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Antibody response against *Pseudomonas aeruginosa* and its relationship with immune mediators in the upper and lower airways of cystic fibrosis patients

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Abstract

Background: The upper airways (UAW) are a niche and a reservoir of *Pseudomonas aeruginosa* strains that cause chronic infection of the lower airways (LAW) in cystic fibrosis (CF). Here, we assessed the role of anti-*P. aeruginosa* immunoglobulin A (IgA) and IgG antibodies in upper and lower airway infections in cystic fibrosis patients.

Methods: Nasal lavage fluid and induced sputum samples of 40 CF patients were microbiologically cultured. We searched for correlations between anti-*P. aeruginosa* IgA and IgG levels, measured by enzyme-linked immunosorbent assay (optical density), and unspecific immune mediators in both specimens.

Results: Anti-*P. aeruginosa* IgA (median optical density: 0.953 vs 0.298) and IgG (0.120 vs 0.059) were significantly higher in nasal lavage than in sputum, but not significantly different between patients with and without chronic *P. aeruginosa* infection in UAW. Matrix metallopeptidase-9 (MMP-9) in nasal lavage and neutrophil elastase (NE) in sputum were predictors of IgA in nasal lavage and IgA in sputum, respectively. IgA was a predictor of myeloperoxidase (MPO) in nasal lavage. Tissue inhibitor of metalloproteinases-1 (TIMP-1) was a predictor of IgG in sputum. IgG, TIMP-1, and NE in sputum were predictors of IgG in nasal lavage.

Conclusion: The anti-*P. aeruginosa* IgA response was more prominent in CF patients' UAW, indicating a lower degree of inflammatory responses. Proteases may play a role in the anti-*P. aeruginosa* humoral response in the upper and LAW, and anti-*P. aeruginosa* IgG may be involved in the crosstalk between upper and lower airways in cystic fibrosis patients.

KEYWORDS

Pseudomonas aeruginosa, IgA, IgG, lower airways, upper airways, nasal

1 | INTRODUCTION

Impaired mucociliary clearance resulting in chronic bacterial airway infection is a hallmark of cystic fibrosis (CF)[1] and chronic infection with *Pseudomonas aeruginosa* is the main cause of pulmonary damage and premature death. Impaired mucociliary clearance also concerns the sinonasal compartment so that nearly all CF patients develop chronic rhinosinusitis (CRS), with or without nasal polyps.[2–4] Besides, the paranasal sinuses are a niche and critical reservoir of *P. aeruginosa* strains that ultimately cause chronic lung infection.[5–8]

Despite such features, CRS is not frequently reported by CF patients[9] and little is known about the pathogen-host interplay in this condition. In a preceding publication including the present cohort, we assessed the complex microbial colonization in both airway compartments, together with inflammatory markers of the unspecific immune response. We found significantly higher amounts of inflammatory markers, such as neutrophil count, interleukin-8 (IL-8), and matrix metallopeptidase-9 (MMP-9) in induced sputum (IS) than in nasal lavage (NL) fluid of CF patients. At the same time, the amount of chemokine (CC-motif) ligand 5 (CCL5)—also known as regulated on activation, normal T cell expressed and secreted (RANTES)—was significantly higher in NL.[10] Sinus colonization with *P. aeruginosa* is also accompanied by a local response mediated by secretory immunoglobulin A (sIgA), with recruitment of macrophages and associated with a comparatively low inflammatory degree, while lung infection is characterized by elevated serum IgG and high neutrophil amount.[11,12] The sIgA-mediated response is detectable in NL and saliva, seems to represent well both the presence and absence of chronic lung infection and has shown to be a potential marker for assessing the risk of exposure to *P. aeruginosa* in the lungs.[13–15]

The comparatively low inflammatory degree of the immune response within the sinuses may contribute to little report of CRS symptoms by CF patients, and quantification of markers like RANTES and sIgA may be interesting alternatives for early *P. aeruginosa* detection in the CF airways. In this scenario, we hypothesized that the levels of IgA and IgG against *P. aeruginosa* are contrasting in NL and IS samples of CF patients and that IgA and IgG levels correlate with unspecific proinflammatory and anti-inflammatory markers of the host immune response, respectively, at both airway levels.

2 | METHODS

2.1 | Patients

Forty CF patients (19 males, 21 females; mean age = 27 years [range, 5–72 years]) were prospectively recruited between August 2010 and May 2011 during regular outpatient visits to the Jena University CF Centre. CF diagnosis relied on two positive sweat tests and/or identification of two disease-causing mutations in the *CFTR* gene, and patients were included if they had not been treated with intravenous antibiotics for at least two weeks. Treatment with azithromycin and other inhaled and oral antibiotics were ascertained but were not regarded as exclusion criteria. Written informed consent was obtained from each patient or his/her legal guardian and the study was approved by the local ethics committee (technical opinion number: 2861-07/10 and 2909-08/10). Chronic bacterial infection was defined according to the Leeds criteria,[16,17] when at least 50% of microbiological examinations were positive during the preceding year, with a maximum interval of three months between examinations. As both airway levels have been routinely assessed during the visits to the Jena University CF Centre for 10 years, such definition was equally applied to the lower airways (LAW) and upper airways (UAW).

2.2 | Nasal lavage collection

Using a syringe, 10 mL of sterile isotonic saline were applied to each nostril. Patients were instructed to hold the head in a slightly reclined position for 10 seconds, lean forward and gently exhale the NL fluid.[18] An aliquot was directly separated for microbiological analysis. For cytological analysis, 5 mL NL were mixed with 500 µL fetal calf serum (FCS) (Biochrom AG, Berlin, Germany) and stored at room temperature (RT) until it was processed. The NL was centrifuged (1000 rpm, 10 minutes, RT), most of the supernatant was discarded, and the pellet was resuspended in 1 mL solution, to which 100 µL FCS were added. For cytokine analysis, 3 to 5 mL of native NL were mixed with protease inhibitors (Protease Inhibitor Mix G; SERVA Electrophoresis GmbH, Heidelberg, Germany) and stored at −80°C until analysis. Total protein concentrations were measured at 280 nm using a NanoDrop spectrophotometer (NanoDrop ND 1000; Thermo Fisher Scientific, Inc, Waltham, MA).

2.3 | Sputum collection

Patients' sputum samples were obtained after spontaneous expectoration or after induction with inhalation of 6% hypertonic saline. An aliquot was separated for microbiological analysis and the remaining volume was diluted 1:5 with sterile phosphate-buffered saline and again diluted 1:4 with 0.1% dithiothreitol, rendering a final dilution of 1:20. After incubation for 10 minutes at 37°C, sputum was filtered with a 40-mm cell strainer to remove debris and mucus. One milliliter of filtrated sputum was mixed with 100 µL FCS and submitted to cytological analysis. The remaining sputum was centrifuged (1000 rpm, 10 minutes, RT), mixed with protease inhibitors as above mentioned, and stored in aliquots at −80°C for cytokine measurement. Total protein concentrations were measured at 280 nm using NanoDrop.

2.4 | Microbiological, cytological, and cytokine analyses

This part was carried out by following a previously described protocol.[10] Total cell counts, including percentages of polymorphonuclear (PMN) neutrophils, were measured by fluorescence flow cytometry (Sysmex XE-5000; Sysmex Deutschland GmbH, Norderstedt, Germany). NL and sputum microbiology were performed according to European standards.

Concentrations of IL-1β, −5, −6, −8, and −10, cathepsin-S (CTSS), RANTES, myeloperoxidase (MPO), tumor necrosis factor-α (TNF-α), MMP-9, neutrophil elastase (NE), secretory leukocyte peptidase inhibitor (SLPI), and tissue inhibitor of metalloproteinases 1 (TIMP-1) were determined using cytometric bead arrays (FlowCytomix Human Basic Kit and FlowCytomix Human Simplex Kits; eBioscience, Inc, Frankfurt, Germany) according to the manufacturer's instructions.

2.5 | IgA and IgG measurement in NLF and IS

P. aeruginosa IgA and IgG were measured using an enzyme-linked immunosorbent assay (ELISA)-based test, as described previously.[13]

2.6 | Statistical analysis

Descriptive statistics were used to present the clinical characteristics of the study population and the results of the tests. The Mann–Whitney *U* test was used for comparison between two groups. Correlations were assessed using the Pearson correlation coefficient. In this case, the values were logarithmically transformed to become homogenous. To search for interactions between variables, which could interfere with the correlation results, we performed multiple stepwise regression analyses. For all tests, a $P < .05$ was considered statistically significant. All statistical analyses were performed with IBM SPSS Statistics 23 (IBM, Armonk, NY), and figures were created using both SPSS Statistics 23 and GraphPad Prism 7 (GraphPad Software, San Diego, CA).

3 | RESULTS

3.1 | Microbiological analyses

S. aureus was the most frequently cultured microorganism in both NL (37%) and IS (55%) samples, while *P. aeruginosa* was recovered from 27% and 45% of NL and IS samples, respectively. Chronic *P. aeruginosa* LAW infection was present in 18 (47%) patients, among whom 12 (67%) also had chronic UAW infection (Table 1).

TABLE 1 Demographic characteristics of patients enrolled in the study (n = 40), *Pseudomonas aeruginosa* colonization/infection status in the upper airways (UAW) and lower airways (LAW) and antibody levels in nasal lavage fluid (NLF) and induced sputum (IS)

Characteristics	N or Value (IQR)
Median age, y	26.68 (14.29)
Gender, male/female	19/21
cftr Mutation	
F508del+/+	14/40
F508del+/−	22/40
Others	4/40
FEV1 (% predicted)	68.3 (26.4)
Antibiotic therapy	
P. aeruginosa (oral)	3/40
S. aureus (oral)	1/40
P. aeruginosa (inhaled)	14/40
Azithromycin	23/40
P. aeruginosa colonization/infection status	
Chronic UAW colonization	12/40
Median IgA levels (NLF)	0.900 (0.52)
Median IgA levels (IS)	0.215 (1.25)
Median IgG levels (NLF)	0.101 (0.11)
Median IgG levels (IS)	0.057 (0.04)
Absence of chronic UAW colonization	28/40
Median IgA levels (NLF)	1.186 (0.96)
Median IgA levels (IS)	0.311 (0.77)
Median IgG levels (NLF)	0.147 (0.63)
Median IgG levels (IS)	0.061 (0.04)
Chronic LAW infection	18/40
Median IgA levels (NLF)	0.783 (0.64)*
Median IgA levels (IS)	0.195 (0.59)
Median IgG levels (NLF)	0.091 (0.12)
Median IgG levels (IS)	0.057 (0.04)
Absence of chronic LAW infection	22/40
Median IgA levels (NLF)	1.315 (0.95)*
Median IgA levels (IS)	0.379 (0.82)
Median IgG levels (NLF)	0.172 (0.28)
Median IgG levels (IS)	0.172 (0.28)

Note: IgA and IgG levels are given in optical density.
Abbreviations: FEV1, forced expiratory volume in 1 second; IgA, immunoglobulin A; IgG, immunoglobulin G; IQR, interquartile range; *cftr* (gene encoding the Cystic Fibrosis Transmembrane Conductance Regulator).
*$P = .02$.

TABLE 2 Levels of *Pseudomonas aeruginosa*-specific IgA and IgG, and concentrations of unspecific immunological markers in nasal lavage fluid and induced sputum samples

	Nasal lavage fluid		Induced sputum		
Marker	Median	IQR	Median	IQR	*p* Value
IgA (OD)	0.953	0.84	0.298	0.79	<.001
IgG (OD)	0.120	0.15	0.059	0.04	<.001
CTSS (ng/mL)	1.33	0.6	38.82	39.5	<.001
IL-8 (ng/mL)	0.92	0.6	49.54	34.1	<.001
MMP-9 (ng/mL)	23.36	77.3	551.70	440.8	<.001
MPO (ng/mL)	298.56	251.7	2165.20	5293.1	<.001
NE (ng/mL)	0.71	0.79	0.63	0.72	.157
RANTES (pg/mL)	14.00	45.5	0	999.3	<.001
SLPI (ng/mL)	118.77	95.8	61 871.31	32 966.96	<.001
TIMP-1 (ng/mL)	3.48	37.4	17.35	90.1	<.001

Abbreviations: CTSS, cathepsin-S; IgA, immunoglobulin A; IgG, immunoglobulin G; IQR, interquartile range; IL-8, interleukin-8; MMP-9, matrix metallopeptidase; MPO, myeloperoxidase; NE, neutrophil elastase; OD, optical density; RANTES, regulated on activation, normal T cell expressed and secreted; SLPI, secretory leukocyte peptidase inhibitor; TIMP-1, tissue inhibitor of metalloproteinases 1.

Among 28 patients without chronic UAW infection, 17 (61%) had a history of *P. aeruginosa* colonization/infection in the LAW.

3.2 | Immunological markers

IS samples showed significantly higher median concentrations of IL-8, MPO, MMP-9, TIMP-1, IL-1β, SLPI, and CTSS, while median levels of RANTES were significantly higher in NL samples (Table 2). All 40 patients had IL-10 levels below the limit of detection in both NL and IS samples, and IL-5, IL-6, and TNF-α were detected in no more than 5% of NL samples and 12.5% of IS samples.

3.3 | IgA and IgG measurement in NLF and IS

The median ODs for IgA and IgG were significantly higher in NL than in IS (Table 1). No statistical difference was seen between patients with or without chronic UAW colonization concerning IgA and IgG levels in both NL and IS samples (Table 1 and Figure 1). Among the 28 patients without chronic UAW infection, the NL IgA levels of patients with history of *P. aeruginosa* LAW colonization were not significantly different of patients without such history. The IgA levels were significantly higher in NL of patients without chronic LAW infection, when compared to patients with this condition. However, no

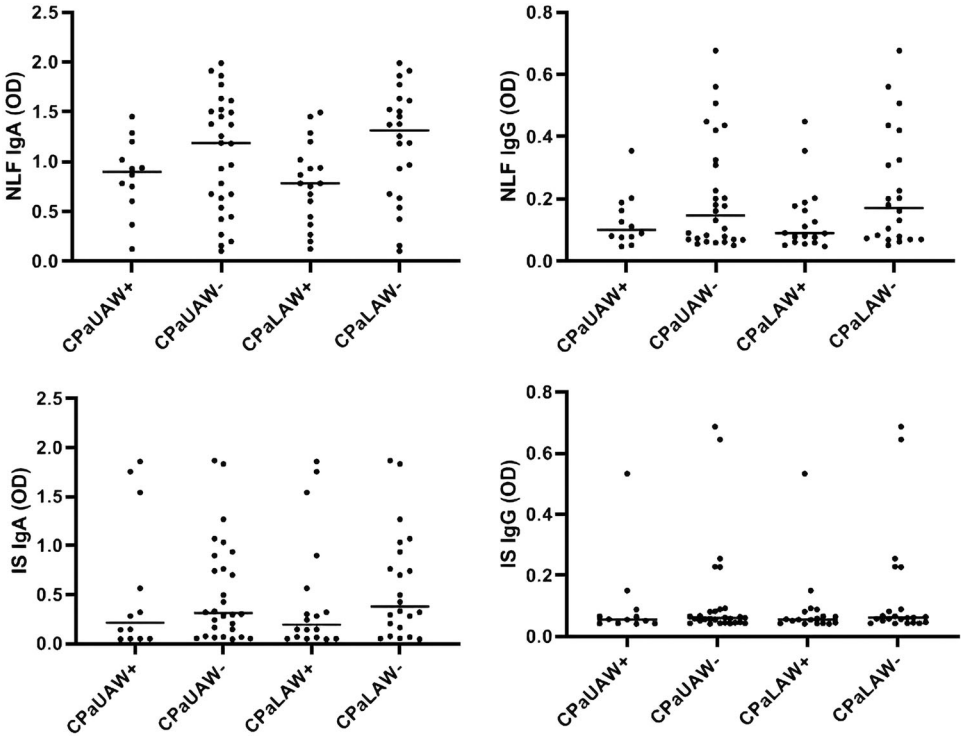

FIGURE 1 Distribution of the anti-*Pseudomonas aeruginosa* immunoglobulin A (IgA) and IgG levels in nasal lavage fluid (NLF) and induced sputum (IS) of 40 patients with cystic fibrosis according to their status of *P. aeruginosa* colonization/infection in the upper and lower airways. The antibody levels were measured using enzyme-linked immunosorbent assay tests and the results are given in optical densities (OD)

TABLE 3 Significant correlations between IgA, IgG, and other immunological markers in nasal lavage fluid (NLF) and induced sputum (IS) after univariate analysis

		IgA (NLF)		IgG (NLF)		IgA (IS)		IgG (IS)	
Markers	Site	*R* value[a]	*p* Value	*R* value[a]	*p* Value	*R* value[a]	*p* Value	*R* value[a]	*p* Value
IgA	NLF	⋯	⋯	0.538	<.001				
IgG	NLF	0.538	<.001	⋯	⋯	0.323	.042	0.522	.001
IL-8	NLF	0.451	.004						
MMP-9	NLF	0.653	<.001	0.395	.012				
MPO	NLF	0.660	<.001						
TIMP-1	NLF	0.604	<.001	0.445	.003				
IgA	IS			0.323	.042	⋯	⋯	0.670	<.001
IgG	IS			0.522	.001	0.670	<.001	⋯	⋯
IL-8	IS	−0.345	.032	−0.449	.004				
MPO	IS	0.426	<.001						
NE	IS			0.399	.015				
TIMP-1	IS							0.368	.042

Abbreviations: IgA, immunoglobulin A; IgG, immunoglobulin G; IL-8, interleukin-8; MMP-9, matrix metallopeptidase; MPO, myeloperoxidase; NE, neutrophil elastase; TIMP-1, tissue inhibitor of metalloproteinases 1.
[a]Pearson correlation test.

differences were observed between these groups concerning IS IgA, IS IgG, and NL IgG (Table 1 and Figure 1).

3.4 | Correlations between antibodies and other immunological markers

Correlations are shown in Table 3 and the results of multiple linear regression analyses are shown in Table 4. IgA and IgG levels were directly correlated in both NL and IS samples (Table 3). IgA levels predicted IgG levels in NL. In IS, IgA and IgG were initially predictors of each other, and IgG was a stronger predictor of IgA than the contrary. However, after adjusting for IS NE and IS TIMP-1 (which were predictors of IS IgA and IgG, respectively—more details in the next section) in a multiple linear regression analysis, NE and TIMP-1 did not significantly change the r^2 of the model with IS IgG as the dependent variable, while NE significantly increased the r^2 of the model with IS IgA as the dependent variable. IgG levels in IS were

TABLE 4 Significant correlations between IgA, IgG, and other immunological markers in nasal lavage (NL) fluid and induced sputum (IS) after stepwise regression analysis.

		IgA (NL)			IgG (NL)			IgA (IS)			IgG (IS)		
Markers	Site	*r*	*β*	*p* Value	*r*	*β*	*p* Value	*r*	*β*	*p* Value	*r*	*β*	*p* Value
IgA	NL	⋯	⋯	⋯	.585	.504	.003						
IgG	NL	.585	.504	.003	⋯	⋯	⋯				.599	.555	.002
MMP-9	NL	.600	.600	.001									
MPO	NL	.672	.759	<.001	−.479	−.457	.013						
IgA	IS										.754[a]	.769[a]	<.001
IgG	IS				.599	.555	.002	.754[a]	.769[a]	<.001			
NE	IS				.420	.311	.041	.425	.302	.03			
TIMP-1	IS				−.414	−.354	.044				.440	.315	.009

Note: r corresponds to the partial Pearson coefficient of correlation between two variables, irrespective of other independent variables; *β* corresponds to the angular coefficient (inclination) in relation to the explanatory variable. Values of all markers were logarithmically transformed to become homogenous.
Abbreviations: IgA, immunoglobulin A; IgG, immunoglobulin G; MMP-9, matrix metallopeptidase; MPO, myeloperoxidase; NE, neutrophil elastase; TIMP-1, tissue inhibitor of metalloproteinases 1.
[a]IS IgA and IS IgG were, initially, predictors of each other. These results were adjusted for IS NE and IS TIMP-1 in another regression analyses, which revealed IS IgA as a stronger predictor of IS IgG.

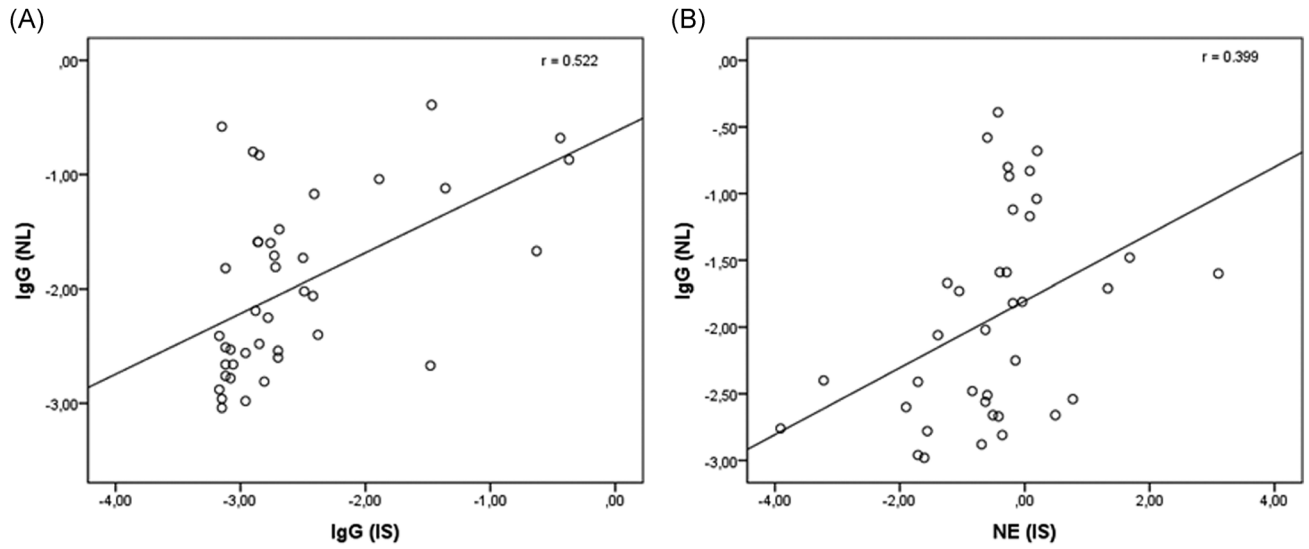

FIGURE 2 Positive correlations given by the Pearson correlation test (univariate analysis) between (A) immunoglobulin G (IgG) levels in the nasal lining (NL) and IgG levels in induced sputum (IS) and (B) NE concentration in IS and IgG levels in NL. More details are shown in Table 3

positively correlated with IgG levels in NL (Figure 2A), and, after regression analysis, they were found to be predictors of IgG levels in NL, while NLF IgA did not significantly correlate either with IgA or IgG in IS (Table 4).

IgA levels had a positive correlation with MMP-9 and MPO in NL, where MMP-9 predicted the IgA levels and MPO concentration was predicted by IgA. On the other hand, IgG levels were negatively correlated with MPO in NL and TIMP-1 in IS, and positively correlated with NE in IS. TIMP-1 concentration in IS was a predictor of IgG levels in both NL (where it predicted lower IgG levels) and IS (where it was positively correlated with IgG). NE concentration in IS were positively correlated with IgG levels in NL (Figure 2B) and were found to be predictors of IgG levels in NL and IgA levels in IS, after regression analysis. IgG levels were predictors of lower MPO levels in NL (Table 4).

4 | DISCUSSION

Although the unified airway hypothesis has been increasingly corroborated, when it comes to the course of the development of *P. aeruginosa* chronic lung infection in CF (see Section 1), little is known about the immunological mechanisms of pathogen-host interaction in the UAW and about mechanisms of UAW-LAW interaction in CF as a whole. The original support for sinus-lung communication in CF relies on microbiological findings.[19] Our findings show that the antibody response supports this theory and may explain the reduced tissue damage seen in the UAW, where the response was predominantly IgA-mediated. We also show a potential role of IgG in UAW-LAW communication, where IgG levels in induced sputum were found to be a predictor of IgG levels in nasal lavage (Figure 2 and Table 4).

The predominance of the anti-*P. aeruginosa* IgA over IgG response both in NL and IS and the finding that IgA was a predictor of IgG in both samples are in agreement with the literature and stresses the IgA role in the local mucosal response, which is elicited to prevent the pathogens' adherence to the mucosa.[11,20] The low IgG levels in these secretions are expected, as the IgG response is essentially systemic, with transudation to the mucosal secretions,[21] a path that is apparently opposite to that followed by IgA.[13] Significantly higher IgA levels in NL, in comparison with IS, corroborate previous findings[12,13] and point to a less inflammatory response against *P. aeruginosa* in the UAW, which is reinforced by the significantly lower levels of inflammatory markers in NL (Table 2). Surprisingly, however, we also found a significantly higher anti-*P. aeruginosa* IgG levels in NL than in IS. The IgG response is commonly associated with neutrophil recruitment, which, in turn, is a remarkable feature of LAW infection in CF,[22] as indicated by the increased levels of proinflammatory markers in IS (Table 2). We and other groups have previously shown that systemic specific IgG levels are increased in CF patients with chronic *P. aeruginosa* lung infection.[23–27] We cannot rule out the influence of the sample type in our results. While NLF was analyzed without being diluted, IS samples are denser and needed to be diluted for a more reliable analysis. Also, the antibodies are likely to be embedded in the dense sputum mass, which can hamper their detection by ELISA. The bronchoalveolar lavage fluid could be more comparable to NLF, but our intention was to focus on noninvasive collection methods for everyday and repeated assessment of both airway levels.

The IgA levels in both compartments are not in agreement with the status of the *P. aeruginosa* infection in both LAW and UAW. There were increased NLF IgA and IS IgA levels in patients without chronic UAW and LAW infection, respectively, which led to the absence of statistical difference in relation to their chronically infected counterparts, in this aspect. This is contrasting with previous studies of our group that showed agreement between IgA levels and *P. aeruginosa* UAW and LAW colonization/infection.[13,14] We can speculate

that these results mean the presence of UAW infection not detected by microbiological NL culture but indicated by increased local IgA response, as most patients without chronic UAW infection had a history of LAW infection with *P. aeruginosa*, and the UAW can be the first niche of infection.[5,19] Moreover, not all sinuses are likely to be sampled by nasal lavage[28]; thus, *P. aeruginosa* detection may be underestimated.[28] Regarding LAW infection, in a recent three-year follow-up, we found a persistent IgA response in saliva even after apparent eradication of *P. aeruginosa* in the LAW, and increased IgA levels in saliva showed association with up to 12.5-fold increased risk of exposure to *P. aeruginosa* in the LAW.[15] In other studies of our group, concentrations of the proinflammatory cytokines IL-1β, IL-6, and IL-8 in NL taken before new detection of *P. aeruginosa* in the LAW were comparable with those of patients who were free of *P. aeruginosa* infection. Most interestingly, at the time of *P. aeruginosa* detection, irrespective of whether *P. aeruginosa* occurred in the UAW or LAW, IL-8 concentrations doubled, and IL-6 concentrations tended to increase in NL.[29] Thus, early UAW assessment may be a window into LAW inflammation. Likewise, a longitudinal analysis with a higher number of patients is necessary to evaluate the diagnostic and prognostic utility of IgA measurement in NL and IS.

The correlation between IgA, MPO, and MMP-9 in the UAW is an interesting finding of our study. MPO is a marker of oxidative stress, is produced mainly by neutrophils and, to a lesser degree, by monocytes and macrophages.[30] However, due to the inverse correlation between MPO and IgG in NL and as IgA was a predictor of MPO, this MPO production is possibly a result from the activity of macrophages produced during the IgA response (or release) against *P. aeruginosa* infection in the UAW, as IgA is associated with a higher macrophage recruitment and reduced neutrophil amount.[12] Conversely, MMP-9 was an isolated predictor of IgA, meaning that it can trigger the IgA response. MMP-9 is a protease produced mostly by PMNs and acts in many ways, especially by degrading components of the extracellular matrix. Its role in the CF lung disease is well described and it has a dramatic increase during episodes of pulmonary exacerbations.[31–34] The knowledge about the role of MMP-9 in UAW disorders is recent. A study showed increased MMP-9 expression in the bone and in the mucosa of patients with CRS and osteitis, despite intranasal and postoperative use of steroids, suggesting a role of MMP-9 in the pathogenesis of osteitis.[35] Recently, an increased MMP-9 expression was shown in advanced stages of nasal polyposis, followed by suppression of the antiprotease TIMP-2.[36] However, the MMP-9 expression seems not to be correlated with the severity of nasal polyposis.[37,38] We have previously shown an increased MMP-9 concentration in the UAW of CF patients when compared to controls, and this concentration decreased after intravenous antibiotic therapy.[39] In the present study, if we consider that MMP-9 causes tissue damage and IgA is a protective antibody,[40] the IgA response in the UAW mucosa may counterbalance the MMP-9 production. Something similar may happen in the LAW, where NE predicted IgA in IS. The role of NE is also well described in the CF lung disease, where free or bound NE is detected very early and predicts the occurrence of bronchiectasis later in life.[41] At the same time, TIMP-1 was a predictor of IgG in IS. As IgG is associated with PMN-mediated inflammatory responses[12] and TIMP-1 acts in the remodeling of the extracellular matrix by repairing damages caused by metalloproteinases,[42] TIMP-1 production may regulate the IgG response. To the best of our knowledge, there are no reports in the literature investigating such a relationship. Taken together, these findings suggest that, both during UAW and LAW infection, innate immune mediators are crucial to elicit and regulate the activity of anti-*P. aeruginosa* antibodies. We also suggest an essential role of specific anti-*P. aeruginosa* IgA against protease-induced tissue damage.

The finding that immune markers in IS, namely NE, TIMP-1, and anti-*P. aeruginosa* IgG predicted anti-*P. aeruginosa* IgG levels in NL is intriguing. This is uncommon not only in CF but in any chronic inflammatory respiratory disease. Interaction between UAW and LAW was previously suggested in allergic rhinitis and asthma, and, apparently, postnasal drips are determinant for the severity of LAW disease.[43,44] However, there is no evidence that LAW disease can influence the host response in the UAW. In a previous study, we did not find a correlation between inflammatory mediators at different airway levels.[10] With the present results, we suggest a potential role of anti-*P. aeruginosa* IgG in the UAW-LAW crosstalk. Although it is difficult for us to explain this possible relationship, due to the lack of studies in the literature, we believe that these findings should not be neglected, and further investigation is needed to better understand the mechanisms of interaction between these two airway compartments.

Our study has several limitations, especially its cross-sectional design and selection bias, as we have assessed a relatively small cohort of patients seen in a single center, which limits our statistical power. This also prevented the determination of positive cutoffs for the anti-*P. aeruginosa* IgA and IgG ELISA tests, hindering a more accurate analysis of their diagnostic utility. Also, as we said previously, there are divergences in the IgA and IgG levels, both in NL and IS, regarding our patients' *P. aeruginosa* infection status, which can be better addressed in a longitudinal analysis. Some immune mediators were not detected, and others were detected only in a small part of our patients, preventing us from assessing their relationship with the anti-*P. aeruginosa* IgA and IgG response in the LAW and UAW. On the contrary, nasal lavages were performed routinely in every patient at every presentation at our CF center, by two nurses, so that UAW sampling methods are performed equally on a high standard. Also, although not all sinuses are likely to be reached by nasal lavage, we collect secretions that are drained from the sinuses, and drainage is the only way to get secretions out of the hollow spaces without a second exit. This is important when considering a noninvasive sampling approach like nasal lavage.

5 | CONCLUSION

We bring findings that may be valuable for understanding the dynamics of UAW-LAW interaction in the CF respiratory disease, an issue that still needs to be better explored. Our study corroborates the role of the humoral immune response against *P. aeruginosa* UAW and LAW infection in CF and highlights the IgA prominence in the

mucosal response. Proteases seem to play a role both in triggering the antibody response in both airway levels—MMP-9 in the UAW and NE in the LAW—and may be useful as diagnostic and therapeutic targets in the CF lung disease. The antiprotease TIMP-1 was a predictor of IgG levels in IS and may be a regulator of the IgG response during LAW infection. The IgA response is more prominent in the UAW than in the LAW, which characterizes a less inflammatory response in the UAW. Three markers in IS—NE, TIMP-1, and anti-*P. aeruginosa* IgG—were predictors of anti-*P. aeruginosa* IgG in NL, which is an interesting result and underlines the UAW-LAW interaction in CF, thereby supporting the unified airway hypothesis. Measuring antibodies and immune mediators in NL and IS may be useful in the CF diagnostic routine, especially for early detection of *P. aeruginosa* infection; however, this should be better assessed in longitudinal studies, with more patients and should include high-throughput analysis, such as gene expression assays, targeting a higher range of immune markers.

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CONFLICT OF INTERESTS

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