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Infections in autoimmune pulmonary alveolar proteinosis : a large retrospective cohort

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ABSTRACT

Background

Autoimmune pulmonary alveolar proteinosis (aPAP) is a rare disease, predisposing to an increased risk of infection. A complete picture of these infections is lacking.

Research question

Describe the characteristics and clinical outcomes of patients diagnosed with aPAP, and to identify risk factors associated with opportunistic infections.

Methods

We conducted a retrospective cohort including all patients diagnosed with aPAP between 2008 and 2018 in France and Belgium. Data were collected using a standardised questionnaire including demographics, comorbidities, imaging features, outcomes and microbiological data.

Results

We included 104 patients, 2/3 were men and median age at diagnosis was 45 years. With a median follow-up of 3.4 years (IQR 1.7–6.6 years), 60 patients (58%), developed at least one infection, including 23 (22%) with opportunistic infections. *Nocardia* spp was the main pathogen identified (n=10). Thirty-five (34%) patients were hospitalised due to infection. In univariate analysis, male gender was associated with opportunistic infections (p=0.04, OR=3.88; 95% CI (1.02 to 22.06)). Anti-granulocyte macrophage colony-stimulating factor antibody titre at diagnosis was significantly higher among patients who developed nocardiosis (1058 (316–1591) vs 580 (200–1190), p=0.01). Nine patients had died (9%), but only one death was related to infection.

Interpretation

Patients with aPAP often presented with opportunistic infections, especially nocardiosis, which highlights the importance of systematic search for slow-growing bacteria in bronchoalveolar lavage or whole lung lavage.

INTRODUCTION

Pulmonary alveolar proteinosis (PAP) is a rare disease, with a prevalence ranging from 4 to 40 cases per million inhabitants.^{1 2} PAP is characterised by an altered activity of alveolar macrophages that inhibits their physiological role of alveolar recycling of pulmonary surfactant and cell debris. This impairment results in an accumulation of intra-alveolar surfactant and can lead to progressive hypoxaemia.

Different classifications of PAP exist, and PAP can be divided into autoimmune PAP (aPAP)—caused by anti-granulocyte macrophage colony-stimulating factor (GM-CSF) antibody-mediated disruption of GM-CSF signalling—secondary PAP (due to haematological diseases, chronic infections, etc), hereditary or congenital.^{3–5} aPAP represents more than 90% of all PAP in adults and is characterised by the presence of autoantibodies neutralizing GM-CSF biological activity.⁶ The diagnosis of PAP is challenging, essentially suggested by bronchoalveolar lavage (BAL), described as ‘milky’, and its cytological analysis reveals periodic acid-Schiff (PAS) positive acellular eosinophilic lipoprotein and foamy alveolar macrophages with PAS-positive intracellular inclusions.^{7 8} aPAP diagnosis is then made on the presence of anti-GM-CSF antibodies. The antibodies testing can be performed by ELISA, which is the gold-standard technique, or by a functional method.^{9 10}

GM-CSF has a pivotal role in the PAP pathophysiology by controlling alveolar macrophage and neutrophil functions.¹¹ Through its transcription factor PU.1, GM-CSF stimulates differentiation, proliferation and survival of alveolar macrophages.^{12 13} Autoantibodies blocking GM-CSF activity result in an impairment of surfactant clearance by alveolar macrophages, which can impact gas exchanges. By affecting macrophages differentiation, GM-CSF antibodies also impair their immune functions such as adhesion, pathogen recognition, chemokine secretion and microbial phagocytosis.^{14 15} Neutrophils functions are also altered with GM-CSF inhibition, by decreasing adhesive, phagocytic and bactericidal capacities.¹¹ Therefore, patients with PAP are at risk for infections.^{2 4 16} In PAP, infections occur in 5%–13% of patients and account for 20% of attributable mortality,^{16 17} with a majority of opportunistic pathogens, such as *Nocardia* spp, mycobacteria and fungi.^{18–22} No predictors of opportunistic infections have been identified in literature so far. Available data on aPAP are scarce, mostly based on small series. A large cohort of aPAP in Japan has increased our knowledge of this disease but very few infections were reported (n=12/223, 5.7%), and more generally, data on non-Asian population are still lacking.¹⁶

In this study, we aimed to describe the characteristics and clinical outcomes of patients diagnosed with aPAP, and to identify risk factors associated with opportunistic infections through a retrospective cohort.

METHODS

Study population and data collection

We conducted a multicentre retrospective cohort study including all patients diagnosed with aPAP between 2008 and 2018 in 25 centres in France and Belgium (available in online supplemental appendix 1). To build this cohort, we systematically screened the medical record of all patients who had detectable anti-GM-CSF antibody in serum. All the anti-GM-CSF antibody testing in serum had been previously performed at the Immunology Department of the Rennes University Hospital, that is, the unique referral centre for this dosage for participating countries. Then, we included only adults (age ≥ 18 years) who fulfilled the diagnostic criteria for PAP defined by the presence of PAS-positive acellular eosinophilic lipoprotein and foamy alveolar macrophages with PAS-positive intracellular inclusions in the BAL or lung biopsy. Data were collected using a standardised questionnaire including demographics, comorbidities, imaging features, outcomes and microbiological data. All imaging features were reviewed by two physicians in a blinded fashion. For survival data, the follow-up was censored in October 2021.

Infection was defined as the combination of compatible symptoms, imaging findings and/or microbiological data. Infections were categorised as opportunistic when related to the following micro-organisms: *Nocardia* spp, *Actinomyces* spp, *Mycobacterium* spp, *Pneumocystis jirovecii*, *Cryptococcus* spp, *Aspergillus* spp or *Mucorales*. Other micro-organisms were categorised as non-opportunistic.

Diagnosis of pneumonia was defined as the combination of pulmonary or systemic symptoms of infection, new opacities on chest X-ray or CT scan and/or proven pathogen on respiratory sample. If the suspected pneumonia occurred at the same time as the aPAP diagnosis, or in the absence of previous radiologic examination performed, the identification of a pathogen was mandatory. Bacteria identified in BAL or sputum samples were considered as causative pathogens by the physicians in charge of the patients. We reported any infectious event mentioned in the patient's medical file, even if prior to aPAP diagnosis.

Complete resolution of PAP was defined as disappearance of any symptom, and normalisation of CT and functional tests. Spontaneous resolution was defined as resolution without any aPAP treatment.

Anti-GM-CSF antibody assessment

Anti-GM-CSF antibodies titre was determined by a functional assay, which is the reference one in France and Belgium, measuring the ability of antibodies to neutralise the effect of recombinant cytokine GM-CSF on the growth of GM-CSF-dependent erythroblastic cell line TF1.^{11 23} Briefly, serial dilutions of sera were incubated with 1 ng/mL GM-CSF (Cellgenix, Freiburg, Germany) and TF1 cells. The proliferation of TF1 cells was measured by incorporation of tritiated thymidine for each dilution. The resulting count-per-minute curve followed a sigmoidal one which allowed for the calculation of a standardised antibody titre inhibiting 50% of TF1 cell proliferation (IC₅₀). The limit of quantification for this technique was a titre of 5. All sera with detectable anti-GM-CSF antibodies using the functional method were also positive with the ELISA technique.

Statistical analysis

We first described the characteristics of patients with and without opportunistic infections. Results were expressed as median and IQR or mean and SD for quantitative variables, and relative frequencies for qualitative variables. Missing data for each variable were excluded from the denominator. The Student's t-test or Mann-Whitney test and Fisher's exact test were used, as appropriate. To assess the risk factors of developing opportunistic infections in aPAP, univariate analysis was performed using logistic regression model. Two-tailed p values were reported, with $p < 0.05$ considered as statistically significant. Statistical analysis was carried out using R-Studio 2020, Integrated Development for R (R-Studio, Boston, Massachusetts, USA).

RESULTS

Baseline characteristics

Of the 375 patients for whom an anti-GM-CSF antibodies dosage was performed in our institution between 2008 and 2018, 141 tested positive. Among them, 104 patients from 25 centres had a confirmed diagnosis of aPAP and were included in the analysis (figure 1). Iterative measurements of the anti-GM-CSF antibodies titre was available for 57% of patients ($n=59/104$). The diagnosis was obtained by BAL in 71% ($n=75/104$) or lung biopsy in 25% ($n=26/104$) of cases. Data were missing in the three remaining cases.

Baseline characteristics of the cohort are summarised in table 1 and online supplemental table S1. Almost two-thirds of patients were men and median age at diagnosis was 45 years. Fifty-nine (58%) patients were smokers and 36 (35%) were exposed to dust inhalation. Twenty-two patients (21%) were immunocompromised, due to immunosuppressant treatments or underlying disease other than aPAP. HIV status was negative for all patients. At the time of diagnosis, 89% of patients were symptomatic and the most frequent symptoms were dyspnoea (77%) and cough (46%). Twenty patients (19%) presented weight loss.

Other symptoms, such as fever (7%) or digital clubbing (7%) were rare. Five out of seven patients with fever at aPAP diagnosis had non-opportunistic pneumonia with proven microbiological documentation in 4 cases (*Staphylococcus aureus*, $n=1$; *Escherichia coli*, $n=1$; *Chlamydia pneumoniae*, $n=1$; *Haemophilus influenzae*, $n=1$; not documented, $n=1$). For the two other patients, fever was not related to an infection.

Median lung volumes on pulmonary function test, such as forced vital capacity, forced expiratory volume in 1 s and total lung capacity, were not impaired. However, median diffusing capacity for carbon monoxide was 56% of predicted values, confirming the gas exchange alteration.

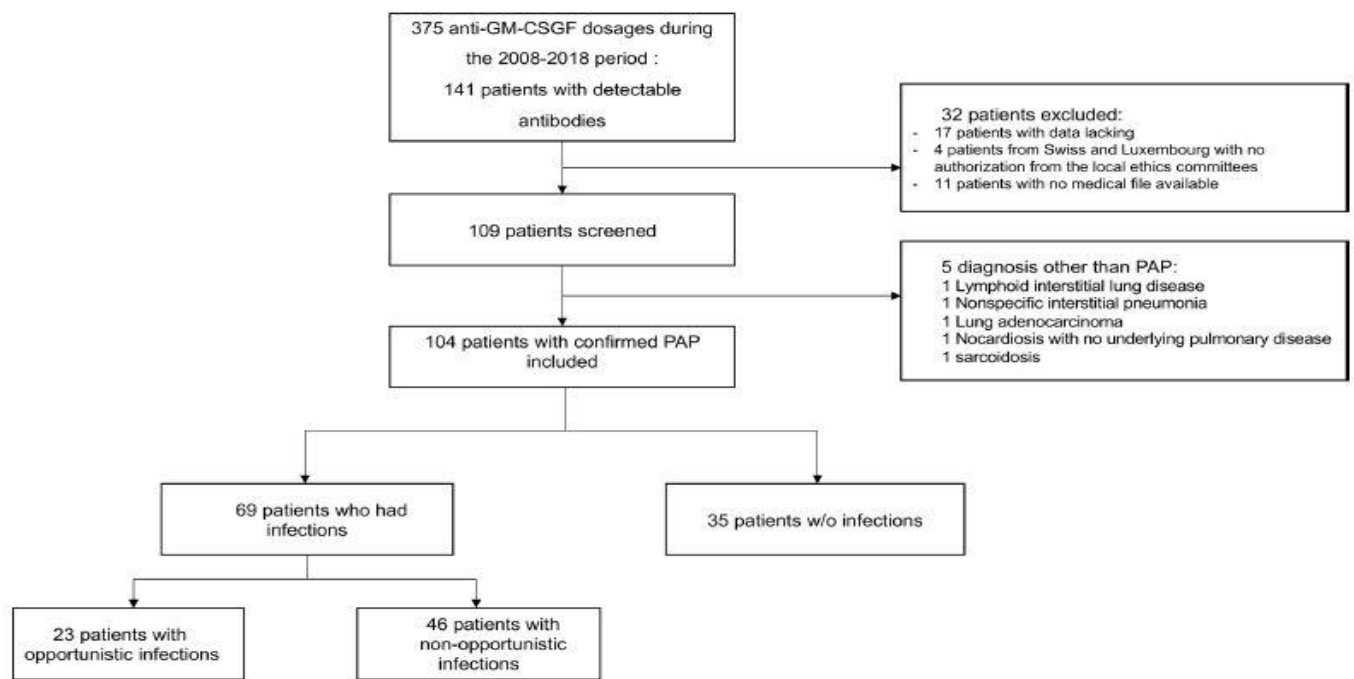


Figure 1 Flow chart of the study. GM-CSF, granulocyte macrophage colony-stimulating factor; PAP, pulmonary alveolar proteinosis.

Characteristics of infections

We identified 74 infections in 60 patients during the follow-up (median follow-up 4.3 years; IQR 1.7–10.8). Twenty-three patients had opportunistic infections (table 2). Sixty-five Per cent of them (n=15/23) occurred at the time of diagnosis of aPAP. Two opportunistic infections (one tuberculosis and one nocardiosis) and seven non-opportunistic infections occurred before aPAP diagnosis.

Nocardia spp was the main pathogen identified (n=10). Only three of these patients required intensive care unit (ICU) admission, and no death related to an opportunistic infection was reported at the end of follow-up. Nocardiosis mostly involved *Nocardia abscessus* (n=4), *Nocardia pneumoniae* (n=2) and *Nocardia paucivorans* (n=2). Pneumonia (n=6) and brain abscesses (n=5) were the two most frequent form of nocardiosis, and only one patient had both lung and cerebral nocardiosis. None of them was immunocompromised. All patients with nocardiosis were hospitalised, including two patients admitted in ICU.

For non-opportunistic infections, 84% of patients (n=31/37) had pneumonia (online supplemental table S2). In less than half of cases, there was no microbiological pathogen identified (46%, n=17/37). Eleven patients presented at least two infectious events. Among them, four presented one opportunistic infection and at least one non-opportunistic infection; five other patients had at least two non-opportunistic infections reported. Two patients developed two different opportunistic infections (nocardiosis and atypical mycobacteriosis; nocardiosis and pneumocystis).

Thirty-five of all patients (34%) were hospitalised due to infection, primarily nocardiosis (n=10) and pneumococcal pneumonia (n=6). Three patients presented an opportunistic infection (two mycobacteriosis and one nocardiosis) and received subcutaneous GM-CSF in addition to anti-infective therapy. One patient died of *Pseudomonas aeruginosa* pneumonia.

Table 1 Comparison of baseline characteristics between patients with and without opportunistic infections

	All patients, n=104	Patients with opportunistic infections, n=23	Patients w/o opportunistic infections, n=81	P value
Baseline characteristics at aPAP diagnosis				
Age, years	45(37–51)	41(35–48)	45(37–51)	0.31
Male gender	70 (67)	20 (87)	50 (62)	0.04
Serum anti-GM-CSF titre	580 (200–1190)	600 (130–1095)	580 (200–1200)	0.73
Comorbidities at aPAP diagnosis				
Active smokers	50 (48)	14 (61)	36 (44)	0.24
Smoking, pack-years	15 (0–26)	17 (0–22)	10 (0–30)	0.93
COPD	4 (4)	1 (4)	3 (4)	1
Asthma	7 (7)	0 (0)	7 (9)	0.20
Dust exposure	36 (35)	9 (39)	27 (33)	0.62
BMI, kg/m ²	25 (22–29)	23 (20–27)	26 (23–30)	0.08
Alcohol abuse	9 (9)	2 (9)	7 (9)	1
Diabetes mellitus	7 (7)	1 (4)	6 (8)	1
Immunosuppressive treatment	3 (3)	0 (0)	3 (3)	0.58
Malignancy	3 (3)	1 (4)	2 (5)	0.54
Symptoms at aPAP diagnosis				
No symptoms	11 (11)	4 (17)	7 (9)	0.25
Cough	48 (46)	9 (39)	39 (48)	0.62
Dyspnoea	80 (77)	16 (70)	64 (79)	0.39
Sputum	21 (20)	4 (17)	17 (21)	0.74
Digital clubbing	7 (7)	2 (9)	5 (6.2)	1
Fever	7 (7)	1 (4)	6 (7.4)	0.65
Weight loss	20 (19)	5 (22)	15 (19)	1
Oxygen supplementation	24 (23)	6 (26)	18 (22)	0.76
Functional data at aPAP diagnosis				
FVC, % predicted	86 (76–99)	88 (69–104)	85 (78–99)	0.99
FEV1, % predicted	87 (77–85)	87 (77–104)	86 (77–93)	0.52
DLCO, % predicted	56 (38–69)	45 (36–67)	58 (41–70)	0.23
6MWD, metres	498 (435–550)	507 (461–546)	485 (432–550)	0.63
Desaturation during 6MWT, % SpO ₂	4 (2–9)	5 (2–9)	4 (2–8)	0.46
PaO ₂ , mm Hg	71 (61–86)	68 (60–87)	71 (61–83)	0.86
Treatments of aPAP				
No specific treatment	34 (33)	7 (30)	27 (33)	1
Whole lung lavage	55 (53)	13(57)	42 (52)	0.80
Inhaled GM-CSF therapy	13 (13)	2 (9)	11 (14)	0.73
Subcutaneous GM-CSF	26 (25)	8 (35)	18 (22)	0.27
Rituximab	21 (20)	2 (9)	19 (23)	0.14
Plasmapheresis	1 (1)	1 (4)	0 (0)	0.22
Clinical outcomes of aPAP				
Spontaneous resolution	11 (11)	4 (17)	7 (9)	0.25
Partial resolution	51 (49)	13 (56)	38 (47)	0.49
Complete resolution	30 (29)	7 (30)	23 (28)	1
Treatment failure	9 (11)	0 (0)	9 (11)	0.20
All-cause mortality	9 (9)	3 (13)	6 (6)	0.69

Data are expressed as median (IQR) or number (%).

aPAP, auto-immune pulmonary alveolar proteinosis; BMI, body mass index; COPD, chronic obstructive pulmonary disease; DLCO, diffusing capacity for carbon monoxide; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; GM-CSF, granulocyte macrophage colony-stimulating factor; 6MWD, 6 m walking distance; SpO₂, peripheral capillary oxygen saturation.

Table 2 Characteristics of infections

	aPAP patients developing infections
Opportunistic infections	n=23
Microbiology	
<i>Nocardia</i> spp* ‡	10 (44)
<i>Mycobacterium tuberculosis</i>	2 (9)
Non-tuberculosis mycobacteria† §	6 (26)
<i>Aspergillus</i> spp	4 (17)
<i>Pneumocystis</i> spp	2 (9)
<i>Mucorales</i>	1 (4)
Outcomes	
Hospitalisation	14 (61)
ICU admission	3 (13)
Mechanical ventilation	1 (4)
Clinical failure of treatment or relapse	0 (0)
All-cause mortality during follow-up	3 (13)
In-hospital mortality related to infection	0 (0)
Non-opportunistic infections	n=37
Infection site	
Pneumonia	31 (84)
Skin and soft tissue infection	2 (5)
Urinary tract infection	1 (3)
Osteitis and joint infections	2 (5)
Sinusitis	1 (3)
Microbiology	
<i>Streptococcus pneumoniae</i>	7 (19)
<i>Haemophilus influenzae</i>	3 (8)
<i>Staphylococcus aureus</i>	3 (8)
<i>Escherichia coli</i>	2 (5)
<i>Pseudomonas aeruginosa</i>	2 (5)
<i>Klebsiella pneumoniae</i>	2 (5)
Respiratory viruses	1 (3)
Not documented	17 (46)
Outcomes	
Hospitalisation	21 (57)
Respiratory failure	10 (27)
ICU admission	5 (14)
Mechanical ventilation	3 (7)
Clinical failure of treatment or relapse	1 (3)
All-cause mortality during follow-up	6 (16)
In-hospital mortality related to infection	1 (3)

Data are expressed as median (IQR) or number (%).

**Nocardia* species identified: *Nocardia abscessus* (n=4), *Nocardia paucivorans* (n=2), *Nocardia pneumoniae* (n=1), *Nocardia* spp (n=3).

†Non-tuberculosis mycobacteria species identified: *Mycobacterium chimerae* (n=2), *Mycobacterium terrae* (n=1), *Mycobacterium xenopi* (n=1), *Mycobacterium parascrofulaceum* (n=1) *Mycobacterium* spp (n=1).

‡Diagnosis of nocardiosis was defined by (1) confirmed microbiological documentation from BAL or biopsy, with positive culture or PCR (16S rRNA or hsp65) and (2) compatible imaging features on chest CT scan, such as nodule(s), pseudonodular consolidation, abscess(es), interstitial infiltrates; or presence of brain abscess(es) on brain imaging.

§Non-tuberculous mycobacterial pneumonia diagnosis was based on the following criteria: (1) pulmonary or systemic symptoms and (2) nodular or pseudonodular lesions on chest CT scan (3) and at least one positive culture from BAL.

aPAP, auto-immune pulmonary alveolar proteinosis; ICU, Intensive care unit.

Risk factors associated with infections

The comparison of patients who developed opportunistic infections and those who did not is summarised in [table 1](#). In univariate analysis, male gender was associated with opportunistic infections ($p=0.04$, OR=3.88; 95% CI (1.02 to 22.06)). Patients with opportunistic infection tended to have lower body mass index than the rest of the population, but it was not statistically significant. All other characteristics, including age, comorbidities or symptoms at diagnosis, were similar between the two groups. The risk of opportunistic infections did not differ in patients previously treated by rituximab, corticosteroids or other immunosuppressive therapy.

We found no correlation between the serum anti-GM-CSF titre and the occurrence of infection ($p=0.49$), nor with the occurrence of opportunistic infection ($p=0.51$). Patients presenting infections requiring hospitalisation tend to have a higher anti-GM-CSF titre, without reaching statistical significance ($p=0.051$). When focusing on patients with aPAP who had developed nocardiosis, anti-GM-CSF titre at diagnosis was significantly higher (1058 (316–1591) vs 580 (200–1190), $p=0.01$).

Treatments and outcomes

For the whole cohort, the median follow-up was 3.4 years (IQR 1.7–6.6 years). Spontaneous improvement of the disease was observed in 28% of patients ($n=29/104$), among which 11% presented complete resolution ($n=11/104$). Seventy patients (67%) received specific treatment of aPAP, primarily whole lung lavage (WLL) ($n=55/70$, 79%), subcutaneous GM-CSF ($n=26/70$, 37%) and rituximab ($n=21/70$, 30%). Outcome was favourable in 81 patients (78%), including 30 complete resolutions. Nine patients died, with a 5-year overall survival of 95% (online supplemental appendix 2). Only one death was related to the progression of aPAP due to a fibrotic evolution. The other causes of death were: malignancy ($n=3$), *P. aeruginosa* pneumonia ($n=1$), terminal respiratory failure secondary to chronic thromboembolic pulmonary hypertension ($n=1$) or to chronic obstructive pulmonary disease ($n=1$) and unknown ($n=2$).

DISCUSSION

The main findings of this large cohort of patients diagnosed with aPAP are as follows: (1) opportunistic infections are common in patients with aPAP (22%, with a median follow-up of 3.4 years), primarily due to *Nocardia* spp ($n=10$); (2) despite important rates of opportunistic infections, the survival remains high; (3) male gender is an independent risk factor for opportunistic infections and (4) a high GM-CSF antibody titre at diagnosis is associated with the risk of nocardiosis.

To the best of our knowledge, this cohort study of aPAP patients is the largest in non-Asian population so far. In this work, population characteristics were consistent with previous studies.^{16 24–26} The increased susceptibility to infections in aPAP has been reported and is supported by a strong rationale.²⁷ The loss of GM-CSF signalling results in altered functions of alveolar macrophages: (1) impaired differentiation, (2) decreased bacterial recognition adhesion and killing and (3) reduced phagocytosis.^{3 12 18 27} We hypothesise that impairment of GM-CSF signalling could increase the susceptibility to ubiquitous

environmental pathogens, such as *Nocardia* or non-tuberculous mycobacteria which were the most frequent pathogens identified in our cohort. Human monocytes are able to produce GM-CSF in presence of *Nocardia* in vitro, suggesting a key role of GM-CSF pathway in nocardiosis pathogenesis.²⁸ In addition, the accumulation of cholesterol into foamy macrophages could represent a source of energy for some pathogens, especially mycobacteria, facilitating persistent infection.^{29 30}

Only small sample size case series has highlighted the risk of opportunistic infections for aPAP patients.^{19 20 31 32} Nevertheless, a large Japanese cohort of 223 patients with aPAP only reported 12 infections (5.6%) over a 6-year follow-up, including four aspergillosis and five mycobacteria pulmonary diseases. This low rate could be explained by ethnic differences or by a selection bias, which has been suggested by the authors themselves. Indeed, their study mainly assessed risk factors for disease severity and progression, while our study focused on infections. As 65% of opportunistic infections occurred at diagnosis, we consider it unlikely that results be affected by the time of follow-up. A literature review from 1950 to 2010 reported 75 cases of opportunistic infections among aPAP patients.¹⁸ Conversely to our study, Punatar *et al* found a larger proportion of tuberculosis and several fungal infections due to *Histoplasma capsulatum*—which is not encountered in Western Europe—or *Cryptococcus* spp. In our work focusing on infectious diseases, the proportion of patients developing opportunistic infections during the follow-up was high (22%), which suggests a causal link between aPAP, lung immune deficiency and respiratory tract infections.²⁷ Although most infections are not eligible for preventive therapy, this study emphasises the need for comprehensive microbiological extensive investigations in these patients—including slow-growing bacteria—in BAL or WLL.

Nocardia was the main opportunistic pathogen reported in our cohort (9%). Nocardiosis is a rare opportunistic infection that generally occurs in immunocompromised patients, particularly in those with phagocyte defects.³³ GM-CSF pathway is involved in the phagocytosis abilities of alveolar macrophages. Rosen *et al* reported seven cases of nocardiosis in patients not known to be immunocompromised, and anti-GM-CSF antibodies were found among five of them.³¹ Interestingly, we found that anti-GM-CSF antibody titre at diagnosis was higher among patients who developed nocardiosis during follow-up. None of the aPAP patients died from nocardiosis, contrasting with the poor outcomes reported in immunocompromised hosts.^{34 35} The high proportion of nocardiosis found in our cohort, and the association with the GM-CSF antibody titre, are in line with a previous study suggesting a tight relationship between this pathogen and the GM-CSF pathway.²⁸

We found a low mortality rate of 9% in the whole cohort, associated with almost 80% of patients with clinical, partial or complete, improvement confirming the good prognosis of aPAP. Only two deaths were related to aPAP: one chronic respiratory failure and one death from *P. aeruginosa* pneumonia. In the literature, the 5-year survival is currently around 95%.^{1 2 16}

Our study has several limitations. First, this study suffers from potential biases inherent to its observational retrospective design. For example, non-opportunistic infections were poorly documented, and some other infections might have been treated on an outpatient basis, without being reported in patient medical records. However, the proportion of missing data remains low in this cohort. Second, due to the long study period and the numerous participating sites, the management of aPAP was not standardised and may differ. Anyway, this limitation did not affect the assessment of risk factors of opportunistic infections. Third,

anti-GM-CSF antibodies titres were quantified using a functional method, whereas ELISA technique is considered as the gold standard. All sera with detectable anti-GM-CSF antibodies using our functional method were subsequently confirmed with the ELISA technique. However, as ELISA assay quantifies antibody concentration against whatever GM-CSF epitope, it might detect antibodies with no pathogenic activity, while our functional technique selects patients with antibodies blocking GM-CSF activity, which may be more relevant and accurate. However, this would make our result of a higher titre of anti-GM-CSF antibodies at diagnosis in patients who developed nocardiosis difficult to confirm worldwide. Fourth, our study was not powered to perform subgroup analysis, especially to assess the impact of additional immunosuppressive therapies on the occurrence of opportunistic infections.

In this large multicentre cohort, the rate of opportunistic infection, especially nocardiosis, was high. These infections did not impact the long-term overall survival rate. This study highlights the importance of systematically searching for slow-growing bacteria when BAL or WLL is performed. Further studies are needed to improve our knowledge on the pathophysiology of aPAP and its infectious complications.

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Contributors

AM fulfilled the role of guarantor. DLP did the analysis. AM, DLP, PT and SJ cowrote the original draft. AM, RB, LW-S, YU, EG, GP, MR-G, JT, E Bergot, JC, SM-A, A Bergeron, E Blanchard, BB, PB, A Bourdin, PRB, SH, CHM, SQ, HN, CC, BC, YG, DM, CM, PT, VC, DLP and SJ contributed substantially to the investigation and data collection, as well to reviewing and editing the manuscript.

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Competing interests

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Patient consent for publication

Not applicable.

Ethics approval

The study was approved by the Ethics Committee of Rennes University Hospital (approval number 19.104). Patients were informed about the study and could refuse to be enrolled. Written informed consent was waived.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

Data can be made available upon reasonable request.

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