



Platelet bound B cells and their role in SSc: Implications for disease subtypes and clinical outcomes

Rubén Osuna-Gómez^a, Ivan Castellví^b, Maria Mulet^a, Jose Luis Tandaipan^b, Carlos Zamora^a, Helena Codes-Mendez^b, M^a Àngels Ortiz^a, Cesar Diaz-Torné^b, Elisabet Cantó^a, Berta Magallares^b, Albert Guinart-Cuadra^a, Patricia Moya^b, Hector Corominas^b, Silvia Vidal^{a,*}

^a Inflammatory Diseases, Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau, Biomedical Research Institute Sant Pau (IIB Sant Pau), Barcelona, Spain

^b Department of Rheumatology and Systemic Autoimmune Diseases, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

ARTICLE INFO

Keywords:

B lymphocytes
lymphocyte-PLT complexes
PLTs
systemic sclerosis

ABSTRACT

Objectives: Systemic sclerosis (SSc) is a complex autoimmune disease characterized by microvascular damage, immune dysregulation, and tissue fibrosis. While lymphocyte-platelet (PLT) complexes have been implicated in autoimmune diseases, their role in SSc is not well understood. **Methods:** In a study of 21 predominantly female SSc patients, 66.7 % had limited SSc (lcSSc), with anti-centromere antibodies (ACA) being the most common autoantibody pattern. We applied flow cytometry to analyze B cells with bound PLTs, enzyme-linked immunosorbent assay (ELISA) to determine plasma levels of activated PLT soluble factors, and co-culture assays to evaluate B cell cytokine secretion and plasma cell differentiation. **Results:** SSc patients had a higher percentage of B cells, but not T cells, with bound PLTs compared to healthy donors (HD). Despite similar PLT counts, SSc patients showed higher plasmatic levels of P-selectin (CD62P), soluble CD40 ligand (sCD40L), platelet-derived growth factor (PDGF), and transforming growth factor- β (TGF- β). Plasma IL-10 levels were also higher in SSc patients, with increased intracellular IL-10 in B cells with bound PLTs. We observed an increased IL-10 production and plasma cell differentiation when B cells were co-cultured with PLTs, especially from SSc patients. B cells with bound PLTs were associated with calcinosis, digital ulcers, and ACA status, with no effect from previous corticosteroid or aspirin therapy. Logistic regression identified B cells with bound PLTs as a predictor for distinguishing lcSSc patients. **Conclusions:** B cells with bound PLTs play a significant role in SSc by modulating B cell function and contributing to disease pathogenesis. Their association with clinical parameters suggests their potential as biomarkers for disease severity and subtype classification in SSc.

Introduction

Systemic sclerosis (SSc) is a connective disease characterized by features of autoimmunity, vasculopathy, inflammation, and fibrosis. However, the pathogenesis of SSc is not completely understood.¹ Autoantibodies are present in 95 % of SSc patients, and are either specific for SSc or have overlap with other connective diseases.² The presence of autoantibodies suggests a pathogenic role of B cells in SSc. Studies have reported hyperactivity of B cells in SSc patients, leading to the overexpression of autoantibodies.³ These autoantibodies can induce a pro-inflammatory and/or fibrotic response in patients with SSc.^{4,5}

Moreover, increasing evidence suggests that B cells contribute to autoimmune responses by a series of antibody-independent mechanisms, which include cytokine secretion, such as IL-10.^{6,7} It has been reported that IL-10 enhanced B-cell proliferation and promoted the generation of plasma cells, contributing to autoimmune disorders, such as systemic lupus erythematosus (SLE).⁸ In SSc patients, particular IL-10 genotypes were associated with SSc-related autoantibodies.⁹ Further, serum levels of IL-6 and IL-10 correlate with total skin thickness score in patients with SSc.¹⁰ However, IL-10 alleviated pulmonary inflammation and fibrosis by reducing IL-17A and IL-4 levels in a bleomycin (Bleomycin)-induced SSc mouse model.¹¹

* Corresponding author at: Department of Inflammatory Diseases, Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau, Biomedical Research Institute Sant Pau (IIB Sant Pau), Barcelona, Spain.

E-mail address: svidal@santpau.cat (S. Vidal).

<https://doi.org/10.1016/j.trsl.2025.07.001>

Received 11 December 2024; Received in revised form 15 April 2025; Accepted 9 July 2025

Available online 9 July 2025

1931-5244/© 2025 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC license (<http://creativecommons.org/licenses/by-nc/4.0/>).

In addition to the contribution of B lymphocytes to the pathogenesis of SSc, numerous studies have also emphasized an important role of platelets (PLTs) in this disease. Many reports have shown an activation of PLTs in SSc patients (10,11) that participates to immune dysregulation associated with SSc. Further, enhanced activation of PLTs and increased tendency to aggregation has long been observed in SSc patients^{12,13} and are generally attributed to the concomitant dysfunction of endothelium.¹⁴ In addition, the elevation of markers of PLT activation like the P-selectin has been shown to correlate with disease activity in SSc,^{15,16} as well as in other diseases characterized by prominent vascular inflammation.¹⁷ Previous studies in our laboratory have shown that B cells with bound PLTs had an increased percentage of IL-10-producing cells compared with those without bound PLTs in SLE patients.¹⁸ In these patients, B lymphocytes with bound PLTs were associated with the presence of immunoglobulin (Ig) G (IgG) and IgA, as well as disease severity.¹⁸ However, the binding of PLTs to B cells in SSc patients has not yet been studied and there is a lack of information about the relationship between PLT-binding to B lymphocytes, their function, and the clinical parameters of these disease.

Therefore, the main goal of this study is to decipher the possible role of lymphocyte-PLT complexes in the altered B cell function and SSc pathogenesis. Firstly, we compared the numbers of lymphocytes with bound PLTs in SSc patients or HD, and established their relationship with plasma PLT activation markers. Secondly, we investigated cytokine secretion and plasma cell differentiation of B lymphocytes with bound PLTs in SSc patients and HD. For this analysis, we co-cultured peripheral blood mononuclear cells (PBMCs) and PLTs from SSc patients and HD. Finally, we established the relationship between B cells with bound PLTs and the clinical and laboratory features of SSc patients.

Material and methods

Patients and samples

A prospective observational study was performed at Hospital de la Santa Creu i Sant Pau from 2020 to 2023. The selected population was SSc patients following our scleroderma program. All of them fulfilled the ACR/EULAR 2013 systemic sclerosis classificatory criteria.¹⁹ The patients were classified as having diffuse (dcSSc) or limited (lcSSc) cutaneous SSc according to LeRoy's classification.²⁰ The extent of their skin involvement was assessed using the modified Rodnan skin score (mRSS).²¹ A peripheral blood sample was obtained from each patient. A summary of patient characteristics is given in Table S1. Written informed consent was obtained from each patient and the ethical approval for the study was granted by the Hospital de la Santa Creu i Sant Pau Institutional Ethics Committee.

Surface staining and analysis by Flow Cytometry

Peripheral blood from 10 healthy donors (HD), as controls, and 21 SSc patients were collected in Vacutainer tubes with heparin (BD Biosciences, San Jose, USA). Whole blood cells (100 µl) of HD and SSc patients were stained with the following anti-human antibodies: anti-CD4-Viogreen (Miltenyi Biotec, Bergisch Gladbach, Germany), anti-CD41a-FITC (Immunotools, Friesoythe, Germany), anti-CD62P-PE (Immunotools), anti-CD8 PerCP (Biolegend, San Diego, CA, USA) and anti-CD19-PE-Cy7 (Biolegend). Then, red blood cells were lysed and leukocytes fixed using BD FACS lysing solution (BD Bioscience), washed with PBS and analyzed by flow cytometry. Data acquisition and analysis was performed on a MACSQuant Analyzer 10 flow cytometer (Miltenyi Biotec). For data analysis, live single cells were analyzed to select lymphocytes based on their morphology by forward- versus side-scatter (FSC-SSC) dot plot. CD4+ and CD8+ T lymphocytes with bound PLT were identified on gated lymphocytes as CD3+ CD8- CD41a+ (CD4+PLT+) and CD3+ CD8+ CD41a+ (CD8+PLT+), respectively, as previously reported in our laboratory.^{22,23} B lymphocytes with bound

PLTs were identified on gated lymphocytes as CD19+ CD41a+ (CD19+PLT+). To analyze PLTs and activated PLTs in blood, samples were acquired using logarithmic FSC and SSC. Blood PLTs were identified as CD41a+ events and activated PLTs as CD41a+CD62P+ events. The percentage and mean fluorescence intensity (MFI) of CD41a+ cells in each subset were obtained using FlowJo version 10 (FlowJo, Ashland, OR, USA). Data acquisition were performed on a MACSQuant Analyzer 10 flow cytometer (Miltenyi Biotec).

Isolation of human PLTs

PLTs were isolated from 10 ml of heparin blood and were centrifuged at 190×g for 20 min at RT. The upper part was PLT-rich plasma (PRP), and it was collected and centrifuged at 190×g for 10 min at RT. The new upper part of the centrifuged PRP was collected and centrifuged at 1000×g for 10 min at RT. PLT pellets were washed with PBS containing 5 mM EDTA to avoid PLT activation at 1000×g for 10 min at RT. The PLTs were then resuspended in RMPI-1640 (Biowest, Nuaille, France) supplemented with 10 % fetal bovine serum (FBS, Biowest) and 1 % Penicillin/Streptomycin (Biowest). Purity (measured as percentage CD41a+ events), activation (measured as percentage CD62P+ of CD41a+ events), and the number of PLTs were assessed by flow cytometry. Data acquisition were performed on a MACSQuant Analyzer 10 flow cytometer (Miltenyi Biotec).

Cell culture and intracellular IL-10 by flow cytometry

To evaluate intracellular production of IL-10, PBMCs were cultured in 96 well-plates (Thermo Fisher Scientific, Vienna, Austria) with complete RMPI-1640 medium and stimulated with 2.5 µg/mL of CPG-B oligodeoxynucleotide-2006 (ODN2006; InvivoGen, San Diego, CA, USA) and 2 µg/mL soluble CD40 ligand (sCD40L) (PeproTech EC, London, UK) during 48 hours in 5 % CO₂ at 37°C as previously described.²⁴ For co-culture experiments, PBMCs without bound PLTs were isolated using a negative magnetic separation with biotinylated anti-CD41a (Miltenyi Biotec) and Magnisort™ positive selection beads (Thermo Fisher Scientific). PBMCs without bound PLTs from HD or SSc patients were cultured in the absence or presence of PLTs from HD or SSc patients at different PLT/PBMC ratios (50:1, 10:1, and 1:1) according to our previous studies.²⁵ Prior to intracellular cytokine analysis, PBMCs were restimulated with 50 ng/ml phorbol myristate acetate (PMA, Sigma Aldrich, St. Louis, Missouri) and 500 ng/ml ionomycin (Sigma Aldrich) for four hours in 5 % CO₂ at 37°C. Cells were collected and stained with anti-CD41a-Viogreen (Miltenyi Biotec) and anti-CD19-PE-Cy7 (Biolegend). PBMCs were then intracellularly stained with anti-IL-10-APC (Miltenyi Biotec) after fixation and permeabilization with BD Cytofix/Cytoperm kit (BD Biosciences) according to the manufacturer's instructions.

For intracellular IL-10 production analysis, B cells with or without bound PLTs were identified on gated lymphocytes as CD19+ CD41a+ (CD19+PLT+) and CD19+CD41a- (CD19+PLT-), respectively. The secretion of IL-10 on CD19+PLT+ and CD19+CD41a- was evaluated. Data acquisition were performed on a MACSQuant Analyzer 10 flow cytometer (Miltenyi Biotec). The percentage of IL-10+ cells (% cells) was obtained using FlowJo version 10 (FlowJo LLC).

Plasma cell differentiation

PBMCs were cultured with complete RMPI-1640 and stimulated with 2.5 µg/mL of ODN2006 (InvivoGen, USA), 2 µg/mL sCD40L (PeproTech EC), 5 µg/mL F(ab)₂ goat anti-human IgM (Sigma-Aldrich) and 100 ng/mL recombinant IL-21 (PeproTech) during 7 days in 5 % CO₂ at 37°C, as previously reported.²⁶ For co-culture experiments, PBMCs without bound PLTs were isolated using a negative magnetic separation with biotinylated anti-human CD41a (Miltenyi Biotec) and Magnisort™ positive selection beads (Thermo Fisher Scientific). PBMCs without

bound PLTs from HD or SSc patients were cultured in the absence or presence of PLTs from HD or SSc patients at different PLT/PBMC ratios (50:1, 10:1, and 1:1). For plasma cell differentiation analysis, cultured cells were analyzed by flow cytometry using anti-CD38-Vioblu, anti-CD3-Viogreen (Miltenyi Biotec), anti-CD27-FITC (Immunotools), anti-CD41a-PE (Immunotools) and anti-CD19-PE-Cy7 (Biolegend) antibodies. Plasma cells (CD19+CD27+CD38+CD3-) with or without bound PLTs were identified on gated lymphocytes as Plasma cell+ CD41a+ (Plasma cell+ PLT+) and Plasma cell+ CD41a- (Plasma cell+ PLT-), respectively. Data acquisition were performed on a MACSQuant Analyzer 10 flow cytometer (Miltenyi Biotec). Data analysis was obtained using FlowJo version 10 (FlowJo, Ashland, OR, USA).

Determination of plasma cytokines

Plasma concentrations of IL-10 (Immunotools), P-selectin (CD62P, R&D Systems, Minneapolis, MN, USA), sCD40L (Peprotech EC), platelet-derived growth factor (PDGF, Peprotech EC), transforming growth factor- β (TGF- β , Mabtech, Stockholm, Sweden), and IL-6 (Mabtech) were determined using specific enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' instructions and using the specific standard curves of recombinant molecules. The limits of detection were as follows: 15.62 – 1000 pg/ml for IL-10, 125 – 1000 pg/ml for CD62P, 31.3 – 2000 pg/ml for sCD40L, 15.62 – 1000 pg/ml for PDGF and 62.5 – 4000 pg/ml for TGF- β .

Statistics

Statistical analyses were performed using GraphPad Prism 7 software. Normal data distribution was assessed by the Kolmogorov-Smirnov test. Variables were presented as mean $\pm$ SEM or median (interquartile range-IQR-) according to normal or non-normal distribution respectively. Comparisons between the two groups were tested with the Student's t-test (paired or unpaired) or the Mann-Whitney test

according to normal distribution. Comparisons of three or more groups were tested with a one-way analysis of variance (ANOVA) and the Bonferroni post-hoc test. Correlation analyses were determined with Pearson's or Spearman's correlation according to normal distribution. All p values were based on a 2-sided hypothesis, and those under 0.05 were considered statistically significant. Correlation matrices were drawn employing an R package called "corrplot".²⁷ A multinomial logistic regression analysis was performed to identify the risk factors associated with limited or diffuse cutaneous pattern in SSc patients. Forest plot analyses were conducted in SSc patients using the JASP 0.17.2 (Amsterdam, The Netherlands).

Results

Population

The characteristics of 21 patients included in the study are shown in Table S1. Mean age was 64 years and there was a predominance of female (20 cases). Fourteen cases (66.7 %) were classified as limited SSc (lcSSc) and the most frequent autoantibody pattern was positive for anti-centromere antibodies (ACA) (57.1 %). The most frequent organ involvement was cutaneous (76.2 %), following by gastrointestinal (47.6 %).

Differential binding of PLTs to lymphocytes in SSc

We firstly compared the percentage of lymphocytes with bound PLTs in HD and SSc patients. We found that the percentage of CD4+ or CD8+ T cells with bound PLTs were comparable in both groups (Fig. 1A-B). In contrast, the percentage of B cells with bound PLTs was higher in SSc patients than HD group (Fig. 1A-B).

The percentages of CD4+ and CD8+ T cells with bound PLTs were correlated in SSc patients (Spearman correlation: $r = 0.95$, $p < 0.001$), while no correlation was found between the percentages of B cells with

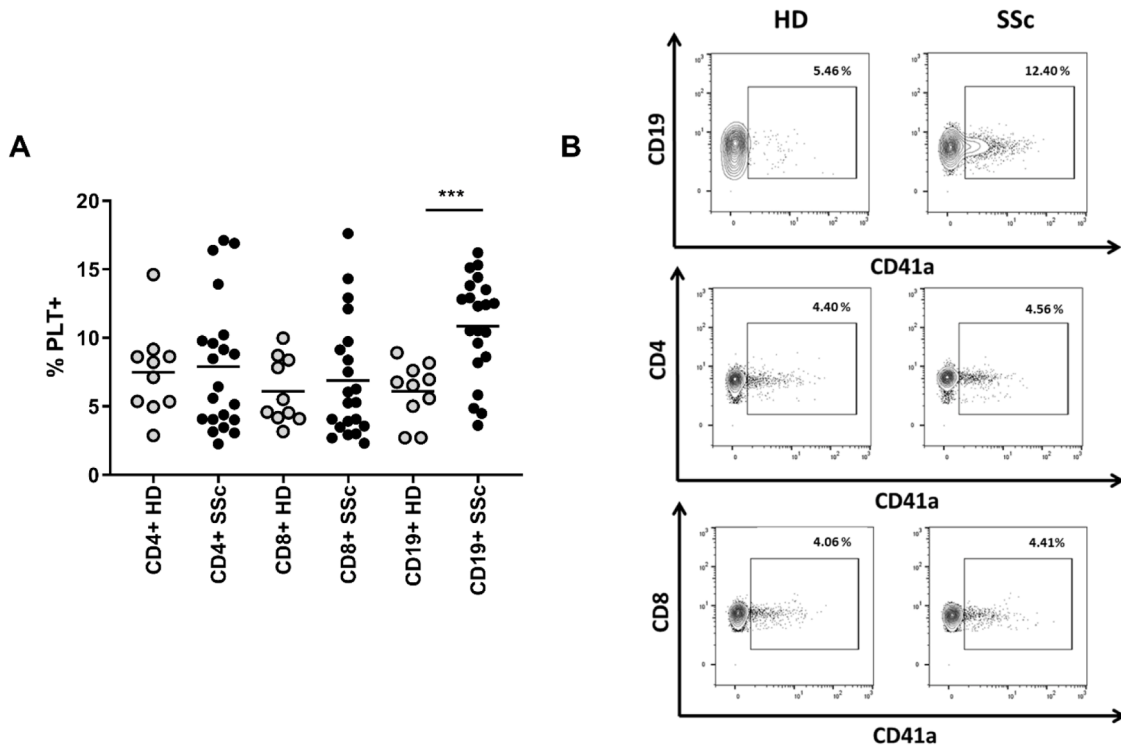


Fig. 1. Percentage of circulating lymphocyte-PLT complexes in healthy donors (HD) and systemic sclerosis (SSc) patients. (A) The dot plot shows the percentage of circulating B cells (CD19+), CD4+ and CD8+ T lymphocytes with bound PLTs in HD ($n = 10$) and SSc patients ($n = 21$). (B) A representative image of B cells, CD4+ and CD8+ T lymphocytes with bound PLTs in HD and SSc patients is shown. Spots in black correspond to SSc patients and spots in grey to HD. *** $p < 0.001$.

bound PLTs and CD4+ or CD8+ T cells with bound PLTs. However, the MFI of CD41a on B cells, CD4+ T cells and CD8+ T cells was similar in both groups, without correlation between the MFIs of CD41a on CD4+ T cells, CD8+ T cells and B cells (**data not shown**).

Enhanced Platelet Activation and plasma PLT-derived Markers in SSc

We found that the number of PLTs count was comparable in both groups (Fig. 2A). However, we found that the percentage of activated PLTs and B cells with activated bound PLTs were higher in SSc patients than HD group (Fig. 2B-C). The percentage of CD4+ and CD8+ T cells with activated bound PLT was similar in both groups (**data not shown**). Further, CD62P, sCD40L, PDGF, and TGF- β levels were higher in SSc patients than HD group (Fig. 2D-G). Only in SSc patients, we found that the percentage of B cells with bound PLTs correlated positively with the percentage of activated PLTs and B cells with activated bound PLTs, but negatively correlated with plasma TGF- β levels (Fig. 2H). In addition, we found a positive correlation between the percentage of activated PLTs with plasma levels of CD62P, PDGF and sCD40L levels in SSc patients (Fig. 2H).

Elevated plasma IL-10 and immunoglobulin correlations with PLT-bound B Cells in SSc

We observed that plasma levels of IL-10 were higher in SSc patients than HD group (Fig. 3A). In contrast, plasma levels of IL-6 were comparable in both groups (**data not shown**). Moreover, in culture experiments, we showed a higher percentage of intracellular IL-10 in B cells with bound PLTs compared to those without bound PLTs in both groups (Fig. 3B), but a higher percentage of intracellular IL-10 in B cells with bound PLTs in SSc patients compared to HD group (Fig. 3B-C). We found a positive correlation between the percentage of intracellular IL-10 in B cells with bound PLTs and plasma levels of IL-10, and the percentage of circulating B cells with bound PLTs in both groups (Fig. 3D-E). Moreover, we found a positive correlation between the percentage of circulating B cells with bound PLTs and plasma levels of IL-10 in both groups (Fig. 3F).

In cell cultures, we found a higher percentage of differentiated plasma cells with bound PLTs compared to those without bound PLTs in both groups (Fig. 4A). This percentage of plasma cells with bound PLTs was higher in SSc patients than in the HD group (Fig. 4A-B). We next found a negative correlation between the percentage of circulating B

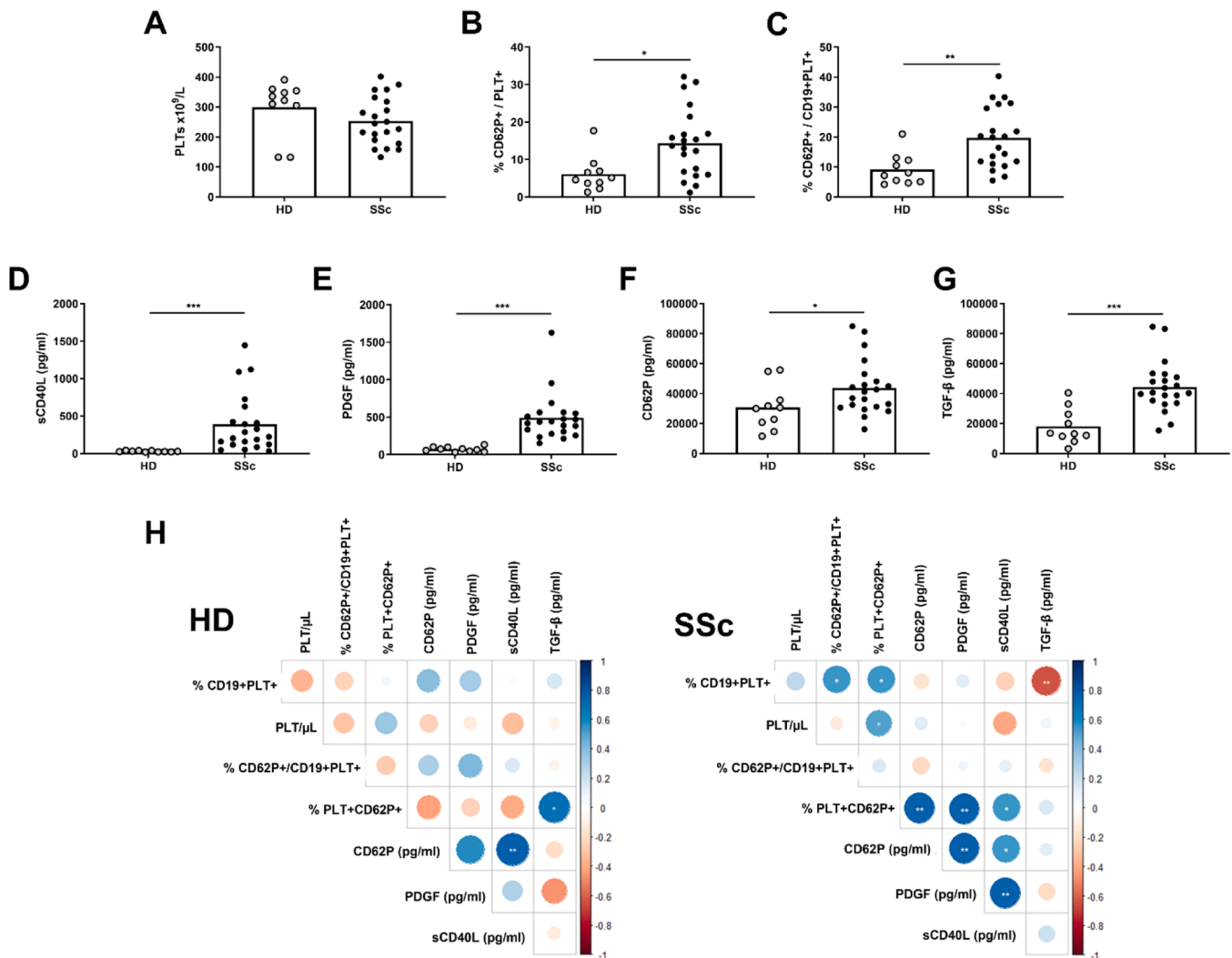


Fig. 2. Association of B cells with bound platelets (PLTs) with PLTs derived factors in healthy donors (HD) and systemic sclerosis (SSc) patients. (A) PLTs count, (B) percentage of circulating activated PLTs (CD62P+PLT+), (C) percentage of circulating B cells with bound activated PLTs (% CD62P+/CD19+PLT+), plasma levels of (D) sCD40L or (E) PDGF, (F) CD62P, and (G) TGF- β in HD ($n = 10$) and SSc patients ($n = 21$) determined by flow cytometry and the corresponding ELISAs. (H) Correlation matrices showing the association between the percentage of circulating B cells with bound PLTs and PLTs derived factors in HD and SSc patients. Spots in black correspond to SSc patients and spots in grey to HD. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

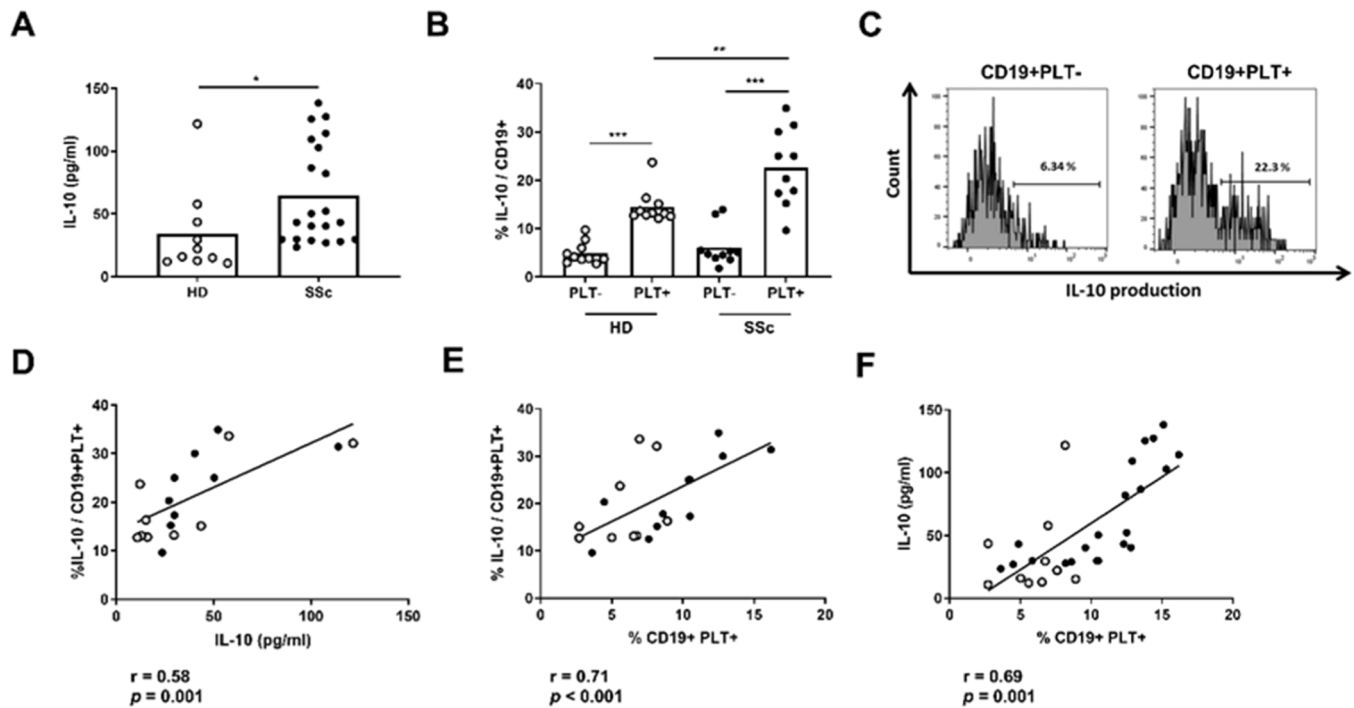


Fig. 3. Association of B cells with bound PLTs with plasma and intracellular production of IL-10 in healthy donors (HD) and systemic sclerosis (SSc) patients. We stimulated PBMCs with ODN2006 and sCD40L during 48 hours. The percentage of intracellular IL-10 in B cells with or without bound PLTs in HD and SSc patients were evaluated by flow cytometry. (A) Plasma levels of IL-10 and (B) percentage of intracellular IL-10 in B cells (CD19+) with or without bound PLTs from HD ($n = 10$) and SSc patients ($n = 10$). (C) A representative image of the percentage of intracellular IL-10 in B cells with or without bound PLTs in SSc patients is shown. Correlation between the percentage of intracellular IL-10 in B cells with bound PLTs and (D) plasma levels of IL-10 or (E) the percentage of circulating B cells with bound PLTs in HD and SSc patients. (F) Correlation between plasma levels of IL-10 and the percentage of circulating B cells with bound PLTs in HD and SSc patients. Spots in black correspond to SSc patients and spots in grey to HD. * $p < 0.05$; *** $p < 0.001$.

cells with bound PLTs and IgM plasma levels, and a positive correlation with IgG and IgA plasma levels in SSc patients (Fig. 4C-E).

PLTs Influence on B Cell IL-10 Production and Plasma Cell Differentiation in SSc

When we co-cultured PBMCs from both groups with PLTs from HD and SSc patients at different PLT/PBMC ratios (50:1, 10:1, and 1:1), we found an increased percentage of intracellular IL-10 in B cells with bound PLTs at 50:1 ratio in both groups (Fig. 5A). However, the percentage of intracellular IL-10 in B cells with bound PLTs, from either group, were higher when co-cultured with SSc PLTs than with HD PLTs (Fig. 5A). Further, the percentage of intracellular IL-10 in B cells with bound PLTs, from either group, were higher when co-cultured with SSc PBMCs than with HD PBMCs (Fig. 5A).

Similarly, we found an increased percentage of plasma cells with bound PLTs when cells were cultured with PLTs at 50:1 ratio (Fig. 5B). Again, the percentage of plasma cells with bound PLTs were higher when cells from either group were cultured with SSc PLTs than with HD PLTs (Fig. 5B). Furthermore, we found a correlation between the percentages of intracellular IL-10 in B cells with bound PLTs and plasma cells with bound PLTs when B cells were co-cultured with PLTs from SSc but not HD PLTs (Fig. 5C-D).

Association of PLT-bound B cells with clinical features and disease subtypes in SSc

We found that the percentage of B cells with bound PLTs were higher in limited (lcSSc) than diffuse (dcSSc) cutaneous disease patients (Fig. 6A). Moreover, we found a positive correlation between B cells with bound PLTs and plasma levels of IgG, IgA, and IL-10, and the percentage of B cells with activated bound PLTs in lcSSc but not in dcSSc

patients (Fig. S1). Only in lcSSc, plasma levels of IgG correlated with plasma levels of IgA and IL-10, and activated PLTs correlated with plasma levels of PDGF and sCD40L (Fig. S1).

We observed that percentage of B cells with bound PLTs were higher in SSc patients with the presence of calcinosis (CC), digital ulcers (DUs), and in ACA+ patients (Fig. 6B-D). Further, we not found differences in the percentage of B cells with bound PLTs when patients were segregated according cutaneous or gastrointestinal disorders, and mRss score (Fig. 6E-G). No effect of corticosteroid or aspirin therapy were found on the percentages of B cells with bound PLTs (Fig. 6H-I).

A multivariate logistic regression model to determine the contribution these factors in differentiating lcSSc and dcSSc identified B cells with bound PLTs as the strongest predictor, followed by plasma levels of IgA and percentage of activated PLTs (Fig. 6G). To find a statistically value, a receiver operating characteristic (ROC) curve analysis with all patients was performed to find a valid cut-off point of the percentage of B cells with bound PLTs to differentiate lcSSc and dcSSc patients leading to >9.10 as the value for limited SSc patients. The area under the ROC curve was 0.892, the sensitivity was 71.4 % and the specificity was 92.8 % (Fig. 6H).

Discussion

This study provides a comprehensive analysis of the differential binding of PLTs to lymphocytes in SSc patients and its potential implications for disease pathology and clinical features. Our findings highlight distinct interactions between PLTs and various lymphocyte subsets in SSc patients compared to HD, underscoring the potential role of PLTs in modulating immune B cell responses in SSc.

Our data reveal that while the percentages of CD4+ and CD8+ T cells with bound PLTs were comparable between SSc patients and HD, B cells with bound PLTs were significantly higher in SSc patients. Additionally,

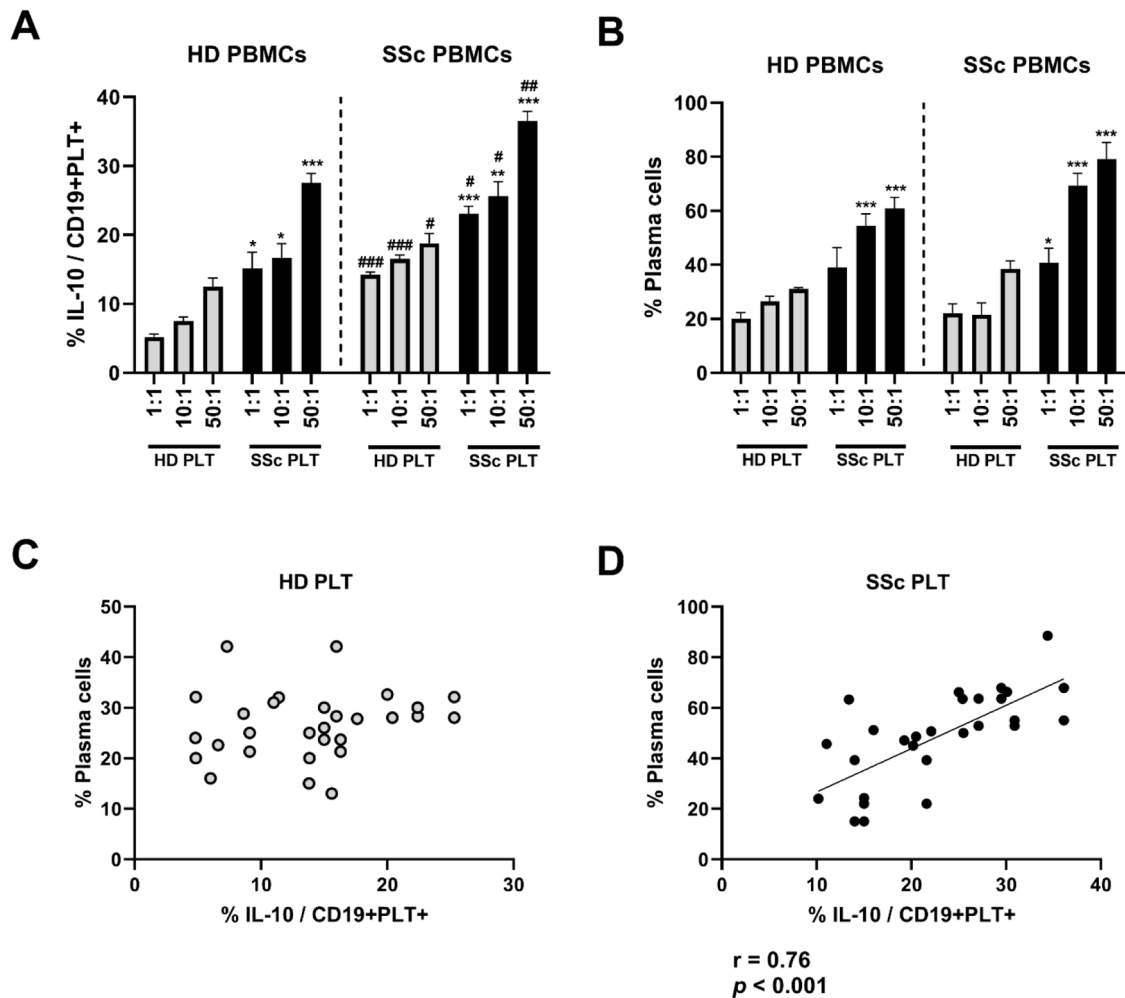


Fig. 5. PLTs from systemic sclerosis patients (SSc) patients altered B cell functions. We co-cultured PBMCs from healthy donors (HD) or SSc patients in the absence or presence of PLTs from HD or SSc patients at different PLT/PBMC ratios (1:1, 10:1, 50:1). In intracellular IL-10 experiments, PBMCs stimulated ODN2006 and sCD40L during 48 hours in 5 % CO₂ at 37°C. The percentage of intracellular IL-10 in B cells with or without bound platelets (PLT) in HD and SSc patients were evaluated by flow cytometry. **(A)** Percentage of intracellular IL-10 in B cells (CD19+) from HD ($n = 5$) or SSc ($n = 5$) patients with or without bound PLTs from HD or SSc patients. In plasma cell differentiation experiments, PBMCs stimulated with ODN2006, sCD40L, F-(ab)2 goat anti-human IgM and IL-21 during 7 days in 5 % CO₂ at 37°C. The percentage of plasma cells in B cells with or without bound PLTs in HD and SSc patients were evaluated by flow cytometry. **(B)** Percentage of plasma cells in B cells from HD ($n = 5$) or SSc ($n = 5$) patients with or without bound PLTs from HD or SSc patients. Correlation between the percentage of plasma cells in B cells with bound PLTs and the percentage of intracellular IL-10 in B cells with bound PLTs in co-culture experiments using PLTs from **(C)** SSc patients or **(D)** HD. Spots in black correspond to SSc patients and spots in grey to HD. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ when compared to their respective ratio in HD PLTs condition. # $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$ when compared to their respective ratio in HD PBMCs condition.

Zamora et al. demonstrated that B cells with bound PLTs had an increased percentage of IL-10-producing cells compared with B cells without bound PLTs in SLE patients and HD.¹⁸ This suggests a potential role of PLTs in modulating B cell function across different autoimmune diseases. However, whether PLTs-B cell interactions contribute to disease pathogenesis in the same way in SSc and SLE remains unclear. Although both conditions share features of autoimmune vasculopathy and immune dysregulation, their distinct clinical and immunological profiles suggest that the functional impact of PLT-B cell interactions may differ. In SLE, where autoimmunity and immune complex-mediated damage are central to disease progression, PLT-B cell interactions may contribute to the dysregulation of B cells and are associated with the presence of autoantibodies and increased inflammatory responses.¹⁸ In contrast, in SSc, where fibrosis and tissue remodeling are more prominent, the role of PLTs in regulating B cell function may be more immunoregulatory. In this context, PLTs could influence B cells in a way that modulates the fibrotic process and immune tolerance, potentially promoting a shift toward a pro-fibrotic phenotype rather than exacerbating autoimmunity. This difference underscores the need for further

studies, including proteomic analysis of B cells and PLTs, to determine whether PLT-B cell interactions lead to disease-specific alterations in immune responses, highlighting the contrasting immunoregulatory roles PLTs might play in each disease. Accordingly, we established an association of PLT-bound B cells with specific clinical features and disease subtypes in SSc. The highest percentages of B cells with bound PLTs were found in SSc patients with calcinosis, digital ulcers and positive ACA autoantibodies. Our results suggest not only that PLT-B cell interactions may be linked to particular disease manifestations but also blocking PLT binding to B cells could be a potential treatment for calcinosis in SSc. No differences in PLT-bound B cells were observed based on treatment, aligning with previous studies showing similar results in SLE patients on antiaggregating drugs.

With a multivariate logistic regression model, we identified PLT-bound B cells as a predictor of limited cutaneous SSc (lcSSc), with high sensitivity and specificity in distinguishing lcSSc from diffuse cutaneous SSc (dcSSc). Increased PLT activation in lcSSc patients may induce higher percentages of B cells with bound PLTs, correlating with elevated Igs and IL-10 plasma levels. This aligns with studies showing

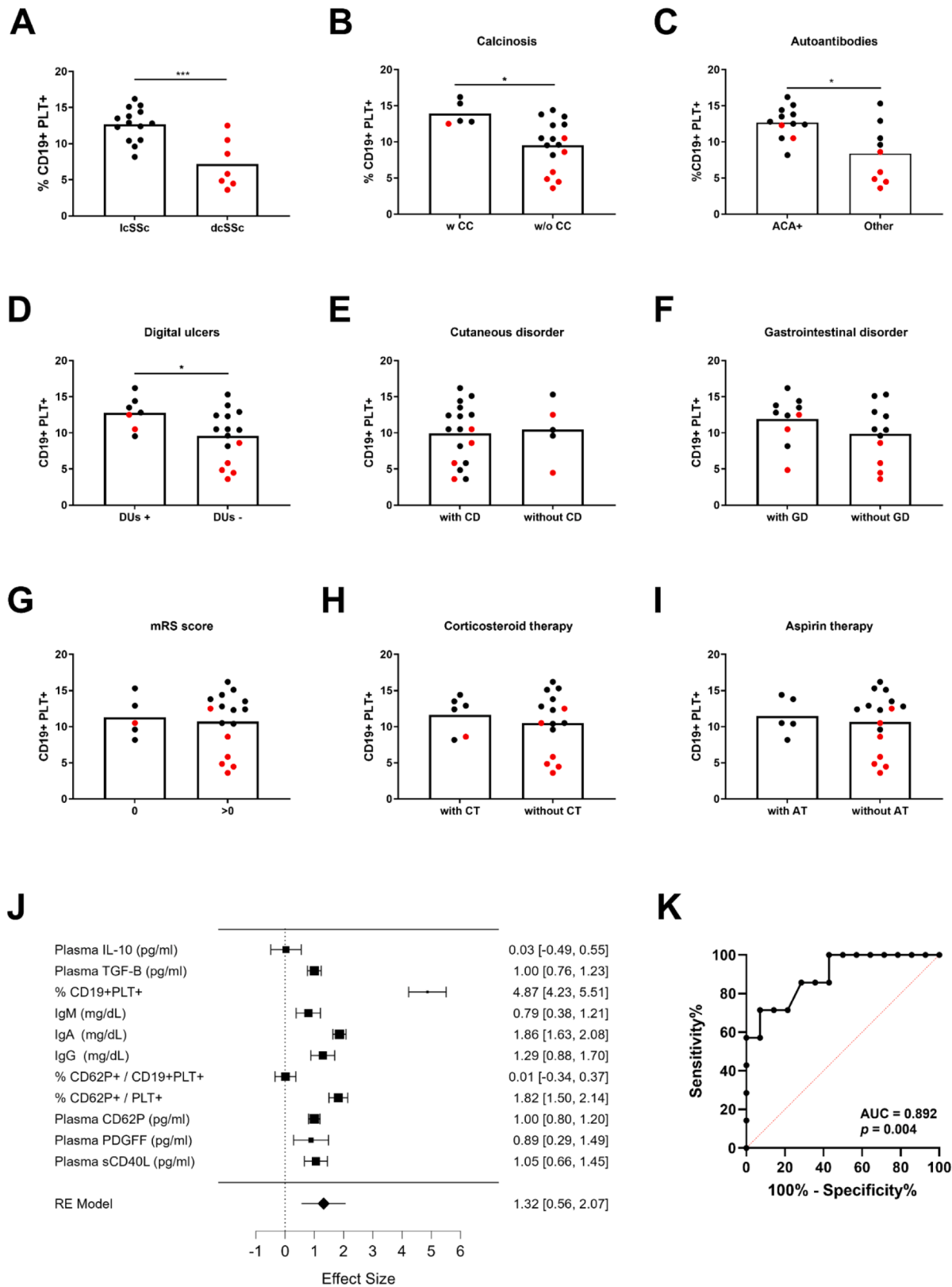


Fig. 6. Association of B cells with bound PLTs with clinical features in systemic sclerosis (SSc) patients. Dot plots of percentage of circulating B cells (CD19+) with bound PLTs in SSc patients segregated according to (A) limited (lcSSc) or diffuse (dcSSc) cutaneous disease, the presence (w) or absence (w/o) of (B) calcinosis (CC), (C) anticentromere (ACA) autoantibodies, (D) digital ulcers (Dus), (E) cutaneous (CD) or (F) gastrointestinal (GD) disorder, (G) mRSS score, and (H) corticosteroid (CT) or (I) aspirin (AT) therapy. (J) Forest plot of logistic regression analysis for factors predicting lcSSc or dcSSc in SSc patients. The reference group was dcSSc patients. Dots represent the odds ratio and the errors bars represent the 95% CI. (K) ROC curve analysis of the percentage of circulating B cells with bound PLTs from lcSSc and dcSSc patients. Spots in black correspond to lcSSc patients and spots in red to dcSSc patients. * $p < 0.05$; *** $p < 0.001$.

higher mean platelet volume in lcSSc patients, reflecting increased PLT activation.³⁸

Despite the insights provided, our study has some limitations. Lymphocyte-PLT complexes were analyzed at a single time point per patient. Longitudinal studies are needed to validate PLT-bound B cells as a tool for monitoring SSc activity. Additionally, a larger cohort study is required to assess the effects of antiaggregating drugs or immunosuppressants on lymphocyte-PLT complexes and their impact on different organ manifestations. Lastly, our findings cannot be extrapolated to tissue, as the phenotype and functional mechanisms of PLTs and B cells may differ in the lung microenvironment. Nonetheless, our findings suggest that controlling PLT binding could be beneficial for therapeutic regulation of autoimmunity in SSc.

Our findings on B cell-PLTs interactions in SSc suggest translational implications. The association between B cells with bound PLTs and specific clinical manifestations, such as calcinosis and digital ulcers, raises the possibility of using these complexes as biomarkers for patient stratification. Moreover, since platelet activation appears to be a key factor in disease pathogenesis, strategies aimed at reducing PLT activation may provide additional immunomodulatory benefits beyond PLT aggregation control. However, further clinical and experimental studies are needed to validate these hypotheses and determine the feasibility of interventions targeting lymphocyte-PLTs interactions in SSc.

Conclusions

This study provides novel insights into the immunopathogenic mechanisms underlying SSc, highlighting the significant role of PLT-bound B cells in contributing to immune dysregulation and inflammation. The findings suggest that SSc-specific PLTs influence B cell function enhancing IL-10 production and plasma cell differentiation. Moreover, B cells with bound PLTs emerged as a predictor for lcSSc, offering a potential biomarker for distinguishing between subtypes. Targeting PLT-bound B cells could present a new avenue for modulating the immune response in SSc.

Data availability statement

All data relevant to the study are included in the article or uploaded as Supplementary Materials. The datasets used and analyzed during the current study are included in the article. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding

n/a.

Conflicts of interest disclosure

The authors declare no conflict of interest.

Ethics approval statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of Research Institute of the Hospital de la Santa Creu i Sant Pau (HSCSP-IR) (protocol approval code IIBSP-COL-2015-40).

Patient consent statement

Written informed consent was obtained from all patients.

Permission to reproduce material from other sources

n/a.

Clinical trial registration

n/a.

CRediT authorship contribution statement

Rubén Osuna-Gómez: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ivan Castellví:** Writing – review & editing, Writing – original draft, Visualization, Validation, Funding acquisition, Conceptualization, Supervision, Resources, Project administration, Methodology, Investigation. **Maria Mulet:** Writing – review & editing, Software, Methodology, Formal analysis. **Jose Luis Tandaipan:** Writing – review & editing, Methodology. **Carlos Zamora:** Writing – review & editing, Methodology, Conceptualization. **Helena Codes-Mendez:** Writing – review & editing, Methodology. **M^a Àngels Ortiz:** Writing – review & editing, Methodology. **Cesar Diaz-Torné:** Writing – review & editing, Methodology. **Elisabet Cantó:** Writing – review & editing, Methodology. **Berta Magallares:** Writing – review & editing, Methodology. **Albert Guinart-Cuadra:** Writing – review & editing, Methodology. **Patricia Moya:** Writing – review & editing, Methodology. **Hector Corominas:** Writing – review & editing, Methodology. **Silvia Vidal:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Acknowledgments

There are no conflicts of interest to disclose, and all authors have read the journal's authorship statement and the manuscript has been reviewed by and approved by all named authors.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.trsl.2025.07.001.

References

1. Sakkas LI, Chikanza IC, Platsoucas CD. Mechanisms of disease: the role of immune cells in the pathogenesis of systemic sclerosis. *Nat Clin Pr Rheumatol*. 2006;2:679–685.
2. Meyer O. Prognostic markers for systemic sclerosis. *Jt Bone Spine*. 2006;73:490–494.
3. Lanteri A, Sobanski V, Langlois Carole, Lefèvre G, et al. Serum free light chains of immunoglobulins as biomarkers for systemic sclerosis characteristics, activity and severity. *Autoimmun Rev*. 2014;13:974–980.
4. Fineschi S, Goffin L, Rezzonico R, et al. Antifibroblast antibodies in systemic sclerosis induce fibroblasts to produce profibrotic chemokines, with partial exploitation of toll-like receptor 4. *Arthritis Rheum*. 2008;58:3913–3923.
5. Walker UA, Tyndall A, Czirják L, et al. Clinical risk assessment of organ manifestations in systemic sclerosis: a report from the EULAR Scleroderma Trials And Research group database. *Ann Rheum Dis*. 2007;66:754–763.
6. Hussein MR, Hassan HI, Hofny ERM, et al. Alterations of mononuclear inflammatory cells, CD4/CD8+ T cells, interleukin 1beta, and tumour necrosis factor alpha in the bronchoalveolar lavage fluid, peripheral blood, and skin of patients with systemic sclerosis. *J Clin Pathol*. 2005;58:178–184.
7. Lafyatis R, O'Hara C, Feghali-Bostwick Carol A, Matteson E. B cell infiltration in systemic sclerosis-associated interstitial lung disease. *Arthritis Rheum*. 2007;56:3167–3168.
8. Ouyang W, Rutz S, Crellin NK, et al. Regulation and functions of the IL-10 family of cytokines in inflammation and disease. *Annu Rev Immunol*. 2011;29:71–109.
9. Hudson LL, Rocca KM, Kuwana M, et al. Interleukin-10 genotypes are associated with systemic sclerosis and influence disease-associated autoimmune responses. *Genes Immun*. 2005;6:274–278.
10. Gourh P, Arnett FC, Assassi S, et al. Plasma cytokine profiles in systemic sclerosis: associations with autoantibody subsets and clinical manifestations. *Arthritis Res Ther*. 2009;11:R147.
11. Huang Y, Zeng W, Liao X, et al. The effects of interleukin-35 and interleukin-10 on pulmonary inflammation and fibrosis in a bleomycin-induced systemic sclerosis mouse model. *Clin Exp Rheumatol*. 2025 Mar 19.
12. Kahaleh MB. Vascular involvement in systemic sclerosis (SSc). *Clin Exp Rheumatol*. 2004;22:S19–S23.

13. Ramirez GA, Franchini S, Rovere-Querini P, et al. The role of platelets in the pathogenesis of systemic sclerosis. *Front Immunol.* 2012;3:160.
14. Pattanaik D, Brown M, Postlethwaite AE. Vascular involvement in systemic sclerosis (scleroderma). *J Inflamm Res.* 2011;4:105–125.
15. Agache I, Rădoi M, Duca L. Platelet activation in patients with systemic scleroderma—pattern and significance. *Rom J Intern Med.* 2007;45:183–191.
16. Yoshizaki A, Komura K, Iwata Y, et al. Clinical significance of serum HMGB-1 and sRAGE levels in systemic sclerosis: association with disease severity. *J Clin Immunol.* 2008;29:180–189.
17. Harris HE, Andersson U, Pisetsky DS. HMGB1: a multifunctional alarmin driving autoimmune and inflammatory disease. *Nat Rev Rheumatol.* 2012;8:195–202.
18. Zamora C, Toniolo E, Diaz-Torné C, et al. Association of platelet binding to lymphocytes with B cell abnormalities and clinical manifestations in systemic Lupus Erythematosus. *Mediat Inflamm.* 2019;2019, 2473164.
19. van den Hoogen F, Khanna D, Fransen J, et al. 2013 classification criteria for systemic sclerosis: an American college of rheumatology/European league against rheumatism collaborative initiative. *Ann Rheum Dis.* 2013;72:1747–1755. <https://doi.org/10.1136/annrheumdis-2013-204424>.
20. LeRoy EC, Black C, Fleischmajer R, et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol.* 1988;15:202–205.
21. Clements P, Lachenbruch P, Siebold J, et al. Inter and intraobserver variability of total skin thickness score (modified Rodnan TSS) in systemic sclerosis. *J Rheumatol.* 1995;22:1281–1285.
22. Osuna-Gómez R, Arqueros C, Galano C, et al. Effector mechanisms of CD8+ HLA-DR + T cells in breast cancer patients who respond to neoadjuvant chemotherapy. *Cancers (Basel).* 2021;13(24):6167. <https://doi.org/10.3390/cancers13246167>.
23. Osuna-Gómez R, Barril S, Mulet M, et al. The immunoregulatory role of IL-35 in patients with interstitial lung disease. *Immunology.* 2023;168(4):610–621.
24. Li X, Zhong H, Bao W, et al. Defective regulatory B-cell compartment in patients with immune thrombocytopenia. *Blood.* 2012;120:3318–3325.
25. Zamora C, Cantó E, Nieto JC, et al. Binding of platelets to lymphocytes: a potential anti-inflammatory therapy in rheumatoid arthritis. *J Immunol.* 2017;198: 3099–3108. <https://doi.org/10.4049/jimmunol.1601708>.
26. Khoenkhoen S, Ádori M, Pedersen GK, et al. Flow cytometry-based protocols for the analysis of Human plasma cell differentiation. *Front Immunol.* 2020;11, 571321.
27. Mulet M, Osuna-Gómez R, Zamora C, et al. Influence of malignant pleural fluid from lung adenocarcinoma patients on neutrophil response. *Cancers (Basel).* 2022;14(10): 2529. <https://doi.org/10.3390/cancers14102529>.
28. Li N, Ji Q, Hjemdahl P. Platelet-lymphocyte conjugation differs between lymphocyte subpopulations. *J Thromb Haemost.* 2006;4:874–881.
29. Matsushita T, Hasegawa M, Yanaba K, et al. Elevated serum BAFF levels in patients with systemic sclerosis: enhanced BAFF signaling in systemic sclerosis B lymphocytes. *Arthritis Rheum.* 2006;54:192–201.
30. Yasuoka H, Sakata K, Yoshimoto K, et al. Phenotype of platelets are altered and activated in circulation of patients with systemic sclerosis. *Blood.* 2018;132:3732.
31. Hawwari I, Rosnagel L, Rosero N, et al. Platelet transcription factors license the pro-inflammatory cytokine response of human monocytes. *EMBO Mol Med.* 2024;16: 1901–1929. –1929.
32. Malabanan KP, Sheahan AV, Khachigian LM. Platelet-derived growth factor-BB mediates cell migration through induction of activating transcription factor 4 and tenascin-C. *Am J Pathol.* 2012;180:2590–2597.
33. Sun Y, Wang T, Lv Y, et al. MALAT1 promotes platelet activity and thrombus formation through PI3k/Akt/GSK-3 β signalling pathway. *Stroke Vasc Neurol.* 2023;8 (3):181–192.
34. Damiani E, Puebla-Orsorio N, Gorbea E, et al. Platelet-activating factor induces epigenetic modifications in Human mast cells. *J Invest Dermatol.* 2015;135: 3034–3040.
35. Levoux J, Prola A, Lafuste P, et al. Platelets facilitate the wound-healing capability of mesenchymal stem cells by mitochondrial transfer and metabolic reprogramming. *Cell Metab.* 2021;33:283–299. e9.
36. Zhang Z, Xu X, Zhang D, et al. Targeting Erbin-mitochondria axis in platelets/ megakaryocytes promotes B cell-mediated antitumor immunity. *Cell Metab.* 2024; 36:541–556. e9.
37. Cognasse F, Hamzeh-Cognasse H, Lafarge S, et al. Human platelets can activate peripheral blood B cells and increase production of immunoglobulins. *Exp Hematol.* 2007;35:1376–1387.
38. Soydu S, Turkbeyler IH, Yavuz Pehlivan, Soydu G, et al. Mean platelet volume seems to be a valuable marker in patients with systemic sclerosis. *Inflammation.* 2014;37:100–106.