Respiratory Medicine (2014) 108, 766–774

Available online at www.sciencedirect.com

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Peripheral CD4+ cell prevalence and pleuropulmonary manifestations in systemic lupus erythematosus patients

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Received 24 October 2013; accepted 10 February 2014
Available online 18 February 2014

KEYWORDS
Systemic lupus erythematosus;
Lung;
Lung function;
T cells;
CD4+CD25hi+

Summary
Introduction: Systemic lupus erythematosus (SLE) is an autoimmune disease involving several organs, including the lungs. Previous results confirmed changes of peripheral T cell subsets in lupus patients; however no data are available about their possible relationship with pulmonary involvement.
Objective: To determine pulmonary manifestations and potential relationship in changes of peripheral CD4+ T cell subsets.
Methods: Patients with SLE ($N = 28$) were enrolled in complex pulmonary examination. Patients were divided into groups with pleuropulmonary manifestations (SLE_{pulm} $N = 13$ age: 44.9 ± 3.3 years, female: male = 11:2) or without (SLE_c $N = 15$ age: 27.2 ± 3.7 years, female: male = 12:3). Peripheral blood was taken for T helper (Th)1, Th2, Th17, CD4+CD25hi+ and regulatory T (Treg: CD4+CD25hi+ CD127−) cell analysis from SLE patients and healthy volunteers (controls, $N = 40$).
Results: SLE_{pulm} patients were older, had more pulmonary symptoms and significantly decreased pO_2 as compared to SLE_c group. Ventilatory disorder was present in 92% of SLE_{pulm} patients, with significantly decreased lung volumes, signs of airway involvement and decrease in DLco. Significant increase in Th1/Th2, while decrease in Th17/Treg ratios was present in all SLE compared to controls. In SLE_{pulm} CD4+CD25hi+ subset without changes in Treg number was

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http://dx.doi.org/10.1016/j.rmed.2014.02.006

significantly increased as compared to SLE_c and this subgroup of T cell showed significant positive correlation with dynamic lung function parameters and DLco ($p < 0.05$).
Conclusion: In lupus patients pleuropulmonary manifestations are prevalent and lung function and blood gas measurements should be regularly performed in the daily clinical assessment. Significant increase of activated CD4+CD25hi+ T cells, but not Treg is associated with decreased lung function parameters in SLE_{pulm} patients.

Introduction

Systemic lupus erythematosus (SLE) is characterized by the presence of autoantibodies and immuncomplexes targeting multiple organs, including skin, joints, kidneys, small vessels and central nervous system. SLE may also affect the pleura and the lungs. According to the affected area pulmonary manifestations include pleuritis, parenchyma destruction, airway disease, vascular or musculoskeletal involvement.

Most often SLE subjects will present with pleuritis (35–50%), which is part of the American College of Rheumatology (ACR)diagnostic criteria [1,2]. It is often asymptomatic but may be accompanied by non-productive cough, dyspnea or thoracic pain. Various studies have shown that high resolution computer tomography (HRCT) reveals interstitial lung disease (ILD) at least in a third of partly asymptomatic SLE subjects, suggesting that subclinical lung disease is even more common in these patients [3]. Adult respiratory distress syndrome (ARDS) may occur in 5–15% of patients [4]. Rare, but clinically severe pulmonary manifestations are diffuse alveolar hemorrhage (1–5%) and acute lupus pneumonitis (1–4%) [5]. Pulmonary arterial hypertension due to SLE has been identified in approximately 4% of subjects, but is present in 75% in patients with Raynaud's syndrome [6,7]. Shrinking lung syndrome, although a lesion of diaphragmatic origin, severely affects lung function and is more prevalent than thought formerly [8].

Concerning airway involvement, only sparse data are available. Bronchial wall thickening and bronchiectasis are present in approximately 20% of subjects with SLE, but it is considered frequently clinically irrelevant. Obliterative bronchiolitis has rarely been described [9]. Reductions in the forced expiratory volume in one second (FEV_1)/forced vital capacity (FVC) ratio and forced expiratory flow rate between 25% and 75% of FVC (FEF_{25-75}) were detected in relative high proportion (5.7–24%) of patients, consistent with small airways disease [10,11].

Although evidence is growing on pathomechanism of systemic autoimmun diseases with multiple manifestations, there is large variance in details. Systemic review of pulmonary manifestations is rare, and possible clinical implications of more accurate observations are missing.

Imbalance of T-helper (Th) cell differentiation and subsequent cytokine dysregulation is implicated in inflammatory and autoimmune diseases. Elevated ratio of Th1/Th2 cytokines correlates positively with disease activity in SLE patients [12,13]. Hoyer and colleagues suggests that Th1 cells drive the early autoantibody response in systemic autoimmune disease [14].

Regulatory T (Treg) cells are playing a critical role in the maintenance of self-tolerance by preventing the activation and function of effector T cells [15,16]. Disturbances in number and suppressive capacity of Treg cells are thought to have a role in development and activity of SLE [17,18,19]. Additionally, activated Treg cells are involved in differentiation of interleukin (IL)-17 producing Th17 cells [20]. Th17 cells are implicated in chronic inflammatory processes, however only few data are available relating to SLE [21,22]. IL-17 produced by Th17 cells may play a role in the amplification and perpetuation of the inflammatory response in organs targeted by SLE [23].

Despite the high prevalence of pleuropulmonary abnormalities which often result in remarkable loss of quality of life and are important causes of SLE mortality, we know little about the mechanisms leading to the development of lung manifestations. In this study we aimed to determine a practical and useful clinical approach for screening pulmonary involvement in SLE. Additionally, differences in peripheral Th1, Th2, Treg and Th17 cell prevalence among patients with and without pleuropulmonary involvement and all SLE patients to healthy controls were analyzed.

Methods

Study subjects included 28 SLE patients with mean age of 35.4 ± 2.9 (mean ± SEM, range 11–64 years) years who were referred to the Department of Pulmonology for pleuropulmonary involvement screening from the I[st]

Table 1 Prevalence of ACR classification criteria in SLE_c ($N = 15$) and SLE_{pulm} ($N = 13$) patients.

ACR criteria	SLE_c *N* (%)	SLE_{pulm} *N* (%)
Malar rash	10 (67)*	3 (23)
Discoid rash	2 (13)	2 (15)
Photosensitivity	11 (73)*	5 (38)
Oral ulcers	4 (27)	2 (15)
Arthritis	11 (73)	9 (69)
Serositis	5 (33)	5 (38)
Renal disorder	9 (60)*	3 (23)
Neurologic disorder	5 (33)	2 (15)
Hematologic disorder	10 (67)	9 (69)
Immunologic disorder	14 (93)	9 (69)
Antinuclear antibody	15 (100)	12 (92)#

Values are number positive (%), #data missing from 1 patient, SLE_{pulm}: SLE pleuropulmonary manifestation group, SLE_c: SLE without pleuropulmonary manifestation group; *: $p < 0.05$ vs. SLE_{pulm}.

Department of Pediatrics, Semmelweis University and from the National Institute of Rheumatology and Physiotherapy. All patients fulfilled the updated ACR criteria for the classification of SLE (Table 1) [24]. Disease activity was scored based on the SLE disease activity index (SLEDAI) [25]. Patient characteristics are listed in Table 2. All patients received immunosuppressive treatment.

Patients were asked for respiratory symptoms, underwent physical examination, chest X-ray, electrocardiography and routine laboratory analysis. FVC, FEV_1, FEV_1/FVC, flow volume curves, FEF_{25-75} and airway resistance (Raw) were measured by means of electronic spirometer and pletysmography (PDD-301/s, Piston, Budapest, Hungary) according to the American Thoracic Society guidelines [26]. Three technically acceptable maneuvers were performed and the highest of them was used. Carbone monoxide diffusing lung capacity (DLco) was measured with single breath method. Pulmonary function variables were expressed as percentage of predicted values. Arterialized capillary blood gases, pH and bicarbonate levels were analyzed at rest at room air (Stat Profile® pHOx Plus®, Nova Biomedical Corporation, Waltham MA, USA).

Peripheral blood was taken from 22 SLE patients at the time of pulmonary screening (age 32.7 ± 3.5 years (range: 11–64 years), female/male:17/5, Table 2) and from a population samples of 40 healthy blood donor adults (age 35.5 ± 5.6 years (range: 20–70 years), female/male: 36/4) for T cell analysis. All cases and controls were of Caucasian ethnic origin. The study was reviewed and approved by the ethics committee of the Department of Pulmonology.

Peripheral T cell subpopulations were analyzed using 6 ml sodium heparinized venous blood. Peripheral blood mononuclear cells were isolated and frozen at −80 °C. Identification of surface markers (CD3, CD4, CD8, CD25, CD127; CXCR3, CCR4, CCR6, BD Biosciences Pharmingen, San Diego, CA, USA) were performed with a BD FACS Aria according to manufacturer's protocols as from isolated PBMC-s described previously [27]. Th1 cells were identified by CD4+CXCR3+, while Th2 cells by CD4+CCR4 positivity [28]. For the detection of Treg cells CD4+CD25hi+ positivity accompanied by low expression of CD127 was used [29], additionally CD4+CD25hi+ cells were also measured. Th17 cells were characterized by CD4+CCR4+ and CCR6+ surface markers [30]. All CD4+ T cell numbers are expressed in G/L accordingly to patients absolute lymphocyte count.

Data are reported as means ± SEM. Statistical analysis was performed with GraphPad software (Graph Pad Prism 5.0 by Graph Pad Software Inc., San Diego, USA). Normally distributed data were analyzed by unpaired *t*-test and ANOVA. After one-way ANOVA, if significant difference ($p < 0.05$) was found, the Newman–Keuls multiple comparison post-hoc test was used for further analysis. Categorical data were analyzed by $\chi 2$ or Fisher's exact test. Parametric data were compared using Student's *t*-test. $p < 0.05$ was considered statistically significant.

Table 3 Prevalence of chest complaints and blood gas parameters in SLE_c ($N = 15$) and SLE_{pulm} ($N = 13$) patients.

Complaints	SLE_c	SLE_{pulm}
Effort dyspnea (N)	1 (7%)*	11 (85%)
Cough (N)	2 (13%)	5 (38%)
Chest pain (N)	1 (7%)	4 (31%)
pH	7.431 ± 0.008	7.460 ± 0.008
pCO_2 (mmHg)	29.85 ± 0.38	30.23 ± 0.53
pO_2 (mmHg)	92.73 ± 2.28*	70.23 ± 3.51
Actual HCO_3^- (mmol/l)	20.21 ± 0.38	21.82 ± 0.48

Values are number positive (%); *: $p < 0.05$ vs. SLE_{pulm}.

Results

Pulmonary manifestations

Patients in the SLE_{pulm} group were significantly older than SLE_c patients; however duration of the disease did not differ between groups. SLE_{pulm} patients were more likely to

Table 2 Patient characteristics and immunosuppressive medication.

	All SLE patients ($N = 28$)		SLE patients with T cell analysis ($N = 22$)	
Characteristic	SLE_c $N = 15$	SLE_{pulm} $N = 13$	SLE_c $N = 13$	SLE_{pulm} $N = 9$
Age (years)	27.2 ± 3.7*	44.9 ± 3.3	24.8 ± 3.7*	44.0 ± 4.7
Gender (female:male)	12:3	11:2	10:3	7:2
BMI ($kg/m^{2)}$	22.0 ± 1.3	23.5 ± 0.9	21.5 ± 1.3	22.8 ± 1.0
Age at onset, years	19.3 ± 3.1*	36.9 ± 2.9	17.9 ± 2.9*	37.2 ± 4.0
Time from diagnosis, years	7.9 ± 1.8	7.0 ± 2.4	7.0 ± 1.7	5.9 ± 2.7
Smoker (*N*)	2 (13%)*	6 (46%)	2 (15%)*	4 (44%)
SLEDAI (>3) (*N*)	3 (20%)*	11 (85%)	2 (15%)*	8 (89%)
Immunosuppressive medication (*N*)	15 (100%)	13 (100%)	13 (100%)	9 (100%)
Systemic corticosteroid (*N*)	8 (53%)*	12 (92%)	7 (54%)*	8 (89%)
Methylprednisolon dose (mg/day)	3.6 ± 1.9*	15.4 ± 2,8	2.0 ± 0.7*	15.6 ± 3.3
Methotrexat (*N*)	2 (13%)	3 (23%)	2 (15%)	3 (33%)
Cyclophosphamid (*N*)	5 (33%)	8 (61%)	4 (30%)	5 (55%)
Azathioprin (*N*)	6 (40%)*	2 (15%)	*5 (38%)**	*1 (11%)*

No significant differences between all patient groups and patients with T cell analysis groups were found. Values are given in mean ± SEM. SLE_{pulm}: SLE pleuropulmonary manifestation group SLE_c: SLE without pleuropulmonary manifestation group; *$p < 0.01$ vs. SLE_{pulm}.

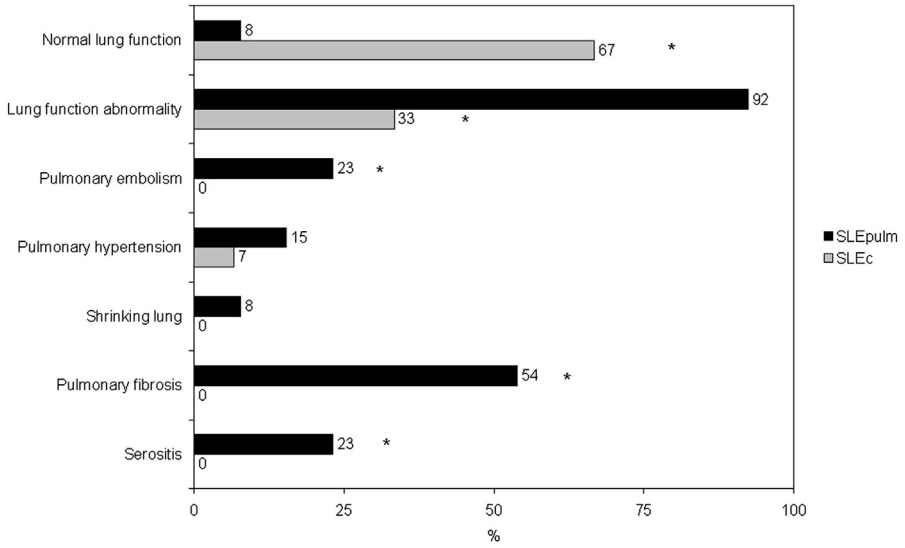

Figure 1 Prevalence of pulmonary manifestations at the time of pulmonary examinations in SLE_c and SLE_{pulm} patients. Pulmonary embolism represents patients on continuous anticoagulant treatment as the result of previously confirmed pulmonary embolism. *$p < 0.0001$ vs. SLE_{pulm} group.

have active disease (Table 2). Immunosuppressive therapy did differ significantly in the daily steroid use, as patients in the SLE_{pulm} had higher doses as SLE_c patients. Azathioprin was more commonly used as compared to other immunosuppressive agents in SLE_c.

SLE_{pulm} patients had significantly more pulmonary complaints than their SLE_c counterparts (Table 3). Dyspnea was the leading symptom in SLE_{pulm} and was associated with significantly decreased pO_2 in these patients. The prevalence of different pulmonary manifestations in all SLE patients is shown in Fig. 1. Significantly increased number of serositis, shrinking lung, pulmonary vascular diseases were noted in SLE_{pulm} patients, however it was the most pronounced in respect to lung function abnormalities (SLE_{pulm}: 92% vs. SLE_c: 33%, $p < 0.0001$) and fibrosis (SLE_{pulm}: 54% vs. SLE_c: 0%, $p < 0.0001$). Two patients in the SLE_c group were formerly treated as a result of interstitial lung disease, however had no signs of pulmonary involvement at the time of analysis. Most patients (69%) had restrictive and mixed type, whereas one fifth in both groups dominantly mild obstructive ventilatory disorder (Fig. 2). Although the mean values for lung function parameters were within the normal range in SLE_c group, decreased FEF_{25-75} and increase in Raw values indicated incipient small airway disease in some of these patients and were regarded as obstructive ventilatory disorder in this group. Significantly decreased values in the SLE_{pulm} group compared to SLE_c were measured for FVC, FEV_1 and FEF_{25-75} (Fig. 3). DLco (96.7 $\pm$ 4.1 vs. 61.8 $\pm$ 5.1% predicted, $p < 0.01$) and lung volume adjusted KL_{CO} (102.9 $\pm$ 7.1 vs. 72.5 $\pm$ 3.4% predicted, $p < 0.01$) were significantly lower in the SLE_{pulm} than in the SLE_c patient group (Fig. 3).

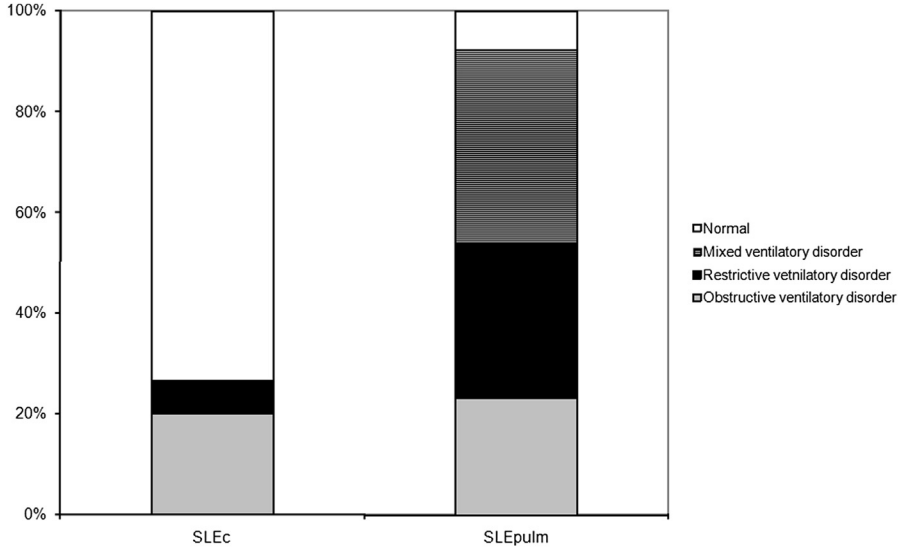

Figure 2 Distribution of lung function abnormalities in SLE_c and SLE_{pulm} patients ($p = 0.0018$ vs. SLE_{pulm} group).

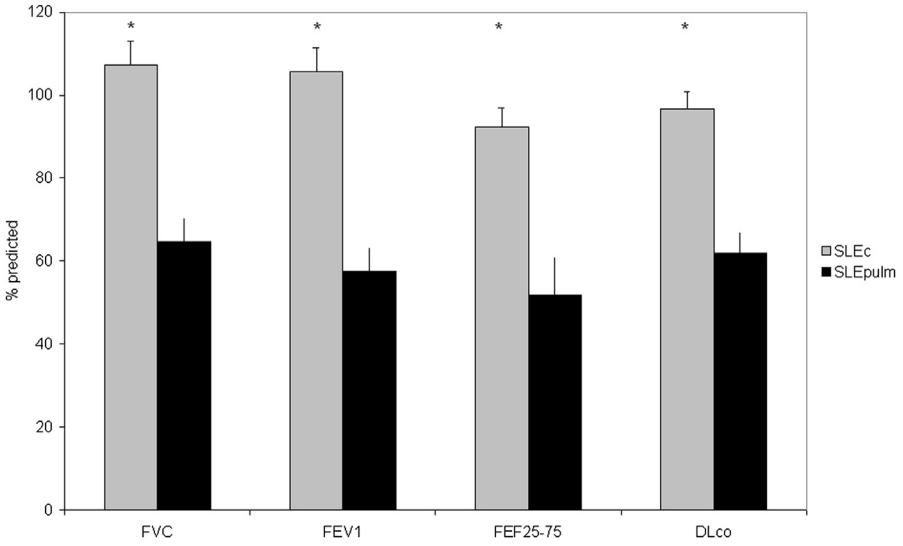

Figure 3 Pulmonary function parameters in SLE_c and SLE_{pulm} patients. Mean values ± SEM of forced vital capacity (FVC % predicted), forced expiratory volume in one second (FEV_1 % predicted), forced expiratory flow (FEF_{25-75}% predicted) and DLco. *$p < 0.05$ vs. SLE_{pulm} group.

Peripheral CD4+ T cell subpopulation prevalence

As a marker of the disease significantly decreased absolute number of lymphocytes was noted in all SLE patients (SLE_{pulm}: 1.55 ± 0.36 G/L; SLE_c: 1.19 ± 0.17 G/L; control: 2.14 ± 0.10 G/L; $p < 0.05$ vs. both SLE groups). To avoid changes resulting from change in lymphocyte number not prevalence but absolute number of respective CD4+ cell populations were analyzed.

No change in absolute circulating number of Th1 cells was measured in SLE patients while significantly decreased number of Th2 cells resulted into elevated Th1/Th2 ratio as compared to controls. There was no difference between SLE_{pulm} and SLE_c subgroups in respect to Th1, Th2 cells or Th1/Th2 ratio (Table 4. Fig. 4(A)).

Significant decrease in Th17 and Treg (CD4+CD25hi+ CD127−) was present in SLE patients as compared to controls, without differences according lung involvement (Table 4). Interestingly, Th17/Treg ratio was also lower in lupus patients as in healthy subjects (Fig. 4(B)). Additional analysis using CD4+CD25hi+ positive cells revealed significantly increased cell number in SLE_{pulm} compared to SLE_c or controls (Table 4). Similarly decreased relative proportion of Treg in CD4+CD25hi+ cells in SLE_{pulm} to SLE_c and controls suggests pleuropulmonary specific increase of other, non "real" Treg cell in SLE_{pulm} probably representing an other subgroup of activated T cells (Fig. 4(C)).

Analyzing relationship between lung function parameters in SLE patients and differences in peripheral Th1, Th2 Th17 or Treg cell subsets no association was observed. In contrast significant association was demonstrated regarding static and dynamic lung function parameters (FVC, FEV_1) or DLco and CD4+CD25hi+ or Treg/CD4+CD25hi+ ratio. This is further emphasizing a possible lung specific CD4+CD25hi+ T cell subset, not incorporating "real" Tregs (Fig. 5).

Discussion

In our analysis high prevalence of lung manifestations in SLE patients was observed. This is in accordance with a recent analysis [8] but higher than detected in earlier studies [2], maybe partly due to patient selection bias, as specific interest on pulmonary manifestation might have resulted in enrollment of more patients with pulmonary complaints. This can influence some of our results — e.g. on correlation between lung manifestations and disease activity — but also allows a more detailed examination of this entity. The most prevalent pulmonary dysfunction

Table 4 Prevalence peripheral CD4+ T lymphocytes in SLE_c ($N = 13$) and SLE_{pulm} ($N = 9$), all SLE ($N = 22$) and control ($N = 40$) patients.

	SLE_c	SLE_{pulm}	All SLE	Control
Th1 (CD4+CXCR3+) G/L	0.655 ± 0.108	0.846 ± 0.199	0.731 ± 0.102	0.865 ± 0.069
Th2 (CD4+CCR4+) G/L	0.023 ± 0.005#	0.023 ± 0.006#	0.023 ± 0.004#	0.138 ± 0.015
Th17 (CD4+CCR4+CCR6+) G/L	0.012 ± 0.003#	0.023 ± 0.006#	0.016 ± 0.003#	0.067 ± 0.007
CD4+CD25hi+ G/L	0.034 ± 0.007*	0.218 ± 0.046#	0.108 ± 0.027	0.093 ± 0.008
Treg (CD4+CD25hi+ CD127−) G/L	0.030 ± 0.006#	0.032 ± 0.012#	0.031 ± 0.005#	0.056 ± 0.005

SLE_{pulm}: SLE pleuropulmonary manifestation group; SLE_c: SLE without pleuropulmonary manifestation group; *$p < 0.05$ vs. SLE_{pulm}; #$p < 0.05$ vs. control.

was detected as decrease in dynamic lung function parameters (FVC, FEV_1) often also accompanied by changes reflecting small airway dysfunction (FEF_{25-75}) and diminished diffusion capacity (DLco). Changes in lung function were observed even without abnormalities on chest X-ray, fluoroscopy or HRCT. Regarding the notably higher proportion of functional than radiological changes among patients, we confirmed recent results from Allen et al. [8] stating that lung function testing is fundamental in screening for lung manifestations in SLE. Additionally to the leading symptoms of dyspnea blood gas analysis can substantiate lung involvement as SLE_{pulm} patients had significantly decreased pO_2 values.

In our cohort of SLE patients significant decrease in the absolute number of Th2 cells without change in Th1 cells resulted in elevated Th1/Th2 as compared to controls irrespective of lung involvement. Similarly, significantly lower Th17 and Treg cell proportions were found in patients with SLE disease. Although investigations of different peripheral $CD4^+$ T cell subsets according to disease activity were performed results remain controversial. Previous investigations confirmed increase in Th1/Th2 ratio in peripheral blood of SLE patients associated with disease activity [12], while Chang et al. did not show association between higher Th1/Th2 ratio and SLEDAI score [31]. Th1 cells seem to drive the early autoantibody response [14] and increase in Th1 cytokines might promote lung fibrosis [32]. In our study all lupus patients had decreased lymphocyte count, so absolute numbers of T cells were analyzed. This revealed that the absolute number of Th1 cells was similar to controls, while Th2 decrease contributed to the increase in Th1/Th2 ratio in all SLE patients without reflecting disease activity or pulmonary involvement.

Th17 cells are a new lineage of effector CD4+ T cells that are characterized by their production of IL-17A. Most analyses use IL-17 labeling, while recent studies confirmed high CCR4 and CCR6 expression on CD4+ cells also corresponding to Th17 cells [30,33,34]. Emerging data support the importance of Th17 cells in inflammatory diseases especially autoimmunity [35]. However, there are controversial data on the number of Th17 cells in the periphery and in the affected organs in lupus patients. Th17 cells and microenvironmental IL-17A has been shown to be involved in vascular inflammation in SLE [43]. Hoyer et al. suggested that Th17 cells might be responsible for the persistent (chronic) inflammation of the tissues in autoimmune mediated inflammation [14]. Th17 derived cytokines (dominantly IL-17) play an important role in lung inflammation by mediating neutrophil recruitment [36]. In a murine model of hypersensitivity pneumonitis and lung fibrosis higher number Th17 derived cytokines were expressed in the lung tissue, however the source of the cytokines were γδT and not Th17 cells [37]. In our SLE patients significantly decreased peripheral Th17 cell number was noted as compared to healthy controls, while no differences according lung involvement were noted.

Treg cells might play an important role in the development of autoimmunity. In our patients decreased Treg number was observed in all SLE patients. The reported findings on Treg cells in SLE are not consistent, however most studies found decreased number of Treg cells in the peripheral blood of SLE patients [17,38]. In addition to low numbers, defects in suppressive capacity of Treg cells might also contribute to development or increased activity of the disease [19,39]. In contrast, increase in Treg cells was observed in SLE patients receiving corticosteroids which act via increase in Treg cell number or activity

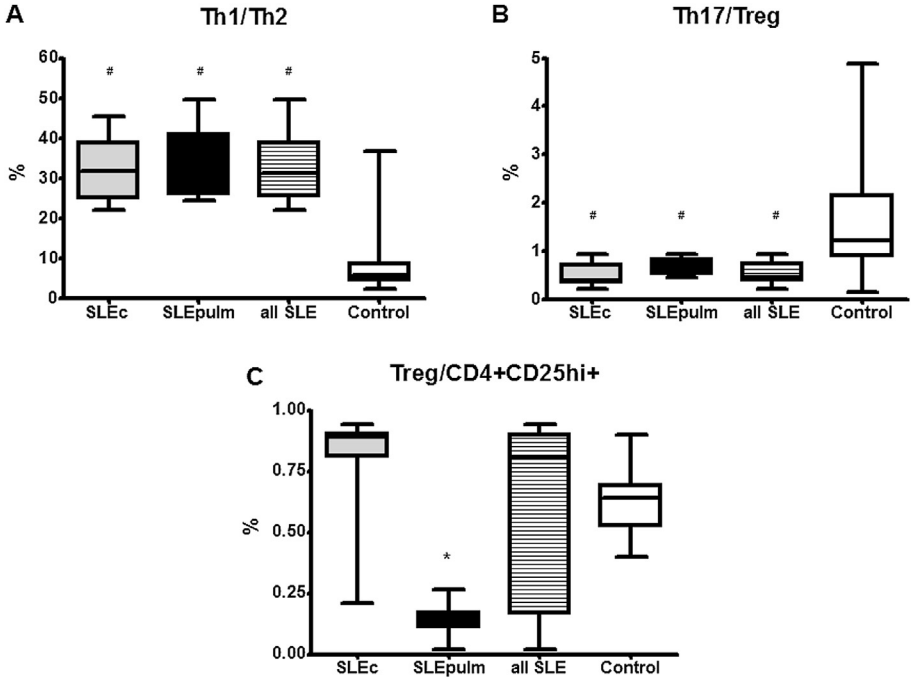

Figure 4 (A): Th1/Th2; (B): Th17/Treg and (C): Treg/CD4+CD25hi+ cell ratio in SLE_c, SLE_{pulm} all SLE and control patients. *$p < 0.05$ vs. SLE_{pulm}; #$p < 0.05$ vs. control.

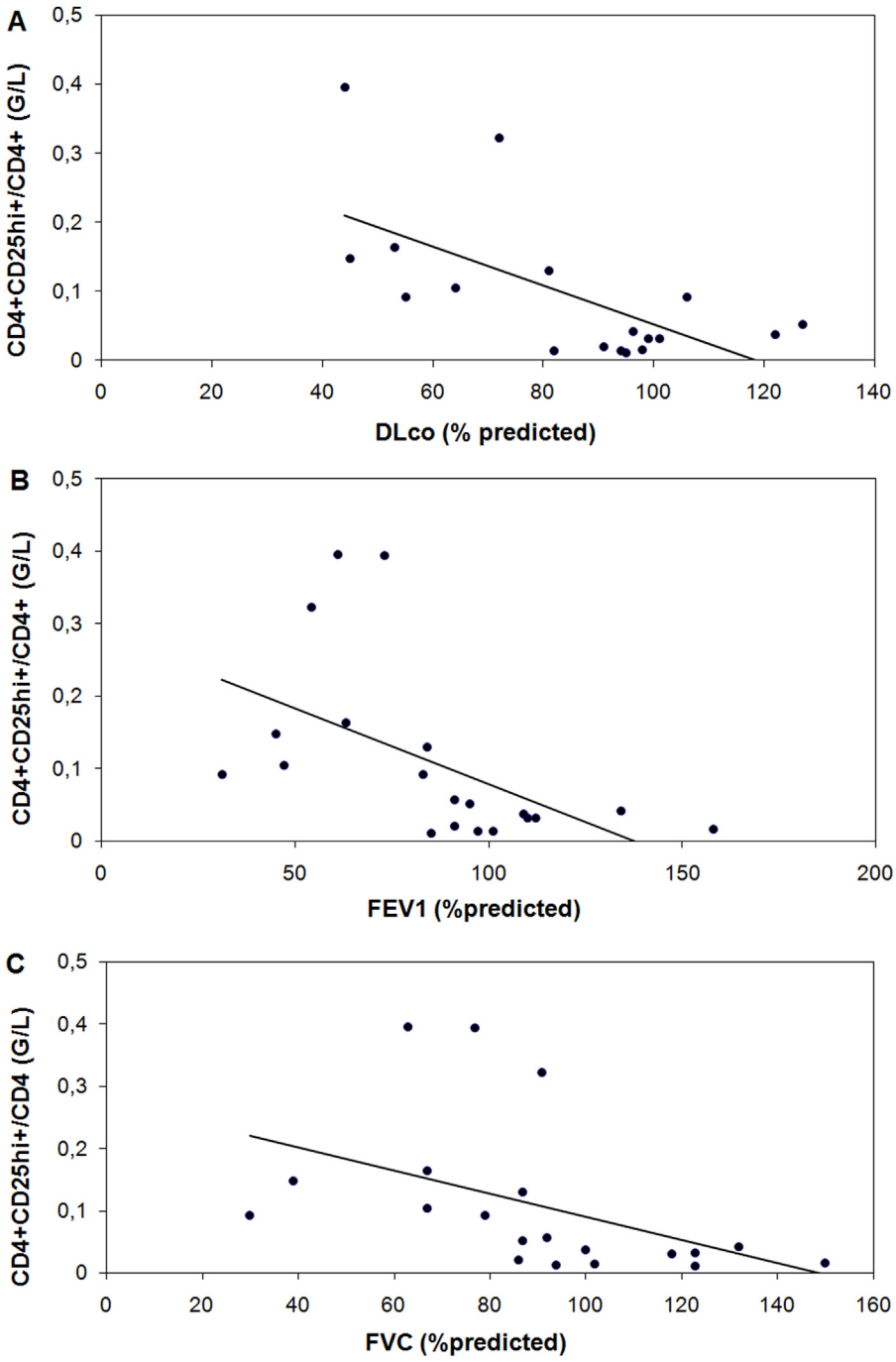

Figure 5 Correlation of lung function parameters and CD4+CD25hi+ cell number and Treg/CD4+CD25hi+ ratio in all SLE patients. A: FVC (% predicted); $r = -0.556$, $p = 0.0109$, B: FEV_1 (% predicted); $r = -0.515$, $p = 0.020$, C: DLco (% predicted); $r = -0.520$, $p = 0.027$.

resulting in better disease control [22,40]. Nearly all of our patients were on corticosteroid therapy, with significantly higher doses administered in SLE_{pulm} patients. Diversity in outcomes concerning Treg cells may result partly from small subject number, different disease phases, various therapy or heterogeneity of the disease itself.

Although multiple types of Treg cells have been described, Treg cells are dominantly characterized by the high expression of the activation surface marker CD25. However, analyzing the CD4+CD25 + lymphocyte subset CD25 is not only expressed on Treg cells, but also on recently activated T helper lymphocytes. The most widely accepted key marker for the identification of true Treg cells is the transcription factor forkhead box (Fox)P3, as it is constitutively expressed at high level in both natural and adaptive CD4+CD25 + cells [41]. As the expression of the surface marker CD127 inversely correlates to that of

FoxP3 it can be alternatively used to identify Treg cells [42]. As previously reported by Yang et al. [43] our data confirmed decreased Treg cell number in the peripheral blood of all SLE patients using Treg cell identification by the combination of CD4+CD25 + CD127− surface markers.

Our analysis of the CD4+CD25hi+ cells confirmed significantly increased number in SLE_{pulm} as compared to SLE_c patients, while no difference in CD4+CD25hi+ CD127− (Treg) cell count was detected. This was the only peripheral CD4+ cell subtype analyzed to show − even at this small sample size − a significant difference according lung involvement in SLE patients and might reflect recent activation of T lymphocytes. Dolff at al. reported the relative proportion of FoxP3 in CD4+CD25hi+ cells to be about 80% in SLE and control groups [44]. The ratio of Treg to CD4+CD25hi+ cell was 87% in our SLE_c and 62% in controls, but showed significant decrease in our SLE_{pulm} group (15%). CD4+CD25hi+ lymphocytes of SLE patients produce IL-4 similarly to healthy controls, while significantly decreased production of interferon gamma and IL-17 were noted in these cells [44].

The CD4+CD25hi+ cell number and Treg/CD4+CD25hi+ ratio correlated to all lung function parameters analyzed pointing out the significance of other than Treg CD4+CD25hi+ T cell subgroups in the development of lung involvement in lupus. Diminished number and suppressive capacity of Treg cells was also shown in patients with other pulmonary diseases including idiopathic pulmonary fibrosis and other systemic autoimmune diseases with pulmonary involvement (rheumatoid arthritis, Sjögren's syndrome, systemic sclerosis and SLE patients) [45]. Smoking and chronic obstructive pulmonary disease increases CD4+CD25hi+ cells in the bronchoalveolar lavage without the increase of Tregs, indicating the expansion of a T cell population without regulatory function [46,47]. More smokers in the subgroup of SLE_{pulm} might have contributed to the increase of this T cell subset, however also non-smokers had higher values in patients with lung involvement.

In conclusion, high prevalence of lung manifestations in SLE, especially in older patients was described. Main respiratory symptom was dyspnea, majority of patients in the SLE_{pulm} group showed lung function abnormalities (92%), including decrease in FVC, FEV_1, FEF_{25-75} and DLco and decrease in pO_2 values. As spirometry including DLco and blood gas analysis is a routine, non invasive, cheap measurement regular assessment should be performed in the daily clinical practice especially in patients with pulmonary complaints to notice pleuropulmonary involvement in time. Changes in Th1/Th2 or Th17/Treg in SLE patients were observed, however no differences according pleuropulmonary manifestations were recorded. In contrast, our data first described a significant positive correlation between peripheral CD4+CD25hi+ T cells and lung function parameters (FVC and FEV_1) and DLco indicating that these cells might represent lung specific immunological activity in SLE. Identification of the increased non-Treg CD4+CD25hi+ T lymphocyte subtype might contribute to the better understanding of lung manifestation development in SLE patients and needs further investigations.

Conflict of interest

Authors have no personal or financial conflict of interest related to the subject matter to disclose.

Acknowledgments

Technical assistance of Mária Bernáth and Beryll Bornemisza is gratefully acknowledged. Work was supported by TAMOP-4.2.2-08/1/KMR-2008-0004, OTKA K108688 and Hungarian Respiratory Society grants.

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