Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Jiawei Song Date

Association between inflammatory cytokine concentration and cognitive function and stress among systemic lupus erythematosus patients

By

Jiawei Song
MSPH

Epidemiology

_____

Brad Pearce
Co-Committee Chair

_____

Laura Plantinga
Co-Committee Chair

Association between inflammatory cytokine concentration and cognitive function and stress among systemic lupus erythematosus patients

By

Jiawei Song

Bachelor of Science
Emory University
2016

Thesis Co-Committee Chair: Brad Pearce, Ph.D.
Laura Plantinga Ph.D.

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Science in Public Health
in Epidemiology
2018

Abstract

Association between inflammatory cytokine concentration and cognitive function and stress among systemic lupus erythematosus patients

By Jiawei Song

Systemic lupus erythematous (SLE) is an autoimmune disease that causes the body's immune system to attack the healthy tissue in many parts of the body, leading to widespread inflammation. To our knowledge, the associations between cytokines and cognitive function and stress among SLE patients have never been systematically evaluated. To address this, we examined plasma concentrations of 10 different immune molecules (CRP, ICAM, VCAM, E-selectin, MIF, VEGF, TNF-α, IL-6, IFN-γ, IL-17a), among SLE patients who participated in a pilot study of functioning and had available samples (*n*=27). In the pilot, cognitive performance on five individual domains (episodic memory, working memory, cognitive flexibility, processing speed, and inhibitory control/attention) was assessed and reported as adjusted t-scores (range 0-100, with 50 representing the average score for individuals of the same age, sex, race/ethnicity, and educational attainment). Overall scores for fluid cognition, or overall capacity to reason and solve novel problems were also calculated. Participants' perceived stress was assessed during the pilot study visit using the 14-item Perceived Stress Scale (PSS-14; range 0-56; higher scores indicating greater perceived stress).

In regression models with cognitive score as the outcome variable, concentrations of E-selectin (ng/ml, beta=0.81, p=0.044) and VCAM (ug/ml, beta=-9.1, p=0.014) were statistically significantly associated with overall fluid cognition. After controlling for the time interval between blood draw and cognitive assessment, E-selectin (ng/ml, beta=0.81, p=0.047) and VCAM (ug/ml, beta=-12.5, p=0.008) remained associated with overall fluid cognition. Concentrations of VEGF (ng/ml, beta=-29, p=0.043) and TNF-α (pg/ml, beta=-7.15, p=0.023) were statistically significantly associated with overall fluid cognition only after this adjustment; however, this association was rendered non-statistically significant after removal of an outlier. All other remaining biomarkers were not associated with fluid cognition. When considering individual cognitive domains, E-selectin was associated with better inhibitory control and attention (beta=0.62, p=0.025), cognitive flexibility (beta=0.86, p=0.036) and processing speed (beta=0.90, p=0.044), while TNF-α (beta=-5.78, p=0.046) and VCAM (beta=-7.69, p=0.038) were associated with worse working memory. We found no association between biomarker concentration and perceived stress score among SLE patients. In conclusion, we found that E-selectin and VCAM levels were associated with the higher and lower, respectively, overall fluid cognition performance in SLE patients. The results provide some specific potential biomarker targets, which may help understand cognitive impairment or decline among SLE patients.

Association between inflammatory cytokine concentration and cognitive function and stress among systemic lupus erythematosus patients

By

Jiawei Song

Bachelor of Science
Emory University
2016

Thesis Co-Committee Chair: Brad Pearce Ph.D.
Laura Plantinga Ph.D.

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Science in Public Health
in Epidemiology

Table of Contents

Introduction

Systemic lupus erythematous (SLE) is an autoimmune disease in which the immune system attacks healthy tissue in many parts of the body, leading to widespread inflammation. The severity of SLE can vary from mild to life-threatening. The female: male ratio among adult SLE patients is 10:1. In the United States, around 160,000 to 320,000 people are suffering from SLE. More specifically, the prevalence of SLE is 100 per 100,000 among white women and 400 per 100,000 among black women (Imboden, Hellmann, and Stone 2007). Although the etiology of SLE is not certain, researchers do believe genetic, lifestyle and environmental factors may play a role in the disease development.

SLE is associated with several comorbid conditions including impairment in physical and cognitive function, such as decreased in muscle strength (Andrews et al. 2015), and increased prevalence of neuropsychiatric syndromes, even in relatively young patients (Ainiala et al. 2001). Previous studies suggest 5-fold higher risk of cognitive impairment among SLE patients when compared with the General U.S. population (Hanly et al. 1992). A prospective cohort study identifying impairment in eight areas of cognitive function among SLE patients and rheumatoid arthritis (RA) patients matched by age and sex suggests that impairment is not irreversible, but impairment persisted longer in the SLE group than in the RA group (Hanly et al. 1994).

Additionally, there are several cytokines and other immune mediators that have been found in previous studies to be associated with SLE. Elevated levels of C-reactive protein

(CRP), endothelial-leukocyte adhesion molecule (E-selectin), macrophage migration inhibitory factory (MIF), vascular endothelial growth factor (VEGF), interleukin 6 (IL-6) and interferon gamma (IFN-gamma) were found to be altered in the blood of SLE patients in previous studies (Rezaieyazdi et al. 2011; Foote et al. 2004; Navarro et al. 2002; Chun et al. 2007; Egerer et al. 2000). A national survey revealed 22 cases of SLE in French patients induced by anti-TNF-α for inflammatory arthritis, which also indicate some association between TNF-α and development of SLE (De Bandt et al. 2005) . Previous study in a mouse model of with lupus nephritis have higher levels of vascular cell adhesion molecule (VCAM) and intercellular adhesion molecule (ICAM) than normal mice (Wuthrich 1992). A study also suggested that IL-17 plays an important role in the pathogenesis of SLE. Specifically, IL-17a amplifies the immune response by inducing the local production of chemokines and cytokines in various cell types augmenting the production of autoantibodies by B cells, and increase damage and inflammation in target organs (Li et al. 2015).

Most of the cytokines that are elevated in SLE patients have also been shown to be associated with cognitive impairment in the general population. In a previous study including 91 overweight and 34 normal BMI U.S. adult women, higher CRP was associated with lower scores on cognitive tests of frontal lobe function in the overweight group (Sweat et al. 2008). A positive correlation between higher baseline IL-6 level and increased risk for subsequent decline in cognitive function was found in a sample of individuals age 70-79 years (Weaver et al. 2002), while another study found IL-6 and ICAM were negatively associated with various cognitive test score after adjusting for age

(Rafnsson et al. 2007). Additionally, VEGF and MIF have both been shown to be elevated in Alzheimer's disease patients and patients with mild cognitive impairment or fast cognitive decline when compared to age-matched healthy controls (Chiappelli et al. 2006; Popp et al. 2009).

To our knowledge, there has not been a prior study focused on the association between inflammatory biomarkers and cognitive performance in SLE patients. Detailed assessments of cognitive functioning were obtained in an ancillary pilot study [Approaches to Positive, Patient-centered Experiences of Aging in Lupus (APPEAL)](Plantinga et al. 2018) among 60 SLE patients recruited from population-based Georgians Organized Against Lupus (GOAL). In another ancillary study, we obtained plasma samples from the GOAL cohort and measured 10 inflammatory cytokines. Our study leverages the linkage of these existing data to examine:

1. The association between different inflammatory biomarker levels (cytokines and adhesion molecules) in serum from SLE patients and cognitive performance.
2. The association between different inflammatory biomarker levels in serum from SLE patients and perceived stress.

Materials and Methods

Study population

We used data from the two ancillary studies to the GOAL cohort in metropolitan Atlanta. GOAL patients were recruited from a population-based registry the Georgia Lupus Registry (Lim et al. 2014). GOAL participants are adults with documented diagnosis of SLE [fulfilled at least 4 criteria for classification of SLE, revised by American College of Rheumatology (ACR)(Hochberg 1997), or 3 ACR criteria plus a diagnosis of SLE by board-certificated rheumatologist]. Inclusion criteria for the APPEAL pilot study were: black and white race, English speaking, sufficient vision and hearing to undergo study testing and ability to travel to an in-person study visit. The Emory Institutional Review Board approved the APPEAL and GOAL study protocols, all the blood draw were drawn under GOAL IRB. All APPEAL participants provided informed consent.

For APPEAL, a total of 107 GOAL participants were contacted by mail and phone to obtain the target sample size of 60 participants (Figure 1). Data were obtained from a series of performance tests and questionnaires administered during study visits (10/16-4/17). In addition to the data collected during the in-person APPEAL visit via REDCap (Harris et al. 2009) and NIH Toolbox (13), biomarker data were linked for APPEAL participants who had had blood samples drawn (n=27).

Study variables

Cognitive Performance. Cognitive performance was assessed via five individual assessments from the NIH Toolbox application (Gershon et al. 2010; Weintraub et al. 2014)).

The five individual assessments:

1. Picture Sequence Memory Test, which measures episodic memory, or ability to remember objects, people, or events experienced at particular times and places.
2. List Sorting Working Memory Test, which measures working memory, or the ability to remember and see connections between items or ideas.
3. Pattern Comparison Processing Speed Test, which measures processing speed, or how quickly one can take in and use information.
4. Flanker Inhibitory Control and Attention Test, which measures attention and inhibitory control, or the ability to focus on relevant stimuli in the presence of irrelevant stimuli.
5. Dimensional Change Card Sort Test, which measures cognitive flexibility, or the ability to shift thoughts and adapt behavior to new conditions.

Raw scores were converted to t-scores, adjusted for age, sex, race/ethnicity, and education. Fully adjusted t-scores (mean=50, SD=10) ranged from 0 to 100, higher scores indicate better cognitive functioning. Individual assessment scores were incorporated into a composite adjusted t-score measuring fluid cognition, or overall capacity to reason and solve novel problems.

Perceived stress. The 14-item Perceived Stress Scale (PSS-14; range 0-56; higher scores indicating greater perceived stress) (Cohen, Kamarck, and Mermelstein 1983) was used to assess perceived stress at the pilot study visit

Biomarkers. Blood samples were drawn from each participant in a separate ancillary study that preceded the APPEAL pilot. Blood was only drawn once. Measured biomarkers measured include common inflammatory factors and some cytokines related to the immune system (E-selectin, ICAM, VCAM, TNFalpha, CRP, MIF, VEGF, IL-6, IFN-gamma, IL-17a, IL4) in the serum were tested using the Meso scale Discovery kits (https://www.mesoscale.com/en/products_and_services/assay_kits).

Other variables. Age at assessment, sex, race, ethnicity, and education, were self-reported at the pilot study visit and time between blood draw and pilot study visit was calculated as the difference between pilot study assessment and blood draw dates.

Statistical analysis

We used overall summary statistics to describe the study population. We used histograms to examine the distributions of biomarkers and plotted biomarker concentrations vs. cognitive performance scores and perceived stress scores. We used linear regression to estimate the associations between targeted cytokines and fully corrected fluid composite cognitive scores which, as explained above, correct for age and other demographic characteristics (education, sex, and race/ethnicity). We further adjusted for the time interval between blood draw and cognitive assessment. In sensitivity analysis, we (1) re-

ran the models removing influential outliers and (2) used linear regression to estimate associations between concentrations of biomarkers,which were associated with composite fluid cognition scores, and scores on individual cognitive domains. The same models were also used to test the association between targeted cytokines and PSS score, adjusting for age, education, sex and race/ethnicity.

The distributions for each biomarker are shown in Figures 2-11. The concentrations for biomarkers (except E-selectin) were not normally distributed, even after log transformation. Hence, rather than introducing complicated units for interpretation due to log transformation, we decided to keep the concentration of biomarkers as they were. All 27 participants have E-selectin data available, while all other biomarker analysis only include 20 observations. IL-17a had 4 observations (20%) below detection range, which were treated as 0 pg/ml for IL-17a concentration.

Results

Participant demographic characteristics and bio-markers levels are summarized in Table 1. The study population include 27 subjects, the majority of whom are female (88.9%) and self-reported as black race (88.5%). Age of the study population ranged from 26 to 70 years old, with an average age of the 51.3 years old, and the average education level is 15.3 years. The average biomarker concentrations for the study population are included in Table 1.The fully corrected cognitive performance scores and PSS-14 scores are summarized in Table 2. Our study population on average had worse cognitive performance (fully corrected scores < 50) than the normal population with similar demographic characteristics across all domains.

The estimated associations between biomarker concentration and fluid cognition composite scores are shown in Table 3. VCAM and E-selectin were statistically significantly associated with the cognitive function (p=0.01, p=0.04). a 1 ug/ml increase in VCAM concentration was associated with a mean difference in fluid cognition score of -9.1 (CI:-16, -2.1), and 1 ng/ml increase in E-selectin concentration was associated with a difference in fluid cognition composite score of 0.807 (CI: 0.023, 1.59).

When we adjusted for the time interval between blood draw and cognitive assessment, VCAM and E-selectin remained associated with the cognitive test score (p=0.008, p=0.047). 1 ug/ml higher VCAM concentration was associated with a mean difference in fluid cognition score of -12.5 (CI:-21.2, -3.8). 1 ng/ml higher E-selectin concentration was associated with a difference in Fluid cognition composite score of 0.807 (CI: 0.01,

1.56). With this adjustment, associations of VEGF and TNF-α with overall fluid cognition were statistically significant as well (p=0.04, p=0.02). 1 ug/ml higher VEGF concentration was associated with a mean difference in fluid cognition score -29 (CI: -56, -1.1). 1 pg/ml higher TNF-α concentration was associated with a mean difference in fluid cognition score of -7.15 (CI:-13.17, -1.13). The scatter plots of biomarker concentration and fluid composite cognitive score and perceived stress score with statistical significant results are shown in Figures 12-16. Figure 12 show a linear trend between VCAM concentration and fluid cognitive composite score. The overall trend for E-selectin concentration and fluid cognitive composite score shown in Figure 13 appears linear, with a few outliers with low performance scores. Figure 15 shows the trend for TNF- α concentration and fluid cognitive composite score was largely influenced by one single observation. After dropping that individual (Table 4), TNF- α was not statistically significantly associated with composite cognitive test (p=0.47). Through looking at the scatter plot (Figure 14) we can see the trend for VEGF was influenced by an observation at the bottom right. After dropping that observation (Table 4), VEGF was no longer associated with fluid cognition (p=0.72).

We also checked associations between performance on individual cognitive domains and biomarkers that were associated with overall fluid cognition (Table 5). E-selectin was statistically significantly associated with better inhibitory control and attention, cognitive flexibility, processing speed. A 1 ng/ml higher E-selectin concentration was associated with a mean difference in inhibitory control/attention score of 0.62 (P=0.0249 CI: 0.08, 1.15), a mean difference in cognitive flexibility score of 0.86 (P=0.0355 CI:0.063, 1.65),

and a mean difference in ‘processing speed score of 0.90 (P=0.0437 CI: 0.028, 1.78). As also shown in Table 5, VCAM and TNF-α were associated with poorer working memory. 1 ug/ml higher VCAM concentration was associated with a mean difference in working memory score of -7.7(p=0.0384 CI: (-14.9, -4.58). 1 pg/ml higher TNF-α concentration was associated with a mean difference in ‘list’ score of -5.78 (p=0.046 CI: (-11.44, -0.126)). VEGF was not associated with any individual cognitive domain.

Table 6 shows the relationship between biomarker concentration and perceived stress. The result indicates MIF was associated with higher stress as indicated by the PSS score, with 1 ng/ml increase of MIF concentration being associated with an average change in PSS score of 0.9 (P=0.0494 CI: 0.0026, 1.8). After further adjusting for the time interval, the association was similar but no longer statistically significant. Figure 16 indicates most of the patients have similar MIF concentration, while a few have extremely high concentrations.

Discussion

We found that E-selectin and VCAM levels were associated with the higher and lower, respectively, overall fluid cognition. After controlling for the time interval between blood draw and cognitive assessment, E-selectin and VCAM remained associated with fluid cognition. VEGF and TNF-α were also statistically significantly associated with poor fluid cognition, but only after adjustment for time interval. We believe the statistically significant result between VEGF and TNF- α and fluid cognition in the study population are possibly by coincidence. The other main outcome of the study is perceived stress score. Only MIF is associated with stress score, but after we adjusted for the time interval between blood draw and assessment, the relationship weakened to be non-significant ($P>0.05$). Overall, our results suggests higher concentration of E-selectin in blood is associated with better cognitive performance, while higher concentration of VCAM in blood is associated with worse cognitive performance among SLE patients.

The levels of circulating soluble adhesion molecules in SLE patients have been addressed by various studies, although results have not been consistent (Spronk et al. 1994; Ikeda et al. 1998). One study suggests at the time of maximal disease activity during exacerbation, plasma levels of VCAM were higher among SLE patients while E-selectin concentration were lower in patients, compared to healthy controls (Spronk et al. 1994). During the disease exacerbation, VCAM was significantly increased, while E-selectin concentrations were not different from the previous time points (Spronk et al. 1994). Another study indicates both E-selectin and VCAM concentration were significantly higher in SLE

patients than in healthy controls, however, only VCAM concentration was significantly associated with disease activity (Ikeda et al. 1998).

The association between biomarkers and cognitive score has been addressed by previous studies of non-SLE populations. According to one previous study, plasma level of VCAM was higher among late-onset Alzheimer's disease and vascular dementia patients than healthy controls, while E-selectin concentration was not significantly different between dementia patients and healthy controls (Zuliani et al. 2008). Another study indicates circulating VCAM and E-selectin concentration were not associated with cognitive function (Yoon et al. 2017).However, there is also study found higher E-selectin concentration was associated with worse cognition among survivors of critical illness (Hughes et al. 2018).

Selectins are cellular adhesion molecules expressed on the cell surface and assist in cell-cell interaction and adhesion. E-selectin is a highly glycosylated protein that is synthesized by activated endothelial cells. The major function of vascular E-selectin was to recruit leukocyte to inflamed sites through the interaction with its counter ligands. E-selectin expression is absent on normal endothelial cells, while the expression is induced in response to cytokines such as TNF-α and IL-1 β (Mann and Tanaka 2011). Similar to E-selectin, VCAM is also a cytokine inducible adhesion molecule on human endothelial cells, which mediates the binding of endothelial cell to leukocyte and melanoma cells (Imhof and Dunon 1995). VCAM is absent on resting endothelial cells but the expression can also be upregulated by IL-1 and TNF-α (Imhof and Dunon 1995). Endothelial

activation has been considered as a central process in the neuropsychiatric manifestations of SLE, in part because of the role played by endothelium in the permeability of the blood-brain barrier (C Sinclair, J Miner, and HJ Kim 2015). Anti-endothelial cell antibodies (AECAs) are thought to activate endothelial cells directly, and potentially lead to blood-brain barrier disruption (Conti et al. 2004; Wong, Dorovini-Zis, and Vincent 2004). AECAs can lead to upregulation of endothelial cell adhesion molecules, including E-selectin, ICAM-1 and VCAM-1, which mediates leukocyte adhesion to the vascular endothelium and promotes extravasation of leukocytes (Conti et al. 2004).VCAM and E-selectin are known to be related with the initiation of atherosclerosis (Cybulsky et al. 2001; Hwang et al. 1997). One study suggests that cardiovascular risk factor and thrombogenic factors are strongly associated with white matter lesion, which are correlated with patients' cognitive function decline (Breteler et al. 1994). So it is reasonable to think VCAM and E-selectin might also be associated with cognitive impairment among SLE patients. However, our results for VCAM are consistent with expectations, while our results for E-selectin are not. Both E-selectin and VCAM play important roles in leukocyte recruitment. The steps that are involved in leukocyte recruitment at site of inflammation are shown in Figure 17A: (1) leukocyte rolling and tethering on vascular endothelium, mediated by the selectin family and their ligands; (2) activation and firm adhesion to endothelium, which depend on chemokine activation of leukocyte integrin and their interaction with ICAM and VCAM; (3) locomotion from the site of site of firm adhesion to endothelial cell junction, which also depends on leukocyte intergrins, ICAM and VCAM; and (4) diapedisis across the endothelial cell and the basement membrane into the tissue (Garton, Gough, and Raines 2006).

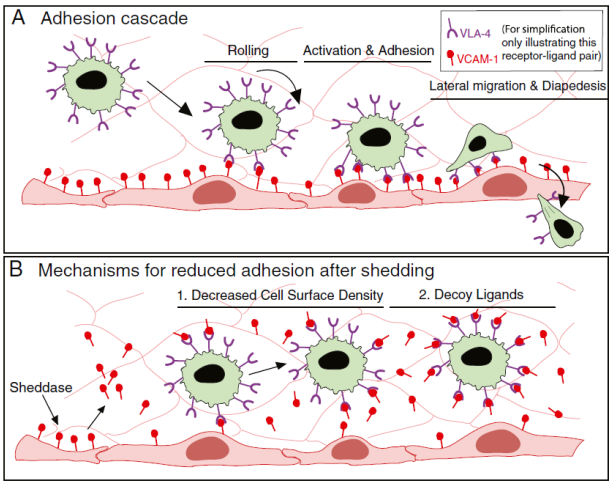

Figure 17. (A)The steps of Leukocyte recruitment at site of inflammation. (B) Shedding of selectin and immunoglobulin superfamily adhesion molecules. Same mechanism apply to both VCAM and E-selectin. For simplicity, only VCAM is shown in the figure (Figure copyright belong to Biomed central journal,(Richter et al. 2003)).

Figure 17B illustrates VCAM as an example for selectin and the immunoglobulin superfamily of adhesion molecules. Shedding of E-selectin into the circulation can affect the ability of endothelial-bound E-selectin to capture flowing leukocytes by both decreasing the cell surface density and by generating a soluble intravascular competitive inhibitor for leukocytes, which can reduce collateral damage in the host (Smith 1997). VCAM shedding can affect the binding between endothelial cell and leukocyte through a similar mechanism, however, VCAM only affects the leukocytes after they are captured by the inflammation site. VCAM shedding can release the leukocytes from the adhesion and facilitate the locomotion process, which can actually lead to more damage to the host (Garton, Gough, and Raines 2006).

The major limitations of the study are listed as follows. First, the sample size of the study is small, as we only have 20 patients with all biomarker data and 27 with E-selectin data, limiting our statistical power. Second, cytokine production is highly dependent on health

conditions; however, we were unable to control for any comorbid conditions. The possibility for residual confounding by these and other factors, such as medications, exists. Third, we assume linear association between biomarkers and cognitive domains, although the scatter plots do suggest roughly linear associations. Fourth, both biomarkers and cognitive domains were only measured once, hence, we were not able to capture change over time.

Despite these limitations, we demonstrate for the first time, to our knowledge, that E-selectin and VCAM may be associated with cognitive function among SLE patients. The results provide some specific potential biomarker targets, which may help prevent cognitive impairment or decline among SLE patients. Studies with larger populations and longitudinal measurements should be done to test the hypotheses generated by these results.

Table and Figures

Table 1. Demographic characteristics and biomarker concentrations among systematic lupus erythematous patients

	SLE patients	N
Age in years, mean (SD)	51.3 (SD=11.8)	27
Gender (% male)	11.1%	27
Education in years, mean (SD)	15.3 (SD=3.1)	27
Race (%)		27
Black	88.5%	
White	11.5%	
CRP ug/ml mean (SD)	9.02 (SD=8.39)	20
VCAM ug/ml mean (SD)	1.17 (SD=0.80)	20
ICAM ug/ml mean (SD)	0.92 (SD=0.64)	20
E-selectin (mean ng/ml)	12.94 (SD=6.84)	27
MIF (mean ng/ml)	3.58 (SD=3.79)	20
VEGF (mean pg/ml)	247.98 (SD=270.02)	20
TNF-α (mean pg/ml)	2.58 (SD=1.02)	20
IL-6 (mean pg/ml)	1.68 (SD=1.39)	20
IFN-γ (mean pg/ml)	9.35 (SD=9.54)	20
IL-17 (mean pg/ml)	1.11 (SD=0.96)	20

Education: 1-6 years (Elementary/Middle School) | 7 to 12 years (Junior High/High School) | 13-16 years (College/University) | 17-23 years (Graduate/Professional School)

Table 2. Fully corrected cognitive performance score and Perceived Stress Scale scores of SLE patients (N=27)

Domain	Mean	Possible score
Picture Sequence Memory*	47.72 (SD=9.24)	0-100
Flanker Inhibitory Control and Attention*	38.8 (SD=8.58)	0-100
Dimensional Change Card Sort*	43.72 (SD=14.22)	0-100
List Sorting Working Memory*	48.58 (SD=11.22)	0-100
Pattern Comparison Processing Speed*	42.55 (SD=14.83)	0-100
Fluid cognition composite score*	41 (SD=12.06)	0-100
Perceived Stress Scale score	23.22 (SD=8.72)	0-56

*The score is adjusted by age, education, gender and race

Table 3. The association between Fluid Cognition composite score and biomarker concentration and further adjusted for time interval

	Biomarker concentration directly				Adjusted by time interval		
Biomarker	Beta	95% Confidence interval	P value		Beta	95% confidence interval	P value
CRP (ug/ml)	0.4	(-0.3, 0.1)	0.279		0.55	(-0.27, 1.38)	0.174
VCAM (ug/ml)	-9.1	(-16, −2.1)	0.014		-12.5	(-21.2, −3.81)	0.008
ICAM(ug/ml)	-5.4	(-1.5, 4.6)	0.270		-7.1	(-21.5, 7.4)	0.315
E-selectin(ng/ml)	0.807	(0.023, 1.59)	0.044		0.807	(0.01, 1.56)	0.047
MIF(ng/ml)	-13	(-3.1, 4)	0.129		-1.6	(-3.5, 0.2)	0.082
VEGF (ng/ml)	-15	(-39, 8)	0.192		-29	(-56, -11)	0.043
TNF-α (pg/ml)	-5.78	(-11.58, 0.012)	0.0504		-7.15	(-13.17, -1.13)	0.023
IL-6(pg/ml)	1.37	(-3.35, 6.10)	0.549		1.20	(-3.73, 6.14)	0.614
IFN-γ (pg/ml)	0.11	(-0.59, 0.80)	0.749		0.09	(-0.62, 0.81)	0.785
IL-17 (pg/ml)	4.39	(-2.18, 10.96)	0.177		5.72	(-1.28, 12.72)	0.103

Table 4. The association between TNF-α, VEGF and overall cognitive test controlling for time interval after dropping one outlier observation.

Biomarker	Beta	Confidence interval	P value
TNF-α (pg/ml)	-2.36	(-9.22, 4.50)	0.48
VEGF (ng/ml)	-5.31	(-36, 25)	0.72

Table 5. The association between biomarkers, which are potentially associated with overall cognitive test, and each individual cognitive test

Cognitive test	Biomarker	Beta	95% confidence interval	P value
Picture	VCAM	-4.6	(-10, 0.97)	0.100
	E-selectin	-0.09	(-0.67, 0.48)	0.746
	MIF	-0.00072	(-0.00204, 0.0006)	0.267
	TNF-α	-2.28	(-6.78, 2.21)	0.300
	VEGF	-3	(-21, 14)	0.684
Flanker	VCAM	-4.6	(-9.25, 0.145)	0.057
	E-selectin	0.62	(0.08, 1.15)	0.025
	MIF	-0.83	(-2, 0.2)	0.139
	TNF-α	-1.12	(-5.12, 2.88)	0.564
	VEGF	-1.6	(-17, 14)	0.834
DCCS	VCAM	-0.615	(-14.1 , 1.76)	0.120
	E-selectin	0.86	(0.063, 1.65)	0.036

	MIF	1	(-3, 0.8)	0.234
	TNF-α	-4.92	(-11.03, 1.19)	0.108
	VEGF	-11	(-35, 14)	0.361
List	VCAM	-7.69	(-14.9, – 4.58)	0.038
	E-selectin	0.33	(-0.44, 1.11)	0.385
	MIF	-0.89	(-30, 0.9)	0.317
	TNF-α	-5.78	(-11.44, -0.126)	0.046
	VEGF	-11	(-3.4, 12)	0.342
Pattern	VCAM	-6.43	(-15.6, 2.77)	0.160
	E-selectin	0.9026	(0.028, 1.78)	0.044
	MIF	-0.7	(-2.9, 1.4)	0.485
	TNF-α	-4.47	(-11.70, 2.75)	0.210
	VEGF	-2	(-4.8, 5.3)	0.110

Unit: VCAM: ug/ml E-selecin: ng/ml MIF: ng/ml TNF-α: pg/ml VEGF: ng/ml

Table 6. The association between perceived stress score and biomarker concentration and further adjusted for time interval

	Biomarker concentration directly				Adjusted by time interval		
Biomarker	Beta	95% Confidence interval	P value		Beta	95% confidence interval	P value
CRP (ug/ml)	-0.291	(-0.745, 0.163)	0.189		-0.288	(-0.766, 0.19)	0.214
VCAM (ug/ml)	-0.78	(-5.8 ,4.25)	0.744		-0.612	(-6.8,5.53)	0.832
ICAM(ug/ml)	-2.49	(-8.42 ,3.4)	0.381		-3.41	(-1.15 ,4.68)	0.376
E-selectin(ng/ml)	0.49	(-0.07, 1.06)	0.0839		0.50	(-0.11, 1.11)	0.1012
MIF(ng/ml)	0.9	(0.0026, 1.8)	0.049		0.9	(-0.5, 1.8)	0.062
VEGF (ng/ml)	10	(-9, 30)	0.2792		10	(-11, 30)	0.307
TNF-α (pg/ml)	2.54	(-1.47, 6.56)	0.194		2.77	(-1.81, 7.36)	0.2122
IL-6(pg/ml)	0.99	(-1.61, 3.60)	0.425		0.97	(-1.84, 3.78)	0.468
IFN-γ (pg/ml)	-0.105	(-0.51, 0.30)	0.583		-0.13	(-0.57, 0.31)	0.528
IL-17 (pg/ml)	-3.40	(-7.03, 0.23)	0.064		-3.40	(-7.25, 0.45)	0.079

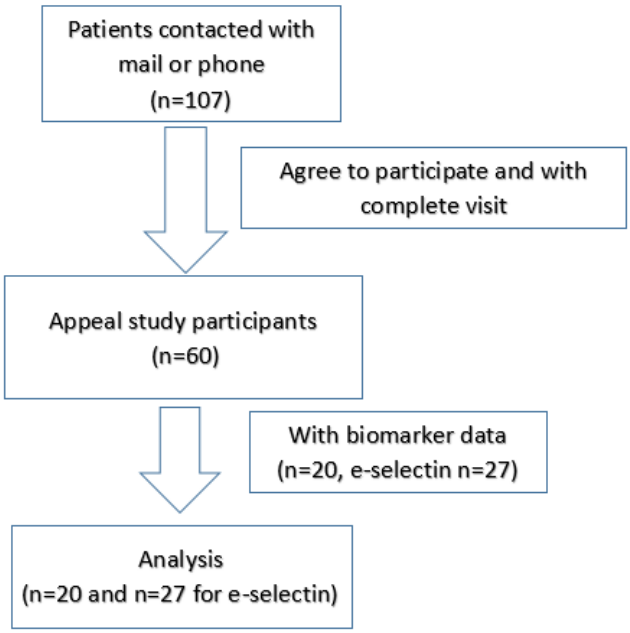

Figure 1. Flow diagram for study population.

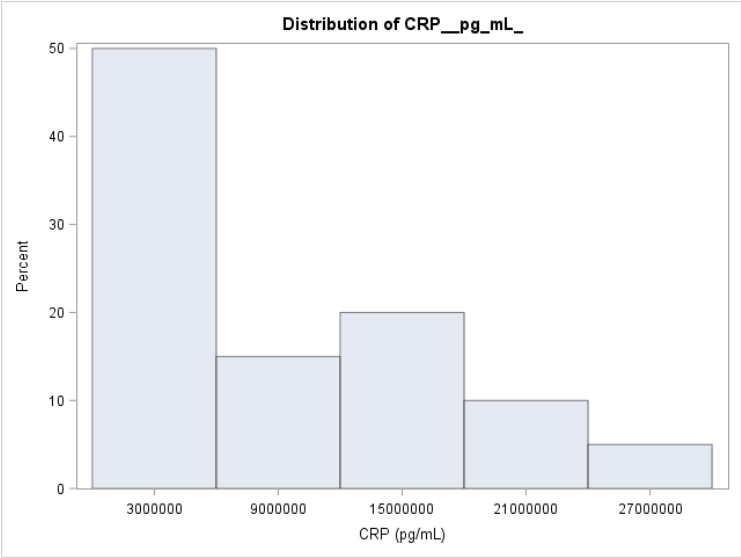

Figure 2. The distribution of CRP concentration in the study population.

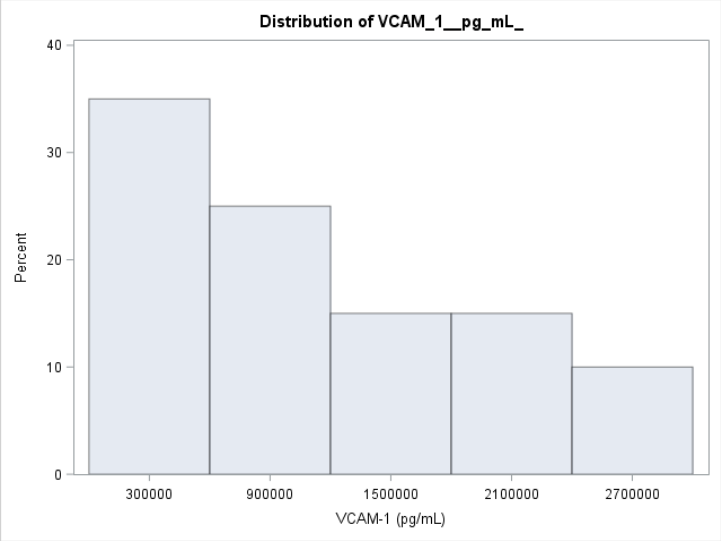

Figure 3. The distribution of VCAM concentration in the study population.

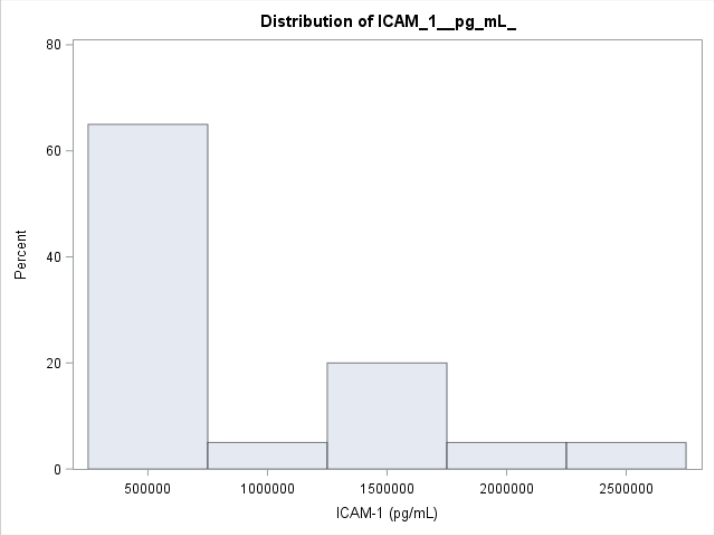

Figure 4. The distribution of ICAM concentration in the study population.

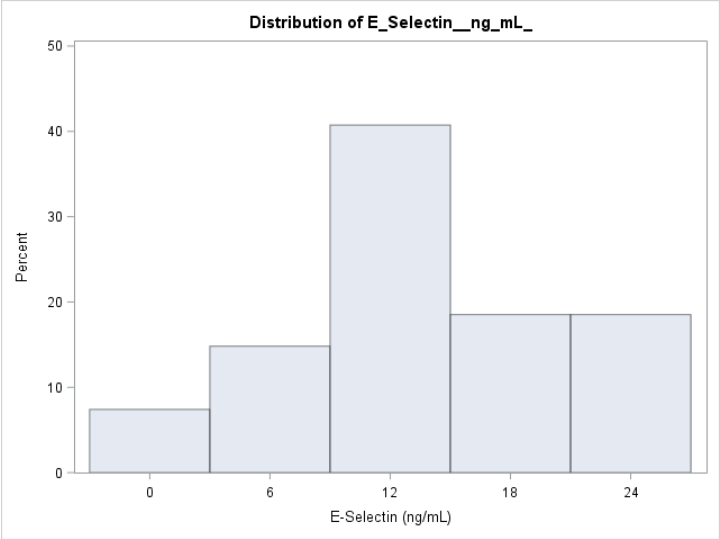

Figure 5. The distribution of E-selectin concentration in the study population.

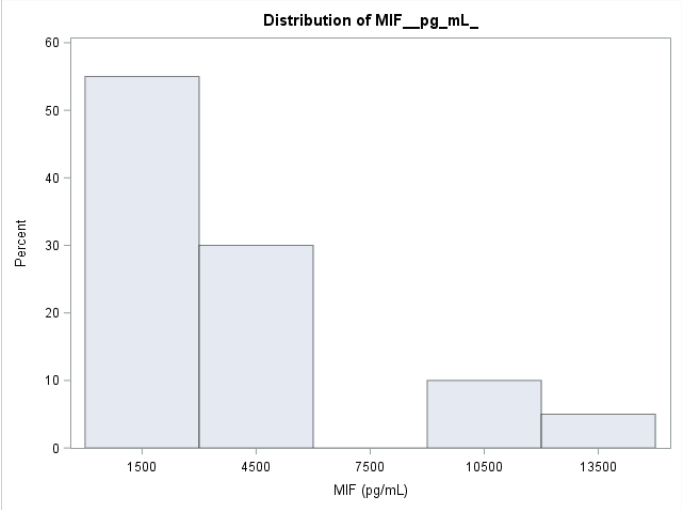

Figure 6. The distribution of MIF concentration in the study population.

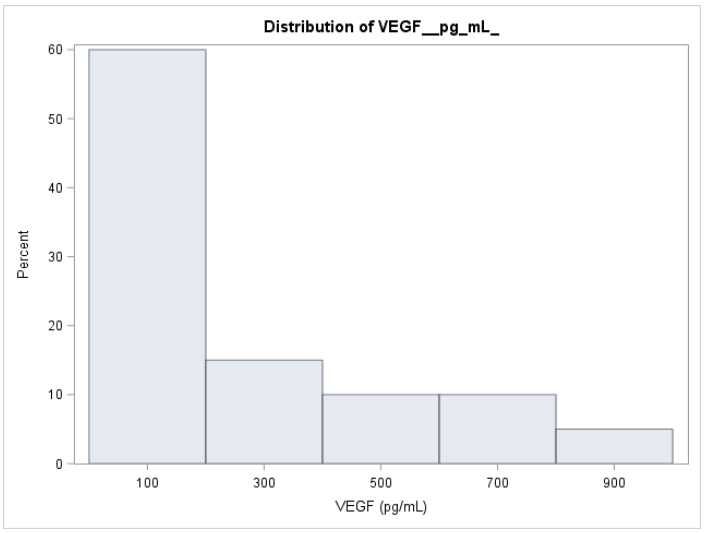

Figure 7. The distribution of VEGF concentration in the study population.

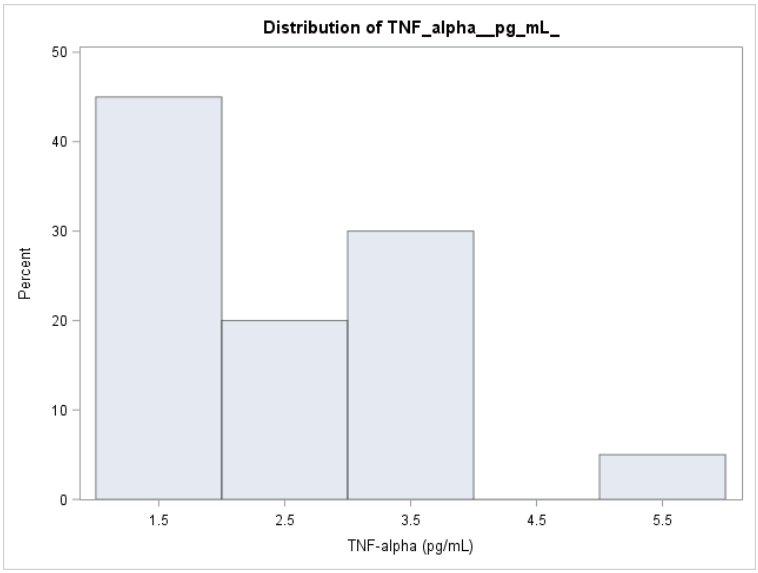

Figure 8. The distribution of TNF-α concentration in the study population.

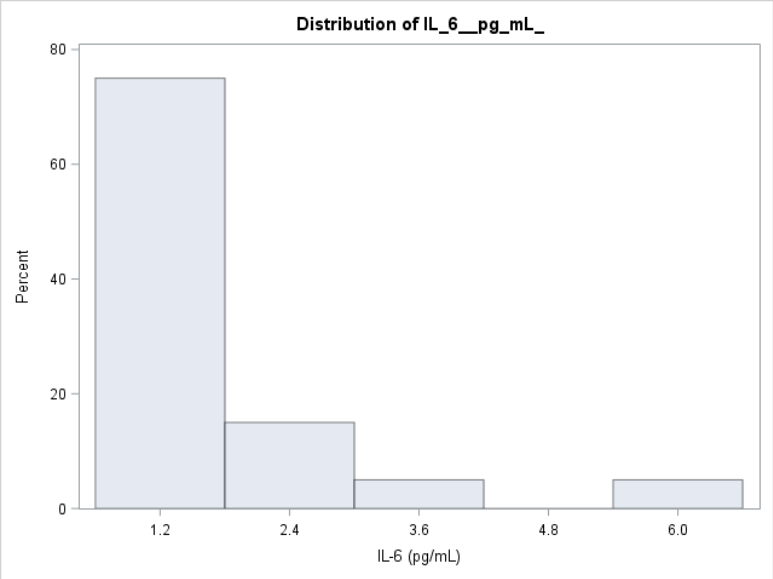

Figure 9. The distribution of IL-6 concentration in the study population.

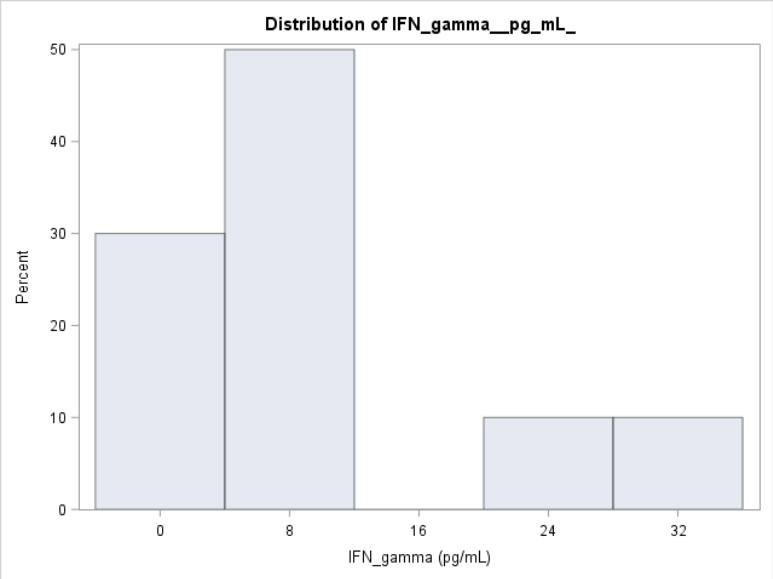

Figure 10. The distribution of IFN-γ concentration in the study population.

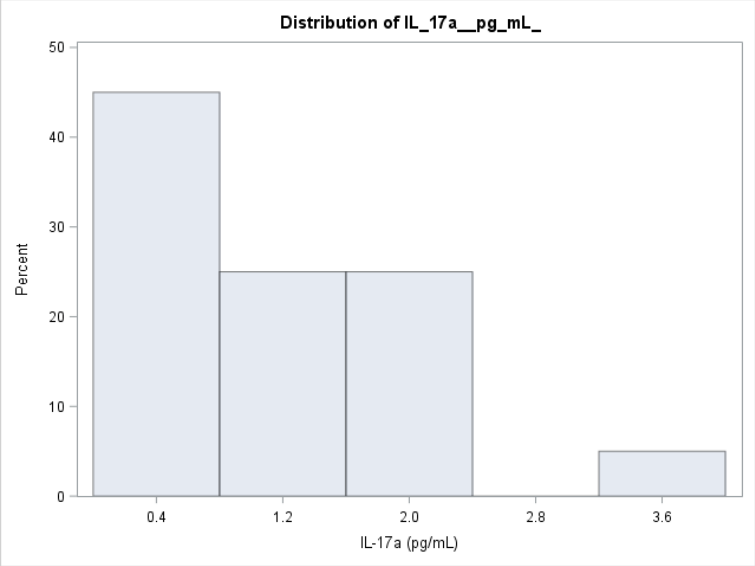

Figure 11. The distribution of IL-17a concentration in the study population.

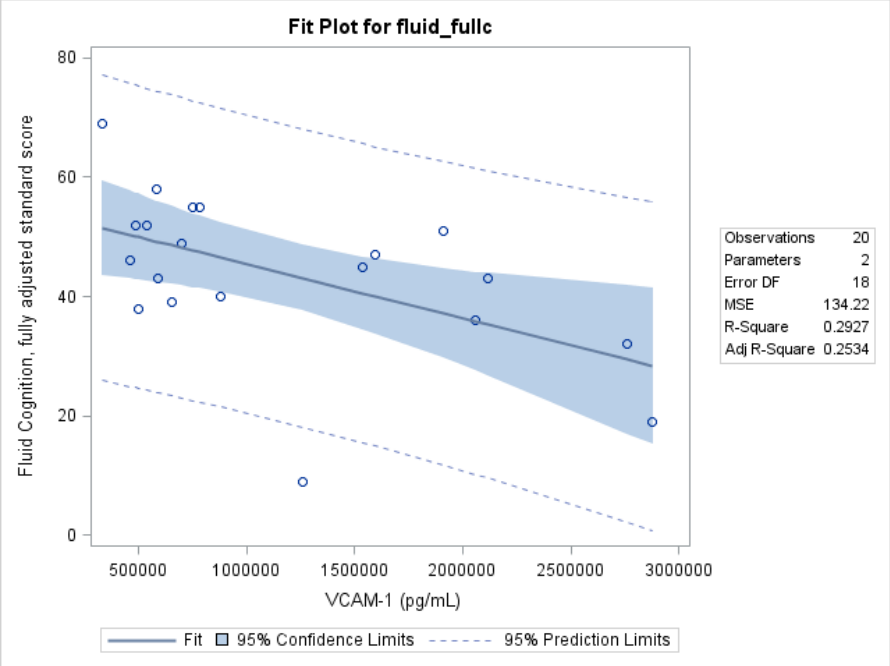

Figure 12. Scatter plot of VCAM concentration and age-, sex-, race-/ethnicity-, and education-corrected fluid cognitive composite score

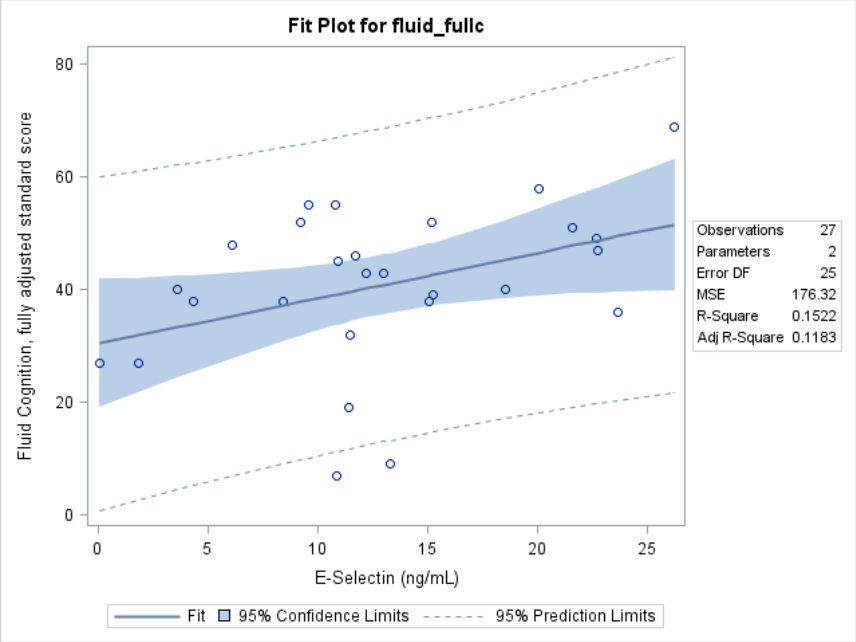

Figure 13. Scatter plot of E-selectin concentration and age-, sex-, race-/ethnicity-, and education-corrected fluid cognitive composite score

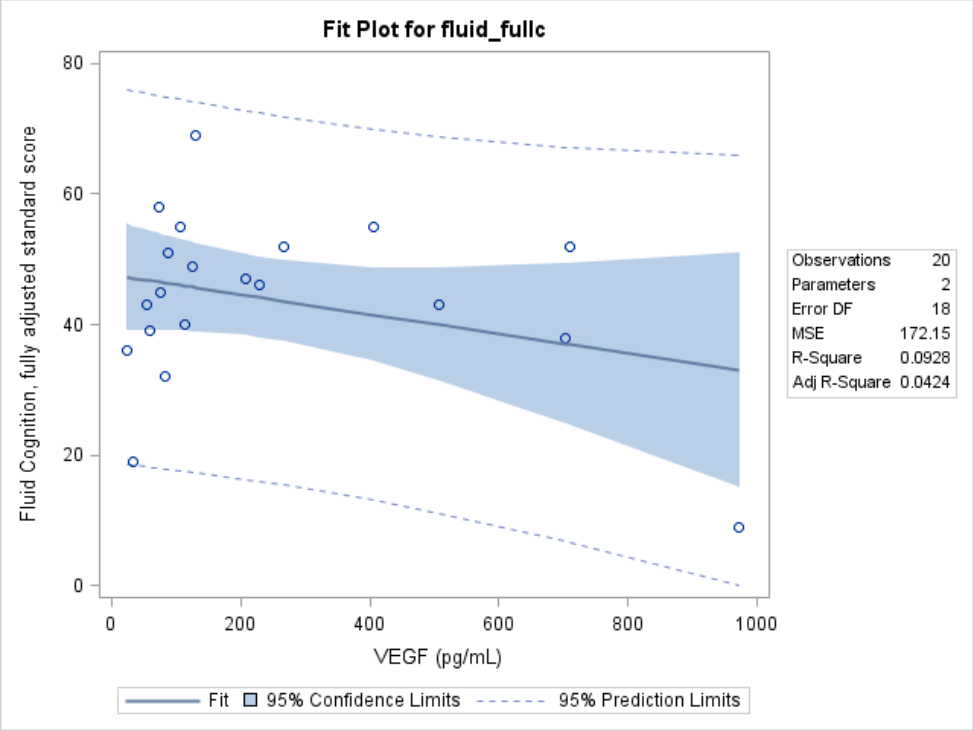

Figure 14. Scatter plot of VEGF concentration and age-, sex-, race-/ethnicity-, and education-corrected fluid cognitive composite score

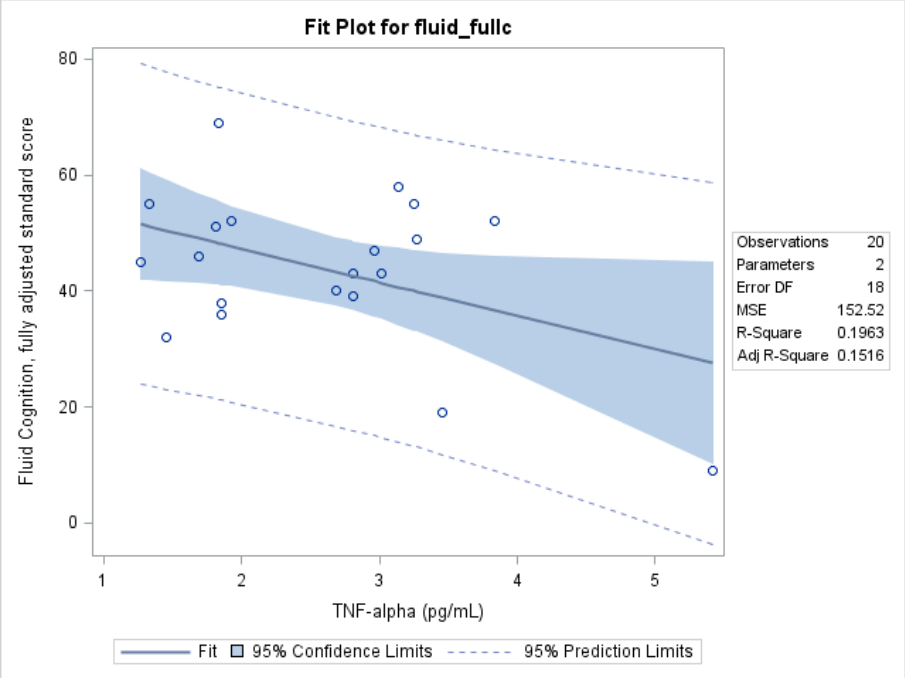

Figure 15. Scatter plot of TNF-α concentration and age-, sex-, race-/ethnicity-, and education-corrected fluid cognitive composite score

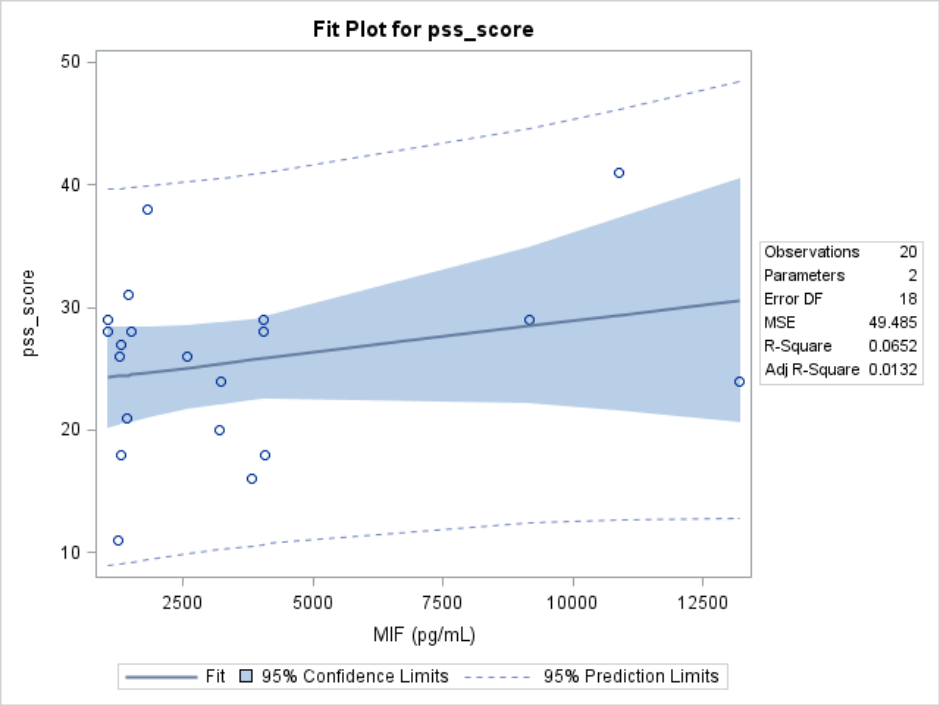

Figure 16. Scatter plot of MIF concentration and Perceived Stress Scale score

References

Ainiala, H., J. Loukkola, J. Peltola, M. Korpela, and A. Hietaharju. 2001. 'The prevalence of neuropsychiatric syndromes in systemic lupus erythematosus', *Neurology*, 57: 496-500.

Andrews, J. S., L. Trupin, G. Schmajuk, J. Barton, M. Margaretten, J. Yazdany, E. H. Yelin, and P. P. Katz. 2015. 'Muscle strength, muscle mass, and physical disability in women with systemic lupus erythematosus', *Arthritis Care Res (Hoboken)*, 67: 120-7.

Breteler, M. M. B., J. C. Vanswieten, M. L. Bots, D. E. Grobbee, J. J. Claus, J. H. W. Vandenhout, F. Vanharskamp, H. L. J. Tanghe, P. T. V. M. Dejong, J. Vangijn, and A. Hofman. 1994. 'Cerebral White-Matter Lesions, Vascular Risk-Factors, and Cognitive Function in a Population-Based Study - the Rotterdam Study', *Neurology*, 44: 1246-52.

C Sinclair, Kyle, Jonathan J Miner, and Alfred HJ Kim. 2015. 'Immunological Mechanisms of Neuropsychiatric Lupus', *Current Immunology Reviews*, 11: 93-106.

Chiappelli, M., B. Borroni, S. Archetti, E. Calabrese, M. M. Corsi, M. Franceschi, A. Padovani, and F. Licastro. 2006. 'VEGF gene and phenotype relation with Alzheimer's disease and mild cognitive impairment', *Rejuvenation Res*, 9: 485-93.

Chun, H. Y., J. W. Chung, H. A. Kim, J. M. Yun, J. Y. Jeon, Y. M. Ye, S. H. Kim, H. S. Park, and C. H. Suh. 2007. 'Cytokine IL-6 and IL-10 as biomarkers in systemic lupus erythematosus', *J Clin Immunol*, 27: 461-6.

Cohen, S., T. Kamarck, and R. Mermelstein. 1983. 'A global measure of perceived stress', *J Health Soc Behav*, 24: 385-96.

Conti, F., C. Alessandri, D. Bompane, M. Bombardieri, F. R. Spinelli, A. C. Rusconi, and G. Valesini. 2004. 'Autoantibody profile in systemic lupus erythematosus with psychiatric manifestations: a role for anti-endothelial-cell antibodies', *Arthritis Res Ther*, 6: R366-72.

Cybulsky, M. I., K. Iiyama, H. Li, S. Zhu, M. Chen, M. Iiyama, V. Davis, J. C. Gutierrez-Ramos, P. W. Connelly, and D. S. Milstone. 2001. 'A major role for VCAM-1, but not ICAM-1, in early atherosclerosis', *J Clin Invest*, 107: 1255-62.

De Bandt, M., J. Sibilia, X. Le Loet, S. Prouzeau, B. Fautrel, C. Marcelli, E. Boucquillard, J. L. Siame, X. Mariette, and Inflammation Club Rhumatismes et. 2005. 'Systemic lupus erythematosus induced by anti-tumour necrosis factor alpha therapy: a French national survey', *Arthritis Res Ther*, 7: R545-51.

Egerer, K., E. Feist, U. Rohr, A. Pruss, G. R. Burmester, and T. Dorner. 2000. 'Increased serum soluble CD14, ICAM-1 and E-selectin correlate with disease activity and prognosis in systemic lupus erythematosus', *Lupus*, 9: 614-21.

Foote, A., E. M. Briganti, Y. Kipen, L. Santos, M. Leech, and E. F. Morand. 2004. 'Macrophage migration inhibitory factor in systemic lupus erythematosus', *J Rheumatol*, 31: 268-73.

Garton, K. J., P. J. Gough, and E. W. Raines. 2006. 'Emerging roles for ectodomain shedding in the regulation of inflammatory responses', *J Leukoc Biol*, 79: 1105-16.

Gershon, R. C., D. Cella, N. A. Fox, R. J. Havlik, H. C. Hendrie, and M. V. Wagster. 2010. 'Assessment of neurological and behavioural function: the NIH Toolbox', *Lancet Neurol*, 9: 138-9.

Hanly, J. G., J. D. Fisk, G. Sherwood, and B. Eastwood. 1994. 'Clinical course of cognitive dysfunction in systemic lupus erythematosus', *J Rheumatol*, 21: 1825-31.

Hanly, J. G., J. D. Fisk, G. Sherwood, E. Jones, J. V. Jones, and B. Eastwood. 1992. 'Cognitive impairment in patients with systemic lupus erythematosus', *J Rheumatol*, 19: 562-7.

Harris, P. A., R. Taylor, R. Thielke, J. Payne, N. Gonzalez, and J. G. Conde. 2009. 'Research electronic data capture (REDCap)--a metadata-driven methodology and workflow

process for providing translational research informatics support', *J Biomed Inform*, 42: 377-81.

Hochberg, M. C. 1997. 'Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus', *Arthritis Rheum*, 40: 1725.

Hughes, C. G., M. B. Patel, N. E. Brummel, J. L. Thompson, J. B. McNeil, P. P. Pandharipande, J. C. Jackson, R. Chandrasekhar, L. B. Ware, E. W. Ely, and T. D. Girard. 2018. 'Relationships between markers of neurologic and endothelial injury during critical illness and long-term cognitive impairment and disability', *Intensive Care Med*, 44: 345-55.

Hwang, S. J., C. M. Ballantyne, A. R. Sharrett, L. C. Smith, C. E. Davis, A. M. Gotto, and E. Boerwinkle. 1997. 'Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases - The atherosclerosis risk in communities (ARIC) study', *Circulation*, 96: 4219-25.

Ikeda, Y., T. Fujimoto, M. Ameno, H. Shiiki, and K. Dohi. 1998. 'Relationship between lupus nephritis activity and the serum level of soluble VCAM-1', *Lupus*, 7: 347-54.

Imboden, John B., David B. Hellmann, and John Henry Stone. 2007. *Current rheumatology : diagnosis & treatment* (McGraw-Hill Medical: New York).

Imhof, B. A., and D. Dunon. 1995. 'Leukocyte migration and adhesion', *Adv Immunol*, 58: 345-416.

Li, D., B. Guo, H. Wu, L. Tan, C. Chang, and Q. Lu. 2015. 'Interleukin-17 in systemic lupus erythematosus: A comprehensive review', *Autoimmunity*, 48: 353-61.

Lim, S. S., A. R. Bayakly, C. G. Helmick, C. Gordon, K. A. Easley, and C. Drenkard. 2014. 'The incidence and prevalence of systemic lupus erythematosus, 2002-2004: The Georgia Lupus Registry', *Arthritis Rheumatol*, 66: 357-68.

Mann, A, and Takemi Tanaka. 2011. 'E-selectin: its role and potential as a biomarker', *Translational Medicine*.

Navarro, C., L. Candia-Zuniga, L. H. Silveira, V. Ruiz, M. Gaxiola, M. C. Avila, and M. C. Amigo. 2002. 'Vascular endothelial growth factor plasma levels in patients with systemic lupus erythematosus and primary antiphospholipid syndrome', *Lupus*, 11: 21-4.

Plantinga, L., B. D. Tift, C. Dunlop-Thomas, S. S. Lim, C. B. Bowling, and C. Drenkard. 2018. 'Geriatric Assessment of Physical and Cognitive Functioning in a Diverse Cohort of Systemic Lupus Erythematosus Patients: A Pilot Study', *Arthritis Care Res (Hoboken)*.

Popp, J., M. Bacher, H. Kolsch, C. Noelker, O. Deuster, R. Dodel, and F. Jessen. 2009. 'Macrophage migration inhibitory factor in mild cognitive impairment and Alzheimer's disease', *J Psychiatr Res*, 43: 749-53.

Rafnsson, S. B., I. J. Deary, F. B. Smith, M. C. Whiteman, A. Rumley, G. D. Lowe, and F. G. Fowkes. 2007. 'Cognitive decline and markers of inflammation and hemostasis: the Edinburgh Artery Study', *J Am Geriatr Soc*, 55: 700-7.

Rezaieyazdi, Z., M. Sahebari, M. R. Hatef, B. Abbasi, H. Rafatpanah, J. T. Afshari, and H. Esmaily. 2011. 'Is there any correlation between high sensitive CRP and disease activity in systemic lupus erythematosus?', *Lupus*, 20: 1494-500.

Richter, V., F. Rassoul, K. Purschwitz, B. Hentschel, W. Reuter, and T. Kuntze. 2003. 'Circulating vascular cell adhesion molecules VCAM-1, ICAM-1, and E-selectin in dependence on aging', *Gerontology*, 49: 293-300.

Smith, C. W. 1997. 'Potential significance of circulating E-selectin', *Circulation*, 95: 1986-8.

Spronk, P. E., H. Bootsma, M. G. Huitema, P. C. Limburg, and C. G. Kallenberg. 1994. 'Levels of soluble VCAM-1, soluble ICAM-1, and soluble E-selectin during disease exacerbations in

patients with systemic lupus erythematosus (SLE); a long term prospective study', *Clin Exp Immunol*, 97: 439-44.

Sweat, V., V. Starr, H. Bruehl, A. Arentoft, A. Tirsi, E. Javier, and A. Convit. 2008. 'C-reactive protein is linked to lower cognitive performance in overweight and obese women', *Inflammation*, 31: 198-207.

Weaver, J. D., M. H. Huang, M. Albert, T. Harris, J. W. Rowe, and T. E. Seeman. 2002. 'Interleukin-6 and risk of cognitive decline: MacArthur studies of successful aging', *Neurology*, 59: 371-8.

Weintraub, S., S. S. Dikmen, R. K. Heaton, D. S. Tulsky, P. D. Zelazo, J. Slotkin, N. E. Carlozzi, P. J. Bauer, K. Wallner-Allen, N. Fox, R. Havlik, J. L. Beaumont, D. Mungas, J. J. Manly, C. Moy, K. Conway, E. Edwards, C. J. Nowinski, and R. Gershon. 2014. 'The cognition battery of the NIH toolbox for assessment of neurological and behavioral function: validation in an adult sample', *J Int Neuropsychol Soc*, 20: 567-78.

Wong, D., K. Dorovini-Zis, and S. R. Vincent. 2004. 'Cytokines, nitric oxide, and cGMP modulate the permeability of an in vitro model of the human blood-brain barrier', *Exp Neurol*, 190: 446-55.

Wuthrich, R. P. 1992. 'Vascular cell adhesion molecule-1 (VCAM-1) expression in murine lupus nephritis', *Kidney Int*, 42: 903-14.

Yoon, C. Y., L. M. Steffen, M. D. Gross, L. J. Launer, A. Odegaard, A. Reiner, O. Sanchez, K. Yaffe, S. Sidney, and D. R. Jacobs, Jr. 2017. 'Circulating Cellular Adhesion Molecules and Cognitive Function: The Coronary Artery Risk Development in Young Adults Study', *Front Cardiovasc Med*, 4: 37.

Zuliani, G., M. Cavalieri, M. Galvani, A. Passaro, M. R. Munari, C. Bosi, A. Zurlo, and R. Fellin. 2008. 'Markers of endothelial dysfunction in older subjects with late onset Alzheimer's disease or vascular dementia', *J Neurol Sci*, 272: 164-70.