polymorphisms of paraoxonase gene and risk of coronary heart disease: a meta-analysis based on 88 case-control studies. Atherosclerosis 2011;214:377–85.

14 Lundberg IE, Tjarnlund A, Bottai M *et al.* 2017 European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. Arthritis Rheumatol 2017;69:2271–82.

15 Stone NJ, Robinson JG, Lichtenstein AH *et al.* 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on practice guidelines. Circulation 2014;129:S1–45.

16 Rider LG, Werth VP, Huber AM *et al.* Measures of adult and juvenile dermatomyositis, polymyositis, and inclusion body myositis: physician and patient/parent global activity, manual muscle testing (MMT), health assessment questionnaire (HAQ)/childhood health assessment questionnaire (C-HAQ), childhood myositis assessment scale (CMAS), myositis disease activity assessment tool (MDAAT), disease activity score (das), short form 36 (SF-36), child health questionnaire (CHQ), physician global damage, myositis damage index (mdi), quantitative muscle testing (QMT), myositis functional index-2 (fi-2), myositis activities profile (MAP), inclusion body myositis functional rating scale (IBMFRS), cutaneous dermatomyositis disease area and severity index (CDASI), cutaneous assessment tool (CAT), dermatomyositis skin severity index (DSSI), skindex, and dermatology life quality Index (DLQI). Arthritis Care Res 2011;63(Suppl 11):S118–57.

17 Travis WD, Costabel U, Hansell DM *et al.* An official American Thoracic Society/European Respiratory Society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med 2013;188:733–48.

18 Kolb M, Collard HR. Staging of idiopathic pulmonary fibrosis: past, present and future. Eur Respir Rev 2014;23:220–4.

19 Charles-Schoeman C, Wang J, Shahbazian A *et al.* Suppression of inflammatory arthritis in human serum paraoxonase 1 transgenic mice. Sci Rep 2020;10:16848.

20 Charles-Schoeman C, Lee YY, Shahbazian A *et al.* Association of paraoxonase 1 gene polymorphism and enzyme activity with carotid plaque in rheumatoid arthritis. Arthritis Rheum 2013;65:2765–72.

21 Mehdi MM, Rizvi SI. Human plasma paraoxonase 1 (PON1) arylesterase activity during aging: correlation with susceptibility of LDL oxidation. Arch Med Res 2012; 43:438–43.

22 Trentini A, Bellini T, Bonaccorsi G *et al.* Sex difference: an important issue to consider in epidemiological and clinical studies dealing with serum paraoxonase-1. J Clin Biochem Nutr 2019;64:250–6.

23 Deakin S, Leviev I, Guernier S, James RW. Simvastatin modulates expression of the PON1 gene and increases serum paraoxonase: a role for sterol regulatory element-binding protein-2. Arterioscler Thromb Vasc Biol 2003; 23:2083–9.

24 Harangi M, Seres I, Harangi J, Paragh G. Benefits and difficulties in measuring HDL subfractions and human paraoxonase-1 activity during statin treatment. Cardiovasc Drugs Ther 2009;23:501–10.

25 Schwarz EG. Estimating the dimension of a model. Ann Stat 1978;6:461–4.

26 Golmanesh L, Bahrami F, Pourali F *et al.* Assessing the relationship of paraoxonase-1 q192r polymorphisms and the severity of lung disease in SM-exposed patients. Immunopharmacol Immunotoxicol 2013;35: 419–25.

27 Bae SS, Lee YY, Shahbazian A *et al.* High- density lipoprotein function is abnormal in idiopathic inflammatory myopathies. Rheumatology 2020;59: 3515–25.

28 Watson AD, Berliner JA, Hama SY *et al.* Protective effect of high density lipoprotein associated paraoxonase. Inhibition of the biological activity of minimally oxidized low density lipoprotein. J Clin Invest 1995;96:2882–91.

29 Aviram M, Rosenblat M, Bisgaier CL *et al.* Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. J Clin Invest 1998;101:1581–90.

30 Rosenblat M, Volkova N, Ward J, Aviram M. Paraoxonase 1 (PON1) inhibits monocyte-to-macrophage differentiation. Atherosclerosis 2011;219:49–56.

31 Aharoni S, Aviram M, Fuhrman B. Paraoxonase 1 (PON1) reduces macrophage inflammatory responses. Atherosclerosis 2013;228:353–61.

32 Bai L, Shi G, Ma Y *et al.* Paraoxonase 1 knockout rats have impaired T cell development at the CD4/CD8 double-negative to double-positive transition stage. Sci Rep 2018;8:14457.

33 Tanimoto N, Kumon Y, Suehiro T *et al.* Serum paraoxonase activity decreases in rheumatoid arthritis. Life Sci 2003;72:2877–85.

34 Bindal UD, Saxena R, Siddiqui MH, Sharma D. Correlation of paraoxonase status with disease activity score and systemic inflammation in rheumatoid arthritic patients. J Clin Diagn Res 2016;10:BC01–5.

35 Tripi LM, Manzi S, Chen Q *et al.* Relationship of serum paraoxonase 1 activity and paraoxonase 1 genotype to risk of systemic lupus erythematosus. Arthritis Rheum 2006;54:1928–39.

36 Husni ME, Wilson Tang WH, Lucke M *et al.* Correlation of high-density lipoprotein-associated paraoxonase 1 activity with systemic inflammation, disease activity, and cardiovascular risk factors in psoriatic disease. Arthritis Rheumatol 2018;70:1240–50.

37 Rozek LS, Hatsukami TS, Richter RJ *et al.* The correlation of paraoxonase (PON1) activity with lipid and lipoprotein levels differs with vascular disease status. J Lipid Res 2005;46:1888–95.

38 Feingold KR, Memon RA, Moser AH, Grunfeld C. Paraoxonase activity in the serum and hepatic mRNA levels decrease during the acute phase response. Atherosclerosis 1998;139:307–15.

39 Litvinov D, Mahini H, Garelnabi M. Antioxidant and anti-inflammatory role of paraoxonase 1: implication in arteriosclerosis diseases. N Am J Med Sci 2012;4:523–32.

40 Aviram M, Rosenblat M, Billecke S *et al.* Human serum paraoxonase (PON 1) is inactivated by oxidized low density lipoprotein and preserved by antioxidants. Free Radic Biol Med 1999;26:892–904.

41 Johnson C, Pinal-Fernandez I, Parikh R *et al.* Assessment of mortality in autoimmune myositis with and without associated interstitial lung disease. Lung 2016;194:733–7.

42 Funauchi M, Shimadsu H, Tamaki C *et al.* Role of endothelial damage in the pathogenesis of interstitial pneumonitis in patients with polymyositis and dermatomyositis. J Rheumatol 2006;33:903–6.

43 Mackness MI, Arrol S, Mackness B, Durrington PN. Alloenzymes of paraoxonase and effectiveness of high-density lipoproteins in protecting low-density lipoprotein against lipid peroxidation. Lancet 1997;349:851–2.

44 Zhou M, Liu XH, Liu QQ *et al.* Lactonase activity, status, and genetic variations of paraoxonase 1 in women with gestational diabetes mellitus. J Diabetes Res 2020;2020: 3483427.

45 Charles-Schoeman C, Hyde C, Guan S *et al.* POS0442 Relationship between paraoxonase-1 genotype, activity, and major adverse cardiovascular events in patients with rheumatoid arthritis receiving tofacitinib . Ann Rheum Dis 2021;80:449–50.