



HAL
open science

Pulmonary alveolar proteinosis

Stéphane Jouneau, Cedric Menard, Mathieu Lederlin

► **To cite this version:**

Stéphane Jouneau, Cedric Menard, Mathieu Lederlin. Pulmonary alveolar proteinosis. *Respirology*, 2020, 25 (8), pp.816-826. 10.1111/resp.13831 . hal-02735851

HAL Id: hal-02735851

<https://univ-rennes.hal.science/hal-02735851>

Submitted on 18 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Pulmonary Alveolar Proteinosis

Jouneau Stéphane, MD, PhD,^{1,2}, Ménard Cédric, PharmD, PhD,³, Lederlin Mathieu, MD, PhD,^{4,5}.

¹Department of Respiratory Medicine, Competence Centre for Rare Pulmonary Diseases, CHU Rennes, Univ Rennes, 35033 Rennes, France

²IRSET UMR1085, Univ Rennes, 35043 Rennes, France.

³Service d'Immunologie, de Thérapie Cellulaire et d'Hématopoïèse, Hôpital Pontchaillou, 35033 Rennes, France

⁴Department of Radiology, CHU Rennes, Univ Rennes, 35033 Rennes, France

⁵LTSI, INSERM U1099, Univ Rennes, 35033 Rennes, France

Correspondence:

Prof. Stéphane Jouneau

Service de Pneumologie, Centre de Compétences pour les Maladies Rares Pulmonaires, Hôpital Pontchaillou, UMR 1085, IRSET, Université de Rennes 1, 2 rue Henri Le Guilloux, 35033 Rennes Cedex 9, France

Stephane.jouneau@chu-rennes.fr

Abstract

PAP is an ultra-rare disease in which surfactant components, that impair gas exchange, accumulate in the alveolae. There are three types of PAP. The most frequent form, primary PAP, includes autoimmune PAP which accounts for over 90% of all PAP, defined by the presence of circulating anti-GM-CSF antibodies. Secondary PAP is mainly due to haematological disease, infections or inhaling toxic substances, while genetic PAP affects almost exclusively children. PAP is suspected if investigation for interstitial lung disease reveals a crazy-paving pattern on chest CT-scan, and is confirmed by a milky looking bronchoalveolar lavage that gives a positive periodic acid-Schiff (PAS) reaction indicating extracellular proteinaceous material. PAP is now rarely confirmed by surgical lung biopsy.

Whole lung lavage (WLL) is still the first line treatment, with an inhaled GM-CSF as second line treatment. Inhalation has been found to be better than subcutaneous injections. Other treatments, such as rituximab or plasmapheresis, seem to be less efficient or ineffective. The main complications of PAP are due to infections by standard pathogens (Streptococcus, Haemophilus, and Enterobacteria) or opportunistic pathogens like mycobacteria, Nocardia, Actinomyces, Aspergillus or Cryptococcus are. The clinical course of PAP is unpredictable and spontaneous improvement can occur. The 5-year actuarial survival rate is 95%.

Key words: Granulocyte-macrophage colony-stimulating factor, infection, pulmonary alveolar proteinosis, rituximab, whole lung lavage.

Short title: Pulmonary Alveolar Proteinosis

1. Introduction

Pulmonary alveolar proteinosis (PAP), first described by Rosen and Castleman in 1958,¹ is an ultra-rare disease in which the alveolar macrophages are dysfunctional, causing surfactant proteins and lipids to accumulate in the pulmonary alveoli, so hindering gas exchange.² Most patients present with increased dyspnea on exertion but appear to be normal on clinical examination when assessed for interstitial lung disease (ILD). Diagnosis is usually based on the presence of a crazy paving pattern on a chest CT-scan plus PAS (periodic acid Schiff) positive eosinophilic material in a bronchoalveolar lavage (BAL). The serum contains anti-GM-CSF antibodies in 90% of cases, leading to a diagnosis of autoimmune PAP. The evolution of PAP varies from spontaneous resolution to death due to respiratory failure or a lung infection. The classification, pathophysiology and therapeutic management of PAP has progressed in recent years, particularly as a result of randomized trials of inhaled GM-CSF.

2. Classification of PAPs

Cases of PAP are assigned to one of three groups:²

- Primary PAP (>90%), caused by disruption of granulocyte–macrophage colony-stimulating factor (GM-CSF) signalling. These, in turn can be either:
 - Autoimmune PAP (> 90% of all PAP), with serum anti-GM-CSF (granulocyte macrophage colony-stimulating factor);
 - Hereditary PAP, due to mutations in the genes encoding GM-CSF receptor subunits (eg: CSF2RA or CSF2RB);
- Secondary PAP (5-10%), caused by reduced numbers and/or functions of alveolar macrophages due to:
 - Haematological disease, particularly myelodysplastic syndromes,

- Chronic infections,
- Inhalation of toxic substances,
- Malignancy
- Immune deficiency
- Chronic inflammation
- Mutations affecting mononuclear phagocytes numbers or function (eg: GATA2 mutations)

Treatment of the associated disease is pivotal in the management of most cases of secondary PAP. Whole lung lavage (WLL) can be used, however the benefit could be only transient.

- Congenital PAP (2%), caused by disorders of surfactant production, and so observed mainly in children, resulting from:
 - Mutations causing a lack of surfactant proteins,
 - Mutations producing a deficient lipid transporter ATP-binding cassette subfamily A member 3 (ABCA3)
 - Mutations affecting lung development.

In PAP patients with no proven serum GM-CSF auto-antibodies and without any sign of disease known to cause secondary PAP, assessment of serum GM-CSF level and GM-CSF signalling pathway integrity could help to differentiate hereditary, congenital and unclassifiable PAP and trigger further testing such as GM-CSF receptor chains or surfactant mutation. More details can be found in the recent and excellent review of Trapnell et al.²

As the vast majority of PAP cases are autoimmune PAP, this review will focus only on them.

3. Epidemiology of autoimmune PAPs

1
2
3 The serum of patients suffering from autoimmune PAP (>90% of all PAP cases) contains anti-
4 GM-CSF antibodies.² PAP is an extremely rare disease whose prevalence varies from country
5 to country. The reported range is from 4 to 40 cases per million inhabitants and its incidence is
6 0.2 per million inhabitants per year.³ Autoimmune PAP is predominantly a male disease, with
7 a sex ratio > 2 and a mean age at diagnosis of around 50 years.⁴⁻⁸ Tobacco is a suspected risk
8 factor and 60% of patients are smokers.^{6, 8} Exposure to dust and vapours is implicated in the
9 genesis of secondary PAP, but many patients with autoimmune PAP are also exposed to these
10 factors: 26% in the Japanese Inoue series and 54% in the German Bonella series.^{4, 6}
11
12
13
14
15
16
17
18
19
20
21
22
23
24

25 **4. Pathophysiology of autoimmune PAP**

26
27
28 Our understanding of PAP pathophysiology has advanced considerably over the past few years.
29
30 Figure 1 illustrates the pathogenesis of autoimmune PAP.
31
32

33 4.1. PAS-positive deposits in alveolar spaces

34
35
36 PAP was first recognized as a distinct disease by Rosen et al. in 1958.¹ It was defined by its
37 pathology: alveolar periodic acid-Schiff (PAS)-positive proteinaceous deposits with no cellular
38 infiltrate and normal interalveolar septa. This was “so characteristic and similar from one case
39 to another that it seems highly unlikely that it could have escaped description previously”.^{1, 8}
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000

48 4.2. Surfactant

50
51 Surfactant is a mixture of proteins and lipids (mainly phosphatidylcholine) synthesized and
52 secreted by type II alveolar epithelial cells². The four main surfactant proteins (SP-A, -B, -C
53 and -D) are encoded by the SFTPA, B, C and D genes. The surfactant lipids are stored as
54 intracytoplasmic lamellar bodies. Surfactant components are recycled by type II alveolar
55 epithelial cells or catabolized by alveolar macrophages. The lipids and proteins of the surfactant
56
57
58
59
60

1
2
3 help reduce the surface tension of the alveoli (prevent tele-expiratory collapse) and are involved
4
5 in the innate defence of the lungs, particularly SP-A and SP-D, which are collectins.² Lipidomic
6
7 analysis of BAL has shown an increase in free cholesterol (by 60-fold), cholesteryl esters (by
8
9 24-fold) and in ceramide (more than 130-fold increase) and other sphingolipids.⁹ The surfactant
10
11 lipid class phosphatidylcholine expanded 17-fold, lysophosphatidylcholine expanded 54-fold,
12
13 and the surfactant proteins A, B, and C expanded 144-, 4-, and 17-fold, respectively.⁹ These
14
15 increases were similar between the different kind of PAP analysed (auto-immune, secondary to
16
17 myeloid leukemia and genetic). Logically, statin therapy has been shown to improve patients
18
19 with autoimmune PAP, as well as to reduce alveolar macrophage cholesterol accumulation and
20
21 PAP lung disease in a mice model of PAP (Csf2rb^{-/-} mice).¹⁰ In other studies also targeting
22
23 cholesterol homeostasis, PPAR γ -agonist therapy has been shown to increase cholesterol
24
25 clearance in macrophages and to reduce disease severity in PAP mice.¹¹

31 4.3. GM-CSF and alveolar macrophages

32
33
34 The granular and monocyte growth factor (GM-CSF) is a major factor in the pathophysiology
35
36 of autoimmune PAP. Stanley et al. found that the haematological profile of GM-CSF (GM-CSF
37
38 ^{-/-}) knockout mice showed little change but that their pulmonary system was severely affected;
39
40 their alveoli were filled with PAS-positive eosinophilic material, lamellar bodies and foamy
41
42 alveolar macrophages indicating an accumulation of surfactant.¹³ These mice also showed signs
43
44 of subclinical bacterial and fungal infections. As this mouse disorder was similar to human
45
46 PAP, the authors concluded that GM-CSF is probably involved in the pathophysiology of PAP.
47
48 Shibata et al. subsequently showed that the alveolar macrophages of these GM-CSF mice ^{-/-}
49
50 also lacked PU.1, a transcription factor that drives the differentiation of alveolar macrophages
51
52 and is activated by GM-CSF.¹⁴ They restored the PU.1 concentration to normal by viral
53
54 transfection and found that the alveolar macrophages had recovered their adhesive and
55
56 phagocytotic capacities, their responsiveness to toll-like receptors (TLRs), and their ability to
57
58
59
60

1
2
3 break down surfactant. Then Bonfield et al. showed that the alveolar macrophages of
4
5 autoimmune PAP patients had the same defects (decreased PU.1, adhesion and phagocytosis)
6
7 and that incubating these macrophages with GM-CSF in vitro restored their PU.1 and other
8
9 functions.¹⁵ Similarly, treating autoimmune PAP patients with GM-CSF (subcutaneous
10
11 injections) restored the PU.1 concentration in their alveolar macrophages. Another study
12
13 showed that injecting healthy primates with anti-GM-CSF antibodies from the sera of patients
14
15 with PAP reproduced in them most features of the disease.¹⁶

16
17
18
19
20 Lastly, alveolar macrophages are not the only cells that are abnormal in patients with
21
22 autoimmune PAP, the phagocytotic, adhesive and bactericidal capacities of their circulating
23
24 neutrophils are also altered.¹⁷

30 31 **5. Diagnosis**

32
33 The diagnosis of PAP mainly relies on chest CT-scans and BAL after exclusion of other causes
34
35 of ILD. The presence of anti-GM-CSF autoantibodies in the serum leads to the diagnosis of
36
37 autoimmune PAP.

40 41 5.1. Clinical manifestations

42
43
44 Around one third of PAP patients are asymptomatic and any symptoms that are present are non-
45
46 specific. Dyspnea is the most common symptom, followed by a chronic cough and chronic
47
48 bronchitis.^{4-6, 18} Chest pain and hemoptysis are rare and suggest complications, particularly
49
50 superimposed infections.^{4-6, 18} Systemic symptoms (fatigue and/or weight loss), often lasting
51
52 many months before evaluation or initial diagnosis, could also lead to PAP diagnosis.²

53
54
55
56 The clinical examination is often normal.⁸ Crackles are found in 50% of cases, digital clubbing
57
58 is uncommon (20% of cases).⁵

5.2. Complementary examinations

5.2.1. Imaging

A chest X-ray generally reveals non-specific features (Figure 2 and 3), such as symmetrical bilateral lung opacity, but multiple presentations are possible.⁸ Abnormalities are often more pronounced around the hilum; they appear like "butterfly wings" or "bat wings" without other signs suggesting heart failure (cardiomegaly, Kerley lines B, pleural effusion). Pleural effusion is evocative of complications. The poverty of the clinical examination often contrasts with the profusion of radiological lesions.⁸

A chest CT-scan is essential for diagnosing PAP (Figure 2 and 3) and typically shows areas of "ground-glass" superimposed on smooth thickening of the inter- and intralobular septal lines resulting in a "crazy paving" appearance. The distribution of these changes is usually patchy or geographic (juxtaposition of healthy and diseased lobules).^{8, 19} Crazy paving is not pathognomonic of PAP but is sufficiently evocative to lead to a BAL and a check for a milky appearance and amorph PAS material.^{19, 20} The differential diagnoses of crazy paving are shown in Table 1.^{18, 20} Pulmonary fibrosis seems to be uncommon in patients with autoimmune PAP; it may appear only in advanced or end-stage disease.²

5.2.2. Bronchoalveolar lavage (BAL)

BAL is the second essential test for PAP diagnosis. Bronchoscopy is macroscopically normal. The BAL fluid is classically described as "milky" (Figure 4) and contains large amounts of PAS-positive acellular eosinophilic lipoprotein.^{3, 5, 8, 18} There are also foamy macrophages with PAS-positive intracellular inclusions. Electron microscopy of BAL shows concentric lamellar phospholipid structures (lamellar bodies).^{3, 13}

5.2.3. Serum anti-GM-CSF antibodies

1
2
3 High titers of anti-GM-CSF antibodies are found in the serum and BAL of patients with
4 autoimmune PAP. These are polyclonal immunoglobulins (IgG, mainly IgG1 or IgG2). They
5
6 bind to different epitopes of GM-CSF to block its interaction with its receptor.²¹ There may be
7
8 neutralizing and non-neutralizing anti-GM-CSF antibodies, depending on the epitope they
9
10 target. Anti-GM-CSF antibodies can be determined by ELISA or by measuring the ability of
11
12 antibodies to neutralize the effect of recombinant cytokine GM-CSF on GM-CSF-dependent
13
14 TF1 erythroblasts cell line growth. Practically, serial dilutions of patients serum, negative (no
15
16 anti-GM-CSF antibody) and positive control serum (from an acknowledged patient with
17
18 autoimmune PAP) are incubated with TF1 cells and 1 ng/mL GM-CSF (Cellgenix®, Freiburg,
19
20 Germany) for 2 days. The proliferation of TF1 cells is measured by the incorporation of tritiated
21
22 thymidine (1mCi/mL added in culture medium for the last 18h hours) for each serum dilution
23
24 and the values plotted against serum dilution. In the case of PAP patients serum, this typically
25
26 results in a sigmoid-shaped curve for which can be calculated a dilution inhibiting 50% of TF1
27
28 cells proliferation (IC50). The titer of anti-GM-CSF antibodies is calculated as the reciprocal
29
30 of this IC50. The presence of anti-GM-CSF autoantibodies in the serum indicates that the
31
32 disorder is autoimmune PAP. The serum concentration of these antibodies is not correlated with
33
34 disease severity.⁶ Serum anti-GM-CSF antibodies are not pathognomonic of PAP, as they can
35
36 be found in healthy subjects and in subjects with other ILD.^{21, 23, 24} Nevertheless, the anti-GM-
37
38 CSF antibody concentrations are significantly higher in patients with PAP.^{22, 25} An anti-GM-
39
40 CSF antibody concentration > 5.0 µg/mL measured by ELISA has both a 100% sensitivity and
41
42 specificity for diagnosis of autoimmune PAP.²⁵ In addition, the antibody detection in the BAL
43
44 is almost 100% specific for autoimmune PAP.²² The BAL anti-GM-CSF antibody
45
46 concentration can be used to monitor disease progression and could be used to predict the need
47
48 for a WLL or the response to GM-CSF treatment.^{23, 26, 27} However, the anti-GM-CSF antibodies
49
50 in BAL are not yet determined routinely.
51
52
53
54
55
56
57
58
59
60

5.2.4. Surgical lung biopsy

Surgical lung biopsy, once considered to be the gold-standard, is no longer required to diagnose PAP. An evocative chest CT-scan combined with a typical BAL is sufficient in almost all PAP cases. Pathological analysis shows pulmonary alveoli and terminal bronchioles filled with PAS-positive acellular eosinophilic material which can be associated with a slight interstitial lymphocytic infiltration.⁸ A major pathological feature is that the pulmonary architecture is generally preserved except in the case of associated pulmonary fibrosis, a late complication of PAP.¹⁵ Electron microscopy shows lamellar bodies in macrophages and type II alveolar epithelial cells.⁸

5.2.5. Pulmonary function tests (PFTs) and gas exchange

PFTs show a restrictive pattern with a disproportionate decrease in carbon monoxide diffusion capacity (DLCO) but only a moderate decrease in functional volumes.^{6, 8} Briens et al. found that the average DLCO was $51 \pm 13\%$ of predicted values (% pred.),⁵ while Inoue et al. found $69 \pm 27\%$ pred.⁶ Radiological abnormalities are correlated with decreased PFT values, both volumes and diffusion capacities.^{8, 28}

Hypoxemia could be assessed by pulse oximetry (SpO₂), more accurately by arterial blood gases analyses (PaO₂). Alveolo-arterial difference in oxygen (A-aDO₂) has been described to be a strong marker of PAP severity,² explaining its use as primary endpoints in recent large clinical trials.^{29, 30}

Desaturation on exercise, assessed by the 6 minutes walking test (6MWT), is also a reliable and reproducible marker of severity of PAP.

5.2.6. Other complementary examinations

1
2
3 The serum lactate dehydrogenase (LDH) level is 2 to 3 times normal in half of PAP cases.⁸ A
4
5 Japanese study found that the severity of PAP was correlated with the serum LDH level and the
6
7 concentrations of Krebs von den Lungen 6 (KL-6), carcinoembryonic antigen (ACE), SP-A and
8
9 SP-D. Patients are often hypoxemic at diagnosis, with an average PaO₂ of 59 ± 16 mmHg, but
10
11 the delay in diagnosis may explain these differences.⁸
12
13

14
15 Although PAP is an autoimmune disease (anti-GM-CSF antibodies), it is rarely (< 2% of cases)
16
17 associated with other autoimmune diseases.⁸
18
19
20
21
22

23 **6. Evolution and complications of Autoimmune PAP**

24
25
26 The evolution of autoimmune PAP is unpredictable. Spontaneous improvement is possible (17-
27
28 25% of cases), but no predictive factors have been identified and secondary aggravation is
29
30 possible.^{5, 6, 8} Spontaneous resolution has also been described after cessation of exposure to a
31
32 wide range of airborne contaminants, such as tobacco or dust, which probably influence the
33
34 pathophysiology of autoimmune PAP.^{5, 8}
35
36

37
38 Actuarial survival at 5 years was 88 ± 4% in 2002,⁸ with most deaths (72%) linked to a
39
40 progression to chronic respiratory failure.⁸ The survival at 5 years is currently around 95%.^{3, 6}
41
42

43
44 Secondary infections, the most common and threatening complication, occur in 5-13% of
45
46 patients, and account for 18–20% of deaths.^{2, 6, 8} The usual pathogens, Streptococci,
47
48 Haemophilus spp., and Enterobacteriaceae, can be involved, but infections may be due to
49
50 opportunistic pathogens such as mycobacteria, Nocardia spp., Actinomyces spp., Aspergillus
51
52 spp. and Cryptococcus. They should be systematically looked for in cases of worsening and
53
54 also during WLL.^{8, 18, 31} Table 2 lists the main opportunistic pathogens associated with PAP.
55
56 These infections may precede or follow PAP diagnosis. Half of the infected PAP patients
57
58 showed no sign of fever.³¹ The other symptoms of infection found by Punatar et al. included
59
60

1
2
3 cough (64%), dyspnea (62%) and weight loss (23%). Infected sites were predominantly
4 pulmonary (75-86% of cases) and brain abscesses were reported during nocardiosis (19% of
5 cases). A quarter of the mycobacterial infections, and half the fungal infections resulted in
6 disseminated (lymph node, hepatic, medullary, cerebral or ocular) infections.³¹
7
8
9

10
11
12
13 Secondary pulmonary fibrosis can also occur, as can honeycombing during follow-up.³²⁻³⁴
14
15
16
17
18

19 **7. PAP: disease monitoring**

20
21
22 Follow-up of patients with autoimmune PAP should be done regularly and be supervised by
23 physicians experienced in this disease. Respiratory symptoms (cough, dyspnea) and general
24 symptoms (fever, weight loss, and fatigue) have to be assessed at each appointment. Lung
25 function parameters (especially DLCO), 6MWT, PaO₂ and A-aDO₂ should be monitored
26 regularly as their degradation may lead to therapeutic changes, especially WLL. Serial thoracic
27 imaging is mandatory, at least by chest X-ray. CT-scan delivers more ionizing radiation but is
28 much more effective for monitoring the disease, evaluating treatment response and diagnosing
29 complications such as infections. Like most chronic pulmonary diseases, PAP will benefit from
30 the increasing use of ultra-low-dose CT,³⁵ which is almost as informative as standard CT for an
31 exposure dose similar to that of a chest radiograph. CT scoring systems have been proposed to
32 enable more quantitative and reproducible evaluation, and may also serve as endpoints in
33 clinical trials.³⁶⁻³⁸ Several serum biomarkers, including KL-6, LDH, YKL40, CCL-2, CCL-18,
34 CEA, CA-19.9, CYFRA 21-1, NSE, have been shown to correlate variably with disease severity
35 but are not specific and are therefore not used in daily clinical practice.³⁹
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53

54 **8. Treatment of Autoimmune PAP**

55
56
57 The therapeutic management of severe and/or disabling autoimmune PAP includes WLL, GM-
58 CSF injections and inhalations, plasmapheresis and rituximab. In contrast, mild forms of the
59
60

1
2
3 disease simply require monitoring, particularly because of the possibility of spontaneous
4 improvement.^{2, 5, 6, 18} Lung transplantation is also an option in selected cases.
5
6
7

8 8.1 Whole lung lavage (WLL) 9

10
11 This is still the gold-standard treatment for autoimmune PAP.
12
13

14 8.1.1 WLL technique 15

16
17 Ramirez et al. first described the technique of "segmented endobronchial flooding" as a way to
18 physically remove accumulated alveolar material in 1963.^{8, 40} Many improvements have been
19 made since this date. WLL is now performed under general anesthesia. Patient are intubated
20 with a selective double lumen intubation tube. Left selective probes should be preferred,
21 regardless of the side to be washed, as positioning is easier (longer main left bronchus, without
22 the lumen of the right upper bronchus). The correct positioning of this intubation probe is
23 verified by flexible bronchoscopy. One of the probe channels is used to ventilate the patient
24 (protective ventilation, $FiO_2 = 100\%$, continuous capnography ($EtCO_2$)) and the other channel
25 is used for lavage. The supine patient is sedated and curarized and 1 to 2L of saline (without N-
26 acetylcysteine or heparin) are instilled at 37°C. The wash liquid is evacuated by gravity
27 ("siphoning").^{5, 18} The first liter of this effluent is routinely analysed for bacteria (standard,
28 *Nocardia*, *Actinomyces*, mycobacteria) and myco-parasites (*Aspergillus*, *Cryptococcus*). The
29 procedure (instillation-retraction) is repeated until the effluent liquid is no longer cloudy (Figure
30 5). PaO_2 increases during the lung "filling" phase by increasing the pressure in the airways and
31 shunting blood to the contralateral ventilated lung.⁴¹ The pressure in the airways decreases
32 during the "emptying" phase and blood returns to the unventilated lung, increasing the shunt
33 and thus decreasing PaO_2 .⁴¹ Invasive hemodynamic monitoring is not required, a standard
34 scope (heart rate, blood pressure and saturation) is sufficient.⁴² Each lung is usually washed
35 with 15 to 20 L saline, but the volume can be as high as 40 L.^{43, 44} Patients are extubated a few
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 hours later and the contralateral lung is washed 1 to 2 days later using the same protocol.^{45, 46}

4
5 WLL can be used together with extracorporeal membrane oxygenation (ECMO) to treat patients
6
7 with severe respiratory insufficiency.
8

9
10 The WLL technique has not been standardized^{2, 44} and many centers have developed adaptations
11
12 to improve the quality of WLL (increase effluent turbidity).¹⁸ Changes may include
13
14 concomitant thoracic percussion (manual percussion is more effective than mechanical
15
16 percussion), washing the contralateral lung during the same anaesthesia, changing positions
17
18 (alternating procubitus-decubitus) during washing, or a specific type of ventilation (repeated
19
20 periods of manual balloon ventilation during WLL). Segment washing under local anesthesia
21
22 and flexible bronchoscopy have also been used successfully to treat patients with less severe
23
24 forms of PAP. These differences in the WLL techniques in current use could be resolved if an
25
26 international consensus were to be reached. All those involved agree, however, that these
27
28 washes should be carried out by a trained team. Perhaps the ideal would be to have one or two
29
30 national WLL reference centers.
31
32
33

34 35 36 8.1.2 WLL indications 37

38
39 There is no consensus.² The indications for WLL are set by expert centers and based on
40
41 worsening dyspnea, gas exchange (PaO₂, desaturation on the 6MWT, A-aDO₂), interstitial
42
43 lesions and PFT parameters. WLL should not be performed if there is any evidence of
44
45 concurrent active bacterial pneumonia, as it increases the risk of disseminated infection and
46
47 sepsis.²
48
49

50 51 8.1.3 Complications and effectiveness of WLL 52

53
54 The complications of WLL are minimal when it is performed in expert centers.⁴⁴ They include
55
56 desaturation, pneumothorax, subcutaneous emphysema, headache, fever, cardiac pulmonary
57
58
59
60

1
2
3 edema, convulsion, pneumonitis, intubation dislocation (with contralateral leakage) and
4
5 prolonged intubation.^{18, 43}
6
7

8 Seymour's 2002 literature review found that survival at 5 years after WLL was $94 \pm 2\%$.⁸ WLL
9
10 improved symptoms, exercise tolerance, chest imaging, and PaO₂ and PFTs parameters.^{8, 43}
11
12 About half of the patients have only needed a single WLL procedure (bilateral), suggesting that
13
14 the presence of anti-GM-CSF antibodies is not sufficient to maintain the disease.³ The other
15
16 patients will need repeated WLL, varying from 1 to 22.⁸ WLL is also associated with fewer
17
18 opportunistic infections.⁴²
19
20
21
22

23 Smoking seems to be linked to failure of WLL. Bonella et al. reported that active smokers
24
25 required an average of 5 WLL to achieve remission, while the non-smokers in their cohort
26
27 required only 2.4.⁴
28
29

30 8.2 GM-CSF Supplementation

31 8.2.1 Subcutaneous GM-CSF

32
33 Several studies have reported that autoimmune PAP patients benefit from subcutaneous
34
35 injections of GM-CSF. Three prospective studies used daily injections of GM-CSF (5-9
36
37 $\mu\text{g}/\text{kg}/\text{day}$ for 12 weeks), with a gradual increase in the dose to 18 $\mu\text{g}/\text{kg}/\text{day}$ depending on the
38
39 clinical response.⁴⁷ Treatment was effective in nearly half of the patients: in 43% of patients in
40
41 the longest study (1 year) and up to 75% in the shortest study (12 weeks).⁴⁷ A few patients have
42
43 been successfully given a maintenance dose for 1 year (20 $\mu\text{g}/\text{kg}/3$ times per week). Adverse
44
45 reactions were minor: edema, erythema and puncture site pain, fever, chills, nausea, vomiting,
46
47 malaise, headache, fatigue, arthralgia and dyspnea.⁴⁷
48
49
50
51
52
53
54

55 8.2.2 Inhaled GM-CSF

1
2
3 Inhaled GM-CSF delivers the drug directly to the lungs with fewer side effects. It also allows
4
5 doses to be less frequent so reducing the cost of treatment. Inhaled GM-CSF therapy results in
6
7 better responses by autoimmune PAP patients, greater increases in PaO₂ and D(A-a)O₂ than
8
9 subcutaneous GM-CSF.⁴⁷

10
11
12
13 A retrospective analysis of the responses of 12 patients with autoimmune PAP treated with
14
15 inhaled GM-CSF (sargramostim, at a dose of 250 µg b.i.d. every other week for 12 week) was
16
17 performed.⁴⁷ The dose can be increased to 500 µg b.i.d. every other week if there is no clinical
18
19 response. Almost all (11/12; 92%) patients responded, with improvements in clinical,
20
21 radiological, biological and/or PFT parameters.⁴⁷

22
23
24 A prospective study of 39 autoimmune PAP patients treated with inhaled GM-CSF
25
26 (sargramostim, 250 µg/d, every other week for 12 week (loading dose) and 125 µg/d, every
27
28 other week for 12 week (maintenance dose) with follow-up for 1 year was also performed.⁴⁷
29
30 Over half (62%) of the patients responded, 17 patients while on the loading dose, 7 patients
31
32 during the maintenance phase. This response persisted in 66% of patients even after follow-up
33
34 for nearly 3 years. Only 12/35 patients required rescue treatment (mostly WLL).⁴⁷

35
36
37
38
39 None of these studies found any specific adverse effects. Blood leukocyte counts remained
40
41 unchanged despite the GM-SCF. No factor predicting the efficacy of inhaled GM-CSF has been
42
43 identified.

44
45
46 Two major randomized trials, IMPALA³⁰ and PAGE²⁹, in which autoimmune PAP patients
47
48 were treated with inhaled GM-CSF have been completed recently.

49
50
51 The IMPALA trial was a randomised, double-blind, placebo-controlled multicenter clinical trial
52
53 on 135 autoimmune PAP patients that investigated the efficacy and safety of inhaled
54
55 molgramostim (rhGM-CSF).³⁰ The primary objective was to assess the effect of a 24-week
56
57 treatment on the alveolar-arterial oxygen difference. The secondary objectives were its effects
58
59
60

1
2
3 on tolerance of exercise, quality of life, time to WLL, pulmonary function, dyspnea and cough,
4 and chest CT-scan score. The number of reported adverse events (AEs), serious AEs, and
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

on tolerance of exercise, quality of life, time to WLL, pulmonary function, dyspnea and cough, and chest CT-scan score. The number of reported adverse events (AEs), serious AEs, and adverse drug reactions were also monitored. The results should be published soon.

The PAGE trial was a double-blind, placebo-controlled trial of daily inhaled that examined the responses of 64 patients with autoimmune PAP to recombinant human GM-CSF (sargramostim).²⁹ The change in the alveolar–arterial oxygen gradient was significantly better in the GM-CSF group (33 patients) than in the placebo group (30 patients), with a mean change from baseline (-4.50 ± 9.03 mm Hg) to 0.17 ± 10.50 mm Hg ($P = 0.02$). The secondary endpoints of dyspnea and CT-scan score were also better in the GM-CSF group.

8.3 Rituximab

Rituximab is a monoclonal antibody directed against the CD20 antigen of B lymphocytes. Rituximab reduces the number of B lymphocytes in patients suffering from autoimmune diseases, which in turn decreases the secretion of antibodies including anti-GM-CSF. Borie et al. first described the effects of rituximab on an autoimmune PAP patient who refused WLL.⁴⁸ Kavuru et al. then treated 10 patients with autoimmune PAP using the same protocol (1000 mg IV infused on days 1 and 14) and found improvements in PaO₂, PFT parameters and chest CT-scan in 7 of the 9 patients who completed the study.²⁶ This clinical improvement was also reflected in improved alveolar macrophage lipid homeostasis.⁴⁹ The main adverse effects of rituximab are due to reactions occurring during infusion, usually during the first injection: fever, chills, flu-like syndrome. Other rarer symptoms are: nausea, pruritus, angioedema, asthenia, hypotension. They can be minimized by premedication (antipyretic and antihistamine) and slowing the rate of infusion.

However, a real-life setting retrospective study has obtained contrasting results.⁵⁰ None of 13 patients with autoimmune PAP treated with rituximab showed any improvement 6 months after

1
2
3 treatment, while the alveolar-arterial oxygen difference of 4 patients (30%) was significantly
4
5 smaller 1 year after treatment. No serious adverse event was observed. The authors concluded
6
7 that their data do not support rituximab as a second line therapy for patients with refractory
8
9 autoimmune PAP.
10

11 12 13 8.4 Plasmapheresis 14 15

16 The rationale for using plasmapheresis is based on the proven pathogenicity of plasma anti-
17
18 GM-CSF antibodies.¹⁶ Plasmapheresis could decrease circulating anti-GM-CSF antibodies
19
20 enough to improve surfactant clearance by restoring alveolar macrophage catabolic functions.
21
22 Unfortunately, when plasmapheresis was used to treat five patients in whom WLL had failed,
23
24 it appeared to be of limited efficacy.^{23, 51-55} The protocol included 10 plasmapheresis sessions,
25
26 over 2 months, in each of which the plasma mass exchanged was 1.5 L. The anti-GM-CSF
27
28 antibody concentration decreased in 3 patients,^{51, 53, 54} no dosage was performed in the two other
29
30 cases. There is also evidence that plasmapheresis induces immunodepression.
31
32
33

34 35 8.5 Lung transplantation 36 37

38 Very few published data are available on lung transplantation and autoimmune PAP in adults.
39
40 One case of PAP recurring on lungs transplanted for pulmonary fibrosis-complicated PAP
41
42 within 3 years of surgery has been reported.⁵⁶ There have also been a few reports of other cases
43
44 of PAP occurring as a complication in lungs transplanted to treat other diseases.⁵⁷⁻⁵⁹
45
46 Immunosuppressive treatment, especially sirolimus, has been incriminated.⁶⁰
47
48
49

50 8.6 Novel therapeutic approaches 51 52

53 As described in the pathophysiology section of this article, targeting lipid homeostasis could be
54
55 a new way to treat patients with PAP. To date, statin therapy has been described to improve two
56
57 patients with autoimmune PAP.¹⁰ *In vitro* and *in vivo* studies have shown a potential role for
58
59 PPAR γ -agonist therapy in PAP patients.¹¹ A pilot phase I/II human clinical trial of oral
60

1
2
3 pioglitazone as therapy for autoimmune PAP is reported on the portal Clin.trial.gov
4
5 (NCT03231033).
6
7

8 Finally, given the recently published positive results of antifibrotics in progressive fibrosis
9
10 interstitial lung diseases (PF-ILD),⁶¹ PAP patients with PF-ILD phenotype could potentially
11
12 benefit from these treatments in the future.
13
14

15 16 **9. Future directions and unmet needs in PAP**

17
18
19 With the recent large randomized clinical trials on inhaled GM-CSF,^{29, 30} the algorithm of
20
21 treatment of autoimmune PAP patients may change in the near future. In addition, the optimal
22
23 dose, timing or duration of administration of inhaled GM-CSF have not been clearly defined,
24
25 so there is still room for improvement in the current response rate.² Assessment of drugs
26
27 targeting lipid homeostasis could also make it possible to increase the therapeutic arsenal
28
29 against PAP. The combination of several of the available treatments should also be evaluated.
30
31 At the moment, we are lacking reliable predictive biomarkers that could be used in daily
32
33 practice. The development of such tools would help to better manage patients with autoimmune
34
35 PAP.³⁹ Standardization of WLL is also a necessary goal as mentioned by experts in the field.
36
37
38
39

40 41 **10. Conclusion**

42
43 Recent advances in the treatment, such as inhaled GM-CSF, of the ultra-rare disease
44
45 autoimmune PAP have led to a better understanding of the processes underlying the disease.
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Acknowledgements:

We thank Dr Owen Parkes for editing the English text. We also thank physicians and colleagues involved in the diagnosis and management of PAP at our site: Bertrand De Latour, Etienne Delaval, Mallorie Kerjouan, Francisco Llamas-Gutierrez, Dominique Lucas, Adel Maamar, and Alexandre Salé. Finally, we thank Dr Alain Lescoat for his great help with the pathophysiological scheme of autoimmune PAP in Figure 1.

Disclosure statement:

SJ co-authored the protocol and was an investigator in the IMPALA trial (NCT02702180) promoted by Savara-Serendex. All other conflict of interests for all authors are outside the PAP field. SJ has received fees, funding or reimbursement for national and international conferences, boards, expert or opinion groups, and research projects during the past 5 years from Actelion, AIRB, Astra Zeneca, Biogen, BMS, Boehringer, Chiesi, Fibrogen, Galactos, Gilead, GSK, LVL, Mundipharma, Novartis, Pfizer, Roche.

CM received one-time advisory board or consultant fees from Bristol-Myers Squibb, Astra-Zeneca and Celgene in addition to research funding from Celgene and Roche.

ML has received fees, funding or reimbursement for national and international conferences, boards, expert or opinion groups, and research projects over the past 5 years from Astra Zeneca, Boehringer, Fresenius-Kabi, Guerbet, Roche, Siemens Healthcare.

References

- 1 S. H. Rosen, B. Castleman, A. A. Liebow. Pulmonary alveolar proteinosis. *N Engl J Med.* 1958; 258: 1123-42.
- 2 B. C. Trapnell, K. Nakata, F. Bonella, I. Campo, M. Griese, J. Hamilton, T. Wang, C. Morgan, V. Cottin, C. McCarthy. Pulmonary alveolar proteinosis. *Nat Rev Dis Primers.* 2019; 5: 16.
- 3 R. Borie, C. Danel, M. P. Debray, C. Taille, M. C. Dombret, M. Aubier, R. Epaud, B. Crestani. Pulmonary alveolar proteinosis. *Eur Respir Rev.* 2011; 20: 98-107.
- 4 F. Bonella, P. C. Bauer, M. Griese, S. Ohshimo, J. Guzman, U. Costabel. Pulmonary alveolar proteinosis: new insights from a single-center cohort of 70 patients. *Respir Med.* 2011; 105: 1908-16.
- 5 E. Briens, P. Delaval, M. P. Mairesse, D. Valeyre, B. Wallaert, R. Lazor, J. F. Cordier. Pulmonary alveolar proteinosis. *Rev Mal Respir.* 2002; 19: 166-82.
- 6 Y. Inoue, B. C. Trapnell, R. Tazawa, T. Arai, T. Takada, N. Hizawa, Y. Kasahara, K. Tatsumi, M. Hojo, T. Ichiwata, N. Tanaka, E. Yamaguchi, R. Eda, K. Oishi, Y. Tsuchihashi, C. Kaneko, T. Nukiwa, M. Sakatani, J. P. Krischer, K. Nakata. Characteristics of a large cohort of patients with autoimmune pulmonary alveolar proteinosis in Japan. *Am J Respir Crit Care Med.* 2008; 177: 752-62.
- 7 U. B. Prakash, S. S. Barham, H. A. Carpenter, D. E. Dines, H. M. Marsh. Pulmonary alveolar phospholipoproteinosis: experience with 34 cases and a review. *Mayo Clin Proc.* 1987; 62: 499-518.
- 8 J. F. Seymour, J. J. Presneill. Pulmonary alveolar proteinosis: progress in the first 44 years. *Am J Respir Crit Care Med.* 2002; 166: 215-35.
- 9 M. Griese, F. Bonella, U. Costabel, J. de Blic, N. B. Tran, G. Liebisch. Quantitative Lipidomics in Pulmonary Alveolar Proteinosis. *Am J Respir Crit Care Med.* 2019; 200: 881-7.
- 10 C. McCarthy, E. Lee, J. P. Bridges, A. Sallese, T. Suzuki, J. C. Woods, B. J. Bartholmai, T. Wang, C. Chalk, B. C. Carey, P. Arumugam, K. Shima, E. J. Tarling, B. C. Trapnell. Statin as a novel pharmacotherapy of pulmonary alveolar proteinosis. *Nat Commun.* 2018; 9: 3127.
- 11 A. Sallese, T. Suzuki, C. McCarthy, J. Bridges, A. Filuta, P. Arumugam, K. Shima, Y. Ma, M. Wessendarp, D. Black, C. Chalk, B. Carey, B. C. Trapnell. Targeting cholesterol homeostasis in lung diseases. *Sci Rep.* 2017; 7: 10211.
- 12 M. J. Thomassen, B. P. Barna, A. G. Malur, T. L. Bonfield, C. F. Farver, A. Malur, H. Dalrymple, M. S. Kavuru, M. Febbraio. ABCG1 is deficient in alveolar macrophages of GM-CSF knockout mice and patients with pulmonary alveolar proteinosis. *J Lipid Res.* 2007; 48: 2762-8.
- 13 E. Stanley, G. J. Lieschke, D. Grail, D. Metcalf, G. Hodgson, J. A. Gall, D. W. Maher, J. Cebon, V. Sinickas, A. R. Dunn. Granulocyte/macrophage colony-stimulating factor-deficient mice show no major perturbation of hematopoiesis but develop a characteristic pulmonary pathology. *Proc Natl Acad Sci U S A.* 1994; 91: 5592-6.
- 14 Y. Shibata, P. Y. Berclaz, Z. C. Chroneos, M. Yoshida, J. A. Whitsett, B. C. Trapnell. GM-CSF regulates alveolar macrophage differentiation and innate immunity in the lung through PU.1. *Immunity.* 2001; 15: 557-67.
- 15 T. L. Bonfield, B. Raychaudhuri, A. Malur, S. Abraham, B. C. Trapnell, M. S. Kavuru, M. J. Thomassen. PU.1 regulation of human alveolar macrophage differentiation requires granulocyte-macrophage colony-stimulating factor. *Am J Physiol Lung Cell Mol Physiol.* 2003; 285: L1132-6.
- 16 T. Sakagami, K. Uchida, T. Suzuki, B. C. Carey, R. E. Wood, S. E. Wert, J. A. Whitsett, B. C. Trapnell, M. Luisetti. Human GM-CSF autoantibodies and reproduction of pulmonary alveolar proteinosis. *N Engl J Med.* 2009; 361: 2679-81.

- 1
2
3 17 K. Uchida, D. C. Beck, T. Yamamoto, P. Y. Berclaz, S. Abe, M. K. Staudt, B. C. Carey,
4 M. D. Filippi, S. E. Wert, L. A. Denson, J. T. Puchalski, D. M. Hauck, B. C. Trapnell. GM-CSF
5 autoantibodies and neutrophil dysfunction in pulmonary alveolar proteinosis. *N Engl J Med.*
6 2007; 356: 567-79.
- 7
8 18 S. Jouneau, M. Kerjouan, E. Briens, J. P. Lenormand, C. Meunier, J. Letheulle, D.
9 Chiforeanu, C. Laine-Caroff, B. Desrues, P. Delaval. Pulmonary alveolar proteinosis. *Rev Mal*
10 *Respir.* 2014; 31: 975-91.
- 11 19 D. M. Hansell, A. A. Bankier, H. MacMahon, T. C. McLoud, N. L. Muller, J. Remy.
12 Fleischner Society: glossary of terms for thoracic imaging. *Radiology.* 2008; 246: 697-722.
- 13 20 S. E. Rossi, J. J. Erasmus, M. Volpacchio, T. Franquet, T. Castiglioni, H. P. McAdams.
14 "Crazy-paving" pattern at thin-section CT of the lungs: radiologic-pathologic overview.
15 *Radiographics.* 2003; 23: 1509-19.
- 16 21 K. Uchida, K. Nakata, B. C. Trapnell, T. Terakawa, E. Hamano, A. Mikami, I.
17 Matsushita, J. F. Seymour, M. Oh-Eda, I. Ishige, Y. Eishi, T. Kitamura, Y. Yamada, K.
18 Hanaoka, N. Keicho. High-affinity autoantibodies specifically eliminate granulocyte-
19 macrophage colony-stimulating factor activity in the lungs of patients with idiopathic
20 pulmonary alveolar proteinosis. *Blood.* 2004; 103: 1089-98.
- 21 22 K. Uchida, K. Nakata, B. Carey, C. Chalk, T. Suzuki, T. Sakagami, D. E. Koch, C.
23 Stevens, Y. Inoue, Y. Yamada, B. C. Trapnell. Standardized serum GM-CSF autoantibody
24 testing for the routine clinical diagnosis of autoimmune pulmonary alveolar proteinosis. *J*
25 *Immunol Methods.* 2014; 402: 57-70.
- 26 23 T. L. Bonfield, M. S. Kavuru, M. J. Thomassen. Anti-GM-CSF titer predicts response to
27 GM-CSF therapy in pulmonary alveolar proteinosis. *Clin Immunol.* 2002; 105: 342-50.
- 28 24 T. Kitamura, K. Uchida, N. Tanaka, T. Tsuchiya, J. Watanabe, Y. Yamada, K. Hanaoka,
29 J. F. Seymour, O. D. Schoch, I. Doyle, Y. Inoue, M. Sakatani, S. Kudoh, A. Azuma, T. Nukiwa,
30 T. Tomita, M. Katagiri, A. Fujita, A. Kurashima, S. Kanegasaki, K. Nakata. Serological
31 diagnosis of idiopathic pulmonary alveolar proteinosis. *Am J Respir Crit Care Med.* 2000; 162:
32 658-62.
- 33 25 K. Uchida, K. Nakata, T. Suzuki, M. Luisetti, M. Watanabe, D. E. Koch, C. A. Stevens,
34 D. C. Beck, L. A. Denson, B. C. Carey, N. Keicho, J. P. Krischer, Y. Yamada, B. C. Trapnell.
35 Granulocyte/macrophage-colony-stimulating factor autoantibodies and myeloid cell immune
36 functions in healthy subjects. *Blood.* 2009; 113: 2547-56.
- 37 26 M. S. Kavuru, A. Malur, I. Marshall, B. P. Barna, M. Meziane, I. Huizar, H. Dalrymple,
38 R. Karnekar, M. J. Thomassen. An open-label trial of rituximab therapy in pulmonary alveolar
39 proteinosis. *Eur Respir J.* 2011; 38: 1361-7.
- 40 27 F. C. Lin, G. D. Chang, M. S. Chern, Y. C. Chen, S. C. Chang. Clinical significance of
41 anti-GM-CSF antibodies in idiopathic pulmonary alveolar proteinosis. *Thorax.* 2006; 61: 528-
42 34.
- 43 28 K. N. Lee, D. L. Levin, W. R. Webb, D. Chen, M. L. Storto, J. A. Golden. Pulmonary
44 alveolar proteinosis: high-resolution CT, chest radiographic, and functional correlations. *Chest.*
45 1997; 111: 989-95.
- 46 29 R. Tazawa, T. Ueda, M. Abe, K. Tatsumi, R. Eda, S. Kondoh, K. Morimoto, T. Tanaka,
47 E. Yamaguchi, A. Takahashi, M. Oda, H. Ishii, S. Izumi, H. Sugiyama, A. Nakagawa, K. Tomii,
48 M. Suzuki, S. Konno, S. Ohkouchi, N. Tode, T. Handa, T. Hirai, Y. Inoue, T. Arai, K. Asakawa,
49 T. Sakagami, A. Hashimoto, T. Tanaka, T. Takada, A. Mikami, N. Kitamura, K. Nakata. Inhaled
50 GM-CSF for Pulmonary Alveolar Proteinosis. *N Engl J Med.* 2019; 381: 923-32.
- 51 30 B. C. trapnell. Clinical features of autoimmune pulmonary alveolar proteinosis from a
52 large international patient cohort: baseline data from the IMPALA trial. *Eur Respir J.* 2019:
53 OA5328.
54
55
56
57
58
59
60

- 1
2
3 31 A. D. Punatar, S. Kusne, J. E. Blair, M. T. Seville, H. R. Vikram. Opportunistic
4 infections in patients with pulmonary alveolar proteinosis. *J Infect.* 2012; 65: 173-9.
- 5 32 M. Akira, Y. Inoue, T. Arai, C. Sugimoto, S. Tokura, K. Nakata, M. Kitaichi, G. Osaka
6 Respiratory Diseases Symposia. Pulmonary Fibrosis on High-Resolution CT of Patients With
7 Pulmonary Alveolar Proteinosis. *AJR Am J Roentgenol.* 2016; 207: 544-51.
- 8 33 M. Luisetti, P. Bruno, Z. Kadija, T. Suzuki, S. Raffa, M. R. Torrisi, I. Campo, F.
9 Mariani, E. Pozzi, B. C. Trapnell, S. Mariotta. Relationship between diffuse pulmonary fibrosis,
10 alveolar proteinosis, and granulocyte-macrophage colony stimulating factor autoantibodies.
11 *Respir Care.* 2011; 56: 1608-10.
- 12 34 S. Marchand-Adam, B. Diot, P. Magro, A. De Muret, C. Guignabert, C. Kannengiesser,
13 A. Londono-Vallejo, I. Draskovic, A. Toutain, P. Diot. Pulmonary alveolar proteinosis
14 revealing a telomerase disease. *Am J Respir Crit Care Med.* 2013; 188: 402-4.
- 15 35 X. Sui, Q. Du, K. F. Xu, X. Tian, L. Song, X. Wang, X. Xu, Z. Wang, Y. Wang, J. Gu,
16 W. Song, Z. Jin. Quantitative assessment of Pulmonary Alveolar Proteinosis (PAP) with ultra-
17 dose CT and correlation with Pulmonary Function Tests (PFTs). *PLoS One.* 2017; 12:
18 e0172958.
- 19 36 S. Tokura, M. Akira, T. Okuma, R. Tazawa, T. Arai, C. Sugimoto, A. Matsumuro, M.
20 Hirose, T. Takada, K. Nakata, H. Ishii, Y. Kasahara, M. Hojo, S. Ohkouchi, Y. Tsuchihashi,
21 M. Yokoba, R. Eda, H. Nakayama, T. Nei, K. Morimoto, Y. Nasuhara, M. Ebina, T. Ichiwata,
22 K. Tatsumi, E. Yamaguchi, Y. Inoue. A Semiquantitative Computed Tomographic Grading
23 System for Evaluating Therapeutic Response in Pulmonary Alveolar Proteinosis. *Ann Am
24 Thorac Soc.* 2017; 14: 1403-11.
- 25 37 T. E. Robinson, B. C. Trapnell, M. L. Goris, L. M. Quittell, D. N. Cornfield. Quantitative
26 analysis of longitudinal response to aerosolized granulocyte-macrophage colony-stimulating
27 factor in two adolescents with autoimmune pulmonary alveolar proteinosis. *Chest.* 2009; 135:
28 842-8.
- 29 38 R. Tazawa, B. C. Trapnell, Y. Inoue, T. Arai, T. Takada, Y. Nasuhara, N. Hizawa, Y.
30 Kasahara, K. Tatsumi, M. Hojo, H. Ishii, M. Yokoba, N. Tanaka, E. Yamaguchi, R. Eda, Y.
31 Tsuchihashi, K. Morimoto, M. Akira, M. Terada, J. Otsuka, M. Ebina, C. Kaneko, T. Nukiwa,
32 J. P. Krischer, K. Akazawa, K. Nakata. Inhaled granulocyte/macrophage-colony stimulating
33 factor as therapy for pulmonary alveolar proteinosis. *Am J Respir Crit Care Med.* 2010; 181:
34 1345-54.
- 35 39 C. McCarthy, M. Kokosi, F. Bonella. Shaping the future of an ultra-rare disease: unmet
36 needs in the diagnosis and treatment of pulmonary alveolar proteinosis. *Curr Opin Pulm Med.*
37 2019; 25: 450-8.
- 38 40 J. Ramirez, W. Nyka, L. J. Mc. Pulmonary alveolar proteinosis. Diagnostic technics and
39 observations. *N Engl J Med.* 1963; 268: 165-71.
- 40 41 J. D. Smith, J. E. Millen, P. Safar, E. D. Robin. Intrathoracic pressure, pulmonary
41 vascular pressures and gas exchange during pulmonary lavage. *Anesthesiology.* 1970; 33: 401-
42 5.
- 43 42 P. L. Shah, D. Hansell, P. R. Lawson, K. B. Reid, C. Morgan. Pulmonary alveolar
44 proteinosis: clinical aspects and current concepts on pathogenesis. *Thorax.* 2000; 55: 67-77.
- 45 43 P. Gay, B. Wallaert, S. Nowak, J. Yserbyt, S. Anevlavis, C. Hermant, A. Lovis, O.
46 Menard, B. Maitre, T. Vandemoortele, H. Dutau, A. Briault, A. Bourdin, J. M. Vergnon, M. E.
47 Froudarakis, F. Groupe d'Endoscopie Thoracique de Langue. Efficacy of Whole-Lung Lavage
48 in Pulmonary Alveolar Proteinosis: A Multicenter International Study of GELF. *Respiration.*
49 2017; 93: 198-206.
- 50 44 I. Campo, M. Luisetti, M. Griese, B. C. Trapnell, F. Bonella, J. C. Grutters, K. Nakata,
51 C. H. Van Moorsel, U. Costabel, V. Cottin, T. Ichiwata, Y. Inoue, A. Braschi, G. Bonizzoni, G.
52
53
54
55
56
57
58
59
60

- 1
2
3 A. Iotti, C. Tinelli, G. Rodi, W. L. L. I. S. Group. A Global Survey on Whole Lung Lavage in
4 Pulmonary Alveolar Proteinosis. *Chest*. 2016; 150: 251-3.
5 45 G. P. Genereux. Lipids in the lungs: radiologic-pathologic correlation. *J Can Assoc*
6 *Radiol*. 1970; 21: 2-15.
7 46 G. Michaud, C. Reddy, A. Ernst. Whole-lung lavage for pulmonary alveolar proteinosis.
8 *Chest*. 2009; 136: 1678-81.
9 47 G. Sheng, P. Chen, Y. Wei, J. Chu, X. Cao, H. L. Zhang. Better approach for
10 autoimmune pulmonary alveolar proteinosis treatment: inhaled or subcutaneous granulocyte-
11 macrophage colony-stimulating factor: a meta-analysis. *Respir Res*. 2018; 19: 163.
12 48 R. Borie, M. P. Debray, C. Laine, M. Aubier, B. Crestani. Rituximab therapy in
13 autoimmune pulmonary alveolar proteinosis. *Eur Respir J*. 2009; 33: 1503-6.
14 49 A. Malur, M. S. Kavuru, I. Marshall, B. P. Barna, I. Huizar, R. Karnekar, M. J.
15 Thomassen. Rituximab therapy in pulmonary alveolar proteinosis improves alveolar
16 macrophage lipid homeostasis. *Respir Res*. 2012; 13: 46.
17 50 B. Soyeze, R. Borie, C. Menard, J. Cadranet, L. Chavez, V. Cottin, E. Gomez, S.
18 Marchand-Adam, S. Leroy, J. M. Naccache, H. Nunes, M. Reynaud-Gaubert, L. Savale, A.
19 Tazi, L. Wemeau-Stervinou, M. P. Debray, B. Crestani. Rituximab for auto-immune alveolar
20 proteinosis, a real life cohort study. *Respir Res*. 2018; 19: 74.
21 51 B. Garber, J. Albores, T. Wang, T. H. Neville. A plasmapheresis protocol for refractory
22 pulmonary alveolar proteinosis. *Lung*. 2015; 193: 209-11.
23 52 A. Jezequel, M. Kerjouan, M. Lederlin, C. Laine-Caroff, C. Camus, P. Delaval, S.
24 Jouneau. Plasmapheresis failure in the treatment of auto-immune pulmonary alveolar
25 proteinosis. *Rev Mal Respir*. 2017; 34: 240-3.
26 53 M. S. Kavuru, T. L. Bonfield, M. J. Thomassen. Plasmapheresis, GM-CSF, and alveolar
27 proteinosis. *Am J Respir Crit Care Med*. 2003; 167: 1036; author reply -7.
28 54 M. Luisetti, G. Rodi, C. Perotti, I. Campo, F. Mariani, E. Pozzi, B. C. Trapnell.
29 Plasmapheresis for treatment of pulmonary alveolar proteinosis. *Eur Respir J*. 2009; 33: 1220-
30 2.
31 55 H. Yu, X. Sun, Y. Wang, Z. Xu, H. Huang. Whole lung lavage combined with
32 Granulocyte-macrophage colony stimulating factor inhalation for an adult case of refractory
33 pulmonary alveolar proteinosis. *BMC Pulm Med*. 2014; 14: 87.
34 56 L. A. Parker, D. B. Novotny. Recurrent alveolar proteinosis following double lung
35 transplantation. *Chest*. 1997; 111: 1457-8.
36 57 A. A. Gal, J. A. Bryan, K. R. Kanter, E. C. Lawrence. Cytopathology of pulmonary
37 alveolar proteinosis complicating lung transplantation. *J Heart Lung Transplant*. 2004; 23: 135-
38 8.
39 58 S. A. Yousem. Alveolar lipoproteinosis in lung allograft recipients. *Hum Pathol*. 1997;
40 28: 1383-6.
41 59 S. Ikeda, A. Sekine, T. Baba, T. Katano, H. Yamakawa, T. Oda, T. Iwasawa, M.
42 Matsumura, T. Takemura, T. Ogura. Secondary pulmonary alveolar proteinosis predominant in
43 the transplanted lung in patients with idiopathic interstitial pneumonia: an autopsy case. *J*
44 *Thorac Dis*. 2018; 10: E351-E4.
45 60 S. L. Pedroso, L. S. Martins, S. Sousa, A. Reis, L. Dias, A. C. Henriques, A. M.
46 Sarmiento, A. Cabrita. Pulmonary alveolar proteinosis: a rare pulmonary toxicity of sirolimus.
47 *Transpl Int*. 2007; 20: 291-6.
48 61 K. R. Flaherty, A. U. Wells, V. Cottin, A. Devaraj, S. L. F. Walsh, Y. Inoue, L. Richeldi,
49 M. Kolb, K. Tetzlaff, S. Stowasser, C. Coeck, E. Clerisme-Beaty, B. Rosenstock, M. Quaresma,
50 T. Haeufel, R. G. Goeldner, R. Schlenker-Herceg, K. K. Brown, I. T. Investigators. Nintedanib
51 in Progressive Fibrosing Interstitial Lung Diseases. *N Engl J Med*. 2019; 381: 1718-27.
52
53
54
55
56
57
58
59
60

Tables**Table 1:** Differential diagnosis of the « crazy paving » pattern (classical / main causes).²⁰

Classical causes	Pulmonary alveolar proteinosis Pulmonary hemorrhage Invasive mucinous adenocarcinoma Pulmonary MALT lymphoma Lipoid pneumonia
-------------------------	---

MALT: mucosa-associated lymphoid tissue.

Table 2: Commonly reported opportunistic infection in patients with PAP (Punatar et al.)³¹.

Pathogen, n (%)	Total (n=75)
Nocardia (n=32)	<i>N. asteroides</i> , 19 (59 %) <i>N. brasiliensis</i> , 1 (3 %) <i>N. farcinica</i> , 1 (3 %) <i>Nocardia spp.</i> , 11 (34 %)
Mycobacteria (n=28)	<i>M. tuberculosis</i> , 21 (75 %) <i>M. kansasii</i> , 4 (14 %) <i>M. avium intracellulare</i> , 3 (11 %)
Fungi (n=15)	<i>Aspergillus spp.</i> , 4 (27 %) <i>Cryptococcus spp.</i> , 5 (33 %) <i>Histoplasma capsulatum</i> , 4 (27 %) <i>Aspergillus spp. et Cryptococcus spp.</i> , 1 (7 %) <i>Zygomycetes</i> , 1 (7 %)

Figure legends:**Figure 1:**

Illustration of pathophysiology of autoimmune PAP adapted from Trapnell et al.² The alveolar surfactant homeostasis is represented in normal subjects (A) and in autoimmune PAP patients (B).

In normal subjects, pulmonary surfactant is synthesized and secreted by type II alveolar epithelial cells (AEC2), and forms a thin layer of polar lipids (mostly phospholipids) with low amounts of neutral lipids (mostly cholesterol) as well as surfactant-associated proteins located at the alveolar air–liquid interface. AEC2 cells also participate in the degradation and recycling of surfactant. Alveolar macrophages are also involved in the clearance and catabolism of surfactant by degrading phospholipids and promoting reverse transport of cholesterol to the liver.²

In PAP patients, neutralizing polyclonal anti-GM-CSF antibodies block GM-CSF binding to its receptor and subsequent downstream signaling altering multiples genes and mechanisms in alveolar macrophages. This leads in a reduction in cholesterol efflux by alveolar macrophages and impaired surfactant clearance from the alveolar surface. Eventually, lipid droplets containing esterified cholesterol accumulate within macrophages, resulting in the formation of foam cells.

GM-CSF: granulocyte-macrophage colony-stimulating factor; SP-B: surfactant protein B; SP-C: surfactant protein C.

Figure 2:

Auto-immune PAP in a 37-year old man with progressive dyspnea, cough and expectoration. A. Plain chest radiograph showing bilateral central symmetrical lung opacities with relative apical and costophrenic angle sparing (“batwing opacities”). B, C. HRCT showing a typical crazy paving appearance (superimposition of ground glass opacities, interlobular and intralobular septal lines).

Figure 3:

Auto-immune PAP in a 46-year old woman with dyspnea on exertion. A. Plain chest radiograph showing a reticulonodular syndrome with a patchy distribution. B, C, D. HRCT showing geographic or lobular distribution of “crazy paving” (i.e. sharply demarcated from the surrounding normal lung).

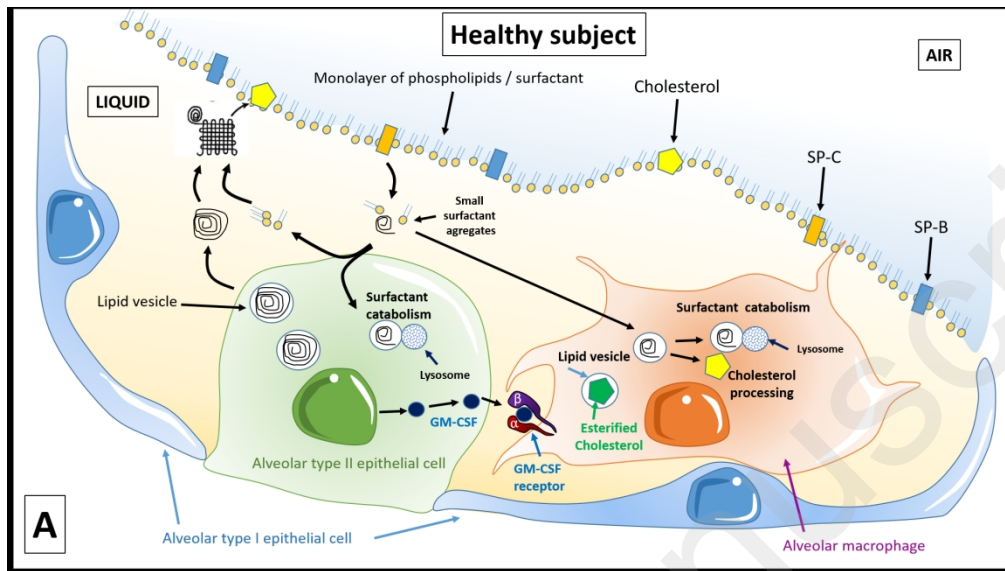
Figure 4:

Milky aspect of broncho-alveolar lavage with sedimentation of lipoproteinaceous material from a patient with PAP.

Figure 5:

Liquid collected by whole lung lavage (WLL) (one lung) from a patient with autoimmune PAP. The turbidity decreases from the right (start of the procedure) to left (end). The amount of lipoproteinaceous material deposited at the bottom of the jars decreases during WLL.

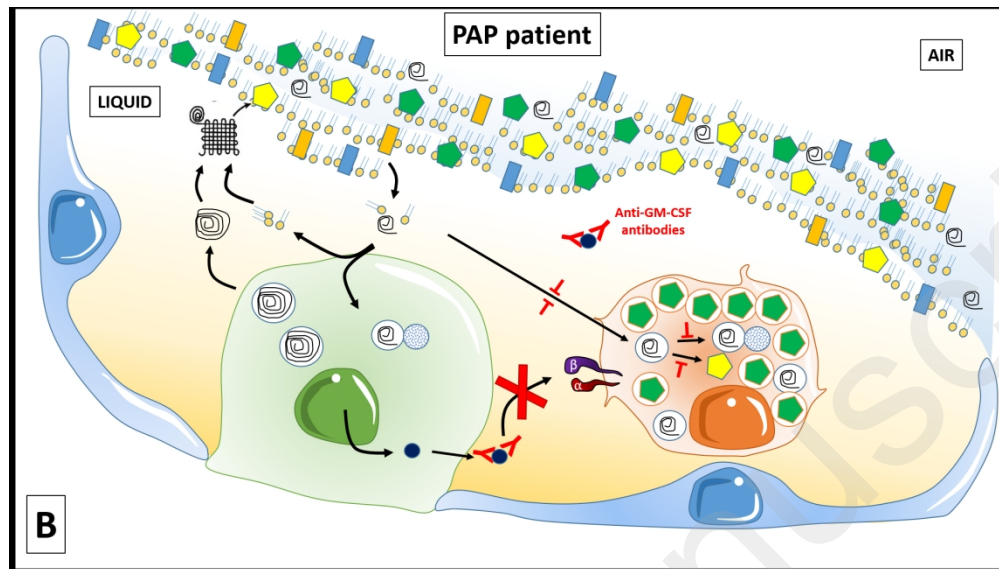
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



New figure 1A

340x193mm (150 x 150 DPI)

Accepted Manuscript

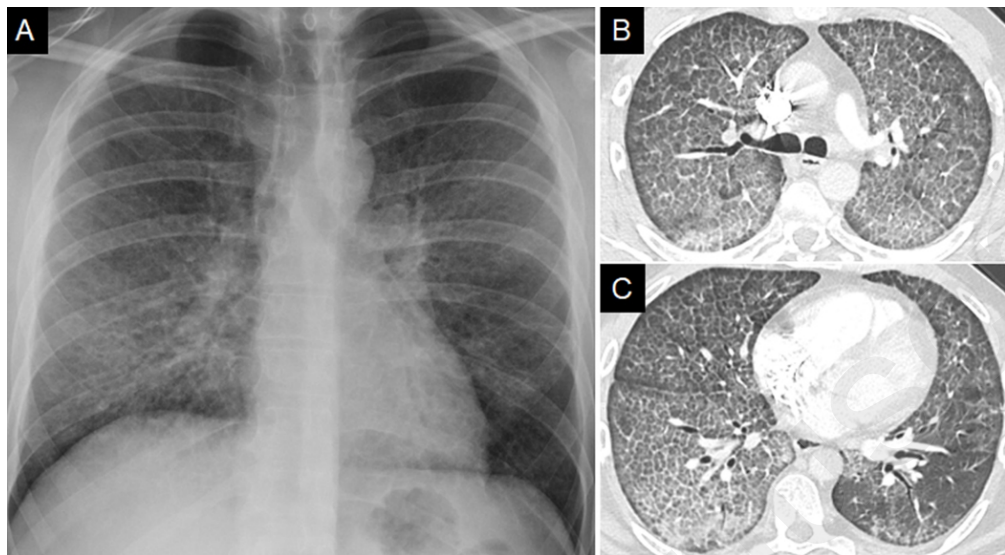


New figure 1B

340x193mm (150 x 150 DPI)

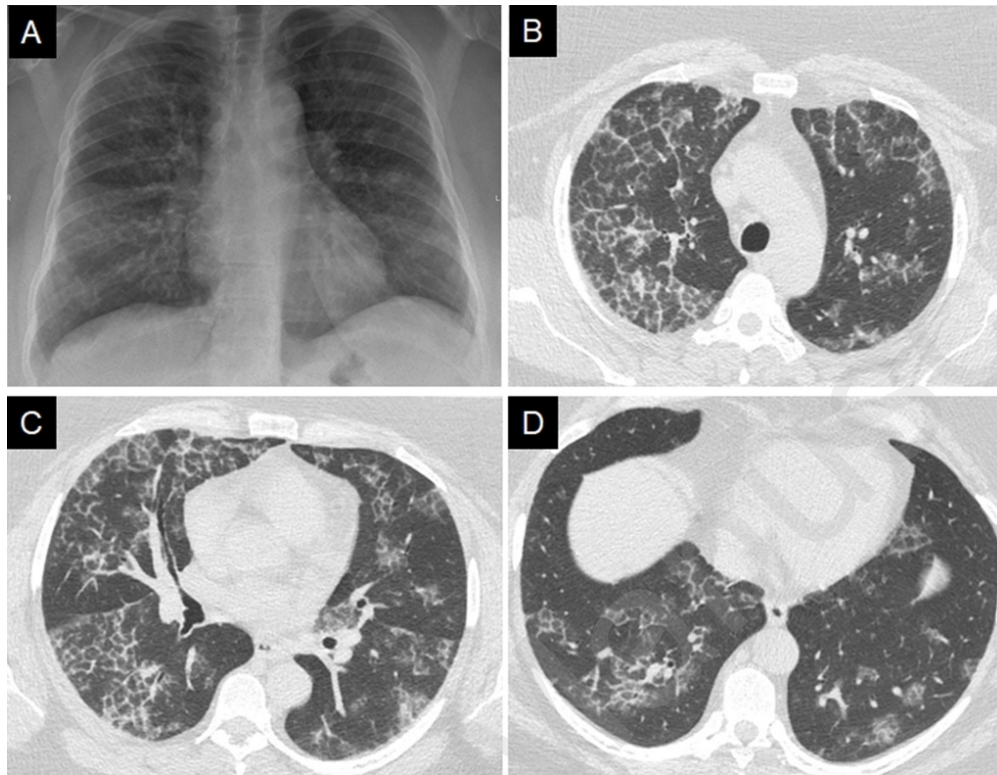
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



177x97mm (150 x 150 DPI)

Accepted Manuscript



173x133mm (150 x 150 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



142x190mm (150 x 150 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



254x190mm (150 x 150 DPI)

Accepted Manuscript