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Principal component analysis reveals disconnect between regulatory cytokines and disease activity in Systemic Lupus Erythematosus

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ABSTRACT

Objective: Cytokine dysregulation contributes to inflammation and organ damage in Systemic Lupus Erythematosus (SLE). Principle Component Analysis (PCA) can determine which groups of cytokines have the most influence across disease activity states.

Material and Method: A cross-sectional study of age- and gender-matched SLE patients (n = 100) and controls (n = 31). SLE patients had a median Systemic Lupus Erythematosus Disease Activity Index – 2000 (SLEDAI-2K) score of 6 (IQR 2, 11). IFN-γ, interleukin (IL)-1β, IL-4, IL-6, IL-10, IL-12, IL-17, BAFF, TNF-α, TGF-β1, MIP-1α, MIP-1β and MCP-1 levels were quantified by sandwich ELISA, and compared non-parametrically between groups. PCA was used to determine the principal components across controls, SLE patients in states of remission (SLEDAI-2K = 0), low disease activity (LDA = SLEDAI-2K from $1 \leq x \leq 4$) or high disease activity (HDA = SLEDAI-2K > 4).

Results: TGF-β1 (Rs −0.266, p = 0.005) and IL-1β (Rs −0.199, p = 0.004) inversely correlated, whereas BAFF correlated with increasing disease activity (Rs 0.465, $p < 0.001$).

IL-1β, IL-4, IL-10, IL-12, IL-17, IFN-γ, MCP-1, and TNF-α were featured consistently in the PC1 of all study groups. PC1 changes from controls to SLE-HDA patients, included: the increased impact of IL-1β (from 0.58 to > 0.95); increased impact of IL-6 in HDA (0.76); increased influence of MIP-1α (0.60) and MIP-1β (0.85); and the uncoupling of TGF-β1 (0.14). PC2 changes from healthy controls to the HDA state, included: the increased influence of BAFF (from −0.18 to 0.88); the oppositional effect of TGF-β1 (−0.36); and, the inclusion of MCP-1 (0.65).

Levels of cytokine profiles were equivalent between controls and SLE patients (p > 0.18). BAFF was not associated with the cytokine profiles. TGF-β1 associated with Th1 (Rs 0.36), Th1 + Th17 (Rs 0.22), and inversely with Th17/Th2 (Rs −0.23) profiles. IL-1β associated with the proinflammatory (Rs 0.47), Th1 (Rs 0.55), Th2 (Rs 0.55), Th17 (Rs 0.51), Th1 + Th17 (Rs 0.56), Th2 + Treg (Rs 0.45), and inversely with the (Th1 + Th17 / Th2 + Treg) (Rs −0.22) and Th17/Th2 (Rs −0.27) profiles (all, $p < 0.05$).

Conclusion: Principal component analysis helped to describe the influence of complex cytokine interactions in SLE in a manner congruent with the wider literature. The typical univariate changes in BAFF and TGF-β1 levels with increasing levels of disease activity, were not the dominant factors (in PC1) in the PCA. The PCA demonstrated that IL-1β did not seem to change its regulatory function in SLE.

1. Introduction

Systemic Lupus Erythematosus (SLE) is an idiopathic disease characterised by multisystem inflammation underwritten by autoimmunity to nuclear antigens [1]. The pathogenesis of SLE is not yet completely defined. Patients experience a chronic, yet unpredictable disease course which leads to organ damage and premature mortality. In SLE, the tools for measuring clinical disease activity, e.g., Systemic Lupus Erythematosus Disease Activity Index – 2000 (SLEDAI-2K), and the definitions of remission in SLE (DORIS), are somewhat limited, in that they represent a one-size-fits-all modality of scoring the multifarious symptoms that a SLE patient can be subjected to [2–4]. These tools were developed as there is a lack of gold-standard biomarkers to support the management of SLE [5]. The identification of gold-standard or

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improved biomarkers would help to optimise the management of SLE patients, by better defining periods that lead up to disease exacerbation ('flares'), characterise the course of a 'flare', or suggest transition to disease quiescence [4].

Targeting hierarchical cytokines has proven helpful in the treatment of inflammatory joint, bowel and skin diseases, but has largely failed in SLE, despite the established cytokine dysregulation in this condition. In SLE, this refers to the aberrant production of TNF ligand super family member 13B, also known as B-cell activating factor or BAFF [6–8]. Overexpression of BAFF in mice lead to increased mature B cells and autoantibodies, with the development of lupus-like disease [6]. The main source of BAFF are monocytes and dendritic cells (DCs), but in human SLE, T cells triggered by autoantigens further increase BAFF production [9,10]. An *in vitro* experiment demonstrated that activated B cells in SLE also release BAFF/APRIL, thus initiating a vicious cycle where BAFF and APRIL act in an autocrine manner that amplifies the humoral immune system [11]. Also, treatment with monoclonal anti-BAFF (Belimumab) is only partially successful in dampening the manifestations of SLE [12], with the greatest efficacy seen in patients with serologic activity [13,14]. This suggests that aberrant BAFF production in SLE is driven by rather than the direct cause of disease activity, as has also been reported in patients with lupus nephritis and scleroderma [15–17].

Transforming growth factor β1 (TGF-β1) is normally a potent immunosuppressant, controlling cellular proliferation through apoptosis [18]. In addition, TGF-β1 reduces differentiation and proliferation of B cells, the production of certain immunoglobulin *iso*-types, and co-stimulates the development of regulatory T cells [19,20]. Reduced TGF-β1 production by lymphocytes is a common feature of SLE, which can amplify pro-inflammatory pathways with activation of autoreactive T cells and subsequent production of autoantibodies [21].

As cytokine production is a complex, cascading process, characterised by the interaction and redundancy of a variety of immune cells, studying cytokine expression in isolation, e.g. univariate analyses, is of limited utility as the clinical significance of cytokine levels in serum is often unclear [22]. For example, proinflammatory IL-17 had seemingly paradoxically effect where it exhibited protective effects on cardiovascular and malignancy outcomes in SLE [23]. Another example of paradoxical cytokine effects can be seen in deficiency of TGF-β1 leading to disease exacerbation in SLE, while the localised production of TGF-β1 to dampen chronic inflammatory processes can cause fibrogenesis and contribute to severe organ damage [18,23–26].

Principal component analysis (PCA) identifies which factors, grouped as principal components (PC), that induce the most variation in an overall model and is particularly useful when the variables within the data set are highly correlated. PCA uses a mathematical algorithm to reduce the multidimensionality of data while retaining most of the variation in the data set and brings out strong patterns in a dataset [27]. Subsequently, PCA can reduce the original variables into a smaller number of new variables, called PC. The variables identified in the PC (in our study, cytokines) can then be plotted to enable a visual assessment of the similarities and differences between samples and determine whether samples can be grouped. This study aimed to demonstrate the feasibility of PCA in detecting the cytokine groups that differentiated between healthy controls and states of disease activity in SLE patients.

2. Methods

In a cross-sectional study, serum was obtained from 100 SLE patients during an extended outpatient visit. Data was simultaneously collected on the relevant clinical, disease activity and laboratory results. A group of 31 healthy volunteers, who were non-smoking and not taking any medication, served as controls for serological measures. All patients fulfilled the American College of Rheumatology's classification criteria for SLE [28]. Disease activity was recorded using the Systemic Lupus Erythematosus Disease Activity Index-2K (SLEDAI-2K) [29]. SLE patients were classified as either being: (i) in remission regardless of treatment status (SLEDAI-2K = 0) [as per the international taskforce DORIS (Definitions of Remission In SLE) [3]]; (ii) a low disease activity state (LDA) with a SLEDAI-2K from $1 \leq x \leq 4$; or (iii) in a high disease activity (HDA) state (SLEDAI-2K score > 4) [30]. Patients provided informed written consented for this study, which was approved by the regional ethics committee (REK: 2015/1400).

Interferon-γ (IFN-γ), interleukin (IL)-1β (IL-1β), IL-4, IL-6, IL-10, IL-12, IL-17A, B-cell-activating factor (BAFF), macrophage inflammatory protein 1 alpha (MIP-1α) (SLE patients only), macrophage inflammatory protein 1β (MIP-1β) (SLE patients only), monocyte chemoattractant protein 1 (MCP-1), tumour necrosis factor-alpha (TNF-α), and transforming growth factor beta 1 (TGF-β1) were measured in stored serum samples by a quantitative sandwich immunoassay (Single Analyte ELISArray™ kit; SuperArray Bioscience Corp., Frederick, MD, USA). All assays were run in duplicate and the results, averaged. The manufacturer's recommendations were followed throughout, and the same lot was used for each cytokine. For statistical purposes, values below the limit of detection (LOD) were replaced by the LOD value.

A PCA was performed using SPSS (Factor Analysis package) to determine the interplay of 11 cytokines for healthy controls (n = 31) and 13 cytokines, after the inclusion of MIP-1α and MIP-1β (described in Table 1, shown in Figs. 1 and 2), in SLE patients either in remission (n = 20), a LDA (n = 22) or a HDA (n = 58). PCA analysis achieved convergence during an Oblimin rotation with Kaiser Normalization [31] with the final PCA iterations including 11 or 13 cytokines depending on the group analysed. To be included in a PC the cytokine must have had a factor correlation coefficient ≥ 0.30 to be deemed sufficiently correlated with the other cytokines in the PC. PC1 represents the group of cytokines that induced the most variation in the data as possible, while PC2 in turn has the highest variance possible under the constraint that it is perpendicular to the preceding component. In healthy controls, the PC1 (57.2%) and PC2 (12.5%) cumulatively accounted for 69.7% of the variance of the data for all 11 cytokines. Similarly, for SLE patients in remission, those in a LDA, and those in a HDA, the PC1 and PC2 cumulatively accounted for 67.0%, 71.8% and 73.9% of the variance of the data within all 13 cytokines, respectively.

Continuous variables are presented as median with inter-quartile range or mean with standard deviation, according to the normality of the data. Categorical variables are described with count and percentage. Differences between groups were assessed with *t*-test, non-parametric tests and chi-square test, wherever appropriate. Mann-Whitney U or Independent-Samples Kruskal-Wallis-tests were used in the comparative analysis of cytokine levels between healthy controls, and the various states of SLE disease activity. Spearman's rho correlation coefficients (Rs) were used to assess correlation between cytokines. A separate post-hoc analysis was performed to investigate whether cytokines, i.e. BAFF and TGF-β1, which differed significantly between disease activity states of SLE patients compared to health controls associated with certain cytokine profiles. Cytokine profiles were defined as described earlier [32] as the sum of the levels of selected cytokines: pro-inflammatory (IL-6 + TNFα) [33]; Th1 profile (IL-12 + IFN-γ) [34]; Th2 profile (IL-4) [34]; Th17 profile (IL-6 + IL-17) [34]; Th1 / Th2 [(IL-12 + IFN-γ)/IL-4]; Th1 + Th17 profile (IL-6 + IL-12 + IL-17 + IFN-γ); (Th1 + Th17)/Th2 profile [(IL-6 + IL-12 + IL17 + IFN-γ)/IL-4]; (Th1 + Th17)/(Th2 + Treg) profile [(IL-6 + IL-12 + IL-17 + IFN-γ)/(IL-4 + IL-10)]; the Th17/Th2 profile [(IL-6 + IL17)/IL-4]; and, the Th2 + Treg profile (IL-4 + IL-10). Statistical significance was set at $\alpha = 0.05$ or 5% level. The statistical analysis was performed on Statistical Package for the Social Sciences (SPSS) Version 22.0 (IBM Corp.; Armonk, NY, USA).

3. Results

SLE patients and controls were well matched for age (49 vs 50 years,

Table 1
Median serum cytokine levels (pg/mL) in controls and SLE patients.

	Study Cohort				Independent-Samples Kruskal-Wallis	Spearman Correlation
	Healthy Controls (n = 31)	Remission (SLEDAI-2K = 0) (n = 20)	LDA (SLEDAI-2K: 1 ≤ x ≤ 4) (n = 22)	HDA (SLEDAI-2K > 4) (n = 58)		
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	p-value	Rs.
BAFF	**0.98 (IQR 0.75, 1.15)**	**1.59 (IQR 1.32, 2.28)**	**1.51 (IQR 1.10, 2.36)**	**1.78 (IQR 1.39, 2.36)**	**< 0.001**	**0.465**
IFN-γ	47.31 (IQR 19.60, 113.90)	58.85 (IQR 19.60, 131.36)	82.02 (IQR 19.60, 108.31)	57.82 (IQR 19.60, 144.36)	0.930	0.048
IL-1β	**17.90 (IQR 17.90, 268.97)**	**17.90 (IQR 17.90, 17.90)**	**17.90 (IQR 17.90, 17.90)**	**17.90 (IQR 17.90, 17.90)**	**0.004**	**−0.199**
IL-4	7.00 (IQR 7.00, 7.00)	7.00 (IQR 7.00, 7.00)	7.00 (IQR 7.00, 7.00)	7.00 (IQR 7.00, 8.82)	0.644	0.094
IL-6	14.00 (IQR 14.00, 15.73)	14.00 (IQR 14.00, 14.00)	14.00 (IQR 14.00, 20.53)	14.00 (IQR 14.00, 24.35)	0.453	0.095
IL-10	5.90 (IQR 5.90, 17.59)	5.90 (IQR 5.90, 7.43)	5.90 (IQR 5.90, 20.81)	5.90 (IQR 5.90, 27.70)	0.290	0.093
IL-12	40.40 (IQR 12.60, 86.20)	35.79 (IQR 12.60, 87.73)	30.21 (IQR 12.60, 61.82)	14.71 (IQR 12.60, 58.40)	0.266	−0.168
IL-17	28.40 (IQR 28.40, 94.92)	28.40 (IQR 28.40, 29.81)	28.40 (IQR 28.40, 71.03)	28.40 (IQR 28.40, 72.89)	0.643	0.037
MCP-1	102.36 (IQR 45.34, 195.66)	158.37 (IQR 86.57, 209.47)	169.78 (IQR 69.81, 228.36)	124.47 (IQR 80.82, 208.65)	0.310	0.111
MIP-1α		18.51 (IQR 15.00, 255.84)	15.00 (IQR 15.00, 69.81)	15.00 (IQR 15.00, 55.35)	0.547	−0.020
MIP-1β		204.29 (IQR 146.12, 307.74)	223.01 (IQR 168.06, 282.52)	195.07 (IQR 163.85, 291.65)	0.909	−0.031
TNF-α	35.89 (IQR 21.40, 92.30)	25.96 (IQR 21.40, 77.06)	33.69 (IQR 21.40, 81.16)	41.18 (IQR 21.40, 92.96)	0.843	0.060
TGF-β1	**746.71 (IQR 606.76, 921.68)**	**769.56 (IQR 581.06, 934.56)**	**454.29 (IQR 306.18, 775.29)**	**559.76 (IQR 312.97, 848.18)**	**0.005**	**−0.266**

Bold text represents statistically significant comparisons. $p < 0.05$.

$p > 0.05$) and gender (87% female vs 77%, $p > 0.05$). The main cohort characteristics reported previously [21] include a median SLEDAI-2K score of 6 (IQR 2, 11) with mean physician and patient VAS of 2.7 ± 2.1 and 3.4 ± 2.5, respectively. Disease activity was predominantly related to lupus headache, arthritis, low complement levels, positive anti-dsDNA, rash, alopecia, and Raynaud's phenomenon. At the time of the study, 57% of patients were on hydroxychloroquine, 37% were on immunosuppressive (IS) medication, i.e. azathioprine, mycophenolate, methotrexate or cyclophosphamide, and 41% were on some form of anticoagulant therapy.

Of the individual cytokines, only levels of BAFF, TGF-β1 and IL-1β were significantly different between healthy controls and lupus patients (Table 1). BAFF levels where highest in HDA patients compared to healthy controls (1.78 vs 0.98 ng/mL, $p < 0.01$), and were associated with increasing SLEDAI-2K score (Rs 0.46). TGF-β1 (746.71 vs 559.76 pg/mL, $p < 0.01$) and IL-1β levels (17.9 vs 17.9 pg/mL, $p < 0.01$) were higher in the healthy controls and both demonstrated an inverse correlation with SLEDAI-2K scores (Rs −0.20 and Rs −0.27, respectively). Within SLE patients the presence of anti-dsDNA was correlated with increased BAFF (2.04 vs 1.63 ng/mL, $p = 0.026$; Rs 0.22, $p = 0.025$) and MIP-1β (259.1 vs 196.3 pg/mL, $p = 0.012$; Rs 0.25, $p = 0.011$). Hypocomplementaemia was not associated with any cytokine in SLE patients. All other cytokines were similar between healthy controls and SLE patients in remission, a LDA or a HDA state (Table 1).

IL-1β, IL-4, IL-10, IL-12, IL-17, IFN-γ, MCP-1 and TNF-α were consistently included in the PC1 across all study groups. The major changes to PC1 were seen in the increase in effect of IL-1β from the control group (0.58) to the SLE groups (> 0.95); the uncoupling of IL-6 from the PC1 cytokines in SLE patients in remission (0.18) and a LDA (0.13), but increasing its influence in SLE patients with HDA (0.76); the increasing influence of MIP-1α from SLE patients in remission (0.05) to those with HDA (0.69) in PC1; the increasing influence of MIP-1β in PC1 from remission (0.53) to a HDA state (0.85); and, the uncoupling of TGF-β1 in the PC1 from a state of remission (0.59) to a HDA (0.14) (Table 2; Fig. 1 and Fig. 2).

The major changes to PC2 across the disease states were related to changes in: IL-6 exhibited an inverse effect (−0.40) in controls compared to the positive correlation with PC2 cytokines in a LDA (0.78). MCP-1 was a component of PC1 and PC2 in the control group, but fell out of the PC2 in SLE patients in remission (0.25) and LDA (−0.22), and became a feature of PC2 in SLE patients with HDA (0.65). MIP-1α and MIP-1β featured in the PC2 in SLE patients in remission but uncoupled from the PC2 in those with LDA and HDA, becoming a part of PC1 in both states. Initially, TGF-β1 was the dominant cytokine in PC2 of controls (0.86), but in SLE, TGF-β1 gradually lost influence on the other PC2 cytokines with increasing disease activity. This decreasing influence was mirrored by a concurrent increase in the influence of BAFF, particularly in the LDA (0.74) and HDA states (0.88) (Table 2; Fig. 1 and Fig. 2).

The post-hoc analysis of theoretical cytokines profiles, showed no differences in proinflammatory, Th1, Th2, Th17, Th1/Th2, Th1 + Th17, (Th1 + Th17) / Th2, (Th1 + Th17) / (Th2 + Treg), Th17 / Th2 or Th2 + Treg profiles, across healthy controls, SLE patients in remission, SLE patients in a LDA or SLE patients with HDA ($p > 0.18$ for all comparisons, Supplementary Table 1). For the SLE patients (n = 100), BAFF failed to correlate with any cytokine profile. Overall, TGF-β1 was associated with associated with the Th1 (Rs 0.36, $p < 0.001$), Th1 + Th17 (Rs 0.22, $p = 0.025$) and inversely with the Th17/Th2 (Rs −0.23, $p = 0.019$). IL-1β was associated with the proinflammatory (Rs 0.47), Th1 (Rs 0.55), Th2 (Rs 0.55), Th17 (Rs 0.51), Th1 + Th17 (Rs 0.56), Th2 + Treg (Rs 0.45) profiles, and inversely associated with the (Th1 + Th17)/(Th2 + Treg) (Rs −0.22) and Th17/Th2 (Rs −0.27) profiles (Supplementary Table 1).

Component matrices of the PCA for healthy controls (blue) vs SLE with low disease activity (LDA: SLEDAI-2K 1 ≤ x ≤ 4) (orange) (Fig. 1)

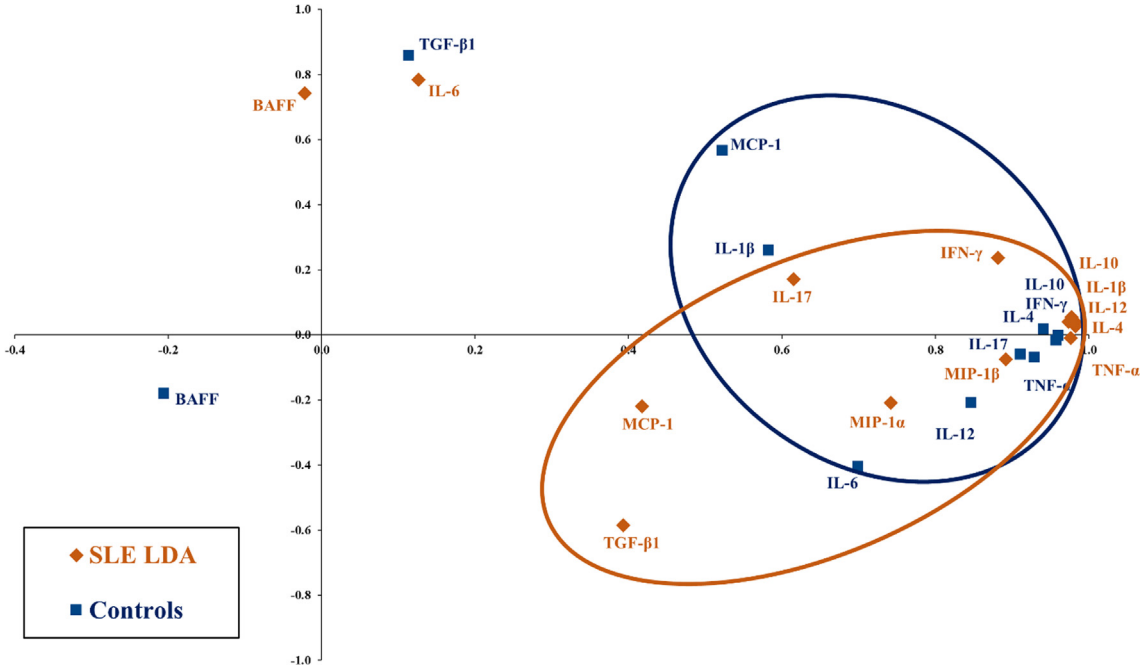

Fig. 1. PCA of Controls vs SLE patients with low disease activity (SLEDAI-2K scores: 1 ≤ x ≤ 4).

and healthy controls (blue) vs lupus patients in a high disease activity state (SLEDAI-2K > 4) (red) (Fig. 2).

Describes for 11 cytokines in healthy controls and 13 cytokines in SLE patients with different levels of disease activity (listed in Table 1), the first two principal components, i.e., PC1 (x-axis) and PC2 (y-axis).

The graphs provide quantitative information on the role of variables as determined by the PCA: 1) the distance of the cytokine (factor) to the null represents the correlation of that factor to the system, i.e., disease activity status. 2) The closer the two or a cluster of cytokines, the more similar their effects are on disease activity.

4. Discussion

Overall, we found that BAFF, TGF-β1 and IL-1β correlated (univariately) with SLE disease activity. In spite of only having a small selection of cytokines, we were able to employ principal component analysis (PCA) to demonstrate the potential influence of cytokines in the context of changing serum levels and disease activity states in SLE versus healthy controls.

Increased BAFF levels in SLE are an independent risk factor for disease flares in association with anti-dsDNA and anti-Sm Ab production, and hypocomplementemia [35–39]. While we reproduced the increased BAFF levels with increasing SLE disease activity, we also found BAFF was not a main driver of variation (i.e. a factor in PC1) in

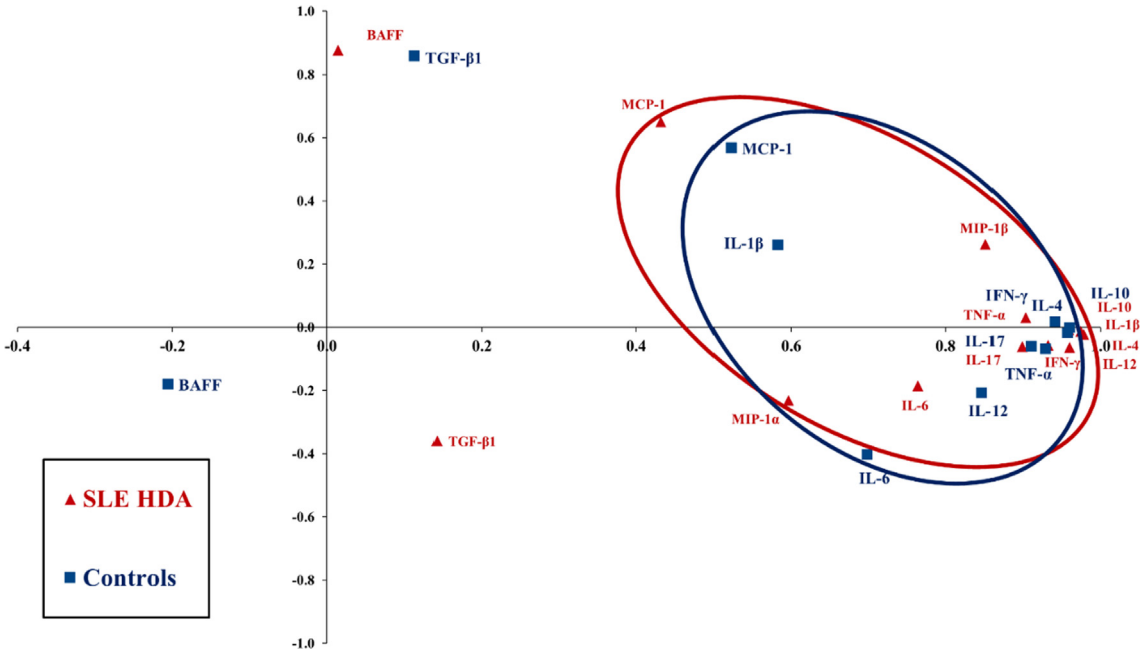

Fig. 2. Principal Component Analysis of healthy controls vs lupus patients in a high disease activity state (SLEDAI-2K > 4).

Table 2
Principal Component 1 and 2 for each patient group for the cytokines in controls and patients with Systemic Lupus Erythematosus with different disease activity states.

	Controls (n = 31)		Remission (SLEDAI-2K = 0) (n = 20)		SLE LDA (SLEDAI-2K: 1 ≤ x ≤ 4) (n = 22)		SLE HDA (SLEDAI-2K > 4) (n = 58)	
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
BAFF	−0.20	−0.18	−0.04	**−0.49**	−0.02	**0.74**	0.01	**0.88**
IL-1β	**0.58**	0.26	**0.95**	−0.25	**0.98**	0.05	**0.97**	−0.01
IL-4	**0.94**	0.02	**0.93**	−0.30	**0.98**	0.03	**0.96**	−0.01
IL-6	**0.70**	**−0.40**	0.18	0.20	0.13	**0.78**	**0.76**	−0.18
IL-10	**0.96**	−0.00	**0.92**	−0.24	**0.97**	0.04	**0.98**	−0.02
IL-12	**0.85**	−0.21	**0.80**	−0.09	**0.92**	0.04	**0.96**	−0.06
IL-17	**0.91**	−0.06	**0.94**	−0.28	**0.62**	0.17	**0.90**	−0.06
IFN-γ	**0.96**	−0.02	**0.97**	−0.03	**0.88**	0.24	**0.93**	−0.06
MCP-1	**0.52**	**0.57**	**0.63**	0.25	**0.42**	−0.22	**0.43**	**0.65**
MIP-1α			0.05	**0.77**	**0.74**	−0.21	**0.60**	−0.23
MIP-1β			**0.53**	**0.64**	**0.89**	−0.08	**0.85**	0.26
TGF-β1	0.11	**0.86**	**0.59**	**0.48**	0.39	**−0.59**	0.14	**−0.36**
TNF-α	**0.93**	−0.07	**0.78**	0.28	**0.98**	−0.01	**0.90**	0.03

Bold text represents statistically significant comparisons. $p < 0.05$.

the PCA nor did we find a correlation between BAFF and the theoretical cytokine profiles. This would suggest that, despite BAFF levels being strongly correlated with SLE activity, the overexpression of BAFF in serum may be a downstream result of localised inflammation or B cell activation, rather than a primary driver of inflammation in SLE [16].

TGF-β1 is the main immune-modulating member of the TGF-β protein family, and shown to be inversely correlated with SLE disease activity [21]. We found lower TGF-β1 in SLE patients compared to healthy controls, and serum TGF-β1 levels were further depleted in those with more active disease. The PCA showed a waning influence of TGF-1β from healthy controls, where it was dominant factor of PC2 (0.86), to SLE patients with HDA where it had a weak oppositional effect in PC2 (−0.36) to the dominant effect of BAFF (0.88). In the post-hoc analysis of cytokine profiles, TGF-β1 associated with the Th1 + Th17 profile, which may reflect the supportive role of TGF-β1 in the differentiation of Th17 cells by IL-6 [40]. Furthermore, we found that TGF-β1 was inversely associated with the Th17/Th2 profile (Rs −0.23), and greater disease activity (Rs −0.27). Our findings suggest that in the context of lower TGF-β1, an imbalance in Th17/Treg cytokines, with uninhibited IL-6 production [41], may contribute to lupus disease activity; and, that this may result from lower TGF-β1 leading to insufficient induction and stabilisation of FoxP3 expression among CD4 + T cells with inadequate formation of regulatory T cells (Tregs) to control pro-inflammatory effects [18,21,32,40,42–46].

Despite being considered an influential cytokine, the role of IL-1β in human lupus is uncertain. Experimental studies have reported lower IL-1β levels in lupus models [47,48], where suppression of IL-1β is reported to prevent progression to overt autoimmune disease and autoantibody-mediated injury [49,50]. Furthermore, mice deficient in IL-1β had milder lupus manifestations and a reduction in the cytokine cascade [51]. In contrast to other studies which reported equivalent IL-1β levels between SLE patients and controls [47,52], we reported lower IL-1β levels in SLE patients ($p = 0.001$), but did not find an association between IL-1β levels and total SLEDAI-2K score (Rs −0.05, $p > 0.05$). However, IL-1β maintained a dominant position in PC1 across all groups, and had greater influence in the PC1 with increasing disease activity. IL-1β was reported to have the ability to increase IFN-γ, induce Th17 differentiation [34,53], and inhibit Treg [49,54]; and in a *post-hoc* analysis we found that IL-1β mostly associated with the pro-inflammatory cytokine profiles, while inversely associated with the (Th1 + Th17)/(Th2 + Treg) and Th17/Th2 profiles [21,33,55]. Therefore, IL-1β functionality in SLE may not be altered, but it may not be capable of inhibiting undesirable effects in the context of lower Treg (Th2 cytokines) or impaired TGF-β1 production.

Across all groups the PC1 consisted of IL-1β, IL-4 (Th2), IL-10 (Treg), IL-12 (Th1), IL-17A (Th17), IFN-γ (Th1), MCP-1, and TNF-α. Aside from the aforementioned changes to IL-1β and TGF-β1, we saw a large increase in the influence of IL-6 in from SLE patients in remission (0.18) to a HDA state (0.76); and, the increased influence of both MIP-1α and MIP-1β from remission to HDA. This might reflect a shift in the balance of pro- and anti-inflammatory cytokines present in SLE patients in remission through to HDA. IL-6 has notable proinflammatory properties and participates in the Th17 cytokine profile (IL-6 + IL-17). The upper-quartiles (upper 75%) of IL-6 levels rose with increased disease activity, from 15.73 to 24.35 pg/mL. Therefore, the inclusion of IL-6 in PC1 of HDA patients, after being uncoupled from the PC1 for both the remission and LDA states, suggests that IL-6 does not underwrite SLE disease activity in quiescent or the LDA, but in a HDA state IL-6 levels may increase and block Treg activity [36,56,57].

Furthermore, the most significant changes to PC2 related to the increasing impact of BAFF from healthy controls (−0.18) to SLE patients with HDA (0.88); and, the waning influence of TGF-β1. However, MCP-1 uncoupled from PC2 in SLE patients in remission and LDA, but became more influential in the HDA state (0.65). Again, MCP-1 levels were not significantly different across disease states, however the upper quartile (75%) of MCP-1, tended to be higher in lupus patients compared to controls. Despite the lack of univariate changes, the association of MCP-1 with BAFF and TGF-β1 in the PCA is congruent with its pro-inflammatory functionality, i.e. attracting leucocytes and other mediators into sites of inflammation [58].

The limitations of this study lie in the fact that our patients were of Northern European descent and were mostly in a state of low disease activity, such that our results cannot be extrapolated to cohorts with different genetic or clinical characteristics. Our results are based on clinical and serological findings and thus cannot confirm the cellular source or causation of cytokine production, for which further experimental studies will be needed. The strength of this study is the availability of a large range of disease characteristics in all patients, the inclusion of a control cohort, and the introduction of PCA to delineate the complexity of cytokine involvement in SLE.

5. Conclusions

Principal component analysis helped to describe the influence of complex cytokine interactions in SLE, and accounted for changing serum levels across disease activity states. Interestingly, the typical univariate changes in BAFF and TGF-β1 levels with increasing levels of disease activity, were not the dominant factors (in PC1) in the PCA.

However, the increasing impact of BAFF and the waning impact of TGF-β1 in the context of increasing disease activity were in PC2. The PCA demonstrated that IL-1β does not seem to change its regulatory function in SLE, and in the presence of TGF-β1 deficiency, IL-1β may amplify inflammation. Ultimately, PCA offered greater insight about the dynamic interplay and influence of cytokines measured in serum across disease activity compared to conventional statistical methods; and, did so in a manner congruent with the wider literature.

Declaration of interests

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. This project was supported by unrestricted grants from the Arthritis and Osteoporosis Foundation of Western Australia, the Norwegian Rheumatism Association and Harstad Reumatiker Forening to JCN.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2018.10.013.

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