Human epididymis protein 4 as a biomarker of interstitial lung disease in patients with idiopathic inflammatory myopathies

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Abstract

Objective

Human epididymis protein 4 (HE4) inhibits the degradation of type I collagen, thus promoting fibrosis. We aimed to investigate serum HE4 levels in patients with idiopathic inflammatory myopathies (IIMs), as potential biomarker of interstitial lung disease (ILD).

Methods

IIMs patients followed in our centre between June 2020 and January 2023 were enrolled. ILD was detected by high-resolution computed tomography (CT) and pulmonary function tests. Serum HE4 levels were measured in patients and controls. Progressive fibrosing (PF-) ILD was evaluated in patients with available 2-year follow-up (INBUILD criteria).

Results

We enrolled 90 consecutive IIMs patients (68% females, mean age 59.5 [52.75- 66.0] years) and 42 healthy, age- and sex-matched controls. ILD was diagnosed in 44 (49%) patients. Serum HE4 levels were higher in IIMs patients than controls: 78.55 [54.6-114.4] vs. 51.05 [41.8-62.8] pmol/L (p=0.001). IIMs-ILD patients had higher levels of HE4 vs. those without ILD (193.7 [78.92-137.42] vs. 58.15 [48.32-79] pmol/L, p<0.0001). Serum HE4 levels correlated inversely with diffusing capacity for carbon monoxide (rho=-0.556, p<0.0001) and total lung capacity (rho=-0.459, p=0.001). Serum HE4 levels were the only variable independently associated with IIMs-ILD in two models of multivariate analysis: OR 1.063 (CI 95% 1.02-1.108), p=0.004, and OR 1.059 (CI 95% 1.020-1.099), p=0.003. PF-ILD was detected in 39.4% of IIMs-ILD patients with available follow-up (33/44), without any significant association with baseline serum HE4 levels.

Conclusion

HE4 might be a useful biomarker in the identification and assessment of ILD in IIMs patients.

Key words

idiopathic inflammatory myopathies, interstitial lung disease, lung fibrosis, connective tissue diseases, biomarkers

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Introduction

Interstitial lung disease (ILD) is one of the most common organ involvements in patients with idiopathic inflammatory myopathies (IIMs), being detectable in about 50% of cases (1-3). In IIMs patients, ILD is associated with high morbidity and mortality, thus it requires prompt treatment (4). Nevertheless, shared screening strategies for IIMs-ILD are still lacking (5), particularly in patients without clinical evidence of ILD at IIMs diagnosis, due to concerns related to costs and ionising radiation exposure of repeat chest high-resolution computed tomography (HRCT), as well as to the intrinsic limitation of pulmonary function tests (PFTs) alone in detecting ILD, especially in the early stages (6).

Although human epididymis protein 4 (HE4) was first identified as a secretory protein in the human epididymis, its expression has been demonstrated in ovarian cancer and in other tissues including respiratory tract, kidney, and prostate (7-9). HE4 is expressed by activated fibroblasts in which it suppresses the activity of serine proteases and metalloproteinases (MMPs) inhibiting their capacity to degrade type I collagen, thereby promoting fibrosis (10). Elevated serum HE4 levels have been found in patients with renal fibrosis and lupus nephritis (11) and HE4 has been proposed as a predictive marker of cardiac remodelling in dilated cardiomyopathy (12). Moreover, serum HE4 levels were found to be higher in patients with lung diseases [*e.g.* idiopathic pulmonary fibrosis and cystic fibrosis (CF)] compared to those without (13, 14).

In the large field of autoimmune rheumatic diseases (ARDs), recent reports have highlighted a potential role for HE4 as a diagnostic biomarker of ILD in patients with rheumatoid arthritis (RA) (15, 16), systemic sclerosis (SSc) (17), and Sjögren's syndrome (SS) (18). The association between HE4 levels and IIMs-ILD has been explored only in one study on Asian patients so far (19). Moreover, there is only limited evidence on its potential role in predicting the occurrence of progressive fibrosing (PF-)ILD (20), a term encompassing a group of pulmonary diseases of various origins which progress despite treatment.

In this study, we aimed to assess the role of HE4 as a potential biomarker of ILD and/or PF-ILD in patients with IIMs.

Material and methods

Study population

Patients affected by IIMs, according to Bohan and Peter (21, 22), ENMC (23) or 2017 EULAR/ACR classification criteria (24), aged >18 years and followed up in our referral centre between June 2020 and January 2023, were consecutively enrolled. At least one HRCT and one PFTs assessment in the 6 months before enrolment were required to be included in the study. Patients affected by group 1 pulmonary arterial hypertension and/or chronic obstructive lung diseases and those with a history of malignant neoplasm were excluded. Healthy volunteers were recruited among healthcare workers (matched for age and sex) and included as controls. The study was conducted in compliance with the principles of the declaration of Helsinki, and approved by our institution's Ethics committee (Azienda Ospedaliera di Padova, 5505/AO/22); all participants gave written informed consent.

Data collection from IIMs patients

Demographic, clinical and serological variables were collected for each patient. We also recorded IIM diagnosis (anti-synthetase syndrome, dermatomyositis or polymyositis), age, sex and disease duration at enrolment, as well as the presence of IIMs-specific clinical signs and symptoms throughout the disease course. Among autoantibodies, serum anti-nuclear antibodies (ANA) were analysed by immunofluorescence (IF) assay on HEp-2 cells, anti-extractable nuclear antigen (ENA) antibodies by enzyme-linked immunosorbent assay (ELISA) and immunoblot, myositis-specific (MSA) and myositis-associated antibodies (MAA) by commercial line blots (Euroline Myositis Profile, Euroimmun, Lübeck, Germany) (25).

ILD was diagnosed in the case of reticular abnormalities, ground glass opacities and/or honeycombing at chest HRCT (26). Three prevalent radiologi-

cal patterns of ILD were identified: usual interstitial pneumonia (UIP), nonspecific interstitial pneumonia (NSIP) and organising pneumonia (OP) (26). The diagnosis of ILD and the prevalent pattern were determined for each patient by a multidisciplinary team, composed of senior rheumatologists (E.Z. and L.I.), pulmonologist (E.B.) and radiologist (C.G.) with expertise in ARDs-ILD.

The following PFTs indices (expressed as the percentage of observed/theoretic values) were recorded: forced vital capacity (FVC), total lung capacity (TLC), diffusing capacity for carbon monoxide (DLCO), carbon monoxide transfer coefficient (KCO) calculated as the ratio between DLCO and alveolar volume.

In patients with available 2-year follow-up (*i.e.* repeat PFTs and HRCT), PF-ILD was defined according to the INBUILD criteria (27).

Quantitative analyses of HE4

Blood samples from patients and healthy controls were collected at enrolment using standardised procedures and processing: serum samples were centrifugated at 3000 rpm for 10 minutes to separate the supernatant and then stored at -80°C until assayed. The HE4 protein was detected by chemiluminescent immunoassay of double-antibody sandwich method: HE4 CMIA (INNODX Biotechnology, Xiamen, China) in a Wan200+ system. Serum samples were assayed in strict accordance with the experimental protocol and the mean value of relative luminous intensity (RLU) was obtained from the calibration curve. The equation has been elaborated according to specific standards provided by the manufacturer, in order to obtain the corresponding antigen content. The range values were from 20.0 pmol/L to 1500.0 pmol/L.

Statistical analysis

Continuous variables were expressed as medians (interquartile range), and categorical variables as frequencies and percentages. Comparisons between groups of patients with IIMs and healthy controls and between patients with IIMs (*i.e.* ILD *vs.* non-ILD, and PF-ILD *vs.* non-PF-ILD) were carried out using Mann-Whitney U-test for continuous variables, and the chi-squared test or Fisher's exact probability test for categorical data, where appropriate. The ability of HE4 to identify patients with IIMs-ILD was assessed by receiver operating char-

Table I. Demographic, serological and clinical features of all patients, and according to the presence of ILD.

	All patients (n = 90)	IIMs-ILD (n=44, 48.9%)	IIMs without ILD (n=46, 51.1%)	*p*-value
Age (yrs)	59.5 (52.75- 66.0)	61.5 (57-66.5)	55.5 (34.75-66)	**0.005**
Female sex, n (%)	61 (68)	31 (71)	30 (65)	0.595
Disease duration (yrs)	4 (2-8)	5 (3-8.75)	6.5 (3-11.25)	0.509
PM, n (%)	21 (23)	7 (16)	14 (30)	0.103
DM, n (%)	36 (40)	11 (25)	25 (54)	**0.004**
ASyS, n (%)	33 (37)	26 (59)	7 (15)	**<0.001**
HE4 (pmol/L)	78.55 (54.6-114.4)	193.7 (78.92-137.42)	58.15 (48.32-79)	**<0.001**
Fever, n (%)	8 (9)	4 (9)	4 (9)	0.947
Weight loss, n (%)	2 (2)	1 (2)	1 (2)	0.975
Muscle weakness, n (%)	37 (41)	20 (46)	17 (37)	0.413
Dysphagia, n (%)	6 (7)	4 (9)	2 (4)	0.367
Heliotropic rash, n (%)	16 (18)	5 (11)	11 (24)	0.120
Mechanic's hands, n (%)	13 (14)	12 (27)	1 (2)	**0.001**
Gottron's sign, n (%)	14 (16)	6 (14)	8 (17)	0.623
Gottron's papules, n (%)	19 (21)	7 (16)	12 (26)	0.237
Skin ulcers, n (%)	4 (4)	3 (7)	1 (2)	0.285
Raynaud's phenomenon, n (%)	22 (24)	12 (29)	10 (26)	0.770
Myalgia, n (%)	14 (16)	6 (13)	8 (17)	0.623
Arthritis, n (%)	19 (21)	14 (32)	5 (11)	**0.015**
Dyspnoea, n (%)	20 (22)	18 (41)	2 (4)	**<0.001**
Cough, n (%)	8 (9)	6 (14)	2 (4)	0.122
CK> 180 U/L, n (%)	43 (49)	18 (42)	25 (56)	0.199
FVC (% pred.)	96 (76.5-109)	90 (73.7-107.5)	106 (92-111)	**0.036**
TLC (% pred.)	83 (71.5-96.5)	80.5 (69.7-91.2)	94 (76-112)	**0.023**
DLCO (% pred.)	70 (56.5-81)	69 (52-77)	80.5 (68-99)	**0.003**
ANA, n (%)	52 (67)	24 (63)	28 (70)	0.522
MSA, n (%)	61 (68)	34 (77)	27 (59)	0.059
MAA, n (%)	39 (44)	25 (57)	14 (31)	**0.015**
Anti ARS, n (%)	36 (40)	26 (59)	10 (22)	**<0.001**
Anti Jo1, n (%)	26 (29)	21 (48)	5 (11)	**<0.001**
Anti PL-12, n (%)	5 (5)	4 (9)	1 (2)	0.159
Anti PL-7, n (%)	4 (5)	2 (5)	2 (4)	0.982
Anti EJ, n (%)	1 (1)	0	1 (2)	0.320
Anti Mi2, n (%)	10 (11)	1 (2.)	9 (20)	**0.009**
Anti MDA5 n (%)	9 (10)	6 (14)	3 (10)	0.276
Anti TIF1γ, n (%)	4 (5)	0	4 (9)	0.043
Anti Ro52, n (%)	32 (40)	20 (54)	12 (28)	**0.017**
Anti SAE, n (%)	2 (2)	2 (4)	0	0.162
Anti PM/Scl, n (%)	5 (6)	2 (4)	3 (7)	0.609
Anti SRP, n (%)	3 (3)	2 (4)	1 (2)	0.570
Anti Ku, n (%)	4 <(5)	3 (7)	1 (2)	0.317
Glucocorticoids, n (%)	64 (71)	31 (70)	33 (72)	0.893
Immunosuppressants, n (%)	62 (69)	30 (68)	32 (70)	0.983
MTX, n (%)	30 (33)	9 (21)	21 (46)	**0.007**
AZA, n (%)	1 (1)	0	1 (2)	0.337
MMF, n (%)	20 (22)	14 (32)	6 (13)	**0.007**
CYC, n (%)	1 (1)	1 (2)	0	0.290
CNI, n (%)	8 (9)	4 (9)	4 (9)	0.881
TCZ, n (%)	1 (1)	1 (2)	0	0.290

Values are expressed as numbers and (%) or medians and interquartile range (IQR) as appropriate.
ANA: anti-nuclear antibodies; ARS: anti-aminoacyl-tRNA synthetase; ASyS: antisynthetase syndrome; AZA: azathioprine; CK: creatine kinase; CNI: calcineurin inhibitors; CYC: cyclophosphamide; DLCO: diffusing capacity of the lungs for carbon monoxide; DM: dermatomyositis; ENA: extractable nuclear antigen; FVC: forced vital capacity; ILD: interstitial lung disease; KCO: carbon monoxide transfer coefficient; MDA5: anti-melanoma differentiation-associated gene; MMF: mycophenolate mofetil; MMT: manual muscle testing; MTX: methotrexate; O2: oxygen; PH: pulmonary hypertension; PM: polymyositis; RNP: ribonucleoprotein; SAE: small ubiquitin-like modifier 1 activating enzyme; SRP: signal recognition particle; TCZ: tocilizumab; TIF1γ: transcription intermediary factor 1-gamma; TLC: total lung capacity.

acteristic (ROC) curve analysis. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. To avoid collinearity, two multivariate models were performed to identify factors independently associated with the diagnosis of ILD by logistic regression. Variables found to be different (p<0.1) at univariate analysis, were included in the multivariate logistic regression models (with backward elimination), adjusted for age and sex. All tests were two-tailed, and p-values <0.05 were considered significant. Bivariate correlations were assessed by the Spearman coefficient (rho). The statistical analysis was performed using the SPSS statistical package, version 28.0.

Results

Study population

We enrolled 90 consecutive IIMs patients (68% females, mean age 55.8±13.8 years) and 42 healthy controls; the two groups were similar in terms of age (p=0.89) and sex (p=0.11). Among IIMs patients, the median disease duration was 4 (2-8) years. Forty-four (48.9%) patients showed signs of ILD on baseline chest HRCT, with a median ILD duration at enrolment of 4 (2–7.5) years. Demographic, clinical, serological and functional features of patients with and without ILD are reported in Table I.

Patients with IIMs-ILD were older than those without ILD (p=0.005), whereas sex and disease duration were similar between the two groups. Compared with those without ILD, patients with IIMs-ILD more frequently exhibited MAA (p=0.015), anti-Ro52 (p=0.017) and anti-synthetase antibodies positivity (p<0.001), as well as arthritis (p=0.015), mechanic's hands (p<0.001) and dyspnoea (p<0.001); whereas anti-Mi2 positivity was more common in patients without ILD (p=0.009). No other differences were found between the two groups as it pertains to the remaining clinical manifestations and the serological profile.

Predictably, IIMs patients with ILD showed lower values of FVC (p=0.036), TLC (p=0.023), and DLCO (p=0.03), compared with those without lung involvement. On HRCT, the most common prevalent pattern was NSIP (65.9%), followed by OP (22.7%), and UIP (11.4%).

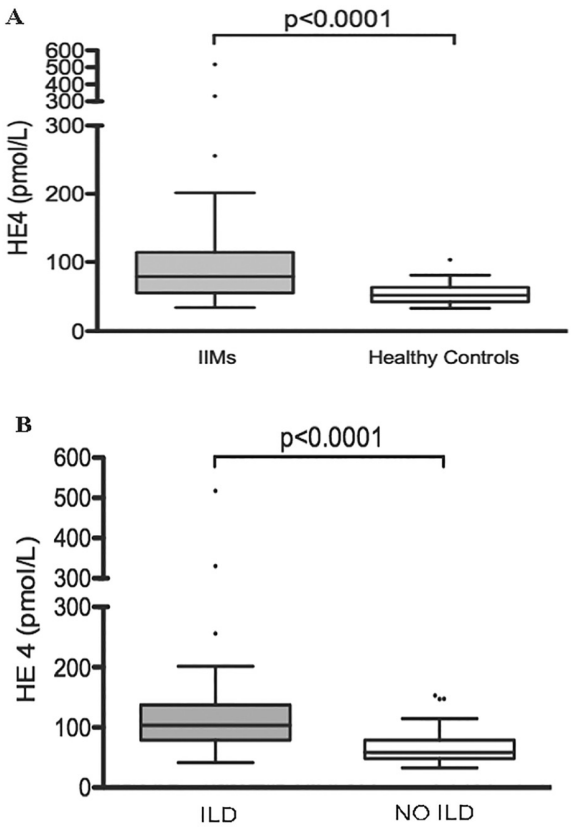

Fig. 1. Serum HE4 levels in healthy controls and in patients with idiopathic inflammatory myopathies (IIMs) (**A**); and in patients with IIMs with and without interstitial lung disease (ILD) (**B**).

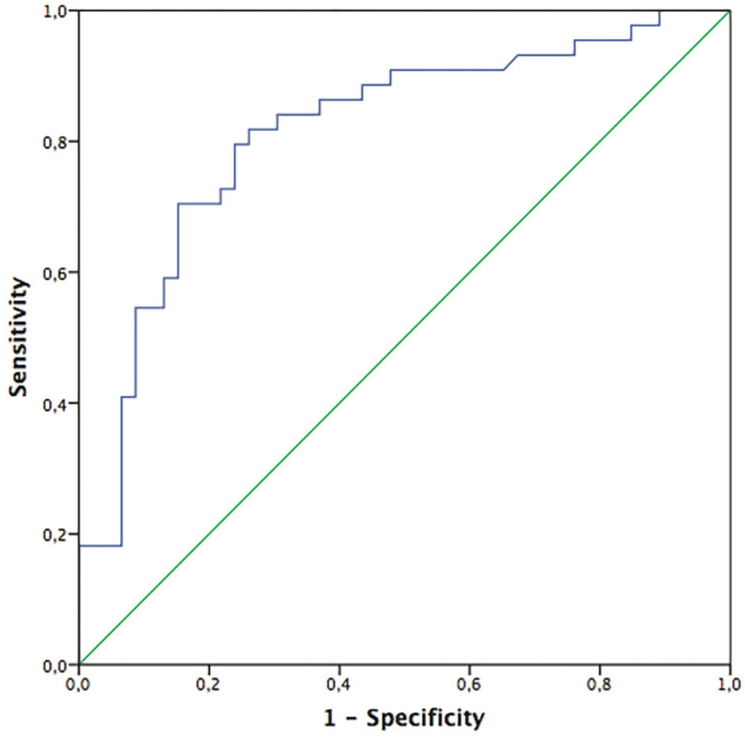

Fig. 2. ROC curve illustrating the diagnostic value of HE4 to detect ILD in patients with IIMs.

Performance of serum HE4 levels as marker of IIMs-ILD

Serum HE4 levels were higher in IIMs patients than in controls [78.55 (54.6-

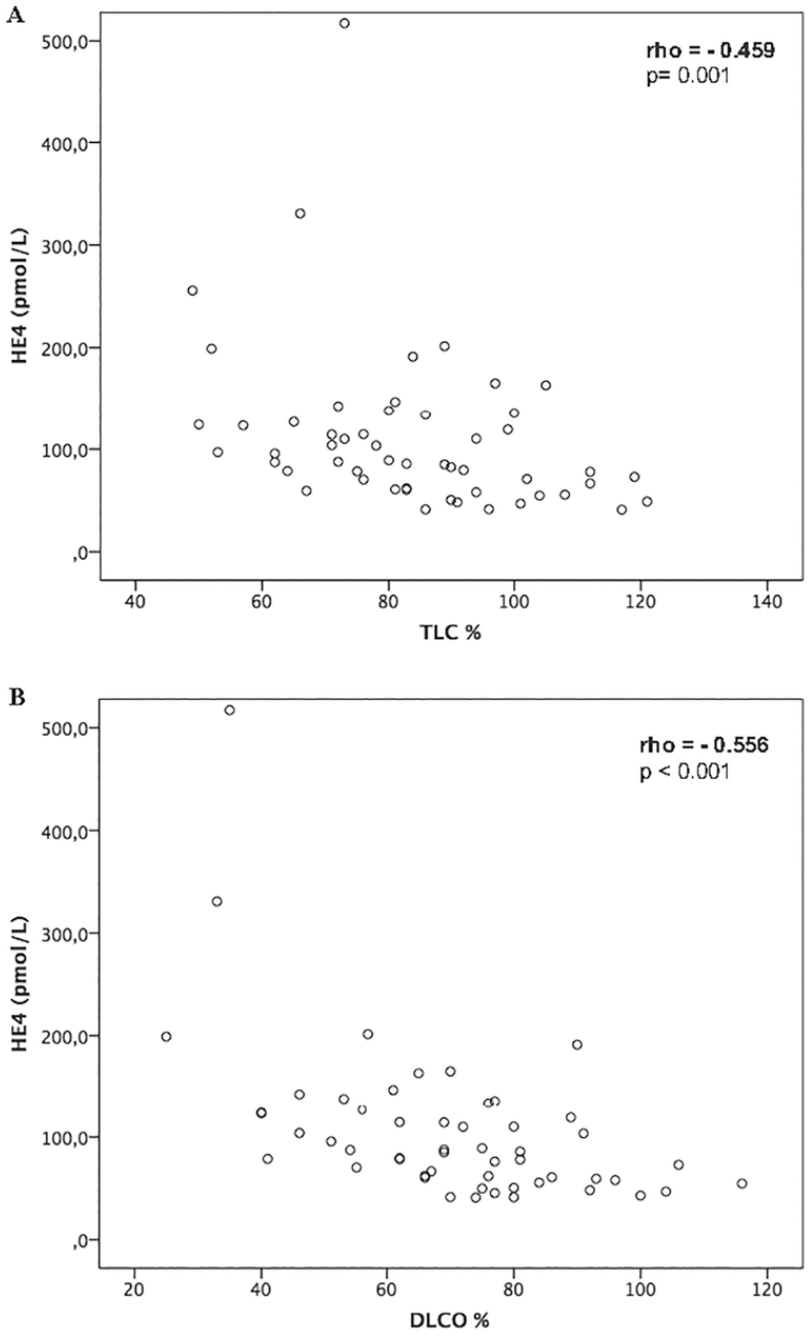

Fig. 3. Correlation between serum HE4 levels and TLC (**A**), and DLCO (**B**).

114.4) *vs*. 51.1 (41.8–62.8) pmol/L, *p*=0.001] (Fig. 1A). Among IIMs patients, those with ILD had higher levels of HE4 than those without [193.7 (78.92–137.42) *vs*. 58.15 (48.32–79) pmol/L, *p*<0.001] (Fig. 1B).

ROC curve analysis to assess the performance of HE4 in identifying IIMs-ILD showed an area under the curve (AUC) of 0.8 (95% CI 0.724–0.906, *p*<0.0001) (Fig. 2). Using a threshold of 84.95 pmol/L, defined by the ROC curve, HE4 showed 81.6% sensitivity, 75% specificity, 70.5% PPV and 84.78% NPV in identifying IIMs-ILD in our cohort.

Correlation between serum HE4 levels and PFTs indices

Using Spearman's correlation analysis, a positive correlation was found between HE4 and age (rho=0.374, *p*<0.001). Serum HE4 levels inversely correlated with TLC (rho=-0.459, *p*=0.001) and DLCO (rho=-0.556, *p*<0.001) (Fig. 3). A trend towards a negative correlation was observed between serum HE4 levels and FVC (rho =-0.261, *p*=0.059).

Multivariate analysis for the diagnosis of IIMs-ILD

We performed two models of multivariate analysis, adjusted for age and sex, to find variables independently associated with the diagnosis of IIMs-ILD. In both models, serum HE4 levels were the only factor independently associated with IIMs-ILD diagnosis: OR 1.063 (CI 95% 1.02-1.108; *p*=0.004) in model 1 and OR 1.059 (CI 95% 1.020-1.099; *p*=0.003) in model 2 (Table II).

Serum HE4 levels in IIMs-ILD patients with and without PF-ILD

Data of two-year follow-up were available in 33/44 (75%) patients with IIMs-ILD and PF-ILD was detected in 13/33 (39.4%). Serum HE4 levels were higher in patients with PF-ILD than in those without [110 (87.50-140.30) vs. 88.25 (76.67-126.12) pmol/L], but the difference was not statistically significant (*p*=0.224).

Discussion

Although ILD is one of the most common organ involvements in patients with IIMs, shared screening strategies are still lacking. Hence the need to optimize the stratification of IIMs patients at risk for ILD (28-30), to improve their management.

In our study serum HE4 levels were significantly higher in IIMs patients compared to healthy controls, and in IIMs-ILD patients compared to those without ILD. Moreover, among several factors associated with ILD in the literature (*e.g*. anti-synthetase antibodies and anti-Ro52 positivity, FVC, DLCO etc.), serum HE4 levels emerged as the only variable independently associated with ILD in two models of multivariate analysis in our cohort. This suggests that high levels of HE4, with a cut-off of 84.95 pmol/L defined by the ROC curve, may help to detect ILD in the early stages and/or of limited extent, when usually PFTs are still normal, better than the traditional aforementioned variables. On the other hand, the high negative predictive value (about 85%)

Table II. Multivariate analysis for the diagnosis of ILD.

	OR (CI 95%)	*p*-value
Model 1		
Age	1.062 (0.982-1.149)	0.133
Sex	0.115 (0.006-2.372)	0.162
Serum HE4 levels	***1.063 (1.02-1.108)***	***0.004***
Anti-ARS	5.588 (0.841-37.133)	0.075
Anti-Ro52	0.062 (0.00-15.94)	0.326
Arthritis	25.48 (0.151-4312.46)	0.216
FVC	0.954 (0.9-1.012)	0.119
DM	61.57 (0.357-10603.15)	0.117
Model 2		
Age	1.039 (0.972-1.111)	0.261
Sex	0.87 (0.105-7.215)	0.897
Serum HE4 levels	***1.059 (1.020-1.099)***	***0.003***
Anti-Ro52	2.84 (0.398-20.24)	0.298
Anti-Mi2	0.086 (0.000-39.105)	0.432
Arthritis	1.9 (0.104-34.693)	0.665
DLCO	0.953 (0.895-1.014)	0.125

Values are expressed as odds ratio (95% CI). CI: confidence interval.
ARS: anti-aminoacyl-tRNA synthetase; DLCO: diffusing capacity of the lungs for carbon monoxide; DM: dermatomyositis; FVC: forced vital capacity.

of our identified cut-off, may help to identify patients in whom HRCT should not be performed, thus avoiding unnecessary radiation exposure.

Although the underlying mechanisms are not yet fully understood, it has been suggested that HE4 may play a key functional role in the development of fibrosis, mainly by inhibiting the activity of several proteases and MMPs, thus avoiding the degradation of type I collagen. A study by Nagy *et al*. (14) found that HE4 correlates with the overall severity of CF, and HE4 mRNA was found in CF lung biopsy specimens compared with no-CF controls. In recent years, serum HE4 levels have been found to be associated with ARDs-ILD in RA (15, 16), SSc (17) and SS (18); only one recent study reported a similar association in IIMs patients of Asian ethnicity (19). Importantly, our results confirmed this association even in Caucasians, further suggesting a role for HE4 in identifying IIMs-ILD patients, independently of their ethnicity. Moreover, it should be noted that the cut-off derived from the ROC curve to identify IIMs-ILD in the study by Sun *et al*. was similar to that observed in our cohort (79.6 pmol/L *vs*. 84.95 pmol/L, respectively) (18).

We were also able to corroborate a correlation between serum HE4 levels and age, as previously reported by some other studies (19). Given that our IIMs-ILD patients were older than those without ILD, it bears noting that both our multivariate models were adjusted for age. Moreover, we found an inverse correlation between serum HE4 levels and both lung volumes (*i.e*. TLC) and DLCO, supporting an additional role for this candidate IIMs-ILD biomarker in stratifying patients according to the severity of lung fibrosis. This finding was also reported in patients with SSc-ILD (17) and in IIMs-ILD Asian patients (19); in RA-ILD, it was demonstrated only for DLCO (15). In our patients we observed only a trend towards correlation between serum HE4 levels and FVC values, which are probably more influenced by concomitant extrapulmonary variables (*i.e*. myositis) compared to TLC, the gold standard for the diagnosis of restrictive lung disease (31).

When evaluating the 2-year follow-up, we did not find any significant differences in serum HE4 levels at baseline between patients who did or did not develop PF-ILD, although the absolute mean value was higher in the former group. Only one study in the literature has endeavoured to assess the potential association of serum HE4 levels with PF-ILD (20). However, it should be noted that PF-ILD patients enrolled in that study were compared to a very heterogeneous group comprising non-ILD patients (*e.g*. bronchial asthma, chronic obstructive pulmonary disease, etc.). Thus, further studies including only ILD patients with and without a progressive fibrosing phenotype are needed to address this issue.

We would remiss not to mention some of the limitations of our study. Our sample size was admittedly rather small, though it may be explained by the fact that IIMs are recognised as a rare condition. Moreover, the lack of a radiological score evaluation did not allow to precisely define the extent of ILD, in addition to functional impairment. By contrast, one strength of our study is that all patients were enrolled from a single, homogeneous, and well-characterized cohort of patients with IIMs.

In conclusion, our results identify serum HE4 as a potential biomarker for the diagnosis of ILD in patients with IIMs. Moreover, the detection of HE4 serum levels might be useful in assessing the functional impairment of IIMs-ILD patients.

Larger studies are needed to assess the clinical significance of HE4 as a biomarker of IIMs-ILD, and ascertain its sensitivity to changes, since only baseline serum levels were evaluated in our cohort. Furthermore, the potential predictive value of monitoring the clinical response to treatment (*i.e*. immunosuppressants and/or antifibrotics) should be assessed, also considering that in other diseases (*e.g*. CF), plasma HE4 levels were inversely associated with lung function improvement after specific treatment.

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