Journal of Inflammation

RESEARCH

Open Access

Auto-antibodies against carbonyl-modified vimentin in COPD: potential role as a biomarker

L. Heinemann[1], I. Adcock[1], K. F Chung[1], W. Lollinga[2,3], M. N. Hylkema[2,3], A. Papi[4], G. Caramori[5] and P. A. Kirkham[1,6*]

Abstract

COPD has many hallmarks of autoimmune dysfunction. Driving this autoimmune response are self-antigens, such as highly abundant structural proteins and cellular proteins, which can lead to the production of auto-antibodies. However, controversy surrounds the detection of some of these auto-antibodies as they have often been screened against native, unmodified proteins. Autoantigens arise as a result of a conformational change in the native protein exposing hidden epitopes or by the creation of neo-epitopes through chemical or enzymatic modifications, often caused by oxidative/carbonyl stress. In this study, we screened for auto-antibodies targeting key structural proteins modified by oxidative/carbonyl stress in peripheral blood from stable COPD patients versus control subjects using ELISA. We found an auto-antibody response against unmodified, carbonyl-modified and citrinylated vimentin, with the highest response observed against carbonyl-modified vimentin. Both the IgG and IgM antibody titres against carbonyl-modified were significantly increased in COPD patients compared to healthy non-smokers. Smokers also displayed increased antibody levels against carbonyl-modified vimentin, but only for the IgG isotype. Selectivity analysis indicated that 70% and 63% of COPD patients had higher IgM and IgG titres, respectively, compared to non-smokers. In contrast only 26% and 48% of smokers had higher IgM and IgG titres, respectively, than non-smokers. ROC analysis gave AUC values of 0.78 ($p < 0.01$) and 0.84 ($p < 0.001$) for IgM and IgG, respectively, for COPD versus non-smokers, which fell to 0.70 ($p < 0.01$) and 0.64 (NS), respectively, when asymptomatic smokers were included. No significant increase in antibody titre against carbonyl-modified elastin or collagen was observed in COPD patients or asymptomatic smokers. We conclude that IgM autoantibody responses against carbonyl modified vimentin could serve as a simple blood-based biomarker for COPD, reflecting the disease's pathophysiology, and could help in patient stratification and diagnosis.

Keywords Respiratory, Oxidative stress, Carbonyl, Biomarker

*Correspondence:
P. A. Kirkham
p.kirkham@wlv.ac.uk
[1] National Heart and Lung Institute, Imperial College London, London, UK
[2] Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, 9713 GZ Groningen, The Netherlands
[3] Groningen Research Institute for Asthma and COPD (GRIAC), University of Groningen, University Medical Center Groningen, 9713 GZ Groningen, The Netherlands
[4] Respiratory Medicine, Centro Interdipartimentale per lo Studio delle Malattie Infiammatorie Delle Vie Aeree E Patologie Fumo-Correlate (CEMICEF), University of Ferrara, Ferrara, Italy
[5] Department of Medicine and Surgery, Pulmonology, University of Parma, Parma, Italy
[6] Department of Biomedical Sciences & Physiology, Faculty of Science & Engineering, University of Wolverhampton, Wolverhampton, UK

Introduction

Chronic obstructive pulmonary disease (COPD) is defined as "a common preventable and treatable disease, characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. Exacerbations and comorbidities contribute to the overall severity in individual patients [1]. COPD is currently one of most important causes of morbidity and mortality worldwide and with over 210 million people diagnosed with the disease, it is predicted to become the third leading cause of death globally by 2030 [2]. Moreover, it has been reported that over 50% of subjects with COPD remain undiagnosed until they present with moderate or severe symptoms [3], suggesting that the actual number of sufferers with COPD is much higher. Forced expiratory volume in one second (FEV_1) is currently the most frequently used measurement of disease severity and progression, but this does not correlate well with symptoms, underlying pathophysiology and other disease markers [4]. Consequently there is a pressing need to identify biomarkers of COPD that can aid diagnosis, improve disease stratification, help monitor therapeutic intervention strategies and importantly identify at risk individuals early before developing more severe disease.

While COPD pathogenesis has historically been viewed in the context of an aberrant innate immune response, there is increasing evidence indicating the involvement of the adaptive immune response [5]. CD8 + and CD4 + T cells as well as B cells are increased in the small airways and lung tissue of COPD patients along with elevated numbers of secondary lymphoid follicles, particularly in severe and very severe COPD, GOLD stages III & IV [6, 7]. However, the nature of the antigen or antigens driving this specific immune response remains unclear, although recent work by Kirkham et al [8] would suggest that oxidative stress-modified self-proteins may act as potent neo-antigens helping to drive an autoimmune response in COPD. COPD, in common with other chronic inflammatory diseases, has hallmarks of autoimmune dysfunction and numerous proteins including the presence in the lungs of highly abundant structural proteins with the potential to be chemically or enzymatically altered could thereby act as auto-antigens [9]. In patients with stable COPD, serum autoantibodies against pulmonary epithelial cells [10], endothelial cells [8, 11] and extracellular matrix proteins such as elastin [12] and collagen [13] have all been reported.

Exposure to environmental oxidants/nitrosants, such as tobacco smoke, is recognised as the single biggest risk factor for developing COPD. Lung inflammation persists long after smoking cessation [14] suggesting that active smoking is not the sole driver of inflammation in COPD [15]. Tobacco smoke is a complex mixture of more than 5000 chemicals including many known to be toxic and/or carcinogenic [16]. In addition the components of tobacco smoke or of oxidative damage to tissues, such as lipid peroxidation, have been shown to readily modify proteins directly. For example, reactive carbonyl products such as the reactive carbonyls malonyldialdehyde or acrolein are capable of irreversibly modifying proteins in vitro and *in vivo* [17–21] or alternatively promote the release of enzymes that are important in the conversion of arginine to citrulline [22]. These modifications can cause sufficient conformational change to render the protein antigen immunogenic by effectively creating "neo-antigens" [9].

It is postulated that high levels of natural IgM antibodies against carbonyl stress-modified proteins acting as DAMPs or neo-antigens may be protective against the damaging effects of autoimmunity by helping to remove these neo-antigens from the circulation [23]. However, IgG antibodies, in particular the IgG1 isotype have a strong cell lysis potential through complement activation [24]. We have previously shown that anti-carbonyl modified auto-antibodies to human serum albumin are elevated in the serum of patients with stable COPD as a consequence of carbonyl stress [8]. Therefore we examined the presence of serum autoantibodies against post-translational modifications of key structural proteins in patients with stable COPD patients and show enhanced titres of immunoglobulin subsets against carbonyl-modified vimentin. By contrast, antibody titres against carbonyl-modified extracellular matrix proteins, such as collagen and elastin, were not altered in COPD.

Methods

Patients and sera

Subjects were recruited from the Section of Respiratory Diseases of the University Hospital of Ferrara, Italy, and from the National Heart & Lung Institute, Imperial College London with approval by the local Ethics Committee. Informed consent was obtained from each participant in accordance with the principles outlined in the Declaration of Helsinki. Venous peripheral blood was collected, processed and stored as described in Kirkham et al. 2011 [8]. Pulmonary function tests and the predicted values for the different measures were performed and calculated as described previously [25, 26]. COPD was defined according to international guidelines (post-bronchodilator FEV1/FVC ratio < 70%) and the severity of COPD was classified according to current GOLD criteria [1]. Subject details are summarised in Table 1.

Table 1 Study subject details

	Age	M/F	Pack years	FEV_1/FVC	% pred FEV1
NS	50 ± 3	10/4	0	0.97 ± 0.03	101 ± 3
Smoker	60 ± 9	14/9	27 ± 16	0.81 ± 0.03	86 ± 3
COPD 21 ex-smokers 5 smokers	70 ± 6	16/10	47 ± 32	0.41 – 0.66	40—88

Data are depicted as Mean ± SD for age and pack years. FEV_1/FVC and % pred FEV1 are displayed as Mean ± SEM,. FEV_1/FVC ratio is pre-bronchodilator

Abbreviations: *pred* predicted, *M* male, *F* Female, FEV_1 Forced expiratory volume in 1 s, *FVC* Forced vital capacity, *NS* Non-smoker

Source of proteins

Full-length native recombinant vimentin was a kind gift from Professor Marlene Rose (Imperial College, London). Elastin and collagen V were purchased from Sigma (Poole, UK).

Malondialdehyde modification of proteins

Malondialdehyde (MDA) modification was performed using a modification of the technique described by Haberland et al [27]. Briefly, a 0.2 M stock solution of MDA was generated by mixing 162 µl of malonaldehyde bis(dimethyl acetal) (Alpha Diagnostics Inc) with 200 µl of 2 M HCl. After incubation at room temperature for 15 min, 4.8 ml phosphate buffer (pH 6.4) was added and the solution neutralised with NaOH.

Equal volumes of elastin or vimentin at 1 mg/ml was mixed with the activated MDA solution and incubated at 37 °C for 24 h. Unreacted MDA was removed from solution by dialysis against PBS. For modification of collagen, protein was coated onto NUNC Maxisorb microtitre plates at 10ug/ml overnight at + 4 °C in PBS and then incubated for 24 h at 37 °C with 0.1 M activated MDA. MDA solution was aspirated and the plates were washed twice with PBS.

ELISA protocol for serum autoantibodies against MDA-modified proteins

Patient serum was screened for antibodies against native or modified proteins by ELISA using 96-well Nunc Maxisorb microtitre plates coated with 100 µl/well of 10 µg/ml target protein. The coated microtitre plates were then treated with 200 µl/well blocking buffer (1% w/v BSA, 0.05% v/v Tween20 in PBS) for 1.5 h at 37˚C. Serial dilutions of serum in blocking buffer (100 µl/well) were added and incubated for 2 h at 37˚C. Bound human antibodies were assessed for IgG or IgM subtype using 100 µl/well of a 1:4000 dilution of HRP labelled goat anti-human IgG or IgM (AbCam, UK) in blocking solution for 1 h at 37˚C. Between all steps up to this point the plates were washed three times with 0.05% v/v Tween20 in PBS. The microtitre plates were then developed using 100 µl/well of OPD substrate solution (Pierce, UK) and the reaction stopped with 100 µl/well 2N sulphuric acid. The absorbance was then read in a microtitre plate reader at 490 nm in a Biotek Synergy HT plate reader. Each ELISA plate had the same control serum sample in quadruplicate, diluted 1000 fold in blocking buffer, so as to allow the data to be normalised between plates.

Data and Statistical analysis

GraphPad Prism was used to perform all statistical analyses. After normalisation of all absorbance readings relative to a control sample on each microtitre plate, all antibody titres were determined as an end point titre and defined as the titre at which the OD signal from a serum dilution curve was equivalent to twice that of background. ANOVA with Fishers post-hoc analysis were used to determine differences between groups and p values < 0.05 were considered statistically significant.

Results

Analysis of the antibody response to carbonylated vimentin revealed that IgM titres were significantly elevated in subjects with stable COPD relative to both asymptomatic smokers and non-smokers with normal lung function (Fig. 1a). In addition, significantly elevated IgG titres were observed in both control smokers with normal lung function and in patients with stable COPD (GOLD I, II and III) compared to non-smokers (Fig. 1b). Interestingly, a small number of smokers (30%) exhibited an elevated IgG/IgM ratio relative to non-smokers, which was attributable to a lack of IgM titre response in these subjects (Fig. 1c). This ratio decreased slightly to 21% within the COPD cohort, again driven by a low IgM response in comparison to the IgG response. Further analysis of the COPD cohort revealed no significant correlation between either IgM or IgG titres and lung function or smoking pack-years (Fig. 2).

Figure 3 illustrates the sensitivity of a positive IgM and IgG response above the levels observed in the non-smoking control group. For the IgM response, a clear distinction is observed between non-smokers, smokers and the COPD cohorts, with 68% of COPD subjects showing a positive IgM response compared to only 26% in smokers (Fig. 3a). In contrast, the IgG response displayed similar sensitivity levels of 63% for COPD and 48% for smokers (Fig. 3b) which is also reflected in the similar mean IgG titre levels observed between these two cohorts in Fig. 1b. Subsequent ROC analysis (Fig. 4) revealed that both the IgM and IgG response had strong selectivity-sensitivity profiles for COPD when compared to non-smokers with AUCs of 0.78

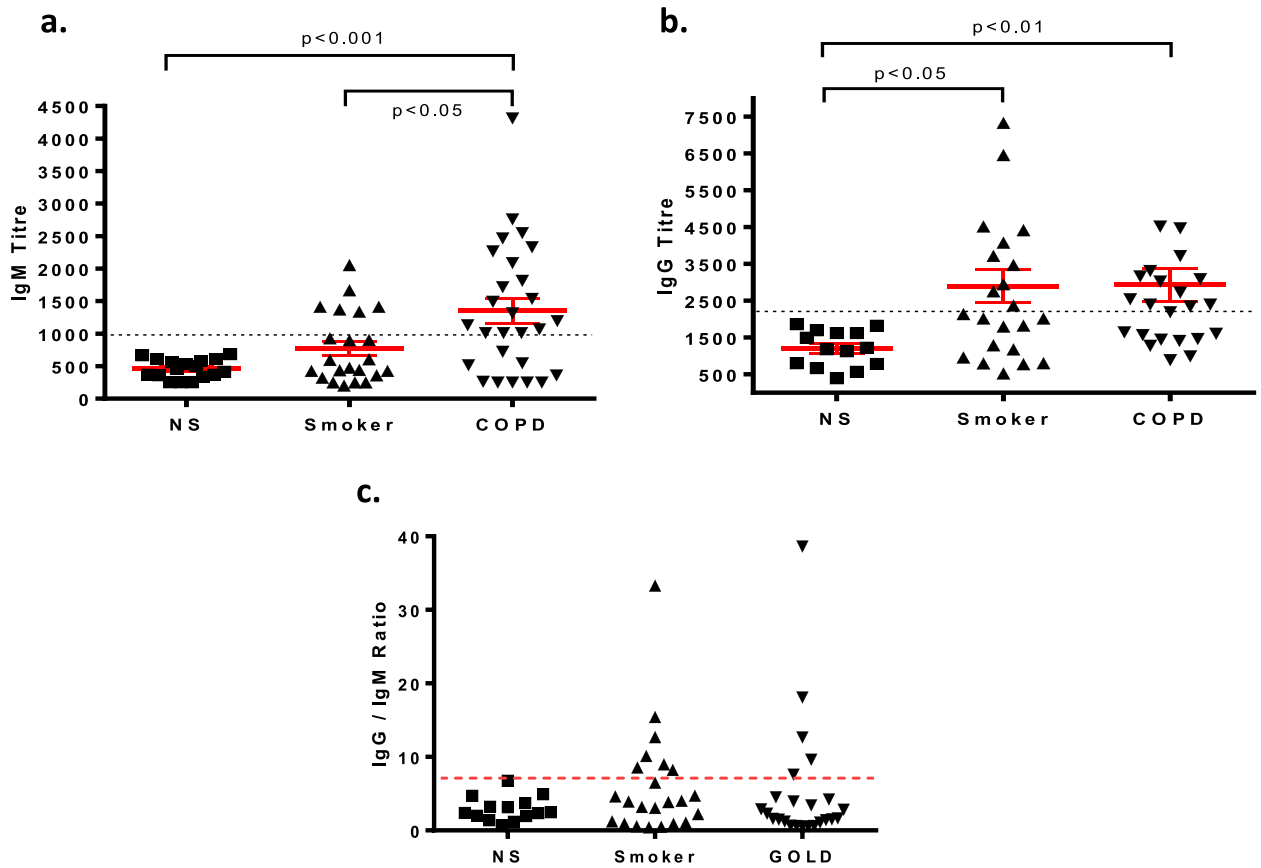

Fig. 1 Isotype specific immunoreactivity towards carbonyl-modified vimentin in control (smokers and non-smokers) versus stable COPD subjects. Serum samples were screened for IgG (**a**) and IgM (**b**) immunoreactivity against MDA-modified vimentin using ELISA. The dotted line in panels (**a**) and (**b**) represents a threshold set at two standard deviations from the mean for non-smoking control subjects, above which immunoreactivity is considered positive. Panel (**c**) displays the ratio of IgG to IgM immunoreactivity for each subject. Antibody titres were determined as detailed in materials and methods. Results are expressed as mean titre ± SEM for each patient group. Statistical analysis was performed using one way ANOVA with multi-comparison Fisher's post-hoc analysis

($p < 0.01$) for IgM and 0.84 ($p < 0.001$) for IgG respectively. However, when COPD was compared against all non-COPD subjects (including both non-smokers and smokers) the AUC fell to 0.70 ($p < 0.01$) for IgM and 0.65 ($p < 0.06$) for IgG, suggesting that only IgM titre against carbonyl-modified vimentin significantly differentiated between COPD and non-COPD subjects.

As shown in Fig. 5a, we were unable to detect any elevated autoantibody response against elastin in our cohort of COPD subjects. Furthermore, as the carbonyl-modified protein has previously been shown to be more immunogenic and a likely instigator of a possible autoimmune response, we screened for antibody responses against carbonyl-modified elastin and carbonyl-modified collagen V. However, we again found no significant elevation in antibody titre response (Figs. 5b and c).

Discussion

We demonstrate, for the first time, the presence of increased autoantibodies against native and carbonyl-modified vimentin in the serum of COPD patients compared to healthy non-smokers and smokers without COPD. In contrast, no similar increase was increase was observed for antibodies against native or modified elastin or collagen V. This data suggests that analysing multiple antibody isotypes against carbonyl-modified vimentin may prove more effective than measuring a single isotype for identifying and stratifying individuals with COPD.

Autoantibodies to mutated citrullinated vimentin have both diagnostic and prognostic value in rheumatoid arthritis, making this an interesting antigen to investigate in other chronic inflammatory settings. Indeed, previous small-scale studies, without the appropriate control groups [28, 29], have demonstrated the presence of these autoantibodies in patients with stable COPD. Although

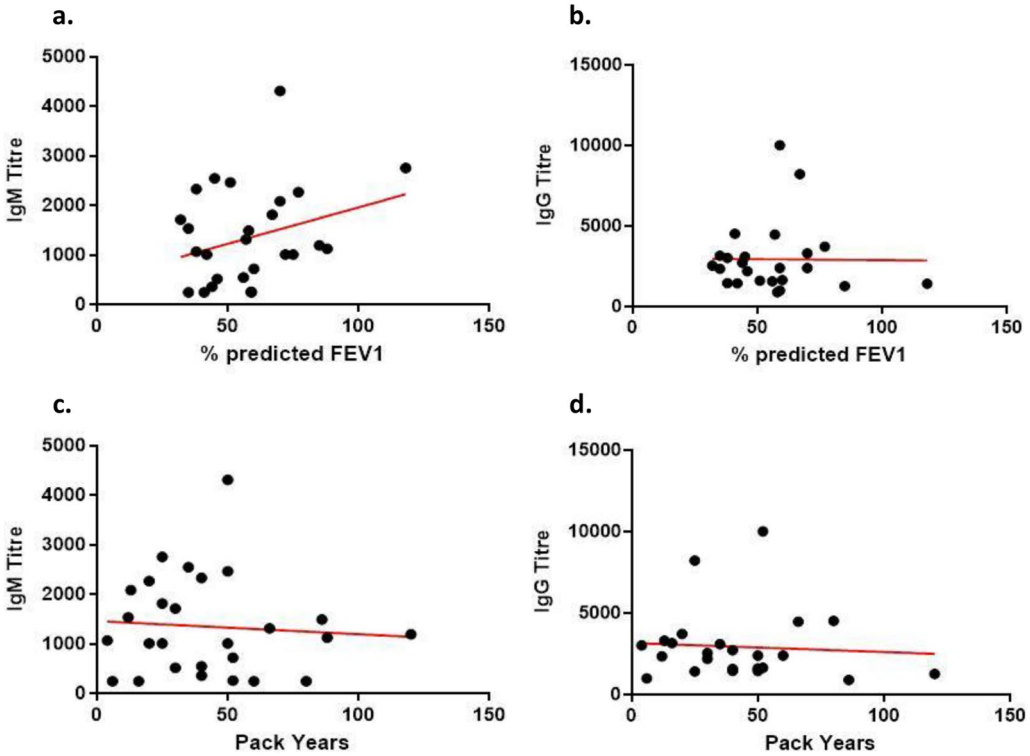

Fig. 2 Scatter plots displaying the lack of correlation between antibody titre for IgG (panels **A** and **C**) or IgM (panels **B** and **D**) versus lung function (panels **A** and **B**) or smoking pack years (panels **C** and **D**) in subjects diagnosed with COPD. Linear regression analysis was performed using GraphPad Prism 6.07 software with the slope found to be not significantly different from zero ($p = 0.14$, 0.96, 0.71, 0.74 for panels **A**, **B**, **C** and **D** respectively)

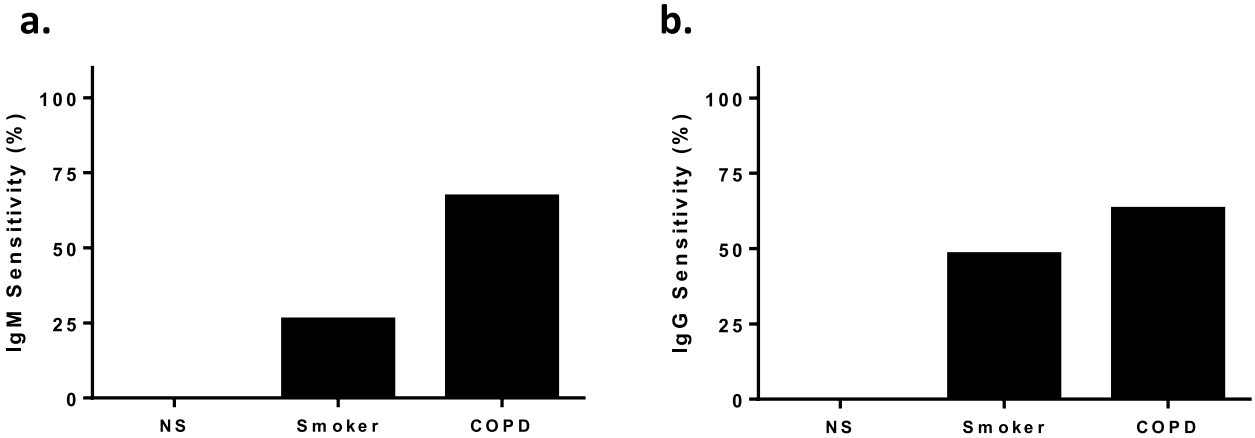

Fig. 3 Sensitivity of immunoreactivity for IgM (panel **A**) and IgG (panel **B**) against carbonyl-modified Vimentin in control (non-smokers (NS) and smokers) and subjects with COPD as defined by lung function. Sensitivity was determined as the percentage of subjects in each group exhibiting antibody titres above the threshold, as defined in Fig. 1, for IgM and IgG, respectively

we detected a small but significant increase in autoantibodies against citrullinated vimentin in stable COPD patients compared to non-smokers (data not shown), a much larger immunoreactivity was noted towards carbonyl-modified vimentin. Consequently, our focus shifted to this response in COPD subjects. There is currently much interest in the identification of suitable biomarkers that accurately reflect COPD severity [30] and these simple blood tests for auto-antibody responses to either native or carbonyl-modified proteins may prove

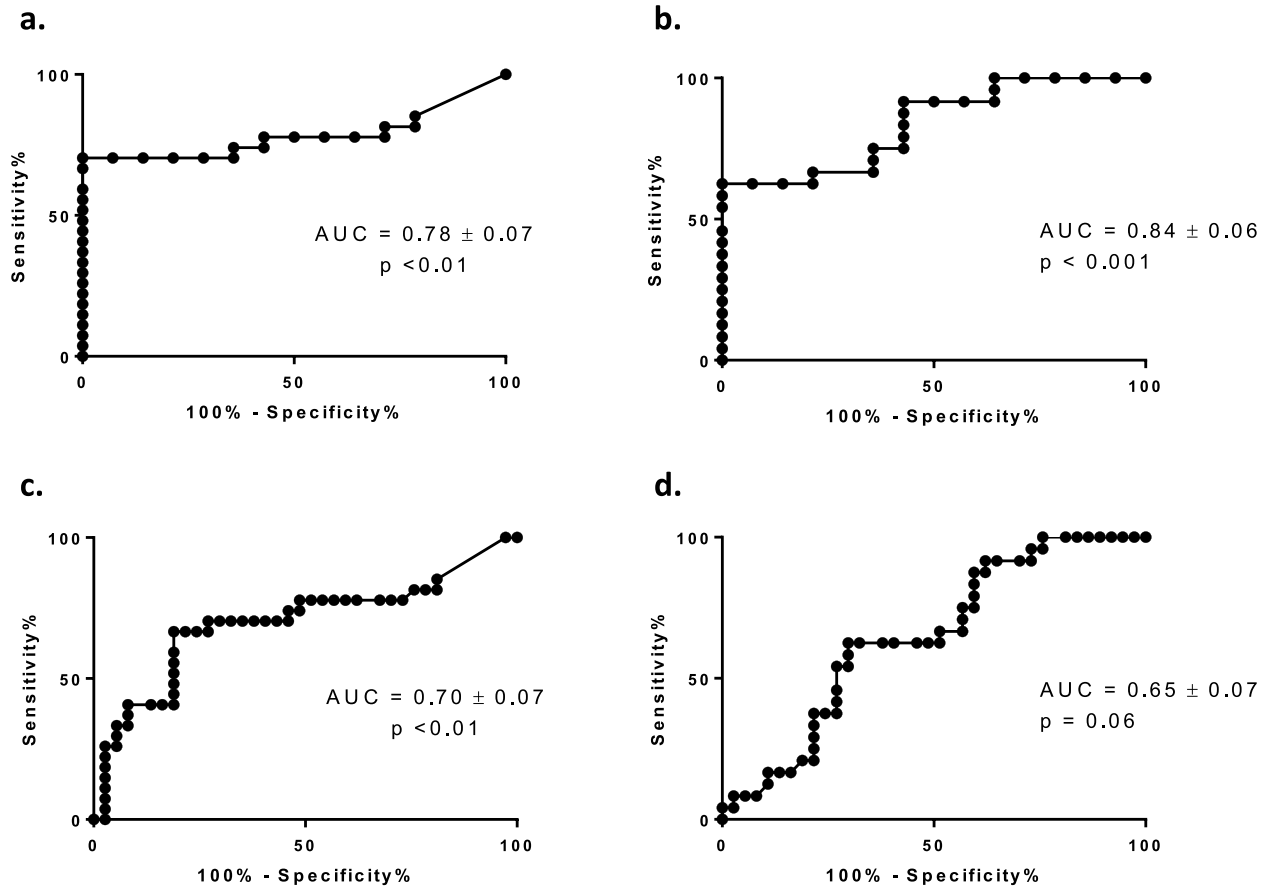

Fig. 4 ROC analysis plots of specificity versus sensitivity for IgM and IgG antibodies against carbonyl-modified vimentin. Panels A and B: ROC analysis for IgM and IgG antibodies, respectively, in control non-smokers and COPD subjects. Panels C and D: ROC analysis for IgM and IgG antibodies, respectively, in non-smokers, smokers and COPD subjects

useful as surrogates in diagnosis, monitoring of disease progression and as surrogate end points in clinical trials.

Carbonylation is a non-reversible, covalent modification of proteins or peptides occurring on residues such as cysteine, histidine and/or lysine through reactions with reactive carbonyls. These carbonyls can be formed as a result of processes such as lipid peroxidation or the combustion of organic matter, (e.g. cigarette smoke). Malondialdehyde (MDA), an end-product of lipid peroxidation serves as a biomarker of oxidative stress [31, 32]. Indeed, carbonyl adducts such as MDA have been shown to be significantly increased in the serum and lung tissues of smokers compared with non-smokers [33]. While non-smoking subjects displayed very low antibody titre responses to carbonyl-modified vimentin, these titres were significantly elevated for both IgG and IgM isoptypes in the COPD cohort. Moreover, IgM titre levels in COPD subjects against carbonyl-modified vimentin were also significantly elevated compared to non-COPD subjects, although this was not the case for IgG.

How these findings relate to clinical phenotypes was beyond the scope of this study, as limited clinical characterisation beyond COPD GOLD status was available. Nevertheless, IgM antibodies are often referred to as natural antibodies and play a role in the homeostatic regulation of immune responses, protecting the host from damaging autoimmune responses and excessive inflammation, particularly under conditions of chronic inflammation [34]. Natural IgM antibodies can recognize and act as sensors for electronegatively charged DAMPs, such as carbonyl epitopes [35]. High levels of natural IgM antibodies have been postulated to provide protection against autoimmunity, as seen in systemic lupus erythematosus (SLE)[23]. Interestingly, no significant increase in IgM antibody titres compared to IgG was observed between certain non-smokers and asymptomatic smokers. This could be due to earlier antibody isotype class switching in those individuals, which might prevent IgM antibodies from countering the harmful effects of IgG responses to carbonyl-modified protein DAMPs,

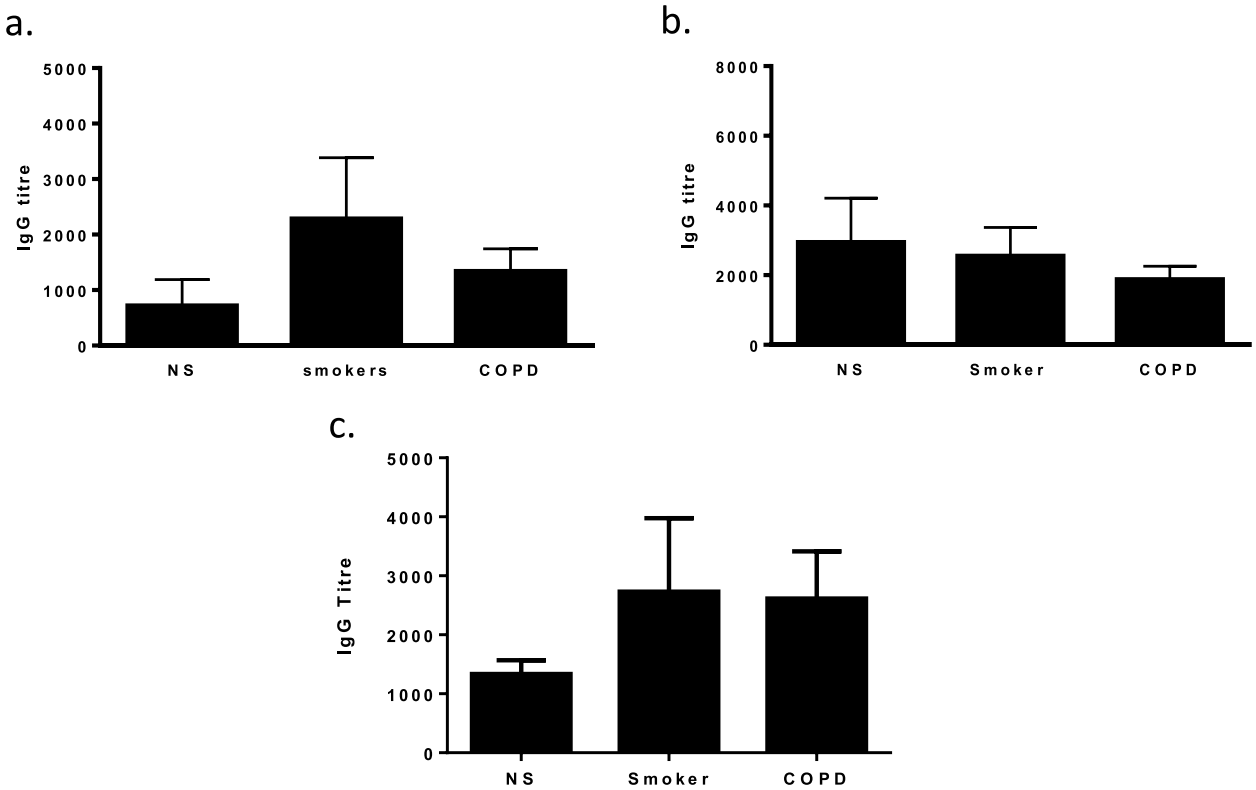

Fig. 5 Immunoreactivity against extracellular matrix proteins. Serum was screened for immunoreactivity towards native elastin (**a**), carbonyl-modified elastin (**b**) and carbonyl-modified collagen V (**c**) using ELISA. Antibody titres were determined as detailed in materials and methods. Results are expressed as mean ± SEM for each patient group. Statistical analysis was performed using the Kruskal–Wallis test with no significant difference identified between the patient groups ($p > 0.05$)

potentially exacerbating inflammation in smokers and COPD patients.

Therefore, the lack of an IgM response in 30% of smokers and some COPD subjects as seen in the raised IgG/IgM ratio in Fig. 1c may signal a precursor to a potentially destructive immune response. Moreover, in Fig. 2a although linear regression analysis suggested there was a trend towards decreased IgM responses in our small cohort of COPD patients as lung function declined, this was not deemed significant and may warrant a larger cohort to identify any significant correlation, if it indeed exists. However, Sullivan et al [36] have recently shown that lung destruction in COPD does not necessarily correlate with COPD GOLD status. Additionally, 20% of smokers without a diagnosis of COPD, as defined by lung function, show signs of emphysema on CT scan. Previously, we reported a significant IgG autoantibody response against endothelial cells in patients with COPD [8]. This is particularly interesting, as endothelial cells can express vimentin on their cell surface [37], and activated macrophages are known to secrete soluble vimentin [38]. These factors might explain the observed deposition of activated complement around the blood vessel walls within the lung parenchyma of stable COPD patients [8], potentially reflecting immune complex formation against carbonyl-modified vimentin. Furthermore, we have previously shown that while IgG auto-antibodies against native proteins belong to the IgG2 subclass, which is non-complement fixing, auto-antibodies against carbonyl-modified proteins were predominantly of the IgG1 subclass, which is complement fixing [8].

Although an early study indicated the presence of anti-elastin autoimmunity in smoke-induced pulmonary emphysema [12] this finding has not been reproduced in subsequent studies[13, 39–41]. In light of this, we screened our cohort for antibodies against both native and carbonylated elastin. No statistically significant difference in antibody titres were observed between any groups for either form of elastin. Collagen remodelling is also another hallmark of COPD, characterized by the release of peptide fragments that could be potentially act as immunogens or as biomarkers. Indeed Leeming et al [42] reported significantly elevated levels of matrix metalloproteinase-degraded collagen types I, III, IV, V and VI in the serum of subjects with mild COPD compared to control subjects. Type IV collagen, the most abundant

non-fibrillar collagen in the basement membrane within the lung [43], has been shown to have elevated protein levels in the lungs of stable COPD patients [44]. Among collagen proteins that have been screened as potential auto-antigens, collagen V has recently aroused particular interest as an auto-antigen in several chronic diseases, including respiratory disease. More specifically, it has been implicated in inducing T-cell immunity, which is more prevalent in smokers [13]. However, in our study, we did not observe any significant difference in serum autoantibody titres against carbonyl-modified type V collagen in COPD patients versus control subjects. While there was a trend towards higher titres in smokers and COPD patients, this finding did not reach statistical significance. Clearly, the limitations of our small cohort size used here warrant a larger study to clarify this issue.

As a blood-borne biomarker, autoantibodies are an attractive and convenient candidate as they can be obtained readily through a minimally invasive procedure, remaining stable in serum for extended periods of time. However, their use is not without limitations. Autoimmune-type responses can be driven by T cells alone, without the involvement of antibodies. Therefore, the absence of elevated antibody titres to some self-antigens in our study does not necessarily exclude their role in disease pathogenesis or progression. Furthermore, systemic levels of autoantibodies may not reflect those seen locally in the airways and lungs of patients with COPD. Additionally, antibody titres can vary widely over time within individuals, presenting another challenge to their consistent use as biomarkers. Our findings for carbonylated vimentin suggest a potential link between antibody responses and disease state. However, these conclusions are based on a small cohort and at a single time point analysis. To substantiate these observations, it would be essential to expand the cohort size and include serial sampling over an extended period of time ideally in both blood and airway samples. Our data indicate that measuring IgG and IgM titres to carbonyl modified vimentin may prove useful in helping to better stratify COPD patients and potentially identifying smokers at risk of lung destruction, as evidenced by CT scans. However, verifying this approach would require further investigation in a larger study cohort.

Conclusions

In conclusion, immunoglobulin isotype titres against carbonyl-modified proteins, such as vimentin, show promise as a potential blood-based biomarker. This approach could provide a simple and effective means for earlier and more stratified phenotyping of COPD patients, offering insights into disease pathophysiology that conventional lung function assessment currently cannot provide.

Acknowledgements
Full-length native recombinant human vimentin was a kind gift from Professor Marlene Rose at Imperial College London.

Authors' contributions
L.H. and W.L. performed the experimental work and analysed the data. G.C. and A.P. provided the clinical samples and provided input into the study design. I.A., F.C. and M.N.H. provided input into the study design and data analysis and helped prepare the manuscript. P.A.K. wrote the manuscript, conceived and designed the study and analysed the data. All authors reviewed the manuscript.

Funding
No grant funding was provided by a research council or charity for this study, but P.A.K. was supported by a Pfizer sponsored Senior Fellowship at Imperial College London whilst conducting this research.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Informed consent was provided by all subjects and the study approved by the institutional local ethics committee at Ferrara University and Imperial College London.

Consent for publication
All authors consent to the publication of this manuscript.

Competing interests
The authors declare no competing interests.

Received: 20 December 2024 Accepted: 29 January 2025
Published online: 12 February 2025

References

1. GOLD - Global Initiative for Chronic Obstructive Lung Disease. (2014). Global Strategy for the Diagnosis, Management and Prevention of Chronic Obstructive Pulmonary Disease. NHLBI/WHO workshop report. Bethesda, National Heart, Lung and Blood Institute, April 2001; NIH Publication No 2701:1–100. http://www.goldcopd.com.
2. Diaz-GUzman E, Mannino DM. Epidemiology and prevalence of COPD. Clin Chest Med. 2014;35(1):7–16.
3. Mannino DM, Braman S. The epidemiology and economics of chronic obstructive pulmonary disease. Proc Am Thorac Soc. 2007;4(7):502–6.
4. Mannino DM. Biomarkers in COPD: the search continues. Eur Respir J. 2015;45:872–4.
5. Brusselle GG, Joos GF, Bracke KR. New insights into the immunology of COPD. Lancet. 2011;378:1015–26.
6. Hogg JC, Timens W. The pathology of chronic obstructive pulmonary disease. Annu Rev Pathol. 2009;4:435–59.
7. Caramori G, Casolari P, Giuffrè S, et al. COPD pathology in the small airways. Panminerva Med. 2011;53:51–70.
8. Kirkham PA, Caramori G, et al. Oxidative stress-induced antibodies to carbonyl-modified protein correlate with severity of chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2011;184(7):796–802.
9. Kurien BT, Scofield RH. Autoimmunity and oxidatively modified autoantigens. Autoimmun Rev. 2008;7(7):567–73.
10. Kuo YB, Chang CA, et al. Identification and clinical association of anti-cytokeratin 18 autoantibody in COPD. Immunol Lett. 2010;128(2):131–6.
11. Feghali-Bostwick CA, Gadgil AS, et al. Autoantibodies in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2008;177(2):156–63.
12. Lee SH, Goswami S, et al. Antielastin autoimmunity in tobacco smoking-induced emphysema. Nat Med. 2007;13(5):567–9.

13. Rinaldi M, Lehouck A, et al. Antielastin B-cell and T-cell immunity in patients with chronic obstructive pulmonary disease. Thorax. 2012;67(8):694–700.
14. Willemse BW, ten Hacken NH, Rutgers B, et al. Effect of 1-year smoking cessation on airway inflammation in COPD and asymptomatic smokers. Eur Respir J. 2005;26(5):835–45.
15. Hogg JC. Why does inflammation persist after smoking stops. Thorax. 2006;61(2):96–7.
16. Talhout R, Schulz T, et al. Hazardous compounds in tobacco smoke. Int J Environ Res Public Health. 2011;8(2):613–28.
17. Cerami C, Founds H, et al. Tobacco smoke is a source of toxic reactive glycation products. Proc Natl Acad Sci USA. 1997;94(25):13915–20.
18. Nicholl ID, Bucala R. Advanced glycation endproducts and cigarette smoking. Cell Mol Biol (Noisy-le-grand). 1998;44(7):1025–33.
19. Nicholl ID, Stitt AW, et al. Increased levels of advanced glycation endproducts in the lenses and blood vessels of cigarette smokers. Mol Med. 1998;4(9):594–601.
20. Negre-Salvayre A, Coatrieux C, et al. Advanced lipid peroxidation end products in oxidative damnage to proteins. Potential role in disaeses and therapeutic prospects for inhibitors. Br J Pharmacol 2008;153(1): 6-20
21. Burcham PC, Raso A, Thompson CA. Intermediate Filament Carbonylation During Acute Acrolein Toxicity in A549 Lung Cells: Functional Consequences, Chaperone Redistribution, and PRotection by Bisulfite. Antioxid Redox Signal. 2010;12(3):337–47.
22. Makrygiannakis D, Hermansson M, et al. Smoking increases peptidylarginine deiminase 2 enzyme expression in human lungs and increases citrullination in BAL cells. Ann Rheum Dis. 2008;67(10):1488–92.
23. Gronval C, Vas J, Silverman GJ. Protective roles of natural IgM antibodies. Front Immunol. 2012;3:66.
24. Binden C, Hale G, Bruggemann M, et al. Human monoclonal IgG isotypes differ in complement activating function at the level of C4 as well as C1q. J Exp Med. 1988;168(1):127–42.
25. Varani K, Caramori G, et al. Alteration of adenosine receptors in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2006;173(4):398–406.
26. Waalkens HJ, Merkus PJ, et al. Assessment of bronchodilator response in children with asthma. Dutch CNSLD Study Group. Eur Respir J 1993;6(5): 645-651
27. Haberland ME, Fogelman AM, et al. Specificity of receptor-mediated recognition of malondialdehyde-modified low density lipoproteins. Proc Natl Acad Sci USA. 1982;79(6):1712–6.
28. Gerardi MC, De Luca N, Alessandri C, et al. Frequency of antibodies to mutated citrullinated vimentin in chronic obstructive pulmonary disease: comment on the article by Demoruelle et al. Arthritis Rheum. 2013;65(6):1672–3
29. Wood AM, de Pablo P, Buckley CD, et al. Smoke exposure as a determinant of autoantibody titre in α_1-antitrypsin deficiency and COPD. Eur Respir J. 2011;37(1):32–8.
30. Stockley RA, Halpin DMG, et al. Chronic Obstructive Pulmonary Disease Biomarkers and Their Interpretation. Am J Respir Crit Care Med. 2019;199(10):1195–204.
31. Rahman I. Oxidative stress in pathogenesis of chronic obstructive pulmonary disease: cellular and molecular mechanisms. Cell Biochem Biophys. 2005;43(1):167–88.
32. Chung KF, Adcock IM. Multifaceted mechanisms in COPD: inflammation, immunity, and tissue repair and destruction. Eur Respir J. 2008;31(6):1334–56.
33. Kirkham PA, Barnes PJ. Oxidative stress in COPD. Chest. 2013;144(1):266–73.
34. Gronval C, Silverman GJ. Natural IgM: Beneficial autoantibodies for the control of inflammation and autoimmune disease. J Clin Immunol. 2014;34(1):12–21.
35. Uchida K. Natural antibodies as a sensor of electronegative damage-associated molecular patterns (DAMPs). Free Radical Biol Med. 2014;72:156–61.
36. Sullivan J-L, et al. B Cell-Adaptive Immune Profile in Emphysema-Predominant Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med. 2019;200(11):1434–9.
37. Xu B, deWaal RM, Mor-Vaknin N, et al. The endothelial cell-specific antibody PAL-E identifies a secreted form of vimentin in the blood vasculature. Mol Cell Biol. 2004;24:9198–206.
38. Mor-Vaknin N, Punturieri A, Sitwala K, et al. Vimentin is secreted by activated macrophages. Nat Cell Biol. 2003;5:59–63.
39. Cottin V, Fabien N, et al. Anti-elastin autoantibodies are not present in combined pulmonary fibrosis and emphysema. Eur Respir J. 2009;33(1):219–21.
40. Greene CM, Low TB, et al. Anti-proline-glycine-proline or antielastin autoantibodies are not evident in chronic inflammatory lung disease. Am J Respir Crit Care Med. 2010;181(1):31–5.
41. Brandsma CA, Kerstjens HA, et al. The search for autoantibodies against elastin, collagen and decorin in COPD. Eur Respir J. 2011;37(5):1289–92.
42. Leeming DJ, Sand JM, et al. Serological investigation of the collagen degradation profile of patients with chronic obstructive pulmonary disease or idiopathic pulmonary fibrosis. Biomarker insights. 2012;7:119–26.
43. Konomi H, Hayashi T, et al. Localization of type V collagen and type IV collagen in human cornea, lung, and skin. Immunohistochemical evidence by anti-collagen antibodies characterized by immunoelectroblotting. Am J Pathol 1984;116(3): 417-426
44. Kranenburg AR, Willems-Widyastuti A, et al. Enhanced bronchial expression of extracellular matrix proteins in chronic obstructive pulmonary disease. Am J Clin Pathol. 2006;126(5):725–35.

Publisher's Note