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Better approach for autoimmune pulmonary alveolar proteinosis treatment: inhaled or subcutaneous granulocyte-macrophage colony-stimulating factor: a meta-analyses

Gaohong Sheng[1†], Peng Chen[2,3†], Yanqiu Wei[1], Jiaojiao Chu[1], Xiaolei Cao[4] and Hui-Lan Zhang[1*]

Abstract

Background: Autoimmune pulmonary alveolar proteinosis (aPAP) is a rare pulmonary disease caused by functional deficiency of granulocyte-macrophage colony-stimulating factor (GM-CSF). GM-CSF therapy in aPAP has been reported effective in some studies. This meta-analyses aimed to evaluate whether GM-CSF therapy, including inhaled and subcutaneous GM-CSF have therapeutic effect in aPAP patients.

Methods: We analyzed 10 studies searched from PubMed, EmBase, Web of Science, Wiley Online Library and Cochrane Collaboration databases to evaluate the pooled effects of GM-CSF treatment in aPAP patients.

Results: Ten observational studies involving 115 aPAP patients were included. The pooled analyses of response rate (81%, $p < 0.001$), relapse rate (22%, $p = 0.009$), PaO_2 (13.76 mmHg, $p < 0.001$) and $P(A-a)O_2$ (19.44 mmHg, $p < 0.001$) showed that GM-CSF treatment was effective on aPAP patients. Further analyses showed that inhaled GM-CSF treatment was more effective than subcutaneous GM-CSF therapy, including a higher response rate (89% vs. 71%, $p = 0.023$), more improvements in PaO_2 (21.02 mmHg vs. 8.28 mmHg, $p < 0.001$) and $P(A-a)O_2$ (19.63 mmHg vs. 9.15 mmHg, $p < 0.001$).

Conclusions: As two routes of exogenous GM-CSF treatment, inhaled and subcutaneous were both proven to have effect on aPAP patients. Furthermore, inhaled GM-CSF therapy showed a higher response rate, more improvements on PaO_2 and $P(A-a)O_2$ than subcutaneous GM-CSF treatment in aPAP patients, suggesting inhaled GM-CSF therapy could have more benefits on aPAP patients. Therefore, GM-CSF therapy, especially inhaled GM-CSF, might be a promising therapeutic option in treating aPAP.

Keywords: Autoimmune pulmonary alveolar proteinosis, Granulocyte-macrophage colony-stimulating factor, Inhaled GM-CSF, Subcutaneous GM-CSF, Meta-analyses

* Correspondence: huilanz_76@163.com
†Gaohong Sheng and Peng Chen contributed equally to this work.
[1]Department of Respiratory and Critical Care Medicine, Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology, No.1095, Jie Fang Road, Han Kou District, Wu Han 430030, Hu Bei Province, China
Full list of author information is available at the end of the article

Background

Pulmonary alveolar proteinosis (PAP), first described by Rosen et al. in 1958 [1], is a rare lung disease characterized by deposition of lipoproteinaceous-rich materials within the alveoli, whose annual prevalence was estimated to be 3.7–6.2 per million [2, 3]. The accumulation of lipoproteinaceous-rich materials was caused by the disability of macrophages to clear alveolar surfactants, which could result in restrictive pulmonary ventilation dysfunction, decreased diffusion capacity, and even could progress to respiratory failure [3–5].

Approximate 90% PAP cases were autoimmune pulmonary alveolar proteinosis (aPAP) characterized by elevated levels of antibodies against granulocyte-macrophage colony-stimulating factor (GM-CSF) in blood and alveoli, and the antibodies could not be detected in health controls [6–9]. In recent years, researchers observed lower ability of alveolar macrophages to clear alveolar surfactants in GM-CSF deficiency mice, and phenotypes similar to aPAP patients histologically were developed in these models [10–12].

Whole lung lavage (WLL) has been used as the standard treatment for aPAP for decades. However, the performance of WLL therapy was limited due to the requirement of anesthesia and invasive procedure [13]. In recent years, some studies reported that inhaled or subcutaneous GM-CSF administration was effective in aPAP patients. Nevertheless, it is debatable whether either or both routes of GM-CSF therapy is effective, and further, which route could achieve better effect.

The purpose of this meta-analyses was to evaluate the effect of GM-CSF therapy including inhaled and subcutaneous routes in aPAP patients.

Methods

Search strategy and study selection

The search flow diagram of included studies for this meta-analyses is shown in Fig. 1. We performed our meta-analyses according to the standards set forth by the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement [14]. We searched PubMed, EmBase, Web of Science, Wiley Online Library
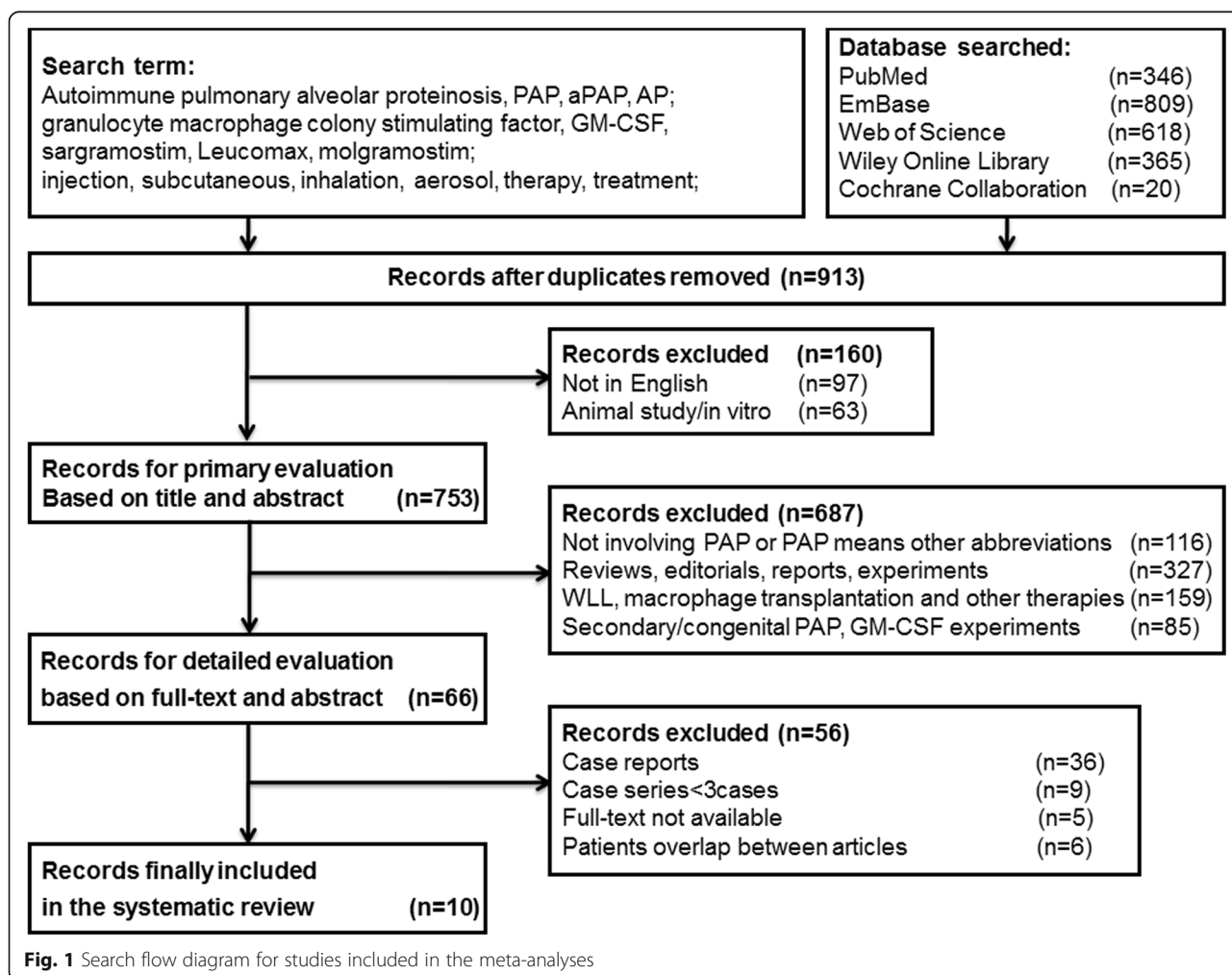

Fig. 1 Search flow diagram for studies included in the meta-analyses

and Cochrane Collaboration databases, conference proceedings, trial registers, and other unpublished studies with the terms "Autoimmune pulmonary alveolar proteinosis", "granulocyte macrophage colony stimulating factor", "PAP", "aPAP", "GM-CSF", "sargramostim", "leucomax", "molgramostim", "injection", "subcutaneous", "inhalation", "aerosol", "therapy", and "treatment". The included studies were published in English from 1 January 1996 to 30 October 2017, since the first patient receiving GM-CSF therapy was reported in the year of 1996 [15]. The relevant studies in references were also searched manually.

Inclusion criteria
We included both controlled and uncontrolled studies in English using GM-CSF therapy in aPAP patients aged 18 years or older. The aPAP patients were diagnosed by open lung biopsy or transbronchial lung biopsy, and anti GM-CSF antibody level was examined. The minimum time for GM-CSF therapy should be more than 4 weeks, because there were delays to observe the effect of GM-CSF therapy [16–19].

Exclusion criteria
Patients diagnosed with secondary or congenital PAP were excluded. In addition, studies not reporting the outcomes of interest, studies with < 3 participants or follow-up time < 3 months were excluded.

A total of 817 studies met the above selection criteria. In the process of screening, 45 case reports were excluded due to low number of participants ($n < 3$). In addition, six studies reporting the same population as other studies were also excluded. And one study was excluded as participants were less than 18-year old. Furthermore, five studies were excluded because their full texts were not available. At last, 10 observational studies satisfying the criteria were included in this meta-analyses.

Data extraction
Two researchers (Gaohong Sheng and Peng Chen) independently performed literature search and extracted the data from the published studies according to prespecified inclusion/exclusion criteria.

The studies were included or excluded when both researchers made the same judgement for inclusion or exclusion. Disagreements between them were resolved by arbitration of principal investigator (Hui-Lan Zhang). Data on study characteristics, baseline characteristics of patients, and clinical endpoints were extracted and analyzed.

Endpoint definition
The endpoints of this study were alveolar oxygen partial pressure (PaO_2), alveolar-arterial oxygen gradient

($P(A-a)O_2$), pulmonary function, and disease severity score (DSS) before and after the treatment of GM-CSF. Through synthesizing these parameters, we evaluated therapeutic effects and calculated the response/relapse rate based on the number of response/relapse patients provided in each study. The response criteria were considered as the improvement in PaO_2, $P(A-a)O_2$, pulmonary function, radiology, or symptoms. During the follow-up period, we considered the responders as relapse when these parameters were pejorative.

Quality assessment
The quality of all included studies was assessed using the Agency for Healthcare Review and Quality (AHRQ) criteria [20]. Each study was assessed by two investigators independently. According to AHRQ score, the included studies were categorized high quality (score > 67), moderate quality (score = 50–66), and low quality (score < 50). Only high and moderate quality studies were eligible for this meta-analyses.

Data analysis
Data on studies and patients were collected by the two investigators independently. The response and relapse rates were assessed by calculating proportion with 95% confidence intervals (CI) for each study. PaO_2 and $P(A-a)O_2$ were analyzed using weighted mean difference (WMD) with 95% CI. Random effects model was performed to compute the pooled analyses because of the large variety in the GM-CSF therapy. The heterogeneity of the studies was assessed using Cochran'Q statistic, and the magnitude of heterogeneity was estimated using the I^2 statistic [21]. The impact of small sample studies on the pooled result was evaluated by comparing the results of the fixed to random effects model. Sensitivity analyses were performed to examine stability of pooled outcome. Publication bias was assessed using the funnel plot, Egger's regression asymmetry test and Begg's adjusted rank correlation test. In addition, we performed Meta regression analyses with single and multiple covariate based on the age of patients, proportions of men, proportions of smokers, and proportions of patients received WLL.

We also realized that different GM-CSF therapy routes might achieve different effects. Therefore, subgroup analyses was performed according to the routes of GM-CSF therapy. Random effects model was performed in each subgroup to compute the pooled analyses, and differences between the subgroups were analyzed. In addition, we also conducted subgroup analyses according to the proportions of WLL therapy, age of patients, proportions of men and proportions of smokers.

All comparisons were two-tailed, and $p < 0.05$ were recognized as statistical significance. STATA software 14.0 was used to perform statistical analyses.

Results

Study characteristics

Ultimately, 10 observational studies were included in this meta-analyses. In these studies, participants in 5 studies received subcutaneous GM-CSF therapy [17, 22–25], and patients in the other 5 studies received inhaled GM-CSF therapy [26–30]. The 10 included studies were published from 2000 to 2016 with follow-up time from 4 months to 10 years. Among them, there were 4 prospective studies and 6 retrospective studies. All the patients in the 10 studies were diagnosed by lung biopsy or bronchoalveolar lavage or computed tomography. More details of the study characteristics were shown in Additional file 1: Table S1. All 10 included studies were eligible to further analyses according to the AHRQ criteria (Additional file 1: Table S2).

Patient characteristics

A total of 115 patients were included in this meta-analyses, the baseline characteristics of the patients in the included studies were listed in Table 1. The mean age of patients was 46.9 years (95% CI, 45.5–48.4). The mean proportion of men was 60%. On average, 52% patients had the history of smoking, and 57% received WLL therapy combined with GM-CSF. No serious side effects were observed during the course of treatment or follow-up period. And the mean follow-up duration was 3.2 years (95% CI, 2.9–3.5). Data on PaO_2, $P(A\text{-}a)O_2$, pulmonary function and the severity of symptoms of the included studies were listed in Additional file 1: Table S3.

Response rate

In a total of 115 aPAP patients, 77 patients were showed response to GM-CSF therapy. The pooled response rate for all 10 included studies was 81% (95% CI: 65–96%, $p < 0.001$) (Fig. 2a). The degree of heterogeneity was I^2: 72.1% and $p < 0.001$. The funnel plot (Additional file 1: Figure S1a) showed no publication bias by visual qualitative evaluation, and the quantitative assessments with Begg's test ($p = 0.47$) (Additional file 1: Figure S1b) and Egger's test ($p = 0.68$) (Additional file 1: Figure S1c). There were no obvious influences caused by small sample studies (Additional file 1: Figure S2) and each single study through sensitivity analyses (Additional file 1: Figure S1d).

Relapse rate

In a total of 115 aPAP patients, 18 patients relapsed during GM-CSF therapy or follow-up period. The pooled relapse rate was 22% (95% CI: 6–39%, $p = 0.009$) (Fig. 2b). The degree of heterogeneity was I^2: 72.2% and $p < 0.001$. The funnel plot (Additional file 1: Figure S3a) showed no visible publication bias by qualitative evaluation, and the quantitative assessments with Begg's test ($p = 0.18$) (Additional file 1: Figure S3b) and Egger's test ($p = 0.17$) (Additional file 1: Figure S3c). There were no obvious influences caused by small sample studies (Additional file 1: Figure S4) and each single study through sensitivity analyses (Additional file 1: Figure S3d).

Alveolar oxygen partial pressure (PaO$_2$)

Five studies provided the data of PaO_2 pre- and post-GM-CSF therapy in total 55 patients. Compared to baseline PaO_2, the PaO_2 after GM-CSF therapy was improved by 13.76 mmHg (95% CI: 7.81–19.72, $p < 0.001$) (Fig. 2c). The degree of heterogeneity was I^2: 89.6% and $p < 0.001$. The funnel plot (Additional file 1: Figure S5a) showed no publication bias by visual qualitative evaluation. And no publication bias was found by Begg's test ($p = 0.639$) (Additional file 1: Figure S5b) and Egger's test ($p = 0.806$) (Additional file 1: Figure S5c). No statistical differences were found by sensitivity analyses (Additional file 1: Figure S5d).

Alveolar-arterial oxygen gradient (P(A-a)O$_2$)

The data on $P(A\text{-}a)O_2$ pre- and post-GM-CSF therapy were available in 8 studies with a total of 103 patients. Through synthetic analyses, one study [24] was obviously different with others, but there was no significant impact of this study on the pooled effect by sensitivity analysis (Additional file 1: Figure S6d). At last, we analyzed the whole 8 studies to evaluate the reduction of $P(A\text{-}a)O_2$. And the $P(A\text{-}a)O_2$ was decreased by 19.44 mmHg (95% CI: 13.42–25.46, $p < 0.001$) (Fig. 2d) between pre- and post- GM-CSF therapy. The degree of heterogeneity was I^2: 95.9% and $p < 0.001$. The funnel plot (Additional file 1: Figure S6a) showed that one study [24] was visually asymmetrical. However, the quantitative assessments showed that no publication bias was found by Begg's test ($p = 0.187$) (Additional file 1: Figure S6b) and Egger's test ($p = 0.266$) (Additional file 1: Figure S6c). Thus, we believed there was no publication bias in the pooled analyses.

We also performed analyses on diffusing capacity of the lung for carbon monoxide (DLCO), total lung capacity (TLC), vital capacity (VC), forced expiratory volume in one second (FEV1), forced vital capacity (FVC), DSS, and 6 min' walk distance (6MWD) (Additional file 1: Figure S8), the details were shown in supplements.

Table 1 Baseline characteristics of all included patients

Study author/ year	No.	Sex, M/F	Age, y	Ever smoking	Anti GM-CSF Ab	GM-CSF dose	GM-CSF duration	WLL	Response	Relapse	Side effect, N
Subcutaneous											
Kavuru et al. [22]	4	4/0	34.3 ± 11.2	3	NA	1-4wk, 250μg/d; 5-8wk, up to 5μg/kg/d; 9th wk, up to 7-9μg/kg/d	12 wk	3	3	0	nausea and emisis, 1
Seymour et al. [17]	14	9/5	33 (14–78)	7	12[a]	1-5d 3.0μg/kg/d; 6th d, up to 5.0μg/kg/d; if no response, up to 7.5-30 μg/kg/d	12 wk.	10	6	5	neutropenia, 1; first dose effect, 4; local erythema, 6; headache, 1; fever, 1; asymptomatic splenomegaly 1
Venkateshiah et al. [23]	25	18/7	45 (21–57)	13	25	1-4wk, 250μg/d; 5-8wk, up to 5μg/kg/d; 9th wk, up to 9μg/kg/d; if no response or suboptimal, up to 9-18μg/kg/d	52 wk	21	12	4	erythema, 18; fatigue, 7; fever, 4; dyspnea, 10; injection-site edema, 12
Khan et al. [25]	4	3/1	40 ± 5.2	NA	NA	5μg/kg/d	12 wk	3	4	0	NA
Hadda et al. [24]	3	0/3	48.3 ± 22	NA	2[a]	3-5 μg/kg/d	6 wk	3	3	0	neutrophilic leukocytosis, 1
Inhaled											
Wylam et al. [26]	12	7/5	42.8 (22–63)	8	6[a]	250μg bid every other week; if no response up to 500μg bid	32 wk	2	11	5	no side effects
Tazawa et al. [27]	39	22/17	56 (46–63)	21	39	high dose: 1-8d, 250μg/d, 9-14d none, six 2-wk cycles; low dose: 1-4d, 125μg/d, 5-14d none, six 2-wk cycles	24 wk	11	24	1	total, 7; fever, 1; respiratory infection,1; otitis media, 1; gastric ulcer, 1; diarrhea, 1; pneumonia, 1; tuberculous lymphadenitis, 1
Papiris et al. [28]	6	1/5	43.8 ± 15.7	2	6	1-4d, 250μg/d, 5-8d none, as long as necessary; if remission, dose down; if relapse, dose up;	14–65 mo	5	6	2	no significant adverse effects
Tazawa et al. [29]	3	1/2	54.7 ± 3.2	2	3	125μg, bid, during alternate weeks	24 wk	1	3	1	no side effects
Ohkouchi et al. [30]	5	4/1	45.8 ± 15.7	4	5	1-8d, 125μg bid, 9-14d, none, six 2-wk cycles; 1-4d, 125μg bid, 5-14d, none, six 2-wk cycles	24 wk	5	5	0	NA

All data are given in median (range) or mean ± SD;

No number, *M/F* male/female, *WLL* whole lung lavage, *GM-CSF Ab* granulocyte macrophage colony stimulating factor antibody;

d day, *wk* week, *mo* month, *NA* not available; [a] the number of detected patients and all were positive
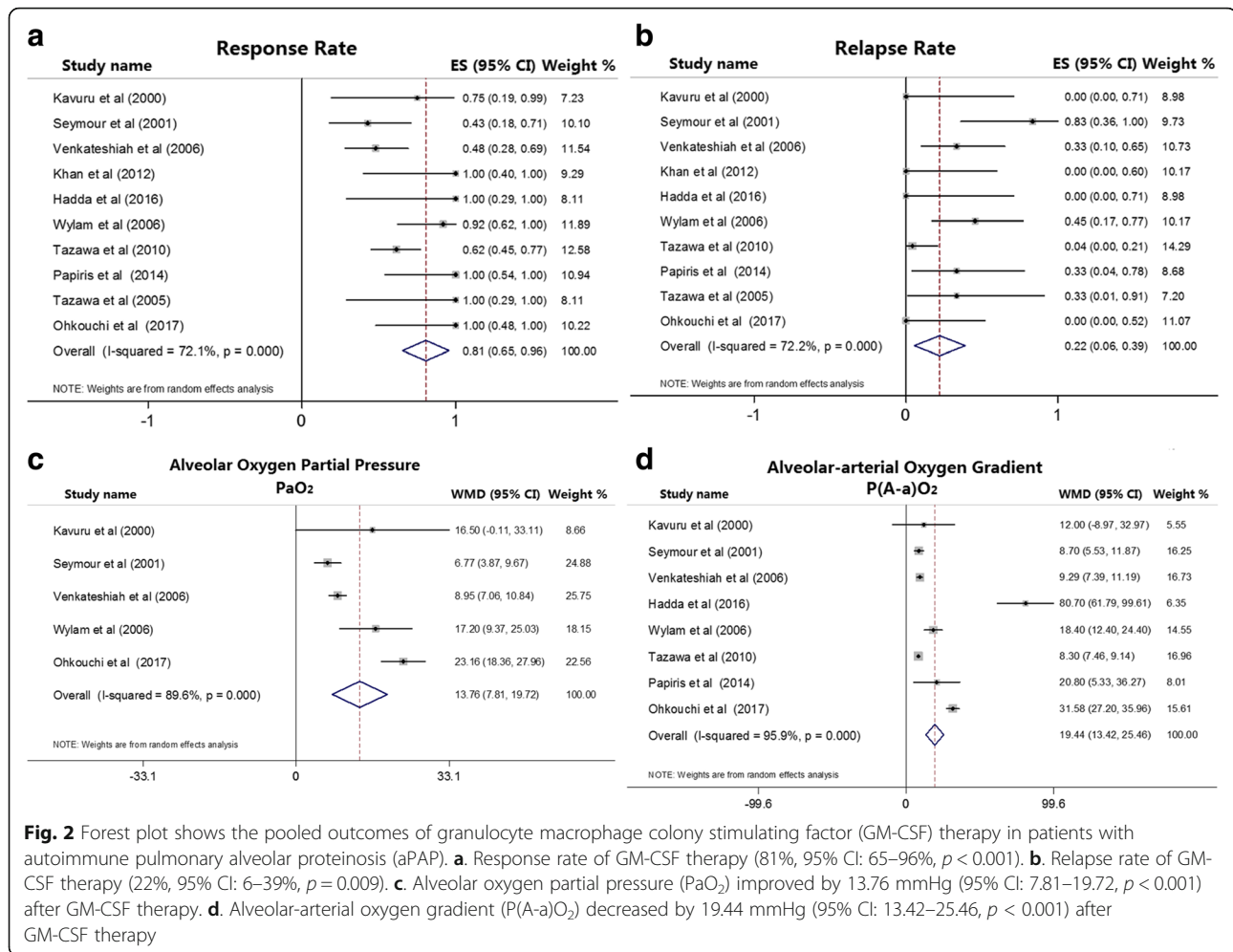
Inhaled versus subcutaneous GM-CSF
Response rate
The subcutaneous subgroup showed a response rate of 71% (95% CI: 46–96%, $p < 0.001$), while the inhaled subgroup was 89% (95% CI: 71–106%, $p < 0.001$) (Fig. 3a), suggesting both inhaled and subcutaneous GM-CSF therapies were effective for aPAP patients. Furthermore, the differences of

response rate between inhaled and subcutaneous subgroups were significant ($p = 0.023$). These data suggested that inhaled GM-CSF was more effective in aPAP patients.

Relapse rate
There were 5 studies assigned to subcutaneous subgroup and the pooled relapse rate was 24% (95% CI: -8-55%, p

Fig. 2 Forest plot shows the pooled outcomes of granulocyte macrophage colony stimulating factor (GM-CSF) therapy in patients with autoimmune pulmonary alveolar proteinosis (aPAP). **a**. Response rate of GM-CSF therapy (81%, 95% CI: 65–96%, $p < 0.001$). **b**. Relapse rate of GM-CSF therapy (22%, 95% CI: 6–39%, $p = 0.009$). **c**. Alveolar oxygen partial pressure (PaO$_2$) improved by 13.76 mmHg (95% CI: 7.81–19.72, $p < 0.001$) after GM-CSF therapy. **d**. Alveolar-arterial oxygen gradient (P(A-a)O$_2$) decreased by 19.44 mmHg (95% CI: 13.42–25.46, $p < 0.001$) after GM-CSF therapy

= 0.14) (Fig. 3b). The other 5 studies were assigned to inhaled subgroup and the pooled relapse rate was 19% (95% CI: 0–37%, $p < 0.05$) (Fig. 3b). The differences between inhaled and subcutaneous subgroups were not significant ($p = 0.262$).

Alveolar oxygen partial pressure (PaO$_2$)

In total, five studies showed data on PaO$_2$ pre- and post-therapy. Significant increase in PaO$_2$ was observed in both subcutaneous group (8.28 mmHg, 95% CI: 6.30–10.26, $p < 0.001$) and inhaled group (21.02 mmHg, 95% CI: 15.41–26.62, $p < 0.001$) (Fig. 3c). The difference between inhaled and subcutaneous subgroups was statistically significant ($p < 0.001$).

Alveolar-arterial oxygen gradient (P(A-a)O$_2$)

In the studies of subcutaneous subgroup, sensitivity analyses showed one study [24] had a significant impact on the pooled effects (Additional file 1: Figure S7). Thus, this study was not included in the further analyses. The reductions in (P(A-a)O$_2$) were significant in both subcutaneous
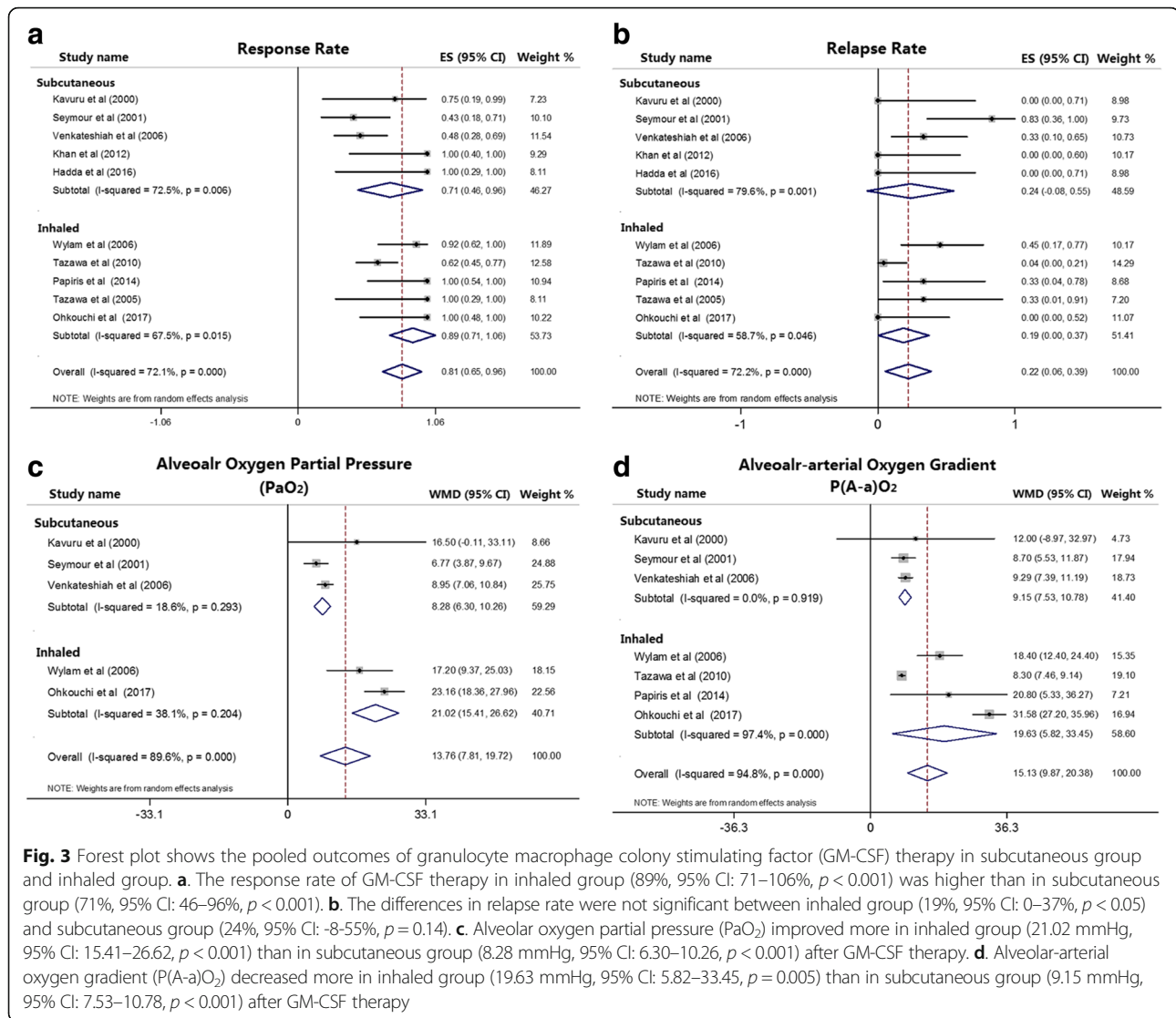
group (9.15 mmHg, 95% CI: 7.53–10.78, $p < 0.001$) and inhaled group (19.63 mmHg, 95% CI: 5.82–33.45, $p = 0.005$) (Fig. 3d). The mean reduction of P(A-a)O$_2$ was larger in inhaled group, and the difference between these two subgroups was statistically significant ($p < 0.001$).

Meta regression

Results of meta regression analyses suggested effects of GM-CSF therapy were independent of the age of patients, proportions of men, history of smoking, and proportions of combined WLL therapy ($p > 0.05$), respectively.

Subgroup analyses

We performed subgroup analyses based on the age of patients, and the proportions of men, smokers, anti GM-CSF antibody titer, initial dose and patients combined with WLL therapy. In the studies with more smokers, higher response rate, more improvements in PaO$_2$ and P(A-a)O$_2$ were found, and the differences between these subgroups were significant. And response rate was significantly higher in the high initial dose

Fig. 3 Forest plot shows the pooled outcomes of granulocyte macrophage colony stimulating factor (GM-CSF) therapy in subcutaneous group and inhaled group. **a**. The response rate of GM-CSF therapy in inhaled group (89%, 95% CI: 71–106%, $p < 0.001$) was higher than in subcutaneous group (71%, 95% CI: 46–96%, $p < 0.001$). **b**. The differences in relapse rate were not significant between inhaled group (19%, 95% CI: 0–37%, $p < 0.05$) and subcutaneous group (24%, 95% CI: -8-55%, $p = 0.14$). **c**. Alveolar oxygen partial pressure (PaO_2) improved more in inhaled group (21.02 mmHg, 95% CI: 15.41–26.62, $p < 0.001$) than in subcutaneous group (8.28 mmHg, 95% CI: 6.30–10.26, $p < 0.001$) after GM-CSF therapy. **d**. Alveolar-arterial oxygen gradient ($P(A-a)O_2$) decreased more in inhaled group (19.63 mmHg, 95% CI: 5.82–33.45, $p = 0.005$) than in subcutaneous group (9.15 mmHg, 95% CI: 7.53–10.78, $p < 0.001$) after GM-CSF therapy

subgroup. Relapse rate was found significantly higher in the subgroup with younger age and higher anti GM-CSF antibody titer. The $P(A-a)O_2$ improvement was more significant in the subgroup which more patients using WLL therapy. The details were shown in Table 2.

Discussion

This meta-analyses including 10 studies enrolling 115 patients aimed to evaluate whether GM-CSF therapy including inhaled and subcutaneous routes were effective on aPAP patients. The pooled outcomes of response rate, relapse rate, PaO_2 and $P(A-a)O_2$ indicated that GM-CSF therapy was effective on aPAP patients. Moreover, inhaled GM-CSF therapy had a higher response rate (89% vs. 71%, $p = 0.023$), more improvements in PaO_2 (21.02 mmHg vs. 8.28 mmHg, $p < 0.001$) and $P(A-a)O_2$ (19.63 mmHg vs. 9.15 mmHg, $p < 0.001$) than subcutaneous GM-CSF therapy, suggesting that inhaled

route was more effective than subcutaneous route in patients with aPAP.

The WLL therapy, whose response rate ranged from 70 to 84%, has been used as standard treatment for aPAP patients for decades [2, 13, 31]. However, compared to the WLL therapy alone, some studies reported that WLL followed by inhaled GM-CSF therapy had better improvements in pulmonary function and radiology [32, 33]. In this meta-analyses, the pooled response rate of GM-CSF therapy (81%) was no less than WLL therapy. The analyses of pulmonary function (including DLCO, TLC, VC, FEV1, FVC), DSS and 6MWD also confirmed the effectiveness of GM-CSF therapy. Furthermore, the subgroup analyses showed no significant differences for response or relapse rate with regard to the proportion of patients using WLL as combination therapy. These results demonstrated that GM-CSF therapy could be recognized as an alternative

Table 2 Subgroup analysis of the effects of GM-CSF therapy on patients with aPAP

Subgroup	Response rate				Relapse rate			
	Studies/patients, n/N	Response rate [95% CI]	*p* value	*p* value for interaction	Studies/patients, n/N	Relapse rate [95% CI]	*p* value	*p* value for interaction
Route of GM-CSF								
Subcutaneous	5/50	0.71 [0.46–0.96]	< 0.001	0.023	5/50	0.24 [−0.08–0.55]	0.14	0.262
Inhaled	5/65	0.89 [0.71–1.06]	< 0.001		5/65	0.19 [0.00–0.37]	0.047	
Age, years								
< 45	5/40	0.83 [0.61–1.04]	< 0.001	0.18	5/40	0.33 [0.01–0.64]	0.04	0.006
≥ 45	5/75	0.79 [0.57–1.01]	< 0.001		5/75	0.10 [−0.03–0.23]	0.13	
Gender, men %								
< 70	6/77	0.81 [0.62–1.01]	< 0.001	0.543	6/77	0.32 [0.05–0.60]	0.02	0.349
≥ 70	4/38	0.80 [0.51–1.09]	< 0.001		4/38	0.09 [−0.08–0.26]	0.293	
Smoker, %								
< 60	4/84	0.63 [0.40–0.86]	< 0.001	0.002	4/84	0.37 [0.01–0.72]	0.041	0.84
≥ 60	4/24	0.93 [0.80–1.07]	< 0.001		4/24	0.19 [−0.06–0.43]	0.136	
Combined therapy with WLL, %								
< 80	6/76	0.77 [0.59–0.96]	< 0.001	0.962	6/76	0.27 [0.00–0.53]	0.047	0.965
≥ 80	4/39	0.86 [0.57–1.15]	< 0.001		4/39	0.16 [−0.03–0.35]	0.098	
Anti GM-CSF antibody titer								
< 40μg/ml	3/50	0.86[0.58–1.14]	< 0.001	0.051	3/50	0.06[−0.06–0.19]	0.313	0.045
≥ 40μg/ml	3/18	0.95[0.80–1.10]	< 0.001		3/18	0.27[−0.02–0.56]	0.066	
Initial dose of GM-CSF								
≤ 250μg/d	7/96	0.74[0.55–0.93]	< 0.001	0.005	7/96	0.25[0.04–0.47]	0.022	0.751
> 250μg/d	3/19	0.95[0.80–1.10]	< 0.001		3/19	0.16[−0.15–0.46]	0.312	
Subgroup	PaO$_2$, mmHg				P[A-a]O$_2$, mmHg			
	Studies/patients, n/N	WMD [95% CI]	*p* value	*p* value for interaction	Studies/patients, n/N	WMD [95% CI]	*p* value	*p* value for interaction
Route of GM-CSF								
Subcutaneous	3/38	8.28 [6.3–10.26]	< 0.001	< 0.001	3/38	9.15 [7.53–10.78]	< 0.001	< 0.001
Inhaled	2/17	21.02 [15.41–26.62]	< 0.001		4/62	19.63 [5.82–33.45]	0.005	
Age, years								
< 45	3/29	12.11 [3.65–20.57]	0.005	0.614	4/35	14.14 [6.85–21.44]	< 0.001	0.725
≥ 45	2/26	15.88 [1.96–29.80]	0.025		4/68	24.56 [15.41–33.72]	< 0.001	
Gender, men %								
< 70	2/25	11.32 [1.19–21.46]	0.029	0.712	4/70	11.36 [7.27–15.45]	< 0.001	0.186
≥ 70	3/30	16.00 [4.31–27.69]	0.007		3/30	18.23 [−0.14–36.59]	0.052	
Smoker, %								
< 60	1/13	6.77 [3.87–9.67]	< 0.001	< 0.001	3/58	8.53 [6.87–10.18]	< 0.001	< 0.001
≥ 60	4/42	16.27[7.16–25.37]	< 0.001		4/42	18.40 [5.10–31.69]	0.007	
Combined therapy with WLL, %								
< 80	3/29	12.11 [3.65–20.57]	0.005	0.614	4/68	10.64 [6.87–14.4]	< 0.001	0.006
≥ 80	2/26	15.88 [1.96–29.80]	0.025		4/35	33.59 [14.54–52.64]	0.001	
Anti GM-CSF antibody titer								
< 40μg/ml	1/5	23.16(18.36–27.96)	< 0.001	-*	3/50	20.11(1.48–38.74)	0.034	0.516
≥ 40μg/ml	1/12	17.20(9.37–25.03)	< 0.001		2/15	48.88(−12.16–109.92)	0.117	

Table 2 Subgroup analysis of the effects of GM-CSF therapy on patients with aPAP *(Continued)*

Initial dose of GM-CSF								
≤ 250μg/d	4/43	13.02(6.36–19.67)	< 0.001	0.715	6/88	14.53(8.85–20.20)	< 0.001	0.466
> 250μg/d	1/12	17.20(9.37–25.03)	< 0.001		2/15	48.88(− 12.16–109.92)	0.117	

GM-CSF granulocyte macrophage colony stimulating factor, *aPAP* autoimmune pulmonary alveolar proteinosis, *PaO₂* alveolar oxygen partial pressure, *P[A-a]O₂* alveolar-arterial oxygen gradient, *WMD* Weighted Mean Difference; * only one study included in each subgroup, *p* value for interaction not available

optimal method rather than a supplement to WLL therapy.

In 2012, one meta-analyses reported that inhaled GM-CSF therapy showed a trend toward higher response rate in aPAP patients comparing with subcutaneous route, suggesting inhaled route might be a better route for GM-CSF therapy [34]. In this meta-analyses, more studies and patients were involved, and the results confirmed a higher response rate in inhaled subgroup with significance ($p = 0.023$). Furthermore, we also compared relapse rate, the values of PaO_2 and $P(A\text{-}a)O_2$ pre- and post-therapy in inhaled and subcutaneous subgroups, the results confirmed that aPAP patients would benefit more from inhaled GM-CSF therapy.

Although the differences in relapse rate were not significant between subcutaneous and inhaled subgroups ($p = 0.262$). We found a significant higher relapse rate in the patients younger than 45 years old than that of older than 45 years old ($p = 0.006$) by subgroup analyses (Table 2). And we also observed that the patients were more prone to relapse with higher titer of anti-GMCSF antibodies ($p = 0.045$) (Table 2). As we know, aPAP is an autoimmune disease, the immunologic function might be associated with the relapse rate of aPAP. In 2011, Frasca et al. reported that the humoral and cellular immune responses were both impaired in aged individuals because of age-related defects in T cells and B cells [35]. And Martin et al. also reported that the age-related dysregulation of B cell might result in lacking of magnitude and significant delay in the responses to challenge in older people [36]. Thus, these results suggested that the higher relapse rate with younger age might be associated with more active immune responses in younger patients.

Although the studies declared GM-CSF treatment was well tolerated, side effects still occurred in some patients during treatment and follow-up inevitably [17, 23, 27, 28]. As multiple side effects could occur in one patient and the duration of follow-up varies from studies, we defined the side effect rate as the number of side effect events divided by the patient-years of follow-up. We performed subgroup analyses based on route, initial dose, and relapse rate, the result showed that side effect rate was higher in subcutaneous subgroup than that of inhaled group ($p < 0.001$) (Additional file 1: Table S4). These results suggested that inhaled GM-CSF therapy, as a local treatment route, would achieve better efficacy with less side effects.

GM-CSF inhaled therapy, as a topical route, might result in higher concentration of GM-CSF deposition in the alveoli, which could achieve a better effect than subcutaneous injection. The size and dissolution of the granular preparation for inhalation therapy were considered as the focus to achieve the optimal effect. Luisetti et al. reported a high efficient nebulizer and described the physical properties, lung deposition modeling, and bioactivity of recombinant GM-CSF [37]. Thus, nebulizer was important for the effect of inhaled route.

Up to now, no consensus have been reached on the treatment of aPAP patients. Each medical institution had their own experiences and applicative treatments on aPAP patients. Only when WLL and GM-CSF therapies were invalid, would other therapies such as rituximab [38], plasmapheresis [39–41], gene therapy [42, 43], pulmonary macrophage transplantation therapy [44–46], and lung transplantation [47] be considered as alternative or adjuvant treatments in some aPAP cases. Although no definite guidelines of GM-CSF therapy were published, GM-CSF therapy, especially inhaled GM-CSF, might be a promising therapeutic option in treating aPAP.

Study limitations
Drawbacks of this study were the differences in baseline characteristics among included studies, containing age, gender, disease severity, treatment dose and duration etc. Second, aPAP is a rare disease with low prevalence, most researches of this disease were studies with small sample. More large-scale samples and long-term follow-up studies are needed in the future. Third, all the studies included were observational studies, 3 abstracts of randomized controlled trials (RCTs) were found, however, full texts of these RCTs were not available.

Conclusions
Pooled analyses of available studies suggests that treatment of GM-CSF were effective on aPAP patients with response rate (81%). However, further analyses demonstrated that inhaled GM-CSF therapy showed a higher response rate (89% vs. 71%), more improvements of PaO_2 and $P(A\text{-}a)O_2$ than subcutaneous route. These results provided a convincing evidence that GM-CSF therapy might be a promising therapeutic option in treating aPAP, furthermore, inhaled GM-CSF would have more benefits on aPAP patients than subcutaneous route.

Additional file

Abbreviations
6MWD: 6 min' walk distance; AHRQ: Agency for healthcare review and quality; aPAP: Autoimmune pulmonary alveolar proteinosis; CI: Confidence interval; DLCO: Diffusing capacity of the lung for carbon monoxide; DSS: Disease severity score; FEV1: Forced expiratory volume in one second; FVC: Forced vital capacity; GM-CSF: Granulocyte-macrophage colony-stimulating factor; $P(A-a)O_2$: Alveolar-arterial oxygen gradient; PaO_2: Alveolar oxygen partial pressure; PAP: Pulmonary alveolar proteinosis; RCTs: Randomized controlled trials; TLC: Total lung capacity; VC: Vital capacity; WLL: Whole lung lavage

Availability of data and materials
All data generated or analysed during this study are included in this published article [and its supplementary information files].

Authors' contributions
HLZ is the guarantor of this study and takes responsibility for this work. GS: contributed to performing meta-analyses and drafting and revising the manuscript. PC: contributed to performing meta-analyses and drafting and revising the manuscript. YW: contributed to performing meta-analyses and revising the manuscript. JC: contributed to performing meta-analyses and revising the manuscript. XC: contributed to revising the manuscript. HLZ: contributed to revising the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The ethics approval of this research was approved by the Institutional Review Board of Tongji Hospital.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of Respiratory and Critical Care Medicine, Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology, No.1095, Jie Fang Road, Han Kou District, Wu Han 430030, Hu Bei Province, China. [2]Division of Cardiology, Departments of Internal Medicine and Genetic Diagnosis Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wu Han, China. [3]Hubei Key Laboratory of Genetics and Molecular Mechanism of Cardiological Disorders, Wuhan 430030, China. [4]Division of Respiratory and Critical Care Medicine, the Second Hospital of Huangshi, Huangshi 435000, China.

References
1. Rosen SH, Castleman B, Liebow AA. Pulmonary alveolar proteinosis. N Engl J Med. 1958;258:1123–42.
2. Seymour JF, Presneill JJ. Pulmonary alveolar proteinosis: progress in the first 44 years. Am J Respir Crit Care Med. 2002;166:215–35.
3. Inoue Y, Trapnell BC, Tazawa R, Arai T, Takada T, Hizawa N, Kasahara Y, Tatsumi K, Hojo M, Ichiwata T, et al. Characteristics of a large cohort of patients with autoimmune pulmonary alveolar proteinosis in Japan. Am J Respir Crit Care Med. 2008;177:752–62.
4. Xu Z, Jing J, Wang H, Xu F, Wang J. Pulmonary alveolar proteinosis in China: a systematic review of 241 cases. Respirology. 2009;14:761–6.
5. Leth S, Bendstrup E, Vestergaard H, Hilberg O. Autoimmune pulmonary alveolar proteinosis: treatment options in year 2013. Respirology. 2013, 18:82–91.
6. Kitamura T, Tanaka N, Watanabe J, Uchida KS, Yamada Y, Nakata K. Idiopathic pulmonary alveolar proteinosis as an autoimmune disease with neutralizing antibody against granulocyte/macrophage colony-stimulating factor. J Exp Med. 1999;190:875–80.
7. Thomassen MJ, Yi T, Raychaudhuri B, Malur A, Kavuru MS. Pulmonary alveolar proteinosis is a disease of decreased availability of GM-CSF rather than an intrinsic cellular defect. Clin Immunol. 2000;95:85–92.
8. Trapnell BC, Whitsett JA, Nakata K. Pulmonary alveolar proteinosis. N Engl J Med. 2003;349:2527–39.
9. Khan A, Agarwal R. Pulmonary alveolar proteinosis. Respir Care. 2011;56:1016–28.
10. Dranoff G, Crawford AD, Sadelain M, Ream B, Rashid A, Bronson RT, Dickersin GR, Bachurski CJ, Mark EL, Whitsett JA, et al. Involvement of granulocyte-macrophage colony-stimulating factor in pulmonary homeostasis. Science. 1994;264:713–6.
11. Stanley E, Lieschke GJ, Grail D, Metcalf D, Hodgson G, Gall JA, Maher DW, Cebon J, Sinickas V, Dunn AR. 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.
12. Reed JA, Whitsett JA. Granulocyte-macrophage colony-stimulating factor and pulmonary surfactant homeostasis. Proc Assoc Am Physicians. 1998;110:321–32.
13. Awab A, Khan MS, Youness HA. Whole lung lavage-technical details, challenges and management of complications. J Thorac Dis. 2017;9:1697–706.
14. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J, Moher D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med. 2009;6:e1000100.
15. Seymour JF, Dunn AR, Vincent JM, Presneill JJ, Pain MC. Efficacy of granulocyte-macrophage colony-stimulating factor in acquired alveolar proteinosis. N Engl J Med. 1996;335:1924–5.
16. Reed JA, Ikegami M, Cianciolo ER, Lu W, Cho PS, Hull W, Jobe AH, Whitsett JA. Aerosolized GM-CSF ameliorates pulmonary alveolar proteinosis in GM-CSF-deficient mice. Am J Phys. 1999;276:L556–63.
17. Seymour JF, Presneill JJ, Schoch OD, Downie GH, Moore PE, Doyle IR, Vincent JM, Nakata K, Kitamura T, Langton D, et al. Therapeutic efficacy of granulocyte-macrophage colony-stimulating factor in patients with idiopathic acquired alveolar proteinosis. Am J Respir Crit Care Med. 2001;163:524–31.
18. Shibata Y, Berclaz PY, Chroneos ZC, Yoshida M, Whitsett JA, Trapnell BC. GM-CSF regulates alveolar macrophage differentiation and innate immunity in the lung through PU.1. Immunity. 2001;15:557–67.

19. Bonfield TL, Raychaudhuri B, Malur A, Abraham S, Trapnell BC, Kavuru MS, Thomassen MJ. 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.
20. West S, King V, Carey TS, Lohr KN, McKoy N, Sutton SF, Lux L. Systems to rate the strength of scientific evidence. Evid Rep Technol Assess (Summ). 2002:1–11.
21. Fleiss JL. Analysis of data from multiclinic trials. Control Clin Trials. 1986;7:267–75.
22. Kavuru MS, Sullivan EJ, Piccin R, Thomassen MJ, Stoller JK. Exogenous granulocyte-macrophage colony-stimulating factor administration for pulmonary alveolar proteinosis. Am J Respir Crit Care Med. 2000;161:1143–8.
23. Venkateshiah SB, Yan TD, Bonfield TL, Thomassen MJ, Meziane M, Czich C, Kavuru MS. An open-label trial of granulocyte macrophage colony stimulating factor therapy for moderate symptomatic pulmonary alveolar proteinosis. Chest. 2006;130:227–37.
24. Hadda V, Tiwari P, Madan K, Mohan A, Gupta N, Bharti SJ, Kumar V, Garg R, Trikha A, Jain D, et al. Pulmonary alveolar proteinosis: experience from a tertiary care center and systematic review of Indian literature. Lung India. 2016;33:626–34.
25. Khan A, Agarwal R, Aggarwal AN, Bal A, Sen I, Yaddanapuddi LN, Puri GD. Experience with treatment of pulmonary alveolar proteinosis from a tertiary care Centre in North India. Indian J Chest Dis Allied Sci. 2012;54:91–7.
26. Wylam ME, Ten R, Prakash UB, Nadrous HF, Clawson ML, Anderson PM. Aerosol granulocyte-macrophage colony-stimulating factor for pulmonary alveolar proteinosis. Eur Respir J. 2006;27:585–93.
27. Tazawa R, Trapnell BC, Inoue Y, Arai T, Takada T, Nasuhara Y, Hizawa N, Kasahara Y, Tatsumi K, Hojo M, et al. Inhaled granulocyte/macrophage-colony stimulating factor as therapy for pulmonary alveolar proteinosis. Am J Respir Crit Care Med. 2010;181:1345–54.
28. Papiris SA, Tsirigotis P, Kolilekas L, Papadaki G, Papaioannou AI, Triantafillidou C, Papaporfyriou A, Karakatsani A, Kagouridis K, Griese M, Manali ED. Long-term inhaled granulocyte macrophage-colony-stimulating factor in autoimmune pulmonary alveolar proteinosis: effectiveness, safety, and lowest effective dose. Clin Drug Investig. 2014;34:553–64.
29. Tazawa R, Hamano E, Arai T, Ohta H, Ishimoto O, Uchida K, Watanabe M, Saito J, Takeshita M, Hirabayashi Y, et al. Granulocyte-macrophage colony-stimulating factor and lung immunity in pulmonary alveolar proteinosis. Am J Respir Crit Care Med. 2005;171:1142–9.
30. Ohkouchi S, Akasaka K, Ichiwata T, Hisata S, Iijima H, Takada T, Tsukada H, Nakayama H, Machiya JI, Irokawa T, et al. Sequential granulocyte-macrophage Colony-stimulating factor inhalation after whole-lung lavage for pulmonary alveolar Proteinosis. A report of five intractable cases. Ann Am Thorac Soc. 2017;14:1298–304.
31. Beccaria M, Luisetti M, Rodi G, Corsico A, Zoia MC, Colato S, Pochetti P, Braschi A, Pozzi E, Cerveri I. Long-term durable benefit after whole lung lavage in pulmonary alveolar proteinosis. Eur Respir J. 2004;23:526–31.
32. Campo I, Mariani F, Paracchini E, Kadija Z, Zorzetto M, Tinelli C, Rodi G, Meloni F, Griese M, Trapnell B, et al. Inhaled sargramostim and whole lung lavage (WLL) as therapy of autoimmune pulmonary alveolar proteinosis (aPAP). Eur Respir J. 2016;48
33. Mariani F, Campo I, Kadija Z, Paracchini E, Piloni D, Pe I, Luisetti M. Interim data analysis of a controlled trial of whole lung lavage followed by inhaled sargramostin in autoimmune pulmonary alveolar proteinosis. Am J Respir Crit Care Med. 2012;185
34. Khan A, Agarwal R, Aggarwal AN. Effectiveness of granulocyte-macrophage colony-stimulating factor therapy in autoimmune pulmonary alveolar proteinosis: a meta-analysis of observational studies. Chest. 2012;141:1273–83.
35. Frasca D, Diaz A, Romero M, Landin AM, Blomberg BB. Age effects on B cells and humoral immunity in humans. Ageing Res Rev. 2011;10:330–5.
36. Martin V, Bryan Wu YC, Kipling D, Dunn-Walters D. Ageing of the B-cell repertoire. Philos Trans R Soc Lond B Biol Sci. 2015;370(1676):20140237.
37. Luisetti M, Kroneberg P, Suzuki T, Kadija Z, Muellinger B, Campo I, Gleske J, Rodi G, Zimlich WC, Mariani F, et al. Physical properties, lung deposition modeling, and bioactivity of recombinant GM-CSF aerosolised with a highly efficient nebulizer. Pulm Pharmacol Ther. 2011;24:123–7.
38. Kavuru MS, Malur A, Marshall I, Barna BP, Meziane M, Huizar I, Dalrymple H, Karnekar R, Thomassen MJ. An open-label trial of rituximab therapy in pulmonary alveolar proteinosis. Eur Respir J. 2011;38:1361–7.
39. Luisetti M, Rodi G, Perotti C, Campo I, Mariani F, Pozzi E, Trapnell BC. Plasmapheresis for treatment of pulmonary alveolar proteinosis. Eur Respir J. 2009;33:1220–2.
40. Kavuru MS, Bonfield TL, Thomassen MJ. Plasmapheresis, GM-CSF, and alveolar proteinosis. Am J Respir Crit Care Med. 2003;167:1036. author reply 1036-1037
41. Garber B, Albores J, Wang T, Neville TH. A plasmapheresis protocol for refractory pulmonary alveolar proteinosis. Lung. 2015;193:209–11.
42. Zsengeller ZK, Reed JA, Bachurski CJ, LeVine AM, Forry-Schaudies S, Hirsch R, Whitsett JA. Adenovirus-mediated granulocyte-macrophage colony-stimulating factor improves lung pathology of pulmonary alveolar proteinosis in granulocyte-macrophage colony-stimulating factor-deficient mice. Hum Gene Ther. 1998;9:2101–9.
43. Korst RJ, Bewig B, Crystal RG. In vitro and in vivo transfer and expression of human surfactant SP-A- and SP-B-associated protein cDNAs mediated by replication-deficient, recombinant adenoviral vectors. Hum Gene Ther. 1995;6:277–87.
44. Nishinakamura R, Wiler R, Dirksen U, Morikawa Y, Arai K, Miyajima A, Burdach S, Murray R. The pulmonary alveolar proteinosis in granulocyte macrophage colony-stimulating factor/interleukins 3/5 beta c receptor-deficient mice is reversed by bone marrow transplantation. J Exp Med. 1996;183:2657–62.
45. Numata A, Matsuishi E, Koyanagi K, Saito S, Miyamoto Y, Irie K, Gondo H, Harada M. Successful therapy with whole-lung lavage and autologous peripheral blood stem cell transplantation for pulmonary alveolar proteinosis complicating acute myelogenous leukemia. Am J Hematol. 2006;81:107–9.
46. Fukuno K, Tomonari A, Tsukada N, Takahashi S, Ooi J, Konuma T, Uchiyama M, Fujii T, Endo T, Iwamoto A, et al. Successful cord blood transplantation for myelodysplastic syndrome resulting in resolution of pulmonary alveolar proteinosis. Bone Marrow Transplant. 2006;38:581–2.
47. Huddleston CB, Bloch JB, Sweet SC, de la Morena M, Patterson GA, Mendeloff EN. Lung transplantation in children. Ann Surg. 2002;236:270–6.