COPD: systemic proteomic profiles in frequent and infrequent exacerbators

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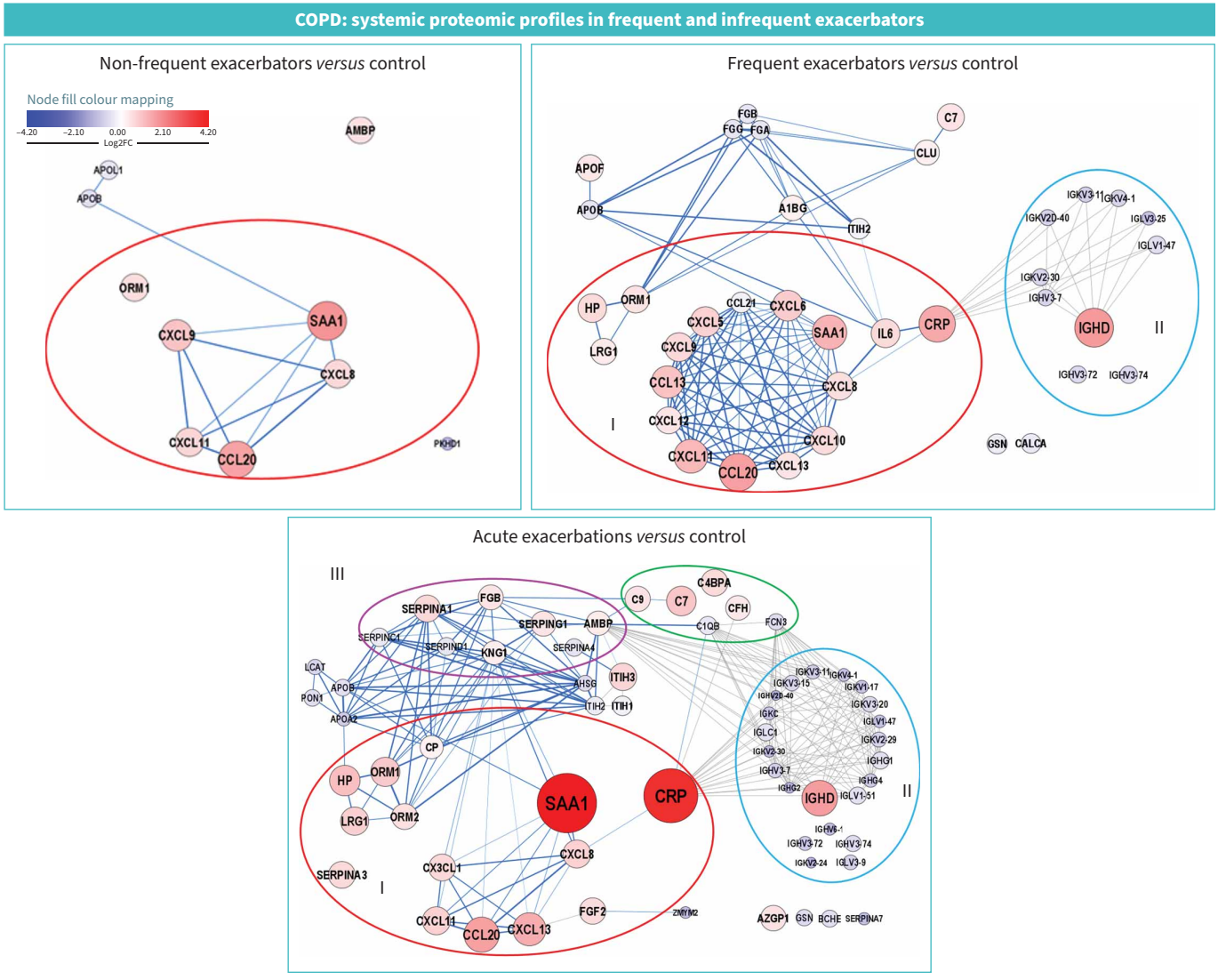

GRAPHICAL ABSTRACT Protein–protein interaction networks. Differentially abundant proteins/peptides (DAPs) are represented by nodes and grouped into inflammatory markers (I), and those involved in antibody-mediated immunity (II), the coagulation cascade (III) and the complement system (IV).

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Shareable abstract (@ERSpublications)
Although COPD exacerbations are characterised by a specific plasma proteomic profile, those patients who suffer frequent acute episodes maintain some of these abnormalities, providing a possible biological basis for this phenotype https://bit.ly/3SbxDz5

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Abstract

Background Some patients with COPD suffer frequent exacerbations (FE). We hypothesised that their systemic proteomic profile would be different from that of non-frequent exacerbators (NFE). The objective of the present study was to contrast the systemic proteomic profile in FE *versus* NFE. As a reference, we also determined the systemic proteomic profile of healthy controls (HC) and COPD patients during an actual episode of exacerbation (AE).

Methods In the analysis we included 40 clinically stable COPD patients (20 FE and 20 NFE), and 20 HC and 10 AE patients. Their plasma samples were analysed by combining two complementary proteomic approaches: label-free liquid chromatography-tandem mass spectrometry and multiplex immunoassays. Gene Ontology annotation, pathway enrichment and network analyses were used to investigate molecular pathways associated with differentially abundant proteins/peptides (DAPs).

Results Compared with HC, we identified 40 DAPs in FE, 10 in NFE and 63 in AE. Also compared to HC, pathway functional and protein–protein network analyses revealed dysregulation of inflammatory responses involving innate and antibody-mediated immunity in COPD, particularly in the FE group, as well as during an AE episode. Besides, we only identified alterations in the complement and coagulation cascades in AE.

Conclusion There are specific plasma proteome profiles associated with FE, which are partially shared with findings observed during AE, albeit others are uniquely present during the actual episode of AE.

Introduction

Patients with COPD can suffer acute episodes of exacerbation (AE) of their respiratory symptoms, which negatively impact their health status and prognosis [1–3]. For reasons still unclear, some COPD patients are particularly prone to suffering frequent exacerbations (FE) while others are not (NFE) [4]. AE can be triggered by viral and bacterial respiratory infections [2, 5] and are associated with lung microbiome

changes and/or airway eosinophilic infiltration [6–8] that can result in a dysregulated host immune response, leading to local and systemic inflammation. Accordingly, we hypothesised that the plasma proteomic profile of FE patients is different from that of NFE and may share some characteristics with an actual episode of AE. To explore these hypotheses, we used a complementary two-way quantitative proteomic approach that combines an unbiased label-free mass spectrometry-based method with a targeted immunoassay-based workflow to compare the plasma proteome of clinically stable COPD patients with and without FE. As a reference, we also determined the proteomic profile observed during an actual episode of AE and in healthy controls (HC).

Methods

Study design and ethics

This analysis is part of the BIOMEPOC project, a prospective, controlled multicentre study, details of which have been published elsewhere [9]. Briefly, BIOMEPOC aimed at identifying circulating biomarkers to better characterise different phenotypes and endotypes in COPD patients. It included a total of 269 patients and 83 controls [9]. The study protocol was approved by local ethics committees from the seven teaching hospitals participating in the study [9]. The investigation was conducted according to the Declaration of Helsinki and informed written consent was obtained from all participants.

Study population

For the current analysis, we included 40 clinically stable COPD patients (20 with FE and 20 with NFE) and 20 HC randomly chosen (computer-generated) from the BIOMEPOC cohort, whereas exacerbated COPD patients were consecutively recruited in the A&E department of our centre in the first 24–36 h of the acute episode and always before administering corticosteroids. The diagnosis of COPD was based on a history of smoking and a post-bronchodilator forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) <0.7 [2, 3]. Clinical stability was defined by the absence of exacerbations in the 3 months before enrolment in the study. AE was defined as an acute worsening of respiratory symptoms requiring the use of specialised tools from the healthcare system and additional therapy [2, 3]. FE was defined by ⩾three moderate-to-severe exacerbations in the previous year, whereas NFE was defined by ⩽one of these acute episodes during the same period of time. Exclusion criteria included lung cancer or other respiratory or chronic inflammatory conditions. The functional assessment of all COPD patients was made during clinical stability.

Blood sample collection

Blood samples were obtained by peripheral venepuncture being placed in K_3-EDTA tubes for plasma analyses. All tubes were centrifuged at 1500 ×g for 15 min at 4°C, and supernatants were transferred to new tubes and stored at −80°C until analyses using two different but complementary approaches: label-free quantification (LFQ) on liquid chromatography/tandem mass spectrometry (LC-MS/MS) and immune-based multiplex analysis.

LFQ on LC/MS-MS

Details of the sample preparation, instrument parameters and protein identification are available in the online supplementary material. Briefly, proteins were subsequently cleaved with endoproteinase LysC (1:100 w:w, overnight; Merck, Wako, Neuss, Germany) and trypsin (1:100 w:w, 8 h; Promega, Madison, WI, USA). All reactions were conducted at 37°C, and total peptides were desalted using C18 Hypersep columns (Thermo Fisher, Waltham, MA, USA). The peptide mixtures were analysed with LC-MS/MS using an LTQ-Orbitrap Fusion Lumos mass spectrometer coupled to an EASY nLC 1000 (Thermo Fisher Scientific). Individual digested samples from each participant were analysed in duplicate. LFQ and database searching was carried out with MaxQuant LFQ software (version 1.6.1.0) integrated with the Andromeda search algorithm and the FASTA File for *Homo sapiens* database downloaded from SwissProt (as in April 2018). Protein abundances were estimated using MaxQuant LFQ values and match-between runs.

Immune-based multiplex analysis

To expand the range of proteins detected with the previous method, the concentration of soluble inflammatory markers in plasma was analysed using three multiplex bead-based immunoassays. These cover a wider range of cytokines, chemokines, growth factors and acute-phase protein biomarkers. Globally, 63 distinct plasma proteins (76 analytes due to some duplications across kits) were assessed simultaneously, using the Bio-Plex 200 array system according to the manufacturer's instructions (Bio-Rad). Further details on immune-based multiplex assays and methodology for immunoglobulin quantification, as well as a reduced validation of our proteomic results using ELISA can all be found in the supplementary material.

Data analysis

Sample size estimation

The sample size for the study population was estimated using the GRANMO software, based on previous studies with similar recruitment approaches [10–14]. Assumptions of 80% power were chosen in all cases to detect differences of >20% in primary outcomes with a level of significance of $p \leqslant 0.05$ and a β-error of 0.2.

Descriptive statistics

Since all clinical variables showed normal distribution (Kolmogorov–Smirnov test), all results are presented as mean±SD. Levene's test was used to determine the homogeneity of variances, and comparisons between groups were performed using an unpaired t-test. Pearson's correlation coefficients were calculated to investigate potential bivariate associations. A $p \leqslant 0.05$ was considered statistically significant. SPSS 3 (SPSS Inc, Chicago, IL, USA) was used for statistical analyses.

Differentially abundant proteins

Protein quantitation values were $\log_2$ transformed to decrease their distribution skewness and make them available for parametric analysis. Levene's test was used to determine variances' homogeneity. Results from both proteomic approaches were merged into a single dataset to generate a more comprehensive profile of the proteome, and the imputation of missing values was omitted to ensure a highly reliable dataset. Quantitative comparisons were restricted to proteins detected in at least 50% of patients in each group.

Initially, proteins/peptides obtained in each COPD study group (NFE, FE and AE) were compared to those from controls. In a second step, differentially abundant proteins (DAPs) were assessed between: 1) FE *versus* and NFE; and 2) each stable COPD group (NFE and FE) *versus* AE. All single pairwise comparisons were analysed using the independent samples t-test when data met the homogeneity assumption, whereas the Welch t-test was employed for data showing unequal variances. The protein fold-change was calculated by subtracting the mean $\log_2$ values between proteins from each comparison group ($\log_2$FC). The p-values obtained in the t-test for comparisons were corrected using the Benjamini–Hochberg false discovery rate (FDR) approach in each pairwise contrast, and without any differentiation or grouping of proteins/peptides. Only DAPs with an FDR $\leqslant 0.1$ were deemed as high confidence and included for further steps of the analysis. All proteomic data were analysed with Python 3.8.3, in a Jupyter notebook v6.0.3 with an Anaconda environment v4.9.1 with SciPy v1.5.0, NumPy v1.18.5, Pandas v1.0.5 and Statsmodels v0.11.1 Python modules.

Functional enrichment and network analysis

Functional enrichment was calculated using: 1) Gene Ontology and PANTHER-GO-slim functional annotations (version 15.0, www.pantherdb.org/panther/goSlim.jsp); and 2) Pathway analysis using gProfiler (https://biit.cs.ut.ee/gprofiler/gost) in the Kyoto Encyclopaedia of Genes and Genomes (KEGG) and REACTOME.

To further assess the biological relevance of DAPs, the existence of previously described functional or physical protein–protein interactions (PPI) was initially surveyed using the STRING platform. This database retrieves a high percentage of total protein interactions available in other databases, but unfortunately, its coverage of immunoglobulin interactions is very skewed. Therefore, to obtain a complete dataset, six additional databases were also examined, and combined interactions were then visualised using the Cytoscape software (v3.8.0, www.cytoscape.org), which generated network representations. Further details are provided in the online supplementary material.

Results

Participant characteristics

Table 1 presents the main characteristics of study participants. Demographics were similar across different groups. By design, lung function was normal in controls and showed similar degrees of airflow limitation in COPD patients. All patient groups consisted of current or former smokers.

Identified proteome

Label-free LC-MS/MS detected 261 proteins and 90 Ig peptides (supplementary table S2) whereas the immune-based multiplex assay identified eight acute-phase proteins and 45 different cytokines, chemokines and growth factors (supplementary table S3). However, 41% of them were not included in the comparisons since they were only present in <50% of participants in each group. Detected proteins belonged to 18 different classes in the PANTHER classification system, including protein-binding activity modulators (16.3%), protein-modifying enzymes (16.3%), defence/immunity proteins (12.9%), transfer/carrier proteins (11.2%), metabolite interconversion enzymes (9.6%) and cytoskeletal proteins (9.6%).

TABLE 1 Clinical characteristics of the different groups

		COPD		
	HC	NFE	FE	AE
Participants n	20	20	20	10
Demographics				
Age years	64±9	69±9	67±9	65±9
Male %	60	70	65	60
BMI kg·m^{-2}	26.7±3.2	26.1±6.2	24.3±6.2*	27.2±4.9
Smoking status				
Current smoker or ex-smoker	9 (45)	20 (100)	20 (100)	10 (100)
Pack-years smoking	9±13	54±23***	55±27***	62±35***
Lung function				
Post-BD FEV_1 % pred	95±13	44±17***	39±12***	39±13***
Post-BD FEV_1/FVC %	83±5	49±13***	42±9***	51±12***
D_{LCO} % pred	96±13	48±22**	47±13**	40±9**
GOLD stage				
II	–	6 (30)	4 (20)	2 (20)
III	–	9 (45)	10 (50)	6 (60)
IV	–	5 (25)	6 (30)	2 (20)
Exacerbations in last year	–	0.7±0.9	3.8±2.3$^{\#}$	1.4±1.6¶

Values are expressed as mean±SD or n (%) unless indicated otherwise. HC: healthy controls; NFE: stable non-frequent exacerbators; FE: stable frequent exacerbators; AE: acute exacerbation; BMI: body mass index; BD: bronchodilator; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; D_{LCO}: diffusion capacity of the lung for carbon monoxide; GOLD: Global Initiative for Chronic Obstructive Lung Disease. $^{\#}$: p<0.001 compared to NFE. ¶: p<0.05 compared to FE. *p<0.05; **p<0.01; ***p<0.001 compared to HC.

Differential proteomic profiles

Compared to HC, stable COPD patients as a whole group (*i.e.* FE plus NFE) showed 28 DAPs (7% of the total protein and peptides quantified), 15 being over-represented (supplementary table S4). When such patients were categorised into NFE and FE, the former showed 10 DAPs (2.5% of the total; seven over- and three under-represented) (supplementary table S5), whereas the number of DAPs reached 40 (10.0% of the total, 22 over- and 18 under-represented) in the latter group when compared with controls (supplementary table S6). A direct comparison between both stable COPD groups added no relevant complementary information. The highest DAPs number was observed in AE patients when compared with HC (63 DAPs, 15.8% of total; 26 over- and 37 under-represented; supplementary table S7). While figure 1a–c show DAP volcano plots, figure 1d presents a Venn diagram of the similarities/differences between groups. Six proteins were consistently different in all comparisons with controls (ORM1, APOB, SAA1, CXCL8, CXCL11 and CCL20), likely representing a global COPD-associated proteomic signature. The largest number of overlapping proteins was found between FE and AE patients. Specifically, 23 protein/peptides were differentially abundant *versus* controls in both situations (OR 10.6, p=1.1e-10). Similar results for intergroup comparisons were obtained with the three proteins used in the validation cohort (C7, HP and LRG1; supplementary figure S2). Only a few DAPs reached statistical significance when comparing AE with either NFE or FE groups (supplementary table S8).

Another remarkable finding of the present study is the reduction in diverse Ig peptides mapping to the heavy- and light-chain variable regions in AE patients, and to a lower extent in the FE group, compared with controls. Moreover, IgG sub-isotypes (IgG1, IgG2 and IgG4) were only lower in AE patients, despite IgG1 and IgG2 also showing a similar tendency in FE. As opposed to this immune markers' reduction, a fraction of IgD (its heavy chain) was overexpressed in these two COPD groups, whereas fractions of IgM showed no difference.

Functional enrichment

PANTHER's functional enrichment showed that binding proteins, molecular function regulators and proteins with catalytic activity were dominant in terms of molecular functions in each pairwise comparison between groups. Proteins involved in the defence/immunity response, cellular and metabolic processes, biological regulation, and response to stimulus predominated in terms of biological process, whereas regarding location, as expected, most identified plasma proteins were extracellular (figure 2, supplementary table S9).

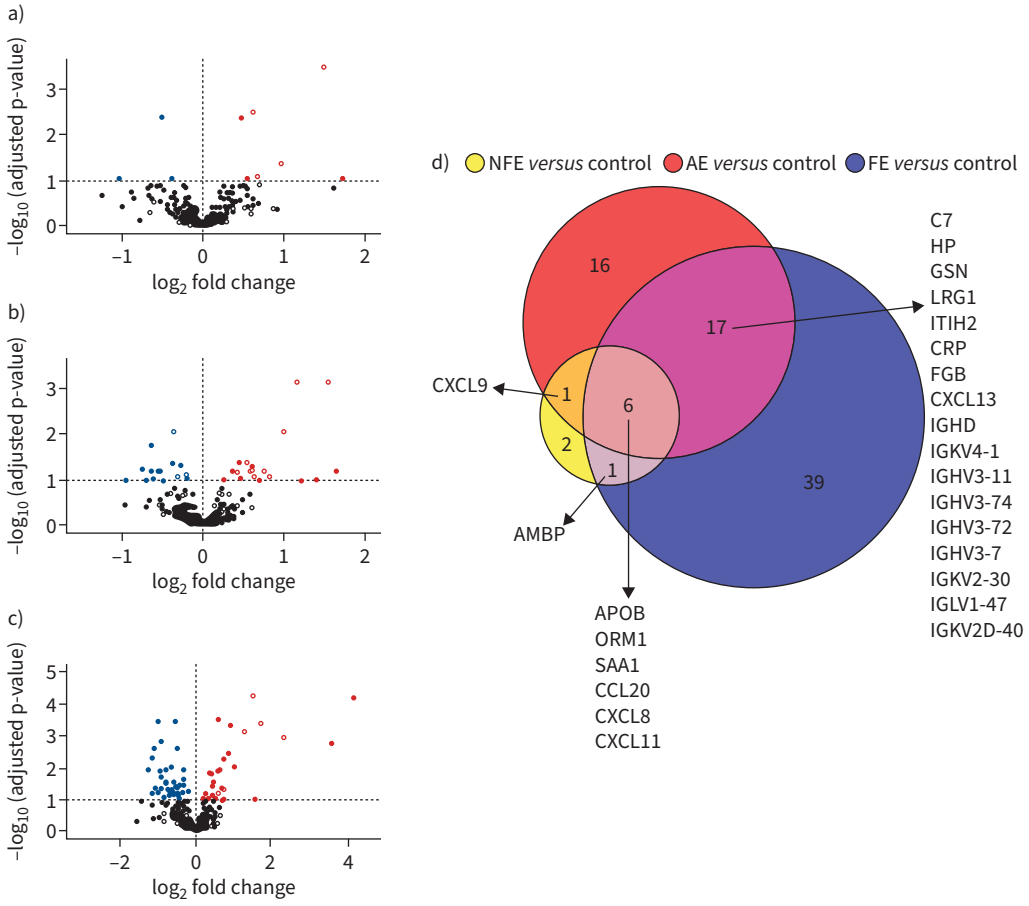

FIGURE 1 Volcano plots and Venn diagram of differentially abundant proteins. Volcano plots showing differentially abundant proteins between a) NFE *versus* control, b) FE *versus* control and c) AE *versus* control. The x-axis represents log2 fold changes of proteins and the y-axis represents $-\log_{10}$ FDR adjusted p-values. The red and blue symbols indicate proteins with significantly different abundance at false discovery rate set at 0.1. Filled circles represent proteins measured by LC-MS/MS and open circles represent proteins measured by immune-based multiplex assays. d) Proportional Venn diagram showing the overlapping DAPs among the different COPD groups compared to control subjects. NFE: stable non-frequent exacerbators; FE: stable frequent exacerbators; AE: patients with an acute exacerbation; FDR: false discovery rate; LC-MS/MS: liquid chromatography/tandem mass spectrometry; DAPs: differentially abundant proteins/peptides.

Complementary pathway enrichment showed that signalling/metabolic pathways, including those related to cytokines–chemokines, were predominant in stable COPD patients, particularly in the FE subgroup. Additionally, immune system-related pathways were prevalent in AE and, to a lesser extent, in the FE groups. Finally, complement and coagulation cascades were quite prominently involved in AE (figure 3, supplementary table S10).

Interactions between DAPs

PPI network analysis revealed that most DAPs were associated with four biological processes, including inflammatory responses, antibody-mediated immune responses, blood coagulation and complement pathways (figure 4). Moreover, some similarities were observed between FE and AE groups, being very different from findings observed in NFE patients.

Discussion

To our knowledge, this is the first study to use two complementary analytical techniques (unbiased mass spectrometry and a hypothesis-driven immune-based assay) to study the plasma proteomic profile of clinically stable COPD patients (FE and NFE) and contrast them with those determined during an actual episode of AE. Main results showed that: 1) clinically stable FE associated with a prominent inflammatory

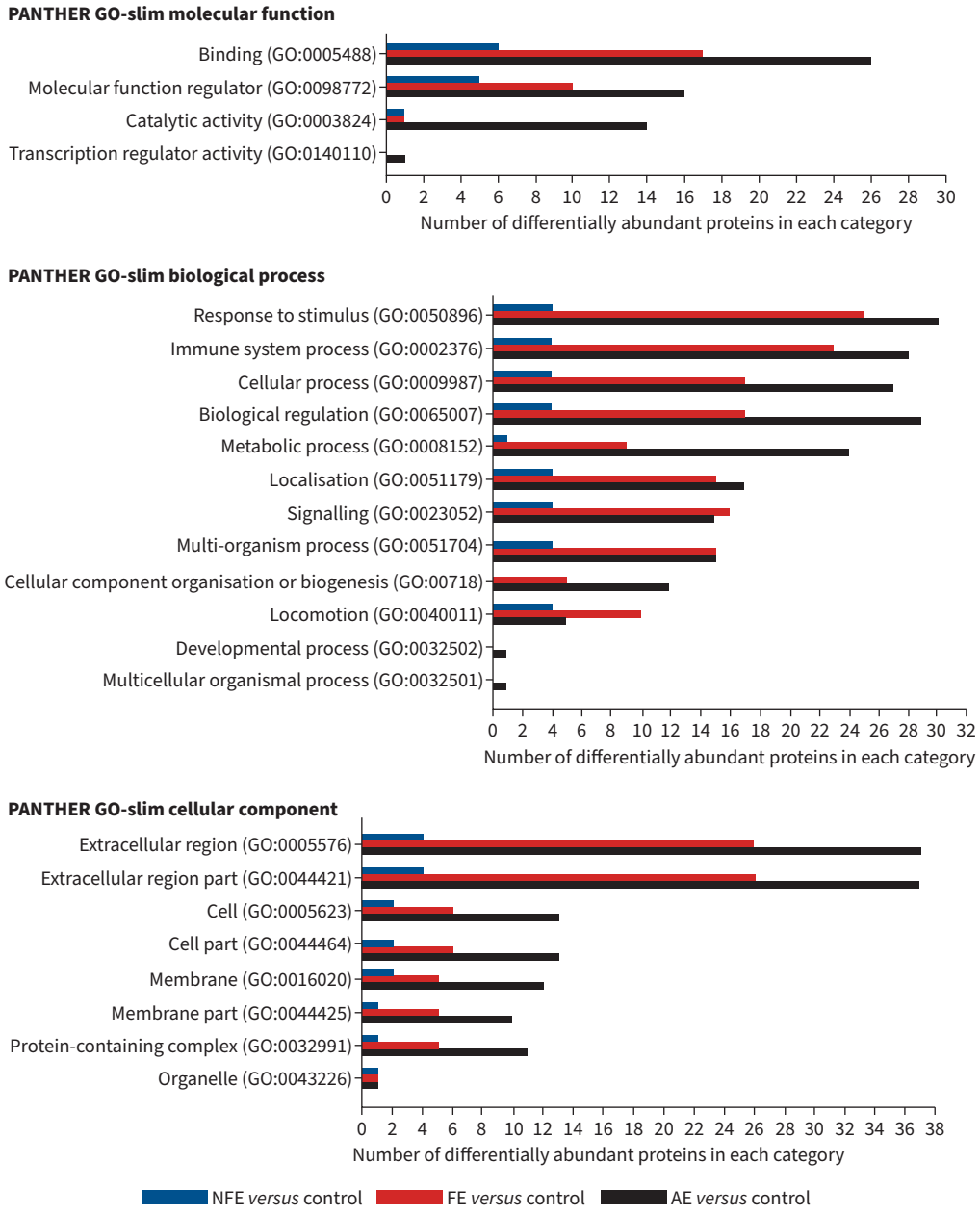

FIGURE 2 GO-slim annotation terms of differentially abundant proteins (DAPs). Protein classification was performed based on the functional annotations of the GO project for molecular function, biological process and cellular compartment categories. y-axis represents each GO-slim term; x-axis represents the number of DAPs associated with the GO-slim term. NFE: stable non-frequent exacerbators; FE: stable frequent exacerbators; AE: patients with an acute exacerbation.

network when compared with NFE; and 2) some of these proteomic alterations could also be observed during AE.

Previous studies

Previous investigations on systemic inflammation in COPD have only assessed a small number of protein markers, such as tumour necrosis factor-α, interleukin (IL)-1β, IL-6, C-reactive protein (CRP) and fibrinogen [15–19]. Some studies showed that stable COPD patients with simultaneously elevated levels of CRP, fibrinogen and leukocyte counts had an increased risk of suffering FE [15, 16]. Taken together, our results and those from previous studies support the notion that the presence of persistent systemic

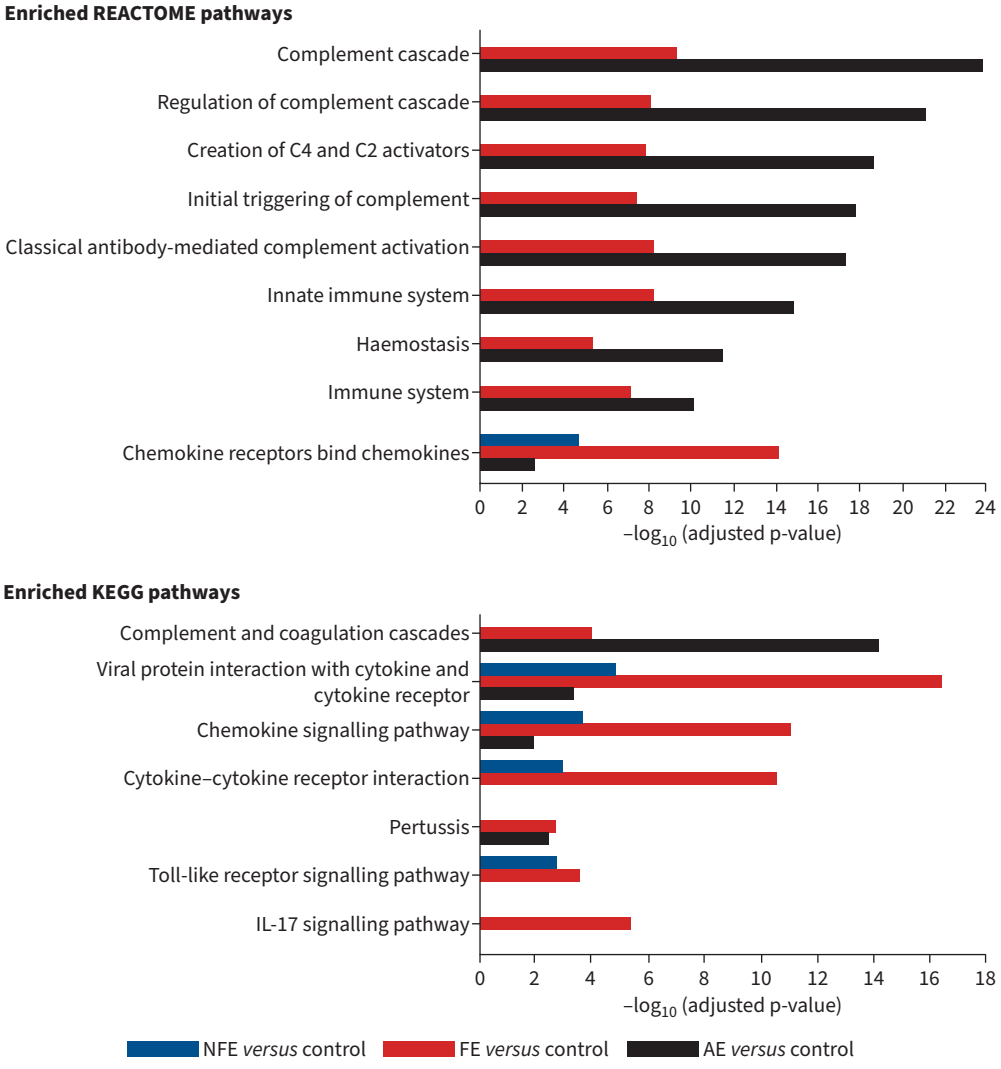

FIGURE 3 Pathway enrichment analyses of differentially abundant proteins. Pathway enrichment analysis of the DAPs was explored by REACTOME and the Kyoto Encyclopaedia of Genes and Genomes (KEGG) Pathway databases using the g:Gost tool in g:Profiler. y-axis represents top KEGG and REACTOME pathways; x-axis represents the $-\log_{10}$-adjusted p-value. A comprehensive list of enriched pathways is available in supplementary table S10. NFE: stable non-frequent exacerbators; FE: stable frequent exacerbators; AE: patients with an acute exacerbation; IL-17: interleukin-17; DAPs: differentially abundant proteins/peptides.

inflammation during clinical stability increases the risk of frequent acute episodes, providing a biological basis for the FE phenotype. Moreover, as demonstrated in the present study, the proteomic profile of the latter has some similitudes with what occurs during an AE episode.

Interpretation of novel findings

The frequent exacerbator phenotype

Multiple studies have shown mild-to-moderate increases of inflammatory mediators in the peripheral blood of stable COPD patients as a whole group, reflecting a low grade of systemic inflammation [20]. However, using very restrictive inclusion criteria to be catalogued as FE and using two advanced and complementary proteomic techniques, we found that, when compared with HC, NFE only showed a few abnormal acute-phase proteins and chemokines levels, whereas FE showed a generalised increase in systemic inflammatory markers.

Proteomic profile during acute exacerbations

It is well established that there is generally a burst of airway and systemic inflammation during AE [21]. However, the identification of blood biomarkers differs among various studies, likely indicating the

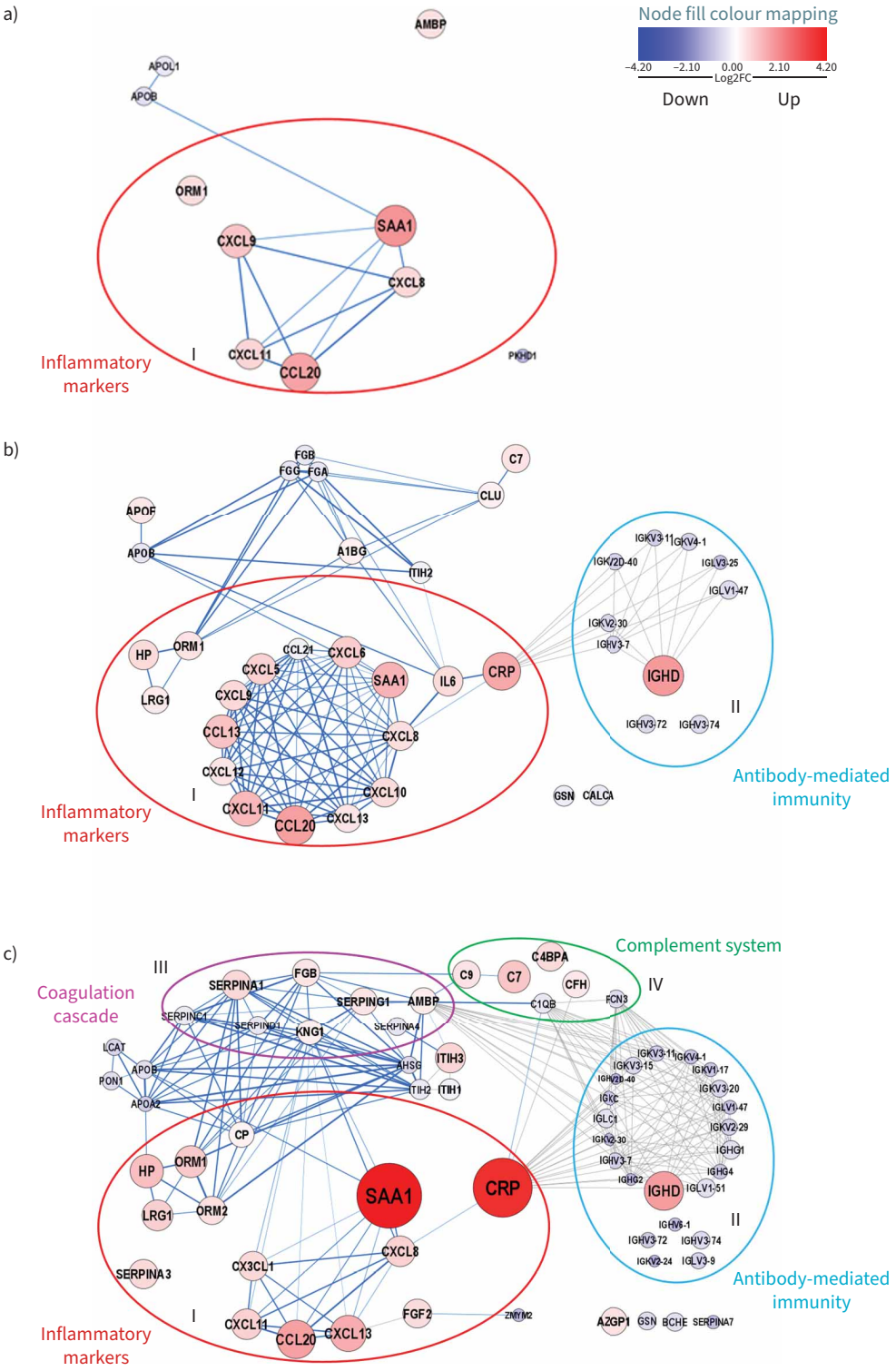

FIGURE 4 Protein–protein interaction networks. PPI networks were performed on DAPs in either a) NFE *versus* control, b) FE *versus* control or c) AE *versus* control. Each node lists the gene name of the identified proteins. The size and shade of the nodes represent the median $\log_2$ fold-change of DAPs with red and blue showing over- and under-represented proteins, respectively. Edges stand for protein interactions. Blue edges represent known and predicted associations by STRING online database using only highest confidence experimental and database evidence (minimum interaction score = 0.9). The width of blue edges positively correlates with the combined score. Grey edges were obtained by using additional public databases. DAPs are grouped into inflammatory markers, including cytokines, chemokines and acute-phase proteins (I), and proteins involved in antibody-mediated immunity (II), the coagulation cascade (III) and the complement system (IV). PPI: protein–protein interactions; NFE: stable non-frequent exacerbators; FE: stable frequent exacerbators; AE: patients with an acute exacerbation; DAPs: differentially abundant proteins/peptides.

heterogeneous nature of exacerbations, which probably reflects diverging underlying mechanisms. The greatest changes detected in AE in the present investigation involved two reactant phase proteins, CRP and serum amyloid A, which have already been consistently reported in other studies [22–30]. However, our results also suggest that disruption of network homeokinetics, characteristic of AE [24], is partially present in stable FE.

Nevertheless, the following novel observations deserve specific discussion. Firstly, our results suggest a potentially compromised adaptive immune response during AE, as shown by a decreased level of IgG sub-isotypes and a reduction in diverse Ig peptides. These findings are consistent with those from some of the previous studies, which showed an inverse association between IgG levels and exacerbations [31]. IgG is a key player in the humoral immune response and allows for the control of body tissue infections through its effector functions: complement activation (see below), opsonisation and antibody-dependent cell-mediated cytotoxicity. Moreover, hypogammaglobulinaemia appears to be more prevalent in FE [32] and has been associated with a higher risk of new acute episodes and hospitalisations [33, 34]. Interestingly, in the present study, the immunoglobulin results observed during AE were similar to those seen in FE, thus suggesting that a compromised adaptive immune response to pathogens may predispose COPD patients to recurrent new AE, probably linked to respiratory infections.

Secondly, we observed protein changes compatible with a reduced activity of the complement system during AE, which were not present in any of the stable groups. The complement system enhances the ability of antibodies to clear microbial pathogens and cell debris. In addition, it can directly induce bacterial lysis, participate in pathogen opsonisation and contribute to the clearance of immune complexes [35]. Activation of the complement cascade occurs primarily *via* one of three major interconnected pathways [35]: 1) the "classical-canonical" pathway, where complement proteins bind to antigen–immunoglobulin complexes; 2) the "MB-lectin pathway", which is triggered by the recognition of carbohydrate patterns exhibited on the surface of foreign particles; and 3) the "alternative pathway", characterised by a persistent state of low-level activation. All three pathways converge to generate the C3 convertase and the terminal membrane attack complex, which causes leaks in the cell membrane inducing cell lysis. AE patients analysed in the present study presented a reduced level of two main triggers of the classical and lectin pathways (C1QB and FCN3), as well as an over-representation of complement inhibitors not only of these two pathways (C4BPA and SERPING1) but also of the alternative one (CFH). These results suggest the presence of a reduced activation efficiency of the complement system during the exacerbation episodes that, along with a reduced humoral immune response, could contribute to an impairment in host defence against respiratory pathogens. In contrast to these changes in the early components of the complement system, we found an increased level of C7 and C9, which are both involved in the final steps of the membrane attack complex formation (C5b–C9), responsible for the lysis of pathogens or infected cells. The factors leading to an increased concentration of these proteins in the presence of a reduced complement activation are not clear and would require further research. The exhaustion of early components of an already activated complement system during exacerbations could be a plausible explanation for our findings. Concordant with our results, increased levels of C7 and/or C9 have been previously described both in patients suffering an exacerbation [36, 37] or in those recovering from these acute episodes [38].

Finally, another subset of differentially abundant proteins in AE patients were those involved in blood coagulation. An imbalance due to reduced anticoagulants (SERPINA4: Kallistatin, SERPINC1: antithrombin, SERPIND1: Heparin cofactor II), or increased activation of pro-coagulants (KNG1: Kininogen 1, FGB: Fibrinogen) may lead to a prothrombotic environment. These findings are consistent with previous studies showing an increased activation of the coagulation system as measured by the assessment of its own factors and inhibitors, as well as markers of thrombin generation and fibrinolysis [39–41]. Systemic thrombogenicity, along with endothelial dysfunction and platelet activation, are critical factors involved in the pathogenesis of atherothrombosis, contributing to cardiovascular events. Indeed, cardiovascular comorbidities are highly prevalent in COPD patients [42], and atherothrombotic complications are particularly augmented during acute exacerbations [43]. Van der Vorm and colleagues [41] have shown that exacerbation is a "pan-thrombotic" state in which coagulation is facilitated and platelets and endothelial cells become activated. Noteworthily, our findings extend these previous observations, and as opposed to other studies that report mild activation of the coagulation system even during the stable status [44], the present findings are restricted only to the exacerbation episodes. This is in accordance with a recent investigation showing that the risk of cardiovascular adverse events associated with exacerbation decreases over time [45].

Strengths and potential limitations

A clear strength of the present study is the complementary use of massive and blinded methods of analysis (mass spectrometry) with more traditional and hypothesis-driven techniques (immune-based assay), which

has provided a wide increase in the spectrum of detected markers. Potential limitations include the relatively small population, the fact that we analysed different patients during clinical stability and AE, and the absence of a validation cohort. However, this limitation would mostly refer to different subgroups of patients since their total number was similar to some of the previous studies. By contrast, criteria for stability, exacerbation and the frequent exacerbator phenotype were very strictly supervised in the present study. The constraint of having different patients for stable and exacerbated groups was due in part to the design of the study (case–control and case–case) and the intrinsic logistical problems for including patients available in both clinical situations. Therefore, a new study with a different design must mandatorily include the same COPD patients in both situations, during an exacerbation and in stable conditions. However, although these restrictions may have reduced the power of the corresponding comparisons, many interesting findings were obtained. Finally, as in many other previous studies using proteomics, a further validation cohort will be necessary to fully confirm our findings.

A potential technical limitation was that the sample preparation method used for the MS analysis did not include the depletion of most abundant plasma proteins. However, this allowed us to identify other differentially abundant proteins such as those of the humoral immune and the complement systems which would otherwise have been removed.

Conclusions

Compared with clinically stable NFE COPD patients, those with a FE history associate with an extensive systemic inflammation and a defective humoral immune system. Since the latter has common elements with those observed during AE, it may be hypothesised that their persistence in periods of relative stability can constitute a biological risk substrate for recurrent exacerbations. By contrast, dysregulation of the complement and coagulation cascades was only observed during AE. Collectively, these results contribute to a better understanding of the pathobiology of AE in patients with COPD.

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