Peripheral CD4+CD28null T-cells as predictors of damage in systemic lupus erythematosus patients

M.F. Ugarte-Gil[1,2], C. Sánchez-Zúñiga[3], R.V. Gamboa-Cardenas[1], M. Aliaga-Zamudio[4], F. Zevallos[1], A. Mosqueira-Riveros[4], M. Medina-Chinchón[1], G. Tineo-Pozo[4], C. Elera-Fitzcarrald[1,2], V. Pimentel-Quiroz[1], O. Sarmiento-Velasquez[1], C. Reátegui-Sokolova[1], J.M. Cucho-Venegas[1], J. Alfaro-Lozano[1], Z. Rodriguez-Bellido[1,5], C.A. Pastor-Asurza[1,5], G.S. Alarcón[6], R. Perich-Campos[1,5]

[1]Rheumatology Department, Hospital Guillermo Almenara Irigoyen, EsSalud, Lima, Perú; [2]Universidad Científica del Sur, Lima, Perú; [3]Diagnostic Support Department, Hospital Grau, EsSalud, Lima, Perú; [4]Molecular Biology Department, Hospital Guillermo Almenara Irigoyen, EsSalud, Lima, Perú; [5]Universidad Nacional Mayor de San Marcos, Lima, Perú; [6]School of Medicine, University of Alabama at Birmingham, AL, USA.

Abstract

Objective

To determine whether the CD4+CD28null T-cells subpopulation predicts the occurrence of damage in SLE.

Methods

This longitudinal study was conducted in consecutive SLE patients seen every six months in our Rheumatology Department since 2012. Patients in whom CD4+CD28null T-cells had been measured and who had at least one subsequent visit were included in the study. Survival analyses (univariable and multivariable Cox-regression models) were performed to determine the risk of overall and domain damage (as per the SLICC Damage Index - SDI) as a function of the frequency of this T-cell subpopulation. The multivariable model was adjusted for pertinent confounders. All analyses were performed using SPSS 21.0.

Results

One hundred and nineteen patients were evaluated; their mean (SD) age was 43.5 (11.9) years, 113 (95.0%) were female. Disease duration was 7.8 (7.0) years, the SLEDAI 5.3 (4.1) and the SDI 1.0 (1.4). The percentage of CD4+CD28null T-cells was 17.4 (14.0). The mean follow-up was 2.1 (0.8) years, and the mean number of visits per patient 3.5 (1.1). Forty-six (38.7%) patients increase at least one SDI point. In the univariable and multivariable analyses, the percentage of CD4+CD28null predicted the occurrence of lung damage [HR: 1.042 (CI95%: 1.001–1.085); p=0.047 and HR: 1.099 (CI95%1.020–1.184); p=0.013, respectively] but neither the total SDI score nor all other SDI domain scores were predicted by the percentage of CD4+CD28null cells.

Conclusion

In SLE patients, CD4+CD28null T-cells predict the occurrence of new lung damage, independently of other risk factors but not of overall damage or damage on other domains.

Key words

systemic lupus erythematosus, disease damage, immunosenescence, T lymphocytes

Manuel F. Ugarte-Gil, MD, MSc
César Sánchez-Zúñiga, MD
Rocio V. Gamboa-Cardenas, MD
Madeley Aliaga-Zamudio, BS
Francisco Zevallos, MD
Ana Mosqueira-Riveros, BS
Mariela Medina-Chinchón, MD
Giannina Tineo-Pozo, BS
Claudia Elera-Fitzcarrald, MD
Victor Pimentel-Quiroz, MD
Omar Sarmiento-Velasquez, MD
Cristina Reátegui-Sokolova, MD
Jorge M. Cucho-Venegas, MD
Jose Alfaro-Lozano, MD
Zoila Rodriguez-Bellido, MD
Cesar A. Pastor-Asurza, MD
Graciela S. Alarcón, MD, MPH
Risto Perich-Campos, MD, MSc

Please address correspondence to:
Dr Manuel F. Ugarte-Gil,
Rheumatology Department,
Hospital Guillermo Almenara Irigoyen,
Av. Grau 800, La Victoria,
Lima 13, Lima, Perú.
E-mail: manuel_ugarte@yahoo.com

Received on September 28, 2017; accepted in revised form on March 5, 2018.

Funding: this work was partially supported by two institutional grants from EsSalud (1483-GCGP-ESSALUD-2013 and 1733-GCGP-ESSALUD-2014) and PANLAR Prize 2015.

Competing interests: none declared.

Introduction

CD4+CD28null T cells are a pro-inflammatory subgroup, expanded in several chronic inflammatory diseases like autoimmune disorders, such as rheumatoid arthritis (RA) (1, 2), cardiovascular disorders (3), chronic kidney disease (4, 5) and some chronic viral infections like cytomegalovirus (CMV) (6). They have an increase capacity for producing pro-inflammatory cytokines like interferon γ (IFN-γ), tumour necrosis factor α (TNF-α) and interleukin (IL) 2; they also express cytotoxic molecules like perforin and granzyme B, markers usually found in natural killer and cytotoxic T cells. Furthermore, they are less sensitive to regulatory T cells-induced apoptosis (7-9).

Given that systemic lupus erythematosus (SLE) is a chronic autoimmune disease with an increased cardiovascular risk, it would be expected to be associated with an expanded population of CD4+CD28null T cells population. Memory CD4+ CCR7- CD27- T cells are increased in SLE patients (10), and this subpopulation is also effector memory, like CD4+CD28null T-cells (11). Furthermore, in SLE patients, another CD4+ T cell subpopulation, with proinflammatory characteristics that express natural killer group 2 member D receptor (NKG2D) is increased (12); this subpopulation is also characterised by the deficiency of CD28 and the secretion of perforin or Granzyme B supporting the idea of an increase of proinflammatory T-cells in SLE. Additionally, other proinflammatory T-cells subpopulations like double negative T cells or angiogenic T cells have been reported to be increased in SLE patients (13). We have previously reported the association between CD4+CD28null and higher global and renal domain scores on the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI) scores (14); however, whether these associations were the result of the presence of organ involvement rather than predictive of their occurrence could not be determined because of the cross-sectional nature of the study.

We have now examined whether this T cell subpopulation predicts overall and specific domains damage utilising data collected longitudinally.

Methods

Patients

The Almenara Lupus Cohort was established in January 2012; all SLE patients presenting to the Rheumatology Department of the Hospital Guillermo Almenara Irigoyen in Lima, Perú were invited to participate. The study had been approved by the Hospital's Institutional Review Board. Details about the constitution of this cohort have been previously reported (14-16). In short, patients who signed the informed consent were recruited into this cohort and followed every six months with an interview, medical records review, physical examination and laboratory tests. For the purpose of these analyses, we have included patients in whom CD4+CD28null T-cells had been measured between September 2013 and April 2014 and who had at least one subsequent visit.

SLE was defined using the revised and updated ACR criteria (17). Variables were evaluated during the same visit in which CD4+CD28null cells were measured (interview, medical records review, physical examination and laboratory tests) with the exception of damage which was evaluated in the subsequent visits. Demographic data included were gender, age at diagnosis, marital status, education level and socioeconomic status. Socioeconomic status was defined using the Graffar's method (18).

Clinical variables included were disease activity [ascertained with the SLE disease activity index (SLEDAI) (19), disease damage [ascertained with the SDI (20)], disease duration, age at diagnosis, comorbidities [ascertained with the Charlson comorbidity index (CCI), and excluding the point assigned to connective tissue disease (21)and others considered risk factors for the occurrence of cardiovascular morbidity such as hypertension and dyslipidaemia].

Laboratory variables included were complete blood cell count, serum creatinine levels, liver tests, random glucose levels, lipid profile, protein and complement levels, coagulation tests, thyroid tests, prolactin levels, 24-hour urinary

protein, antiphospholipid and other autoantibodies [antinuclear antibody (ANA), anti-Ro, anti-La, anti-Sm, anti-RNP, anti-histone and anti-dsDNA].

Therapeutic variables included were glucocorticoid, antimalarial and immunosuppressive drugs use. Glucocorticoids were recorded as current prednisone-daily dose and time of exposure to prednisone. Antimalarial and immunosuppressive drug use was recorded as current, past or never.

Flow cytometry

EDTA-treated peripheral blood was processed using a lyse-wash-method. To examine the expression of CD4 and CD28, peripheral blood cells were stained with peridinin chlorophyll protein complex anti-CD45, Alexa Fluor 647-labelled anti-CD4, and R-phycoerythrin anti-CD28 were used to define $CD4^+CD28null$ subpopulation (all above reagents obtained from AbD Serotec, Oxford, UK). Acquisition and analyses were performed using the flow-cytometer FACS Calibur (BD, San Jose, CA, USA) and BD CELLQUEST TM Pro software (BD, San Jose, CA, USA). Negative controls consisted of staining with appropriate Ig isotype control anti-bodies. $CD4^+CD28null$ T cells were calculated as percentage of $CD4^+$ T cells.

Statistical analyses

Categorical variables were summarised as frequencies and percentages while continuous variables are presented as means and standard deviation (SD).

Variables were evaluated during the same visit in which $CD4^+CD28null$ cells were measured (interview, medical records review, physical examination and laboratory tests) with the exception of damage which was evaluated in the subsequent visits.

Survival analyses using univariable and multivariable Cox-regression models were performed to determine the risk of overall and domains damage as a function of the frequency of this T-cell subpopulation. The multivariable model was adjusted for age at diagnosis, gender, disease duration, socioeconomic status, SLEDAI, SDI, prednisone, antimalarial and immunosuppressive drugs use at the intake visit. As applicable, that is if this T-cell population predicted the risk of damage (overall or domain), a receiving operating curve was obtained to determine the cut-off predictive of damage.

For the analysis of the renal domain, those patients with end-stage renal disease at baseline were excluded. For the analysis of the diabetes domain, those patients with diabetes at baseline were excluded, and for the analysis of premature gonadal failure, only premenopausal patients were included.

All analyses were performed using SPSS 21.0.

Results

One hundred and nineteen patients were evaluated; their mean (SD) age was 43.5 (11.9) years, 113 (95.0%) were female; all patients were Mestizo (mixed Caucasian and Amerindian ancestry). Socioeconomic level was low in 50 (42.0%), medium in 48 (40.3%) and high in 21 (17.6%) patients. Disease duration was 7.8 (7.0) years. The SLEDAI was 5.3 (4.1) and the SDI 1.0 (1.4). Overall, these features were comparable to those of the entire cohort.

At baseline, the dose of prednisone was 6.9 (5.1) mg/day and the total time of exposure to prednisone was 7.3 (6.8) years; 93 (78.2%) and 13 (10.9%) patients were current and former antimalarial users. Fifty-six (47.1%) and 28 (23.5%) patients were current and former users of immunosuppressive drugs. The mean lymphocyte count was 1499.76 (692.81), the mean $CD4^+$ T-cell count was 595.96 (357.97) and the mean $CD4^+CD28null$ T-cell count was 105.11 (146.54) per mm^3. The percentage of $CD4^+CD28null$ T-cells was 17.4 (14.0). A representative flow cytometry dot plot is shown in Figure 1.

The mean follow-up was 2.1 (0.8) years, and the mean number of visits per patient was 3.5 (1.1). Forty-six (38.7%) patients increase at least one point in the SDI. Domain increases are depicted in Table I.

Patients who accrued new lung damage had a higher percentage of $CD4^+CD28null$ T-cells compared to those who did not [27.6 (13.6) *vs*. 16.8 (13.9); 0.021; Fig. 2], but this percentage was lower in those who accrued new gonadal damage compared to those who did not [2.3 (2.1) *vs*. 17.3 (12.9); p=0.015]; however, no differences were observed for the other domains of the SDI as well as for the global SDI score. These data are depicted in Table II. Given the small number of events for the individual components of the lung domain individual analyses for each one of them could not be performed: pulmonary hypertension 1 patient (0.8%), shrinking lung 2 (1.7%) and interstitial lung disease 5 (4.2%).

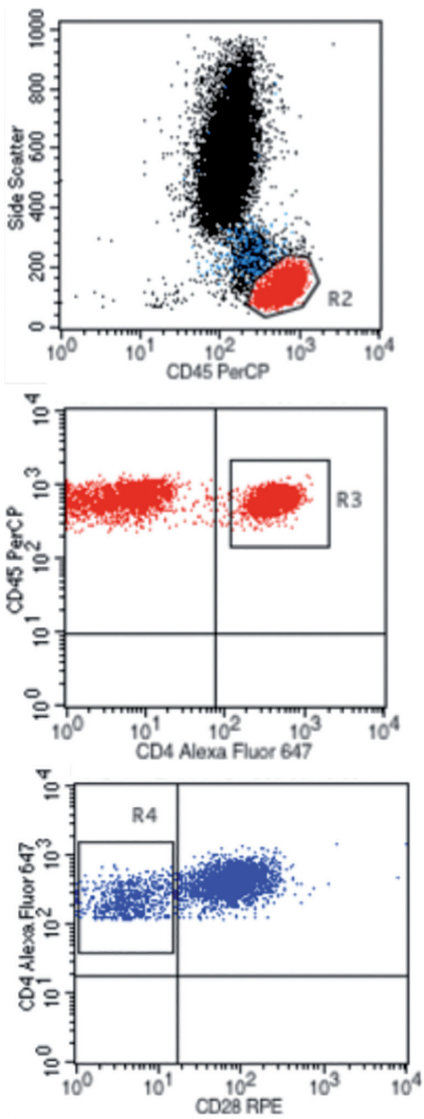

Fig. 1. Expression of CD28 on $CD4^+$ T cells to analyse percentage of $CD4^+CD28null$ flow cytometry, using a mixture of monoclonal antibodies to CD28 and CD4 in one of the patients studied. The representative dot plots show the gates followed to obtain $CD4^+CD28$ on lymphocytes. At least 20000 events were analysed for each sample.

Table I. Patients with an increase in the damage score.

	n (%)*
Global	46 (38.7)
Ocular	9 (7.6)
Neuropsychiatric	21 (17.6)
Renal	6/116 (5.2)
Lung	7 (5.9)
Cardiovascular	2 (1.7)
Peripheral vascular	2 (1.7)
Gastrointestinal	4 (3.4)
Musculoskeletal	9 (7.6)
Skin	0 (0.0)
Premature gonadal failure	2/57 (3.5)
Diabetes	1/111 (0.9)
Malignancy	1 (0.8)

*Percentage over 119 patients unless otherwise stated.

In the univariable and multivariable analyses, the percentage of CD4+CD28null predicted the occurrence of lung damage [HR: 1.042 (CI95%: 1.001–1.085); p=0.047 and HR: 1.099 (CI95%1.020–1.184); p=0.013, respectively] but that was not the case for the total and all other domain SDI scores (Table III). The corresponding ROC provided an area under the curve of 0.761; the best cut-off for CD4+CD28null as predictor of lung damage was 20%, which had a sensitivity of 71.4% and a specificity of 72.3% (Fig. 3). Using this cut-off, HRs for the univariable and multivariable analyses were 5.38 (0.98–29.46); p=0.053 and 135.02 (2.01–9064.52); p=0.022, respectively.

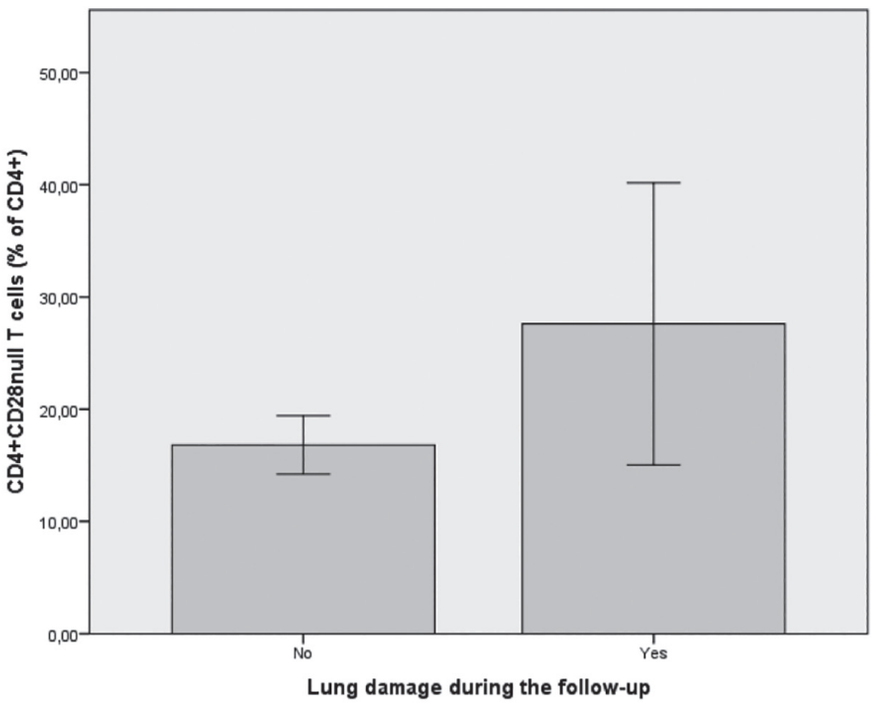

Fig. 2. Percentage of CD4+CD28null T cells according to the SDI.
*Lines reflect 95% confidence interval. p=0.021.

Table II. Percentages of CD4+CD28null T-cells according the presence or absence of new damage.

	No new damage	New damage	p-value
Global	17.1 (13.5)	18.0 (15.1)	0.754
Ocular	17.6 (14.5)	15.3 (6.6)	0.802
Neuropsychiatric	17.5 (13.5)	17.4 (16.7)	0.794
Renal	16.8 (13.3)	19.0 (16.3)	0.814
Lung	**16.8 (13.9)**	**27.6 (13.6)**	**0.021**
Cardiovascular	17.5 (14.1)	17.2 (11.7)	0.862
Peripheral vascular	17.1 (13.1)	38.9 (49.7)	0.831
Gastrointestinal	17.3 (14.1)	21.4 (13.6)	0.452
Musculoskeletal	17.9 (14.3)	12.3 (9.6)	0.308
Premature gonadal failure	**17.3 (12.9)**	**2.3 (2.1)**	**0.015**
Diabetes	17.4 (13.8)	39.19	0.216
Malignancy	17.5 (14.0)	9.5	0.689

Discussion

We have examined if CD4+CD28null subpopulation was predictive of damage in a primarily Mestizo SLE cohort, and we have found it to be predictive of lung damage, after adjustment by other well-known predictors of damage; however, that was not the case for the other domains or the total score of the SDI. A cut-off of 20% was obtained from the corresponding ROC.

We have previously reported that these more differentiated effector memory cells are increased in SLE patients (10), and in particular in those patients who already have developed some damage (14); this subpopulation is increased after chronic stimulation, due either to a lower threshold for T-cell activation (22) or to a greater exposure to auto-antigens(23). But if they are predictors of inflammation and subsequent damage or if they are consequence of both, has not been previously evaluated.

The association between CD4+CD28null and lung involvement in SLE patients has not been previously reported. In primary lung disease, like bronchiolitis obliterans syndrome (BOS) (24), asthma (25), and chronic obstructive pulmonary disease (COPD) (26), however, the CD4+CD28null subpopulation is increased. And, CD28 down regulation in CD4+ T-cells has been associated with an increased incidence of BOS and poor outcomes in a study of 65 patients from Pittsburgh after lung transplantation (27); furthermore, in a study from Australia of 20 BOS patients undergoing lung transplant, CD4+CD28null and CD8CD28null were increased compared to 38 stable transplant patients and 10 healthy controls. An increased production of TNF-α, IFN-γ and IL-2 (24) by these T cells subpopulations and resistance to the corticosteroids has also been shown. CD4+CD28null subpopulation have also been shown to predict a worse prognosis (either death or the need for lung transplantation) in 89 predominantly Caucasian patients from Pittsburgh with idiopathic pulmonary fibrosis (28). Additionally, the pro-

Table III. Impact of CD4+CD28null subpopulation in damage accrual. Univariable and multivariable analyses.

	Univariable	*p*-value	Multivariable*	*p*-value
Global	1.00 (0.97-1.02)	0.778	1.00 (0.97-1.02)	0.706
Ocular	0.97 (0.90-1.04)	0.360	0.96 (0.87-1.06)	0.430
Neuropsychiatric	1.00 (0.96-1.04)	0.816	0.98 (0.94-1.02)	0.292
Renal	1.00 (0.94-1.07)	0.902	1.00 (0.91-1.10)	0.969
Lung	**1.04 (1.00-1.09)**	**0.047**	**1.09 (1.02-1.18)**	**0.013**
Cardiovascular	1.00 (0.91-1.10)	0.998	0.94 (0.03-32.75)	0.974
Peripheral vascular	1.08 (1.00-1.16)	0.050	1.20 (0.01-111.29)	0.939
Gastrointestinal	1.02 (0.96-1.09)	0.580	1.14 (0.01-180.67)	0.960
Musculoskeletal	0.93 (0.85-1.01)	0.083	0.86 (0.69-4.90)	0.167
Skin				
Premature gonadal failure	0.62 (0.32-1.19)	0.152	0.80 (0.01-48.28)	0.914
Diabetes				
Malignancy	0.91 (0.69-1.20)	0.511	0.91 (0.03-24.61)	0.955

*Variables adjusted for in the multivariable analyses were age at diagnosis, gender, disease duration, socioeconomic status, SLEDAI, SDI, use of prednisone, antimalarials and immunosuppressive drugs at the intake visit.

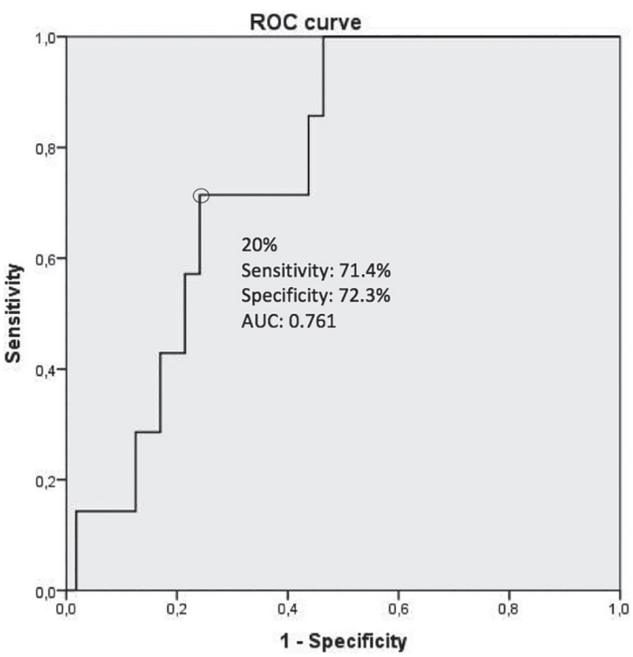

Fig. 3. Receiver-operating characteristic (ROC) curve of CD4+CD8null T-cells for predicting new lung damage.

inflammatory profile of Natural Killer cells has been associated with a higher prevalence of interstitial lung disease and pulmonary hypertension in patients with systemic sclerosis (29) supporting the role of proinflammatory cells in lung disease.

The impact of this subpopulation in lung diseases is supposed to be due to the production of proinflammatory cytokines like IFN-γ or TNF- (25); these cytokines in turn would stimulate lung macrophages, leading to secretion of matrix metalloproteinases and tissue destruction (30). In fact, CD4+CD28null subpopulation has been found to be increased in lungs of COPD patients, and these lung-resident cells shown a proliferative response after being stimulated with components of the extracellular matrix (26). Finally, CD4+CD28null T-cells expressing of CD56, and CD56+ T-cells were found in lung infiltrates of patients with RA-related interstitial lung disease (ILD), and CD4+CD56+CD28null subpopulation was higher in those with extraarticular RA than in those with only joint involvement (31).

Taken together, the role of CD4+CD28 null subpopulation in primary lung diseases and in RA-related ILD there is enough evidence to support the role of this subpopulation in lung damage in SLE patients.

Our study has several limitations, first, as this is not an inception cohort, patients with severe disease succumbing to it could have not been part of it (immortal bias). Second, due to the relatively short follow-up after flow-cytometry measurement, the impact of this subpopulation in other SDI domains may yet to be realised. Third, in order to determine the impact of this subpopulation in other domains, a larger number of patients may be needed. Fourth, due to the fact that we ascertain pulmonary damage using the SDI, asymptomatic patients with pulmonary involvement may have been missed/ likewise, we could not categorise the damage present into mild, moderate or severe as the SDI only defines whether a manifestation is present or not. Fifth, individual analyses for the different components of the lung domain could not be performed due to the small number of events; further studies may also validate the cut-off value we have obtained from the ROC. Nevertheless, we consider important to report the predictive value of CD4+CD28null subpopulation for the accrual of damage, as these data may lead to studies of longer duration, and in other ethnicities and geographical regions; this will allow determining the real impact of immunosenescence in damage accrual.

In conclusion, we describe, for first time, the possible role of CD4+CD28null T cells subpopulations as a predictor of new lung damage independently of age at diagnosis, gender, disease duration, socioeconomic status, SLEDAI, SDI, use of prednisone, antimalarials and immunosuppressive drugs at the intake visit.

References

1. THEWISSEN M, SOMERS V, VENKEN K *et al.*: Analyses of immunosenescent markers in patients with autoimmune disease. *Clin Immunol* 2007; 123: 209-18.
2. QUANDT D, ROTHE K, SCHOLZ R, BAERWALD CW, WAGNER U: Peripheral CD4CD8 double positive T cells with a distinct helper cytokine profile are increased in rheumatoid arthritis. *PLoS One* 2014; 9: e93293.
3. LIUZZO G, BIASUCCI LM, TROTTA G *et al.*: Unusual CD4+CD28null T lymphocytes and recurrence of acute coronary events. *J Am Coll Cardiol* 2007; 50: 1450-8.

4. BETJES MG, HUISMAN M, WEIMAR W, LITJENS NH: Expansion of cytolytic CD4+CD28- T cells in end-stage renal disease. *Kidney Int* 2008; 74: 760-7.
5. YADAV AK, JHA V: CD4+CD28null cells are expanded and exhibit a cytolytic profile in end-stage renal disease patients on peritoneal dialysis. *Nephrol Dial Transplant* 2011; 26: 1689-94.
6. VAN LEEUWEN EM, REMMERSWAAL EB, VOSSEN MT *et al*.: Emergence of a CD4+ CD28- granzyme B+, cytomegalovirus-specific T cell subset after recovery of primary cytomegalovirus infection. *J Immunol* 2004; 173: 1834-41.
7. MALY K, SCHIRMER M: The story of CD4+CD28- T cells revisited: solved or still ongoing? *J Immunol Res* 2015; 2015: 348746.
8. BROUX B, MARKOVIC-PLESE S, STINISSEN P, HELLINGS N: Pathogenic features of CD4+CD28- T cells in immune disorders. *Trends Mol Med* 2012; 18: 446-53.
9. DUMITRIU IE: The life (and death) of CD4+ CD28(null) T cells in inflammatory diseases. *Immunology* 2015; 146: 185-93.
10. FRITSCH RD, SHEN X, ILLEI GG *et al*.: Abnormal differentiation of memory T cells in systemic lupus erythematosus. *Arthritis Rheum* 2006; 54: 2184-97.
11. KOCH S, LARBI A, DERHOVANESSIAN E, OZCELIK D, NAUMOVA E, PAWELEC G: Multiparameter flow cytometric analysis of CD4 and CD8 T cell subsets in young and old people. *Immun Ageing* 2008; 5: 6.
12. YANG D, WANG H, NI B *et al*.: Mutual activation of CD4+ T cells and monocytes mediated by NKG2D-MIC interaction requires IFN-gamma production in systemic lupus erythematosus. *Mol Immunol* 2009; 46: 1432-42.
13. LA PAGLIA GMC, LEONE MC, LEPRI G *et al*.: One year in review 2017: systemic lupus erythematosus. *Clin Exp Rheumatol* 2017; 35: 551-61.
14. UGARTE-GIL MF, SANCHEZ-ZUNIGA C, GAMBOA-CARDENAS RV *et al*.: Circulating CD4+CD28null and extra-thymic CD4+CD8+ double positive T cells are independently associated with disease damage in systemic lupus erythematosus patients. *Lupus* 2016; 25: 233-40.
15. UGARTE-GIL M, GAMBOA-CARDENAS R, ZEVALLOS F *et al*.: High prolactin levels are independently associated with damage accrual in systemic lupus erythematosus patients. *Lupus* 2014.
16. UGARTE-GIL MF, SANCHEZ-ZUNIGA C, GAMBOA-CARDENAS RV *et al*.: Circulating naive and memory CD4+ T cells and metabolic syndrome in patients with systemic lupus erythematosus: data from a primarily Mestizo population. *Rheumatology* (Oxford) 2015; 54: 1302-7.
17. HOCHBERG MC: Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1725.
18. MENDEZ CASTELLANO H, DE MÉNDEZ MC: Sociedad y estratificación: método Graffar-Méndez Castellano. Caracas, Venezuela: Fundacredesa; 1994.
19. BOMBARDIER C, GLADMAN DD, UROWITZ MB, CARON D, CHANG CH: Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 1992; 35: 630-40.
20. GLADMAN D, GINZLER E, GOLDSMITH C *et al*.: The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. *Arthritis Rheum* 1996; 39: 363-9.
21. CHARLSON ME, POMPEI P, ALES KL, MACKENZIE CR: A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987; 40: 373-83.
22. MAK A, KOW NY: The pathology of T cells in systemic lupus erythematosus. *J Immunol Res* 2014; 2014: 419029.
23. COLONNA L, LOOD C, ELKON KB: Beyond apoptosis in lupus. *Curr Opin Rheumatol* 2014; 26: 459-66.
24. HODGE G, HODGE S, AHERN J, HOLMES-LIEW CL, REYNOLDS PN, HOLMES M: Up-regulation of alternate co-stimulatory molecules on proinflammatory CD28null T cells in bronchiolitis obliterans syndrome. *Clin Exp Immunol* 2013; 173: 150-60.
25. HODGE S, HODGE G, SIMPSON JL *et al*.: Blood cytotoxic/inflammatory mediators in non-eosinophilic asthma. *Clin Exp Allergy* 2016; 46: 60-70.
26. HOETZENECKER K, MITTERBAUER A, GUENOVA E *et al*.: High levels of lung resident CD4+CD28null cells in COPD: implications of autoimmunity. *Wien Klin Wochenschr* 2013; 125: 150-5.
27. STUDER SM, GEORGE MP, ZHU X *et al*.: CD28 down-regulation on CD4 T cells is a marker for graft dysfunction in lung transplant recipients. *Am J Respir Crit Care Med* 2008; 178: 765-73.
28. GILANI SR, VUGA LJ, LINDELL KO *et al*.: CD28 down-regulation on circulating CD4 T-cells is associated with poor prognoses of patients with idiopathic pulmonary fibrosis. *PLoS One* 2010; 5: e8959.
29. PUXEDDU I, BONGIORNI F, CHIMENTI D *et al*.: Cell surface expression of activating receptors and co-receptors on peripheral blood NK cells in systemic autoimmune diseases. *Scand J Rheumatol* 2012; 41: 298-304.
30. PARK W, WEYAND CM, SCHMIDT D, GORONZY JJ: Co-stimulatory pathways controlling activation and peripheral tolerance of human CD4+CD28- T cells. Eur J Immunol. 1997; 27: 1082-90.
31. MICHEL JJ, TURESSON C, LEMSTER B *et al*.: CD56-expressing T cells that have features of senescence are expanded in rheumatoid arthritis. *Arthritis Rheum* 2007; 56: 43-57.