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Early View

Research letter

COVID-19 in patients with Pulmonary Alveolar Proteinosis A European multicenter study

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COVID-19 in patients with Pulmonary Alveolar Proteinosis

A European multicenter study

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Take home message

Adult PAP patients experienced similar COVID-19 rates with the general population and high rates of hospitalizations and deaths underscoring their vulnerability and the need for measures to prevent infection. The impact of iGM-CSF must be considered.

Key words: pulmonary alveolar proteinosis; COVID-19; adults; children; i-GM-CSF; outcome

Introduction

Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) signaling is essential in both alveolar macrophages (AMs) differentiation and activation of lung immune cells [1]. Differentiated AMs are crucial in both the elimination of alveolar microbes and surfactant clearance. The disruption of the GM-CSF axis in alveolar macrophages leads to the development of pulmonary alveolar proteinosis (PAP) [1]. In the majority of patients this relates to the presence of autoantibodies against GM-CSF autoimmune (a)PAP but there are multiple other causes [1, 2, 3]. GM-CSF deficient animals may have impaired lung inflammatory response to commensal microbes and humans with PAP may occasionally develop opportunistic lung infections [4]. The mainstay of pharmacological treatment in aPAP is inhaled GM-CSF which is off-label but increasingly used worldwide [5, 6, 7 8, 9].

Severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), a new infection causing COVID-19 is clearly associated with worse prognosis in adults with preexisting lung disorders and might represent an additional risk factor for severe pneumonia in PAP patients [10, 11]. However, controversy exists regarding the relationship between GM-CSF signaling and outcome of COVID-19 [12] [Figure 1]. There are no studies in patients with PAP who also have COVID-19. We hypothesized that patients with PAP would be at increased risk and have poor outcomes. This European collaborative study aimed to investigate prevalence and clinical consequences of COVID-19 in PAP patients and the impact of prior iGM-CSF treatment on outcome.

Methods

This multi-center, observational, retrospective, European collaborative study includes all adult and pediatric PAP patients with COVID-19 diagnosed in referral centers from 11 European Countries from January 24th 2020 to August 31st 2021. PAP diagnosis was based on chest computerized tomography (CT) findings and the results of either lung biopsy or cytologic analysis of bronchoalveolar lavage (BAL) fluid. Further characterization of autoimmune or hereditary PAP was based on increased levels of GM-CSF autoantibodies or a positive genetic analysis for CSF2RA, CSF2RB or MARS mutations respectively [1]. PAP patients were eligible if COVID-19 was confirmed either by RT-PCR or by compatible clinical (acute onset of fever, flu-like symptoms, headache, and anosmia), radiological and serology findings [10]. Generally, hospitalization was considered in patients with dyspnea or increased respiratory rate (≥30 breaths per min), or oxygen saturation ≤ 94% on room air or decrease in saturation to < 90% with ambulation and also on the basis of overall clinical concern by Emergency Department staff, including perceived risk of high risk for complications from severe COVID-19". [https://www.bumc.bu.edu/id/covid-19-response/clinical-algorithms-for-admission-and-discharge/]. The prevalence of COVID-19 in PAP patients was calculated and demographic, clinical and functional characteristics closest to the time of COVID-19 infection as well as outcomes were collected using anonymized data forms. Since the prevalence of COVID-19 has differed between countries during the pandemic, the median prevalence (IQR) of COVID-19 in all European countries participating in the study was calculated at 14.8% (11.09-17.15), taking into consideration the population of each country and the cumulative cases of COVID-19 officially

reported [https://www.ecdc.europa.eu/en/covid-19/data] (footnote Table 1).The study was approved by all the Medical Ethics Committees, first that of the General University Hospital “Attikon”, Athens, Greece (ΒΠΝΕΥΜ, ΕΒΔ657/30-11-2021).

Statistical Analysis

Normality of distributions was checked with Kolmogorov-Smirnov test. Categorical variables are presented as n (%), whereas numerical variables are presented as median (interquartile ranges) or as mean (minimum, maximum value) for the results in the adult and children population respectively. Comparisons between groups were performed using chi-square tests for categorical data and Mann-Whitney U test for numerical data. Cox regression univariate and multivariate analyses were performed to evaluate predictors of outcome. Data were analyzed using SPSS 18.0 for Windows (SPSS Inc, Chicago, IL, USA), p-values <0.05 were considered statistically significant.

Results

COVID-19 infection was diagnosed in 34 out of 255 PAP patients (13.3%), 31/34 were adults (91.2%), 30/31 with aPAP, 19/31 (61.3%) male, median age at inclusion (IQR) 47 years (35-56), median disease duration 57 (35-115) months, ever-smokers 61.3%, FVC% 78 (64.3-86), DLCO% 62.9 (48.8-70.75) of predicted, long-term oxygen therapy (LTOT) 7/31 (22.6%). Whole lung lavage (WLL) had been performed in 27/31 (87.1%) and iGM-CSF prescribed in 15 (48.4%) (Table 1).

In aPAP patients COVID-19 presented mostly with fever (77.4%) and dyspnea (61.3%). All patients were infected before the preventive option of vaccination was available. 11/31 patients (35.5%) needed hospitalization, 5/31 patients (16.1%) in the ICU. All patients with mild disease treated at home survived. Among hospitalized patients, two died and one patient underwent lung transplantation (3/11, 27%). These three patients had worse DLCO% predicted (p=0.019) and had more often arterial hypertension (AH) (p=0.012) and a smoking history (p=0.002). Treatment with iGM-CSF was withheld during hospitalization in all patients. Gender, age, disease duration, spirometry results, LTOT, comorbidities, history of WLL and of iGM-CSF treatment had no impact upon hospitalization or outcome. Among seven children (5 hereditary PAP), 3 developed COVID-19 (2 hereditary PAP), all needed hospitalization and all survived (Table 1).

Discussion

To our knowledge this is the only study of COVID-19 disease in PAP. The major findings are: 1) COVID-19 developed in 13.3% of patients, the majority were adults (91.2%), all with aPAP, all unvaccinated, 2) one third needed hospitalization (35.5%), almost fifty percent in the ICU 3) although the numbers are small, 27% hospitalized patients either died or were lung-transplanted, whereas all patients with mild disease treated at home survived 4) poor prognosis was related to lower DLCO% predicted, AH and smoking history, 5) previous treatment with iGM-CSF had no impact upon hospitalization or outcome 6) all pediatric patients were hospitalized but survived.

The prevalence of COVID-19 in PAP patients was found to be similar to the calculated median prevalence of COVID-19 in the general population of participating European Countries (13.3% vs 14.8%, p=0.256). However, previous studies demonstrated that other groups of ILD experienced lower than expected COVID-19 most probably due to better use of prophylactic measures and remote contact with physicians for electronic prescription of treatment [13]. The younger age of PAP patients and presumably their higher sociability might partly explain discrepancy.

During the pandemic it soon became clear that adults with chronic respiratory diseases, especially ILD, were at increased risk of developing severe COVID-19 and dying [10, 11]. There are very few data regarding COVID-19 and ultra-rare diffuse parenchymal lung diseases. Lymphangioleiomyomatosis (LAM) patients with COVID-19, compared to an age-matched general population (30-59 years), had increased rates of hospitalization but not mortality [14]. In the present study, PAP patients showed increased rates of mortality and lung transplantation, however similar to the upper limits of in-hospital mortality ranges recently reported for certain European Countries [15]. This could be explained by the presence of known risk factors such as AH and smoking and possibly also due to macrophage immunosuppression in PAP [4, 10]. It is known that GM-CSF facilitates AMs and other immune cells to clearance of pathogens including viruses. Based on positive results from animal models, recombinant forms of iGM-CSF are under investigation in patients with COVID-19-related acute hypoxic respiratory failure (NCT04326920). Conversely, inhibition of GM-CSF pathways could reduce the exuberant lung

inflammation in COVID-19. This study cannot distinguish between these possibilities.

Only a small number of children were included in the study because of the rarity of pediatric PAP [3]; half of the patients were COVID-19 with good outcome. The effects of COVID-19 on children are less severe than in adults, but the effects on children with chronic rare respiratory diseases is unknown [16].

The present study has limitations. The number of PAP patients with COVID-19 might be underestimated because asymptomatic or newly diagnosed patients could have been missed; however, the close contact of patients with this ultra-rare disease with specialized centers means that it is likely that at least all patients with a known diagnosis were included. Multivariate analysis was not performed to investigate predictors of mortality because univariate analysis did not demonstrate any significant association probably due to the limited number of patients and events. Finally, the level of enforcement of prophylactic measures may have varied between countries, and the effects of this on the interpretation of the data is challenging and beyond the scope of the present study. However, this collaboration permitted the analysis of data of multiple specialized centers for both children and adults providing a unique opportunity for examining COVID-19 in this ultra-rare disease.

In conclusion, PAP patients experienced similar rates of COVID-19 with the general population and high rates of hospitalizations and deaths, underscoring the vulnerability of this population and the necessity of

preventive measures to avoid infection. If infected, secondary prophylaxis with monoclonal antibodies and the impact of iGM-CSF must be considered.

Table 1. Demographic, clinical, functional characteristics and outcomes of patients with PAP who had COVID-19 (n=34) *

Demographic and clinical data for PAP adults who had COVID-19 (n=31)

Parameter	*All (n=31)*	*Non-Hospitalized (n=20)*	*Hospitalized (n=11)*	*p-value*
Gender (Male, n%)	19 (61.3)	12 (60.0)	7 (63.6)	p=0.700
Age at inclusion, years	47 (35.0-56.0)	40 (28.5-50.7)	51 (45.0-56.0)	p=0.060
Duration of disease, months	57 (36.0-115.0)	74.5 (38.0-127.5)	43 (28.0-98.0)	p=0.256
Autoimmune PAP, n (%) * **	30 (96.8)	19 (95)	11 (100)	p=0.451
GM-CSF autoantibody level (mcg/ml)	89.5 (52.3-154.7)	69.01 (21.1-187.7)	107.6 (62.3-125.2)	p=0.625
PAP documented by lung biopsy, n (%)	9 (29)	3 (15)	6 (54.5)	**p=0.020**
Smoking (no/yes/ex), (%)	38.7/9.7/51.6	45/5/50	27.3/18.2/54.5	p=0.391
BMI	26.9 (23.5-30.0)	26.9 (23.4-29.3)	27 (22.6-34.0)	p=0.714
LTOT, n (%)	7 (22.6)	2 (10)	5 (45.5)	**p=0.029**
Arterial hypertension, n (%)	8 (25.8)	2 (10)	6 (54.5)	**p=0.007**
History of treatment with WLL, n (%)	27 (87.1)	18 (90)	9 (81.8)	p> 0.950
Number of WLL	2 (1-4)	2 (1-3)	3 (1-11)	p=0.283
History of treatment with iGM-CSF, n (%)	15 (48.4)	10 (50)	5 (45.5)	p=0.809
FVC % predicted	78 (64.3-86.0)	81.5 (71.0-89.0)	73 (55.0-81.0)	p=0.175
DLCO % predicted	62.9 (48.8-70.7)	69.4 (53.0-75.5)	53 (35.5-69.3)	p=0.137
SpO_2% at rest	96.0 (95.5-98)	97.0 (94.0-98.0)	95.5 (94.5-96.2)	p=0.257
Distance walked at 6MWT, meters	481 (360-525)	501 (437.5-560.0)	407 (333.7-483.3)	p=0.060
Fever, n (%)	24 (77.4)	14 (70)	10 (90.9)	**p=0.053**
Dyspnea, n (%)	19 (61.3)	10 (50)	9 (81.8)	**p=0.032**
Fatigue, n (%)	18 (58.1)	10 (50)	8 (72.7)	p=0.114
Cough, n (%)	16 (51.6)	8 (40)	8 (72.7)	**p=0.038**
Anosmia, n (%)	9 (29)	6 (30)	3 (27.3)	p>0.950
Oxygen therapy or increase of oxygen	11 (35.5)	0 (0)	11 (100)	**p<0.001**
HFNC	7 (22.6)	0 (0)	7 (63.6)	**p<0.001**
Corticosteroids systemic	18 (58.1)	8 (40)	11 (100)	**p=0.001**

Macrolides	14 (45.2)	6 (30)	8 (72.3)	**p=0.031**
Anticoagulants	13 (40)	4 (20)	9 (81.8)	**p=0.020**
Remdesivir	2 (6.4)	0 (0)	2 (18.2)	p=0.118
Other (Plasma therapy, Bamlavinmab)	2 (6.4)	0 (0)	2 (18.2)	p=0.118
iGM-CSF	2 (6.4)	2 (10)	0 (0)	p=0.527
ICU admission, n (%)	5 (16.1)	0 (0)	5 (45.5)	**p=0.003**
Death or lung transplantation, n (%)	3 (9.7)	0 (0)	3 (27.3)	**p=0.037**

Demographic and clinical data for children who had PAP (n=7)

Parameter	***All (n=7)***	***Without COVID-19 (n=4)***	***With COVID-19 (n=3)***	***p-value***
Gender, male (%)	4 (57.1)	1 (25)	3 (100)	p=0.100
Age at inclusion, years	12 (5-17)	6 (11-17)	9 (5-14)	p=0.108
Duration of disease, months	33 (3-36)	23 (3-26)	22 (14-36)	p=0.154
Autoimmune PAP, n (%)	2 (28.6)	1 (25)	1 (33.3)	p=0.809
Congenital PAP *, n (%)**	5 (71.4)	3 (75)	2 (66.7)	p=0.809
BMI [kg/m^2]	13.1 (14.4-27.5)	13.1 (14.4 (27.5)	7 (14.6-21.6)	p=0.724
LTOT, n (%)	5 (71.4)	3 (75)	2 (66.7)	p=0.809
History of treatment with WLL, n (%)	5 (71.4)	3 (75)	2 (66.7)	p=0.809
Number of WLL	96 (0-96)	96 (0-96)	13 (0-13)	p=0.372
History of treatment with iGM-CSF, n (%)	1 (14.3)	1 (25)	0 (0)	p=0.325
Other (Methionine, AZM, simvastatin, bromhexine)	5 (71.4)	3 (75)	2 (66.7)	p>0.950
FVC % predicted	47 (35-82)	19 (39-58)	47 (35-82)	p>0.950
DLCO % predicted	61 (31-92)	18 (31-49)	0 (92)	p=0.221
SpO_2% at rest	22 (75-97)	22 (75-97)	12 (85-97)	p>0.950
Hospitalization**, n (%)**	3 (42.9)		3 (100)	
Death or lung transplantation	0 (0)		0 (0)	

* 21/31 adult patients had SARS-CoV-2 infection documented by RT-PCR, only 1 adult patient had received one dose of the vaccine against SARS-CoV-2 before developing COVID-19, 3/3 children had SARS-CoV-2 infection documented by RT-PCR

** The median prevalence (IQR) of COVID-19 for European countries participating in the study was calculated taking into consideration the population of each country and the cumulative cases of COVID-19 officially reported for each one as per January 7th, 2022 and was found 14.8% (11.09-17.15) [https://www.ecdc.europa.eu/en/covid-19/data], in detail: Greece 14.02%, Italy 11.74%, Germany 8.85%, Denmark 15.68%, France 17.14%, Turkey 10.81%, Poland 11.09%, Spain 14.8%, Ireland 18.13%, United Kingdom 20.74%, Portugal 15.15%). Regarding adult PAP patients the prevalence was calculated by COVID-19 patients /Active PAP patients followed-up in each center (Italy, 10/50, Greece 3/27, Turkey 3/11, Poland 3/9, Germany 2/38, France 3/38, Spain 2/9, United Kingdom 2/50, Denmark 1/10, Ireland 1/4 Portugal 1/2: 31/255=12.5%. Children included in the study were as follows: 4 in Germany, 1 in the United Kingdom, 1 in France and 1 in Greece; 3 children (Germany) presented COVID-19. Overall prevalence: 34/255=13.3%.

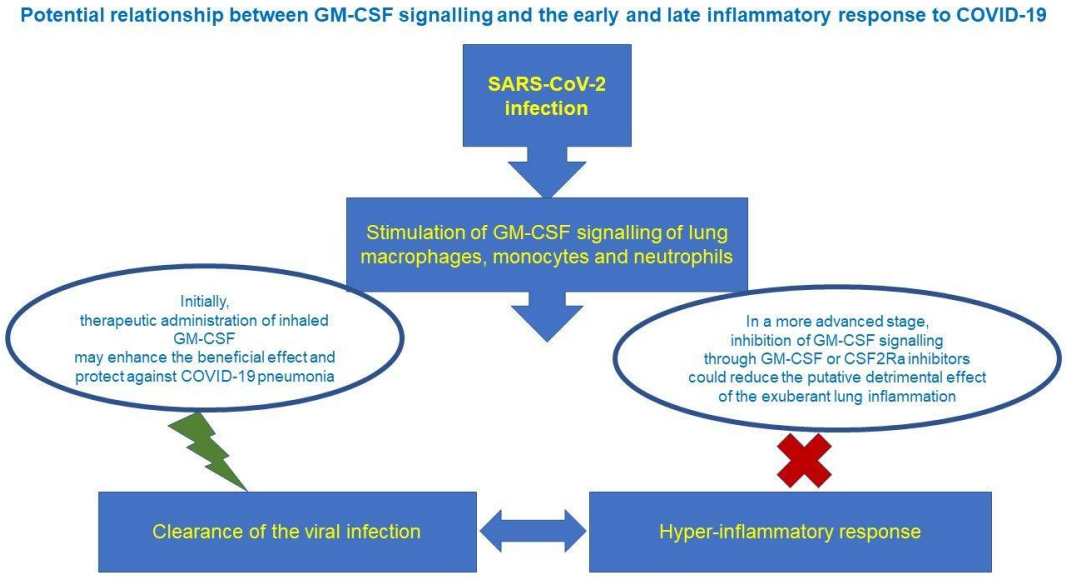
*** In adults, only one patient had disease related to *CSF2RA* mutation; in children, 1 had disease related to *CSF2RA* (Granulocyte-Macrophage colony-stimulating factor receptor subunit alpha) mutation, 1 related to *CSF2RB* (Granulocyte-Macrophage colony-stimulating factor receptor subunit beta) mutation and 3 to *MARS1,* out of them, 2 brothers with *MARS1* mutation contracted SARS-CoV-2 infection **** details on treatment upon hospitalization are available for 1 child and included HFNC oxygen treatment, systemic corticosteroids, anticoagulants, no iGM-CSF

For adults, values are presented as median (IQR) unless otherwise indicated; for children values are presented as range (minimum-maximum) unless otherwise indicated; IQR= interquartile range; p-values <0.05 were considered statistically significant, PAP: pulmonary alveolar proteinosis; GM-CSF=Granulocyte-Macrophage colony stimulating factor; BMI= Body Mass Index; LTOT=Long-term oxygen therapy; WLL= Whole lung lavage; SpO_2 = Arterial Oxygen Saturation; 6MWT=6minute walking test; COVID-19=Corona Virus Disease 2019; HFNC=High Flow Nasal Canula; ICU=Intensive Care Unit; MARS= methionyl-transfer-RNA-synthetase; M=male; FVC= forced vital capacity; DLCO= diffusing capacity of the lung for carbon monoxide.

Figure legend

Figure 1. Potential relationship between GM-CSF signaling and the early and late inflammatory response to COVID-19.

References



1 Trapnell BC, Nakata K, Bonella F, et al. Pulmonary alveolar proteinosis. Nat Rev Dis Primers. 2019; 5:16.

2 Hadchouel A, Wieland T, Griese M, et al. Biallelic Mutations of Methionyl-tRNA Synthetase Cause a Specific Type of Pulmonary Alveolar Proteinosis Prevalent on Réunion Island. Am J Hum Genet. 2015; 96:826-831.

3 Bush A, Pabary R. Pulmonary alveolar proteinosis in children. Breathe (Sheff). 2020;16:200001.

4 Tazawa R, Hamano E, Arai T, et al. Granulocyte-macrophage colony-stimulating factor and lung immunity in pulmonary alveolar proteinosis. Am J Respir Crit Care Med. 2005; 171:1142-1149.

5 Tazawa R, Trapnell BC, Inoue Y, et al. Inhaled granulocyte/macrophage-colony stimulating factor as therapy for pulmonary alveolar proteinosis Am J Respir Crit Care Med. 2010;181:1345-1354.

6 Papiris SA, Tsirigotis P, Kolilekas L, et al. Long-term inhaled Granulocyte Macrophage-Colony Stimulating Factor in Autoimmune Pulmonary Alveolar Proteinosis: Effectiveness, safety, lowest-effective dose. Clin Drug Invest 2014; 34: 553-564.

7 Papiris SA, Tsirigotis P, Kolilekas L, et al. Pulmonary alveolar proteinosis: time to shift? Expert Rev Respir Med. 2015; 9 :337-349.

8 Tazawa R, Ueda T, Abe M, et al. Inhaled GM-CSF for Pulmonary Alveolar Proteinosis. N Engl J Med. 2019 ;381 :923-932.

9 Trapnell BC, Inoue Y, Bonella F, et al ; IMPALA Trial Investigators. Inhaled Molgramostim Therapy in Autoimmune Pulmonary Alveolar Proteinosis. N Engl J Med. 2020; 383:1635-1644.

10 Gallay L, Uzunhan Y, Borie R, et al. Risk Factors for Mortality after COVID-19 in Patients with Preexisting Interstitial Lung Disease. Am J Respir Crit Care Med. 2021; 203:245-249.

11Beltramo G, Cottenet J, Mariet AS, et al. Chronic respiratory diseases are predictors of severe outcome in COVID-19 hospitalized patients: a nationwide study. Eur Respir J. 2021 ; 58 :2004474.

12 Bonaventura A, Vecchié A, Wang TS, et al. Targeting GM-CSF in COVID-19 Pneumonia: Rationale and Strategies. Front Immunol. 2020; 11:1625

13 Papiris SA, Bouros D, Markopoulou K, et al. Early COVID-19 lockdown in Greece and idiopathic pulmonary fibrosis: a beneficial "impact" beyond any expectation. Eur Respir J. 2021 ; 57 :2003111.

14 Baldi BG, Radzikowska E, Cottin V et al. COVID-19 in lymphangioleiomyomatosis: an international study of outcomes and impact of mTOR inhibition. Chest. 2021: S0012-3692(21)05166-7.

15 Gray WK, Navaratnam AV, Day J, et al. COVID-19 hospital activity and in-hospital mortality during the first and second waves of the pandemic in England: an observational study. Thorax. 2021 Nov 24:thoraxjnl-2021-218025. doi: 10.1136/thoraxjnl-2021-218025]

16 Rasmussen SA, Thompson LA. Coronavirus Disease 2019 and Children: What Pediatric Health Care Clinicians Need to Know JAMA Pediatr. 2020; 174:743-744.