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# Circulating lung biomarkers in idiopathic lung fibrosis and interstitial lung diseases associated with connective tissue diseases: Where do we stand?

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1 **Circulating lung biomarkers in idiopathic lung fibrosis and interstitial lung diseases**  
2 **associated with connective tissue diseases: where do we stand ?**

3 CIRCULATING BIOMARKERS IN INTERSTIAL LUNG DISEASES: A SYSTEMATIC  
4 REVIEW AND META-ANALYSIS

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19 **HIGHLIGHTS:** CTD-ILD and IPF share common biomarkers suggesting common  
20 pathways.

- 21
- 22 • KL-6, SP-D and MMP7 are sensitive but not specific to diagnose lung fibrosis in IPF
  - 23 • KL-6, SP-D and CCL18 are sensitive but not specific for SSc-ILD diagnosis
  - 24 • KL-6 is the most sensitive circulating biomarker for diagnosis
  - 25 • KL-6 and CCL18 can predict lung involvement worsening in IPF and SSc-ILD
- 26
- 27
- 28

1 **ABSTRACT**

2 Interstitial lung diseases (ILDs) are complex diseases with various courses where personalized  
3 medicine is highly expected. Biomarkers are indicators of physiological, pathological  
4 processes or of pharmacological response to therapeutic interventions. They can be used for  
5 diagnosis, risk-stratification, prediction and monitoring of treatment response. To better  
6 delineate the input and pitfalls of biomarkers in ILDs, we performed a systematic review and  
7 meta-analysis of literature in MEDLINE and Embase databases from January 1960 to  
8 February 2019. We focused on circulating biomarkers as having the highest generalizability.  
9 Overall, 70 studies were included in the review and 20 studies could be included in the meta-  
10 analysis. This review highlights that ILD associated with connective tissue diseases (CTD-  
11 ILD) and idiopathic pulmonary fibrosis (IPF) share common biomarkers, suggesting common  
12 pathophysiological pathways. KL-6 and SP-D, could diagnose lung fibrosis in both IPF and  
13 CTD-ILD, with KL-6 having the strongest value (OR: 520.95[110.07-2465.58],  $p < 0.001$  in  
14 IPF and OR:26.43[7.15-97.68],  $p < 0.001$  in CTD-ILD), followed by SPD (OR: 33.81[3.20-  
15 357.52],  $p = 0.003$  in IPF and 13.24 [3.84-45.71] in SSc-ILD), MMP7 appeared as interesting  
16 for IPF diagnosis ( $p < 0.001$ ), whereas in SSc, CCL18 was associated with ILD diagnosis. Both  
17 CCL18 and KL-6 were predictive for the outcomes of ILDs, with higher predictive values for  
18 CCL18 in both IPF (OR:10.22[4.72-22.16],  $p < 0.001$  and in SSc [2.62[1.71-4.03],  $p < 0.001$ ).  
19 However, disease specific biomarkers are lacking and large longitudinal studies are needed  
20 before the translational use of the potential biomarkers in clinical practice. With the recent  
21 availability of new effective therapies in ILDs, further studies should assess response to  
22 treatment.

23 **KEY WORDS:** biomarker, interstitial lung disease, rheumatoid arthritis, systemic sclerosis,  
24 idiopathic pulmonary fibrosis.

25

1 **INTRODUCTION**

2

3 Interstitial lung diseases (ILDs) are a group of heterogeneous disorders, either idiopathic  
4 (idiopathic pulmonary fibrosis (IPF)) or associated with other diseases, particularly  
5 connective tissue diseases (CTDs) (CTD-ILD) or sarcoidosis.

6 IPF affects around 3 million people worldwide, with incidence increasing dramatically with  
7 age [1]. The prognosis for patients with IPF is poor, with a median survival of 3–5 years, if  
8 untreated [1].

9 Lung involvement is a common extra articular complication in CTDs, such as systemic  
10 sclerosis (SSc), rheumatoid arthritis (RA) or dermatomyositis [2–5]. As a further measure of  
11 impact, ILD represents the most common cause of death in patients with underlying RA and  
12 SSc and is a significant contributor to morbidity [6,7].

13 Management of ILDs is challenging because individual prognosis is unpredictable: there is a  
14 wide spectrum of disease courses ranging from stability or slow progression over several  
15 years to rapid deterioration, with, particularly in IPF, acute exacerbations, which are leading  
16 causes of mortality [1]. Furthermore, IPF and CTD-ILD present challenges to diagnosis, often  
17 leading to delays that might augment morbidity and mortality. With the recent development of  
18 new and effective treatments for lung fibrosis, it is critical to identify patients with lung  
19 disease at an earlier stage and to rapidly identify those who will progress to extensive lung  
20 disease [8–13]. This earlier detection at a preclinical stage and the stratification of individual  
21 risk of mortality might potentially rely on combined models that include biomarkers,  
22 demographics and imaging data [1].

23 Biological markers, often referred as biomarkers, are commonly defined as objectively  
24 measured elevated indicators of physiological, pathological processes or pharmacological

1 response to therapeutic interventions [14]. Their applications include (i) diagnosis, (ii) staging  
2 of disease (severity), (iii) prediction of the progression of the disease (prognosis) and (iv)  
3 prediction and monitoring of treatment response.

4 Until now, despite a large number of publications on this topic, routine use of biomarkers is  
5 not recommended in clinical practice in IPF or CTD-ILD [15]. To better delineate advantages  
6 and pitfalls of biomarkers use in ILDs, we aimed to perform a systematic review and a meta-  
7 analysis of the current literature on biomarkers in IPF and CTD-ILD. Ideal biomarkers would  
8 be easily sampled, analyzed and generalizable. Therefore, we focused on circulating  
9 biomarkers.

10

## 11 **MATERIALS AND METHODS**

12 The Meta-analyses of Observational Studies in Epidemiology (MOOSE) guidelines were  
13 followed [16]. Eligible studies were studies (i) reporting use of biomarkers in ILDs (IPF and  
14 CTD-ILD), the latter being defined by imaging (high-resolution computed tomography  
15 (HRCT)) or histology), and (ii) controlled (with at least 20 patients per group). Selected  
16 biomarkers had to be confirmed in at-least two independent cohorts in a single or more  
17 studies. We searched MEDLINE and Embase databases between January 1960 and February  
18 2019 using the terms (pulmonary fibrosis OR lung diseases, interstitial OR fibrosing alveolitis  
19 OR diffuse parenchymal lung disease OR idiopathic pulmonary fibrosis) AND (serum  
20 biomarkers OR blood biomarkers). Reference lists of the papers initially detected were  
21 searched by hand to identify additional relevant reports. Only articles in English and reporting  
22 the number of patients with concentration of the studied biomarker below or above the cut-off  
23 value were included in the meta-analysis. Eligibility of references retrieved by the search was  
24 assessed independently by two authors (Y.A. and M.E.) and disagreements resolved at each  
25 step. Data were extracted from the selected studies using a predefined standardized form.

1 Quality assessment of individual studies was performed using the Newcastle–Ottawa Scale.  
2 Only studies of good quality were included in the meta-analysis.

### 3 **Statistical analysis**

4 Statistical heterogeneity was tested by Q-test ( $\chi^2$ ) [17]. This test allows description of the  
5 percentage of total variation across trials that is attributable to statistical heterogeneity rather  
6 than chance. I<sup>2</sup>-values of 25, 50 and 75% correspond to low, moderate and high between-trial  
7 heterogeneity of results, respectively [18]. Fixed and random effects models based on Q-  
8 statistics for heterogeneity were used for homogeneous and heterogeneous trials, respectively.  
9 We used MedCalc software (v19.0.5) to perform the statistical analysis. We calculated an  
10 odds ratio (OR) based on the number of patients with concentration of the studied biomarker  
11 above the cut-off value for ILD diagnosis or progression compared to the number of patients  
12 with concentration of the studied biomarker below the cut-off value. This analysis was  
13 performed in IPF and in CTD-ILD. For KL6 we performed an additional analysis in SSc-ILD,  
14 whereas for other biomarkers CTD-ILD only included SSc-patients. We obtained OR with a  
15 confidence interval of 95% (IC95%). When our data were heterogeneous (i.e.  $p < 0.05$ , where  
16  $p$  is the  $p$  value of the Cochran test), we used the random effects to provide an OR, otherwise  
17 we used the fixed effects. We used the Mantel-Haenszel method for calculating the weighted  
18 summary OR. Publication bias was assessed using a funnel plot.

19

### 20 **RESULTS**

21 Among 3252 identified references, 3050 were excluded based on their title or abstract,  
22 resulting in 168 articles being examined for the full text (Figure 1).

23

24

## 1 **I Literature data**

2 Overall, 70 studies fulfilled the inclusion criteria and are presented in Table I and II and in  
3 Supplementary Tables I and II. Biomarkers and their sources are represented in Figure 2.  
4 Biomarkers were classified into seven main categories according to their source of production  
5 and their biology.

### 6 1. ALVEOLAR EPITHELIAL CELL DAMAGE

7 Molecules connected to alveolar epithelial cell damage were the most studied biomarkers and  
8 provided the most convincing data. The increase in serum levels of these markers can be  
9 attributed to an increase in the production of these proteins by regenerating alveolar type II  
10 cells and/or to an enhanced permeability following the destruction of the alveolar–capillary  
11 barrier [19,20].

#### 12 1.1 KL6

13 KL-6 is a high-molecular-weight mucin-like glycoprotein, also known as human mucin-1  
14 (MUC1). It is expressed mainly on type II pneumocytes in alveoli and bronchiolar epithelial  
15 cells, particularly on proliferating and regenerating type II pneumocytes [21]. *In vitro*, KL-6  
16 exerts profibrotic and antiapoptotic effects on lung fibroblasts [22], suggesting a possible  
17 pathogenic role of KL-6 in ILD.

18 Several studies have highlighted the value of KL-6 for IPF diagnosis (Table 1), with higher  
19 levels found in patients compared to controls, but without significant differences among ILDs  
20 connected to different etiologies [23,24,24–28]. In two studies, KL-6 was identified as the  
21 best performing biomarker for diagnosis of lung fibrosis as compared to CCL18, SP-A and  
22 SP-D [23,24].

23 Besides its interest for diagnosis of IPF, KL-6 has been mostly studied as a prognostic  
24 biomarker [24,26,29–33]. Two independent studies have shown that KL-6 could predict acute

1 exacerbations [29,34,35], the leading cause of death in IPF [36], with concentrations >1000  
2 U/mL and 1300 U/mL (hazard ratio [HR] 11.8, 95% CI 1.43 to 97.8, P=0.022) after  
3 adjustment for total vital capacity [25,29]. Consistently, a staging system including KL-6  
4 among four parameters has been recently proposed to predict the occurrence of acute  
5 exacerbations [37].

6 However, data regarding the interest of KL6 for assessing response to treatment are still  
7 lacking: three small studies suggested the interest of KL-6 in this context [30,38,39]  
8 proposing that monitoring KL-6 may contribute to an early decision for changing treatment in  
9 progressive IPF. However, in one study, KL-6 did not decrease following anti-fibrotic therapy  
10 [40].

11 Serum KL-6 is also elevated in CTD-ILD [23,28,31,41–55]. Most of the studies have been  
12 performed in SSc with a good ability of KL-6 in staging disease severity with moderate to  
13 high correlations with pulmonary function tests and HRCT [41,56,57]. KL-6 levels were also  
14 correlated with the severity of parenchymal involvement in sarcoidosis and RA [42,43].  
15 However, the predictive value of KL-6 levels on decline in pulmonary function tests and  
16 survival is still controversial [25,41,58].

17 Besides these promising data on KL-6, the major issue is a marked inter-individual variability  
18 in serum levels. This is explained by a polymorphism on MUC1, which has been found to  
19 influence serum KL-6 levels in Caucasian and Japanese subjects [59]. Therefore,  
20 standardization about cut-off values remains to be determined.

## 21 1.2 SP-A and SP-D

22 SP-A and SP-D are lipoprotein complexes secreted by type II pneumocytes and Clara cells to  
23 decrease surface tension at the air–liquid interface. They are also involved in lung host  
24 defense. The existence of familial forms of IPF associated with mutations of surfactant

1 protein C highlights the relevance of surfactant proteins in this context [60]. Consistently with  
2 what was observed for KL-6, polymorphisms in the SP-D gene have been shown to affect the  
3 levels of SP-D [61].

4 In IPF, SP-A and SP-D are elevated as compared to controls [23,24,27,62–67]. In one study,  
5 SP-D distinguished IPF from other ILDs with sensitivity, specificity and accuracy of 70%,  
6 65% and 68.5%, respectively [62]. However, in most studies, SP-D and SP-A were elevated  
7 in ILD (including lung cancers or infections) without specificity for one disease [68–71],  
8 suggesting that these biomarkers are rather general markers for alveolar damage than for  
9 specific diseases.

10 Increased serum SP-A and SP-D were strong predictors of mortality in IPF in three  
11 independent studies [63–65], with better performance of a model combining both [65].  
12 Interestingly, SP-D levels were predictors of disease progression and prognosis in patients  
13 with IPF treated with pirfenidone [72].

14 In CTD-ILDs, most of studies were performed in SSc and assessed SP-D, which demonstrated  
15 a good correlation with HRCT abnormalities and pulmonary function tests  
16 [23,41,44,45,56,65,66]. SP-A and SP-D have also demonstrated their interest in preliminary  
17 studies as diagnostic biomarkers in RA-ILD and dermatomyositis/polymyositis-ILD (18, 41,  
18 68). Until now, studies assessing the prognostic value of surfactant proteins are negative [41]  
19 or preliminary [44,45].

### 20 1.3 CC16

21 Clara cell 16-kDa protein (CC16) is a 15.8-kDa homodimeric protein secreted throughout the  
22 tracheobronchial tree, especially in the terminal bronchioles where Clara cells are localized.  
23 Only 2 studies could be included in this literature review, suggesting an interest in CC16 for  
24 diagnosis and staging of ILD [45,73].

## 1.4 Ca19-9 and Ca-125

Both Ca19-9 and Ca-125 recognize mucous-associated antigens. In a large prospective longitudinal cohort of treatment-naive patients with IPF, an unbiased multiplex immunoassay assessment of 123 biomarkers identified Ca19-9 and Ca-125 as relevant prognostic factors for IPF [63]. In this study, there was an increase in Ca19-9 and Ca-125 staining throughout the metaplastic epithelium in fibrotic lesions, whereas Ca19-9 and Ca-125 were only detected in the apical aspect of bronchial epithelium in normal lungs, underlining a possible role in IPF pathogenesis. The value of Ca19-9 and Ca-125 to diagnose ILD was also raised by a preliminary study in RA [74].

## 2. CYTOKINES/CHEMOKINES

### 2.1 MCP-1

CC-chemokine ligand (CCL) 2, also known as MCP-1, is a chemoattractant for T-cells, natural killer cells and fibrocytes and has been shown to play a role in fibrosis by *in vivo* and *in vitro* studies [75,76]. CCL2 is elevated in the blood and bronchoalveolar lavage of IPF patients [23,77,78]. However, the role of CCL2 in IPF pathogenesis is controversial, since a phase 2 trial, evaluating carlumab, inhibiting CCL2, in IPF did not show any significant effects on pulmonary function tests [79].

In CTD-ILD, despite positive results, CCL2 appears as less accurate and sensitive than KL-6 for diagnosis [23,46,47,77,80].

### 2.2 CCL18

CCL18 is a chemotactic factor produced by alveolar macrophages which has profibrotic effects.

As observed for other biomarkers, CCL18 levels are higher in IPF as compared to controls, but without significant difference between ILD subtypes [24]. In a longitudinal study of 72 IPF-patients, CCL18 levels of >150 ng/ml were independently associated with death [81].

1 Interestingly treatment with pirfenidone led to a significant suppression of CCL18 expression  
2 by alveolar macrophages in IPF [82], suggesting a possible interest in this biomarker to assess  
3 treatment response.

4 The prognostic value of CCL18 has been underlined in SSc, where it was found to be  
5 associated with lung fibrosis progression [41,83,84] and death [83]. However, in the  
6 GENISOS study, focused on early SSc patients, CCL18 was not a long-term predictor of  
7 forced vital capacity course [85].

### 8 2.3 CXCL13

9 CXCL13 is produced by macrophages and recruits B cells to secondary and tertiary lymphoid  
10 structures. CXCL13 has been shown to be a biomarker of advanced disease in IPF and to be  
11 associated with a poorer prognosis [86,87]. Some recent data also suggest that CXCL13 could  
12 be a biomarker for diagnosis and staging of ILD in SSc [88].

### 13 2.4 IL-6

14 In SSc, serum IL-6 levels were predictive of early disease progression in patients with mild  
15 ILD [78]. A preliminary study suggested that IL-6 could be interesting to detect lung  
16 involvement in dermatomyositis [89].

### 17 2.5 Soluble receptor of IL-2

18 The interest of soluble receptor of IL-2 was suggested in sarcoidosis for diagnosis and staging  
19 of disease severity [90,91]. This receptor is released by activated T cells in active sarcoidosis.

20

## 21 3. GROWTH FACTOR AND ADHESION MOLECULES

### 22 3.1 YKL-40

23 YKL-40 is a member of the highly conserved family of chitinases and chitinase-like proteins,  
24 regulating cell proliferation and survival. YKL-40 is elevated in inflammatory conditions and  
25 could be involved in tissue remodeling [92].

1 YKL-40 was studied for diagnosis by few studies in IPF, CTD-ILD and sarcoidosis [93–96].  
2 Besides some studies have suggested a worse prognosis associated with higher values [94–  
3 96]. However, its clinical use is limited by the inter-individual variability of serum levels of  
4 YKL-40, related to a SNP in the YKL-40-encoding gene [93].

### 5 3.2 ICAM-1

6 Intracellular adhesion molecule 1 (ICAM-1) is overexpressed on pulmonary epithelial cells in  
7 IPF. Serum levels of ICAM-1 are increased in IPF [97]. Some preliminary data suggest an  
8 interest of ICAM-1 in the prognosis of SSc-ILD [98].

## 9 4. FIBROGENESIS AND EXTRACELLULAR REMODELLING

### 10 4.1 MMP

11 Matrix metalloproteinases (MMPs) regulate the remodeling of extracellular matrix. Among  
12 the numerous MMPs, elevated serum levels of MMP1 and MMP7 have been detected in IPF,  
13 with more sensitivity for IPF diagnosis by combining the two biomarkers [24,62,63,67,99–  
14 103]. MMP-1 is involved in collagen degradation, but also in the regulation of cell migration  
15 and, potentially, of cell growth.

16 MMP-7 is the smallest member of MMP family, capable of degrading multiple components of  
17 the extracellular matrix in IPF. This is the most studied MMP as a biomarker. *In vivo* and *in*  
18 *vitro* data support a role of MMP7 in IPF pathogenesis: mice lacking MMP7 are protected  
19 from pulmonary fibrosis [104], MMP7 is detected in fibrotic lung tissue on the surface of  
20 epithelial cells and alveolar macrophages. Several studies have demonstrated that MMP7 is a  
21 valuable diagnostic biomarker for IPF [24,62,63,67,99,101–103]. Of most interest, two  
22 studies have suggested that MMP7 could be specific to IPF with sensitivities of 71% and  
23 72.3% and specificities of 63% and 66.3%, respectively in distinguishing IPF from other ILDs  
24 [62,100]. However, MMP7 could also be interesting in detecting subclinical ILD in RA [105].  
25 In IPF, MMP7 could also predict survival [67].

## 1 4.2 Osteopontin

2 Osteopontin is a glycoprotein involved in immune response and tissue repair. *In vivo*,  
3 osteopontin, localized on epithelial cells, induces migration and proliferation of both  
4 fibroblasts and epithelial cells and promotes extracellular matrix deposition. A study has  
5 suggested an interest of osteopontin for distinguishing patients with IPF from patients with  
6 other types of ILD [62].

7 One preliminary study suggests that osteopontin could be an interesting prognostic factor in  
8 CTD-ILD [106].

## 9 4.3 Periostin

10 Periostin is an extracellular matrix and intracellular protein, localized in fibroblasts. Two  
11 small size studies have suggested that periostin could predict disease progression in IPF  
12 [107,108]. Some data suggest an interest of periostin in SSc and in ILD-SSc, but its interest as  
13 a biomarker has not been studied so far [109,110].

## 14 4.4 Extracellular matrix neopeptides

15 In the multicenter PROFILE study, concentrations of protein fragments generated by MMP  
16 activity were increased in the serum of individuals with IPF compared with healthy controls  
17 [111]. Furthermore, increased neopeptide concentrations were associated with disease  
18 progression and survival. Other collagen fragments have been associated with diagnosis and  
19 prognosis of IPF and CTD-ILD [112,113]. In SSc, markers of collagen degradation were  
20 associated with ILD diagnosis and staging, whereas cartilage oligomeric matrix protein could  
21 predict survival [114–116].

## 22 5. OXIDATIVE STRESS MARKERS

### 23 5.1 Lysyl oxidase-like 2 (LOXL2)

24 LOXL2 is an enzyme promoting cross-linking of fibrillary collagen, leading to stabilization of  
25 the extracellular matrix. In IPF, LOXL2 levels were shown to predict disease progression and

1 mortality [117]. However, its role in IPF pathogenesis is challenging since in a phase II study,  
2 simtuzumab, an anti-LOXL2 antibody, did not improve progression-free survival [118]. In  
3 RA, some data suggest its interest to diagnose early ILD [119].

#### 4 5.2 Autoantibodies directed against HSP70

5 The heat shock protein 70 (HSP 70) is a molecular chaperone, which is expressed in response  
6 to stress. Autoantibodies against HSP70 have been detected in IPF and are associated with a  
7 worse prognosis [120]. Besides, some data suggest that in RA, anti-Hsp70 antibodies could be  
8 associated with smoking-related lung disease in humans and mice [121].

#### 9 6. CIRCULATING CELLS

10 CD45+Col-1+ fibrocytes are circulating bone marrow-derived mesenchymal progenitor cells  
11 which can differentiate into fibroblast and myofibroblasts. The detection of >5% fibrocytes  
12 has been associated with a worse prognosis in IPF [122]. Besides in RA, a preliminary small  
13 size study has suggested a correlation between the level of circulating fibrocytes and ILD  
14 diagnosis and severity [123].

#### 15 7. Micro-RNA

16 In Yang study, miR-21, miR-199a-5p, miR-200c, miR-31, let-7a and let-7d could differentiate  
17 slow versus rapid progressors in two independent cohorts in IPF [124]. Some other micro-  
18 RNAs have been identified as diagnostic biomarkers in IPF and RA-ILD [125,126]. One  
19 preliminary study suggests that miR-155 could be interesting to assess disease severity in  
20 SSc-ILD [127].

#### 21 8. COMBINATION OF BIOMARKERS

22 Few studies have assessed the interest of combining biomarkers in ILD. In two independent  
23 cohorts of IPF (n=86 and n=63), combining SP-D, MMP-7 and osteopontin was more  
24 performant in distinguishing IPF-patients from patients with alternative ILD than using one  
25 biomarker alone [62]. Similarly, Rosas et al. proposed a five-protein signature (MMP-7,

1 MMP-1, MMP-8, IGFBP1 and TNF receptor superfamily member 1A) to distinguish IPF  
2 patients from controls with a sensitivity of 98.6% and specificity of 98.1% [99]. Song et al.  
3 suggested that at least three biomarkers (MMP-7, SP-A, and KL-6) were necessary to improve  
4 predictability of mortality in IPF as compared to clinical parameters [67].

5 In RA, a peripheral blood biomarker signature composed of MMP- 7, CCL18, and SP-D was  
6 proposed to diagnose subclinical ILD [105].

7

## 8 **II. Meta-analysis**

9 In all, 20 studies provided enough data to be included in the meta-analysis (Table III and  
10 Supplementary Table III). Among them, 10 concerned IPF: 704 patients could be included,  
11 73% of males, mean age: 68 years, whereas 10 concerned CTD-ILD: 1716 patients, 16% of  
12 males and mean age: 55 years. Heterogeneity of the studies included according to each  
13 outcome is presented in Supplementary Table IV. Funnel plots regarding diagnosis, are  
14 presented in Supplementary Figures 1 and 2.

15 Among the CTD-ILD studies, 8 included only SSc-patients corresponding to 1296 patients:  
16 208 (16%) of males, 439/1230 with available data (35.7%) of diffuse cutaneous form,  
17 368/1230 (29.9%) positive for anti-Scl70 antibodies and 460/1230 (37.4%) positive for anti-  
18 centromere antibodies.

19 In IPF, four biomarkers, KL-6, SP-D, SP-A, and MMP7 could be studied for diagnostic  
20 performance, whereas in CTD-ILD, studies concerned KL-6, SP-D, and CCL18. For SP-D  
21 and CCL18, data were only obtained in SSc-ILD.

22 In IPF, KL-6 had the strongest association with diagnosis of lung fibrosis (OR:  
23 520.95[110.07-2465.58],  $p < 0.001$ ), followed by SP-D (OR: 33.81 [3.20-357.52],  $p = 0.003$ ),  
24 MMP7 (OR: 23.67 [5.83-96.06],  $p < 0.001$ ) and SP-A (OR: 7.94[4.36-14.46],  $p < 0.001$ ) (Figure  
25 3). KL-6 was also the most performant to diagnose CTD-ILD (OR: 26.43[7.15-97.68],

1 p<0.001) and also when considering only SSc-ILD (OR: 21.86[5.07-94.24], p<0.001). SP-D  
2 and CCL18 could also diagnose SSc-ILD (OR: 13.24[3.84-45.71], p<0.001 and 3.31[1.25-  
3 8.77], p=0.016, respectively) (Figure 4)

4 For prognostic studies (decline in forced vital capacity and/or mortality), funnels plot are  
5 presented in Supplementary Figure 3. In IPF, KL-6 and CCL18 showed significant prognostic  
6 value with OR equal to 2.79 [1.65-4.71], p<0.001 and 10.22[4.72-22.16],p<0.001,  
7 respectively, whereas MMP7 was not statistically significant (P=0.108) (Figure 5). Data  
8 obtained in SSC-ILD confirmed the prognostic value of KL-6 and CCL18 (OR: 1.80[1.02-  
9 3.17], p=0.042 and 2.62[1.71-4.03], p<0.001)

10

## 11 **DISCUSSION**

12

### 13 **Inputs of biomarkers and pitfalls for translation in clinical practice**

14 In clinical practice, the physician is facing three main questions, for which biomarkers could  
15 be helpful.

#### 16 1. Diagnosis

17 Diagnosis of IPF remains challenging and relies on imaging and/or histology after exclusion  
18 of other differential diagnoses.

19 Our review and meta-analysis highlights a high value of alveolar epithelial cell damage  
20 biomarkers and MMP7 for diagnosis of ILD, with KL-6 being the most sensitive and specific  
21 biomarker and with the most consistent data. Whereas alveolar epithelial cell damage markers  
22 have been studied in IPF and CTD ILD, data regarding MMP7 concern mostly IPF.

23 However, one major limitation for use of alveolar cell damage markers in ILD diagnosis in  
24 practice is the ethnic variability of serum levels of some of these biomarkers dependent on  
25 polymorphisms. This raises questions about the generalizability of the results obtained in

1 Japanese cohorts as well as about the determination of an optimal cut-off [59,61]. This might  
2 be the reason for the current recommendation against measurement of serum biomarkers in  
3 IPF [15].

4 In CTD-ILD, the aim is to detect the disease at a subclinical stage in order to adapt  
5 monitoring and treatment. In this context, biomarkers could for example help to determine  
6 which patient should benefit from HRCT and pulmonary function tests. KL-6 has shown the  
7 strongest sensibility and accuracy for ILD diagnosis, but SP-D and CCL18 also appear to be  
8 sensitive biomarkers. However, some limitations must be taken into account: most of the  
9 studies were cross-sectional and small-sized.

10 Some other biomarkers, such as Th22 circulating cells or endothelial progenitors could be  
11 interesting to diagnose SSc-ILD, but their interest remains to be confirmed in prospective  
12 larger cohorts [128,129].

### 13 2. Disease severity

14 For this purpose, most of the data were obtained for KL-6 and SP-D. KL-6 appears as the best  
15 biomarker to reflect disease severity according to extent of parenchymal involvement on  
16 HRCT and impact on pulmonary function tests. However, we could not meta-analyze these  
17 data because of missing values (only one study reported enough data). Other studies only  
18 provided correlation with forced vital capacity and HRCT scores as continuous variables and  
19 did not use a cut-off to define severe disease (such as the staging system proposed in SSc  
20 [130]). Therefore, there is a need of standardization of criteria for severe disease on  
21 pulmonary function tests and HRCT for future studies aiming to identify biomarkers for  
22 staging purposes.

### 23 3. Prognosis

24 Convincing data were obtained for KL-6 and CCL18 with CCL18 identifying as having the  
25 most predictive value both in IPF and in SSc. However, these results need to be confirmed in

1 prospective studies. Some newly identified biomarkers, such as leptin and semaphoring 7a+  
2 regulatory T cells could be interesting to predict disease worsening in IPF and should be  
3 further studied [131,132].

4 The use of biomarkers in identifying patients more likely to benefit from a treatment has not  
5 been studied so far. With the availability of new treatments in IPF and CTD-ILD, it can be  
6 anticipated that stratification according to biomarkers will be investigated [12,13,133].

#### 7 4. Response to treatment

8 This domain has not been the matter of large studies despite its huge interest with regards to  
9 precision medicine. IGFBP-2 could be interesting for IPF diagnosis, but also to assess  
10 response to antifibrosing therapy [40]. Future studies should assess the use of biomarkers as  
11 surrogate endpoints in clinical trials to assess response, but also to predict tolerance, to  
12 treatments.

#### 13 5. A better understanding of pathogenesis

14 Interestingly biomarker studies could also reveal relevant targets in disease pathogenesis: for  
15 many years, IPF was considered to be a principally inflammatory disease, given the increase  
16 in inflammatory cells in the lungs. However, as previously showed, many targets identified  
17 are related to alveolar epithelial cell damage. These data highlight that IPF is rather an  
18 epithelial-driven disease whereby an aberrantly activated lung epithelium produces mediators  
19 of fibroblast migration, proliferation and differentiation into active myofibroblasts that  
20 produce high amounts of extracellular matrix leading to fibrosis. Besides our review and  
21 meta-analysis highlights that CTD-ILD and IPF share common biomarkers suggesting  
22 common pathways shared between different subtypes of ILD.

#### 23 **Limitations/strenghts**

1 First, most studies were small-sized, of retrospective design, cross-sectional and mostly  
2 obtained in Japanese cohorts (40%). Furthermore, we could not exclude a bias of publication  
3 leading to over-representation of positive studies.

4 Our meta-analysis could only include 20 articles because of the small sample size of the  
5 studies, the lack of data to perform meta-analysis and the lack of definition of ILD based on  
6 histological or HRCT. Diagnostic criteria for IPF have recently changed and most of the  
7 studies published before did not systematically use HRCT or histology [15]. However, using  
8 these stringent criteria, we could obtain confident data regarding biomarkers value.

9 Furthermore, in CTD-ILD, our meta analysis could only include SSc for most of the data  
10 obtained (except KL6 for diagnosis). Therefore, our data might not apply to other CTD.

11 Our study has also strengths: this is the first meta-analysis on this topic analyzing data on IPF  
12 and CTD-ILD and studying several biomarkers, using stringent inclusion criteria. Therefore,  
13 we could obtain confident results, which confirmed the high value of KL-6 for diagnosis and  
14 prognosis of ILD, both in IPF and CTD-ILD. Our study also highlights the sensitivity of SPD  
15 to diagnose lung fibrosis in IPF and SSc and of CCL18 as prognostic biomarker. This  
16 extensive literature review and meta-analysis allowed better delineating the place of different  
17 biomarkers in ILDs and highlighted that most of the biomarkers were shared by IPF and  
18 CTD-ILD, suggesting common pathways in both diseases. This is supporting the potential of  
19 drugs being effective in various subsets of ILDs.

## 20 **Future directions**

21 ILDs are complex and multifactorial diseases with a wide inter-individual variability: one  
22 may not believe that one biomarker might in the future allow diagnosis/staging or prognosis  
23 of all these different patients/diseases. Therefore, future research should work on the  
24 development of multiparameter models including combination of biomarkers, but also other

1 relevant clinical/biological/imaging parameters. Furthermore, large longitudinal studies with  
2 serial measurements of biomarkers are needed to confirm these results and assess sensitivity  
3 to change of the biomarkers. Response to treatment should be studied with the development of  
4 new effective therapies in ILDs.

## 5 **CONCLUSION**

6 ILDs are heterogeneous diseases associated with a poor prognosis. Recent advances have  
7 been achieved regarding treatments. However, earlier diagnosis and risk-stratification leading  
8 to individual treatment are still lacking to improve prognosis and management of the patients.  
9 Despite their heterogeneity, ILDs share common biomarkers, suggesting common pathways.  
10 KL-6, SP-D, SP-A and MMP7 appear as the most promising biomarkers for diagnosis,  
11 whereas KL-6 and CCL18 are prognostic factors. Prospective longitudinal studies are  
12 warranted to confirm these results and also to identify new biomarkers, which are disease  
13 specific and to determine biomarkers to use as surrogate endpoints in clinical trials and to  
14 monitor response to treatments.

15

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## 19 FIGURE LEGENDS

20 Figure 1: Flow-chart of the study

21 Figure 2: Emerging biomarkers in IPF and CTD-ILD with their cellular source. IPF:  
22 idiopathic pulmonary fibrosis, CTD: connective tissue disease, ILD: interstitial lung disease.

23 Figure 3: Forest plot of odd ratio based on the number of patients with concentration of the  
24 studied biomarker above the cut-off for diagnosis of IPF for KL-6 (A), SP-D (B), SP-A (C)  
25 and MMP7 (D). Each square represents an individual odds-ratio estimate, the size of the  
26 square being proportional to the weight given to the study. The lines represent the 95% CI for  
27 the point estimate in each study.

28 Figure 4: Forest plot of odd ratio based on the number of patients with concentration of the  
29 studied biomarker above the cut-off for diagnosis of CTD-interstitial lung disease for KL6  
30 (A), KL-6 in SSc (B), SPD (C) and CCL18 (D). Each square represents an individual odds-  
31 ratio estimate, the size of the square being proportional to the weight given to the study. The  
32 lines represent the 95% CI for the point estimate in each study.

1 Figure 5 Forest plot of odd ratio based on the number of patients with concentration of the  
2 studied biomarker above the cut-off for prognosis of interstitial lung disease: (A-C):  
3 prognosis of IPF: KL-6 (A), CCL18 (B), MMP7 (C); (D-E) prognosis of SSc-ILD: KL-6 (D)  
4 and CCL18 (E). Each square represents an individual odds-ratio estimate, the size of the  
5 square being proportional to the weight given to the study. The lines represent the 95% CI for  
6 the point estimate in each study.

7 Table I: Circulating biomarkers associated with idiopathic pulmonary fibrosis  
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Biomarker	Study	Country	Diagnosis	Severity	Prognosis
KL-6	[23,24,26–28,31–33,118,119]	Japan, Germany	X		X
SP-D	[23,24,27,62–66,120]	Japan, USA, The Netherlands, UK	X	X	X
SP-A	[23,24,27,65–67]	Japan, USA, South Korea	X		X
CC16	[73]	Mexico	X		
Ca19-9	[63]	UK	X		X
Ca-125	[63]	UK			X
MCP-1	[23,77,78]	Japan, UK	X		X
CCL18	[24,81]	Italy, Germany, Japan	X		X
CXCL13	[86,87]	USA	X	X	X
YKL 40	[93,94]	The Netherlands	X	X	X
ICAM1	[97]	Japan	X		
MMP1	[99]	USA	X		
MMP1 and MMP7	[100]	Portugal	X		
MMP7	[24,62,63,67,97,99–101]	USA, Italy, Korea, Japan, UK, Australia, Austria, Belgium, Canada, Croatia, Czech Republic, France, Germany, Ireland, Israel,	X	X	X

		Netherlands, Serbia, Spain, Switzerland,			
Osteopontin	[62]	USA	X		
Periostin	[107,108]	USA, Japan	X	X	X
serum type IV collagen 7S	[112]	Japan	X		
collagen degradation biomarkers	[111]	UK	X		X
Laminin, type IV collagen, PIIINP, and hyaluronic acid	[113]	China	X	X	X
LOXL2	[117]	USA			X
AntiHSP 70	[120]	USA	X		X
Oxydative stress markers	[136]	Japan	X	X	X
Fibrocytes	[122]	Canada			X
microRNA (miR-21, miR-199a-5p, miR-200c, miR-31, let-7a, let-7d, miR- 25-3p)	[124,125]	China	X		X

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Table II: Circulating biomarkers for CTD-ILD (interstitial lung disease associated with connective tissue disease)

Biomarker	Study	Disease	Country	Diagnosis	Severity	Prognosis
KL-6	[23,28,31,41,46-55,121-123]	CTD-ILD, RA, SSc, DM, PM, Antisynthetase syndrome, sarcoidosis	Japan, Turkey, Hungary, USA, Japan, Italy, France, Norway, China	X	X	X
SP-D	[23,41,45,46,49,66,105,137]	CTD-ILD, RA, SSc, DM, PM	Japan, USA, France, Norway, China	X	X	
SP-A	[46,65,66],	SSc, DM, PM	USA, China	X		X
CC16	[45]	SSc	Japan	X	X	
MCP-1	[23,46,47,77,80]	CTD-ILD, sarcoidosis, SSc, DM, PM	Japan, USA, Canada, China, Japan	X		X
CCL18	[41,83,84,105]	RA, SSc	USA, Japan, Norway, France	X	X	X
IL-6	[78,89]	SSc, DM (including CDAM)	UK, China	X		X
sIL2 receptor	[90,91]	Sarcoidosis	The Netherlands	X	X	

YKL 40	[92–94]	CTD-ILD, DM, PM, Sarcoidosis	The Netherlands, Japan, Denmark	X	X	X
ICAM-1	[98]	SSc	Japan			X
MMP7	[105]	RA	USA	X	X	
Laminin, type IV collagen, PIIINP, and hyaluronic acid	[113]	CTD-ILD	China	X	X	X
hsa-miR-214-5p and hsa-miR-7-5p	[126]	RA	Japan	X		

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ILD: interstitial lung disease, HRCT: high resolution computed tomography, SSc: systemic sclerosis, DM: dermatopolymyositis, PM: polymyositis, CTD: connective tissue disease, CDAM: clinically amyopathic dermatomyositis.

6 Table III Studies included in the meta-analysis (by alphabetical order)

Author	Biomarker	Disease	n	Smoker*	Males	Age	Outcome
Benyamine[53]	KL-6	SSc	75		9	59.3±14	Diagnosis
Elhai [41]	KL-6, SP-D and CCL18	SSc	427	170	78	59.6 ±13.6	Diagnosis and progression
Hamai[24]	KL-6, SP-A, SP-D, MMP7 and CCL18	IPF	65		50	69.3±8.5	Diagnosis
Hant[137]	KL-6 and SP-D	SSc	66		9	48±12	Diagnosis
Hasegawa[98]	KL-6, SP-D	SSc	92		15	52.3 ±13.5	Diagnosis
Hoffmann-Vold[83]	CCL18	SSc	298	107	55	53.9	Progression
Hu[28]	KL-6	CTD-ILD	373			59.9 ±10.9	Diagnosis
Ishii[33]	KL-6	IPF	59		43	68 [44-82]	AE and mortality
Kinoshita[138]	KL-6	RA	47		14	63.5 [37-79]	Diagnosis
Kodera[84]	CCL18	SSc	123		17	51±14	Diagnosis
Kumánovics[52]	KL-6	SSc	173		19	57.6	Mortality

						±11.3	
Morais[100]	MMP7	IPF	47	28	30	70.6±9.5	Diagnosis
Prasse[81]	CCL18	IPF	72	33	49	67.2±8.6	Mortality and progression
Rosas[99]	MMP7	IPF	74	58	49	65.9±9.4	Diagnosis
Samukawa[27]	KL-6,SP-A and SP-D	IPF	20		18	68.9±9.4	Diagnosis
Song[67]	MMP7	IPF	118	88	95	62.8±8.1	Mortality
Tzouvelekis[102]	MMP7	IPF	97	70	76	70±8	Diagnosis and mortality
Wakamatsu[135]	KL-6	IPF	66	38	44	73 [51-86]	AE and mortality
White[62]	SP-D and MMP7	IPF	86	60	62	63±8.8	Diagnosis
Yanaba[49]	KL-6 and SPD	SSc	42		6	49±18	Diagnosis

1 Values are median [interquartile range] or mean ± SD or numbers of observations. SSc: systemic sclerosis, RA:  
2 rheumatoid arthritis, IPF: idiopathic pulmonary fibrosis. ILD: interstitial lung disease, AE: acute exacerbation. \*  
3 smoker: past or current.

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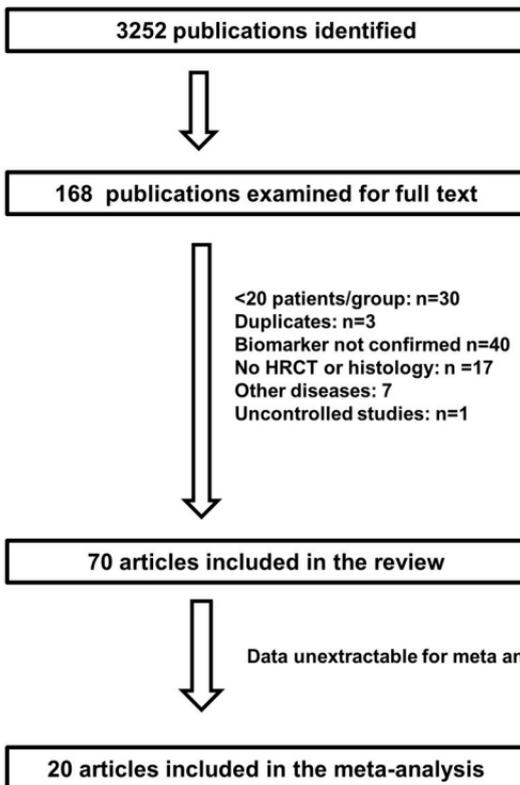
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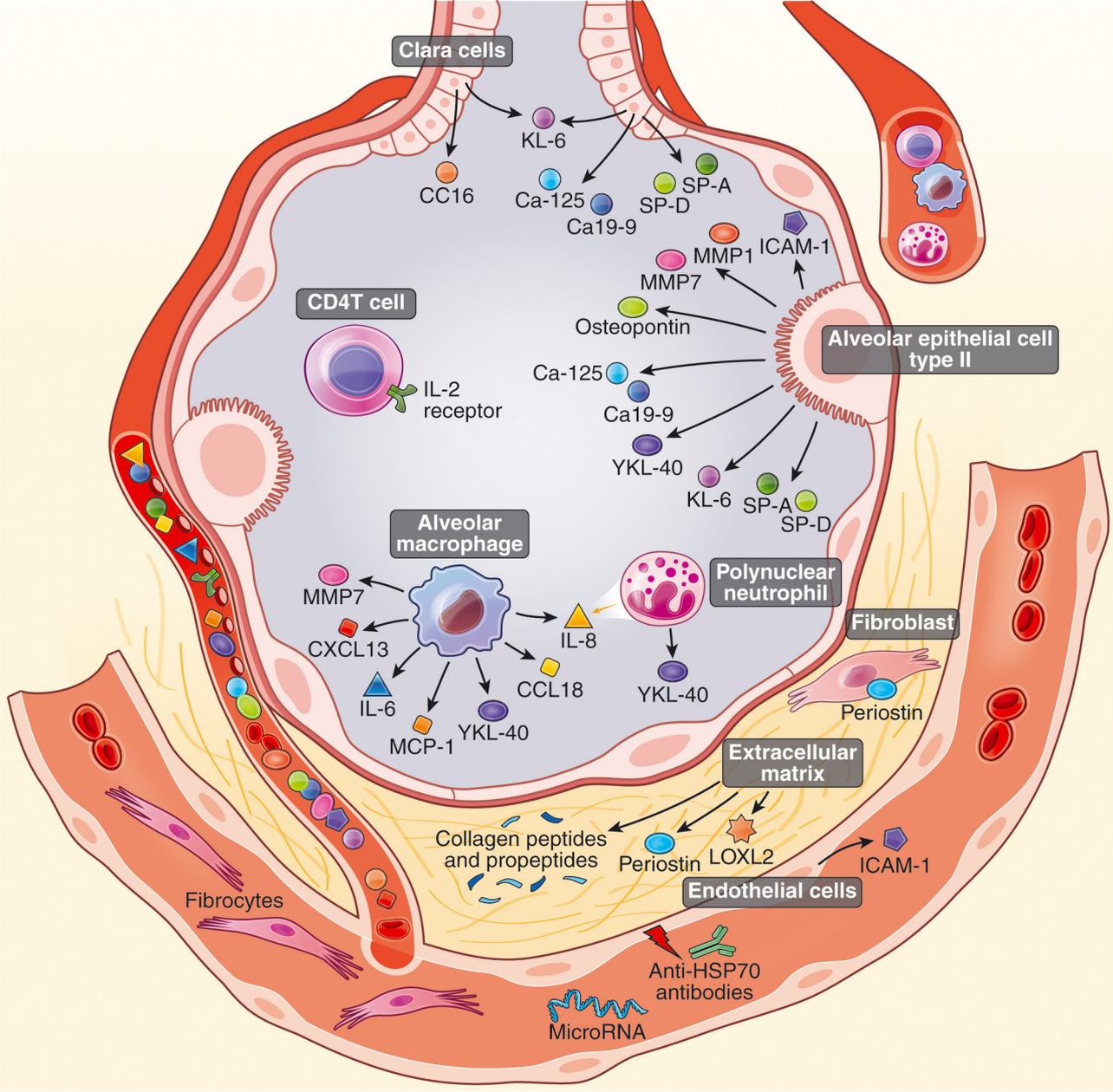
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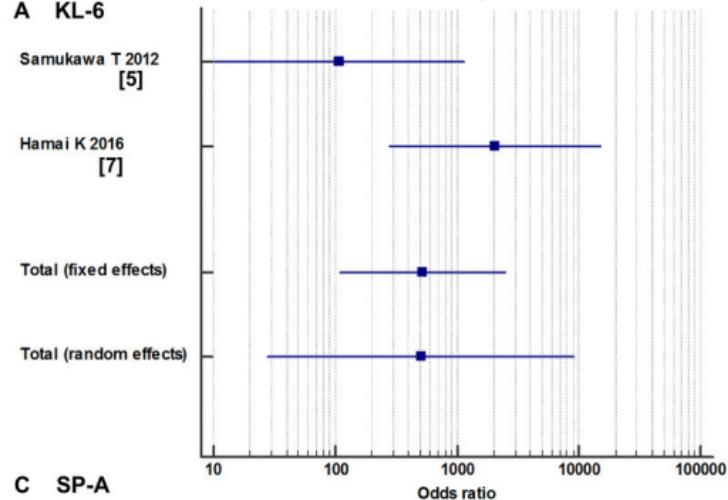
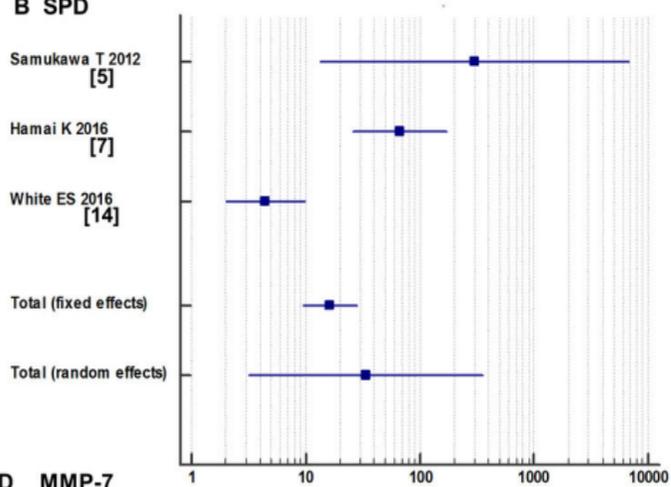
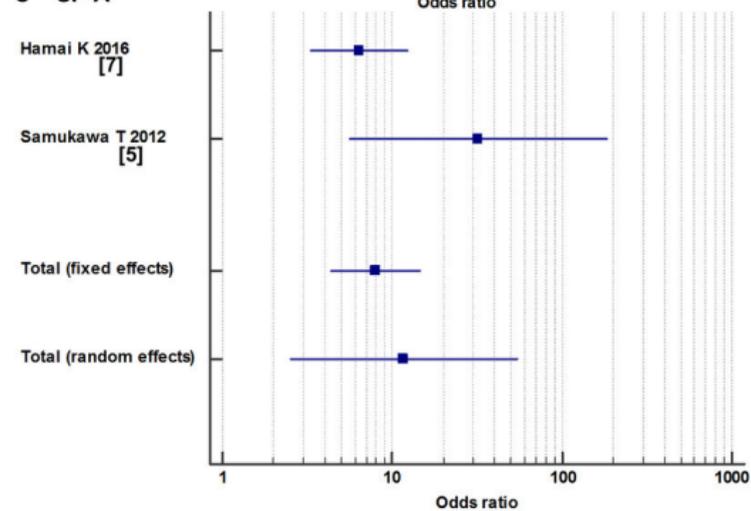
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**A KL-6****B SPD****C SP-A****D MMP-7**