Arthritis Research & Therapy

RESEARCH ARTICLE

Open Access

CrossMark

Impact of heart rate variability, a marker for cardiac health, on lupus disease activity

Aikaterini Thanou[1][*], Stavros Stavrakis[2], John W. Dyer[2], Melissa E. Munroe[1], Judith A. James[1,3] and Joan T. Merrill[1]

Abstract

Background: Decreased heart rate variability (HRV) is associated with adverse outcomes in cardiovascular diseases and has been observed in patients with systemic lupus erythematosus (SLE). We examined the relationship of HRV with SLE disease activity and selected cytokine pathways.

Methods: Fifty-three patients from the Oklahoma Lupus Cohort were evaluated at two visits each. Clinical assessments included the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), British Isles Lupus Assessment Group (BILAG) index, physician global assessment (PGA), and Safety of Estrogens in Lupus Erythematosus National Assessment-SLEDAI Flare Index. HRV was assessed with a 5-minute electrocardiogram, and the following HRV parameters were calculated: square root of the mean of the squares of differences between adjacent NN intervals (RMSSD), percentage of pairs of adjacent NN intervals differing by more than 50 milliseconds (pNN50), high-frequency power (HF power), and low frequency to high frequency (LF/HF) ratio, which reflects sympathetic/vagal balance. Plasma cytokine levels were measured with a multiplex, bead-based immunoassay. Serum B lymphocyte stimulator (BLyS) and a proliferation-inducing ligand (APRIL) were measured with an enzyme-linked immunosorbent assay. Linear regression analysis was applied.

Results: Baseline HRV (pNN50, HF power, LF/HF ratio) was inversely related to disease activity (BILAG, PGA) and flare. Changes in RMSSD between visits were inversely related to changes in SLEDAI ($p = 0.007$). Age, caffeine, tobacco and medication use had no impact on HRV. Plasma soluble tumor necrosis factor receptor II (sTNFRII) and monokine induced by interferon gamma (MIG) were inversely related with all baseline measures of HRV ($p = 0.039$ to <0.001). Plasma stem cell factor (SCF), interleukin (IL)-1 receptor antagonist (IL-1RA), and IL-15 showed similar inverse relationships with baseline HRV, and weaker trends were observed for interferon (IFN)-α, interferon gamma-induced protein (IP)-10, and serum BLyS. Changes in the LF/HF ratio between visits were also associated with changes in sTNFRII ($p = 0.021$), MIG ($p = 0.003$), IFN-α ($p = 0.012$), SCF ($p = 0.001$), IL-1RA ($p = 0.023$), and IL-15 ($p = 0.010$). On the basis of multivariate linear regression, MIG was an independent predictor of baseline HRV after adjusting for plasma IL-1RA, SCF, IFN-α, IP-10, and serum BLyS. In a similar model, the sTNFRII impact remained significant after adjusting for the same variables.

Conclusions: Impaired HRV, particularly the LF/HF ratio, is associated with lupus disease activity and several cytokines related to IFN type II and TNF pathways. The strongest association was with MIG and sTNFRII, expanding previous immune connections of vagal signaling.

Keywords: Systemic lupus erythematosus, Disease activity, Heart rate variability, Cytokines

Abbreviations: anti-dsDNA, anti-double-stranded DNA; APRIL, A Proliferation-Inducing Ligand; AZA, Azathioprine; BILAG, British Isles Lupus Assessment Group; BLyS, B Lymphocyte Stimulator; CS, Corticosteroids; ECG, Electrocardiogram; HCQ, Hydroxychloroquine; HF power, High-Frequency Power; HIPAA, Health Insurance Portability and Accountability Act;

(Continued on next page)

* Correspondence: aikaterini-thanou@omrf.org
[1]Arthritis and Clinical Immunology Research Program, Oklahoma Medical Research Foundation, 825 N.E. 13th Street, Oklahoma City, OK 73104, USA
Full list of author information is available at the end of the article

Thanou *et al. Arthritis Research & Therapy* (2016) 18:197

Page 2 of 10

Background

Neural influence on immune function can been profound, raising the question of neuroimmune modulation in chronic inflammatory conditions such as systemic lupus erythematosus (SLE) [1]. Autonomic dysfunction is well documented in SLE, with increased sympathetic and decreased parasympathetic activity reported by different studies [2–9]. Such autonomic aberrations can be the result of inflammatory cytokines acting on the central nervous system and may reciprocally modulate inflammatory responses in the periphery, exerting a pathogenic role in chronic inflammation [10].

Both sympathetic and parasympathetic pathways are relevant in acute and chronic inflammation, based on in vitro studies and animal models. Upon initiation of an acute inflammatory process, the body adopts a nonspecific "inflammatory configuration" with increased systemic sympathetic activity and hypothalamic-pituitary-adrenal axis activation [11]. At the same time, sympathetic neurotransmitters act directly on immune cells, promoting primarily anti-inflammatory mechanisms at the tissue level. As inflammation becomes chronic, tissue regulation is uncoupled from central influence by repulsion of sympathetic nerve fibers that promotes an inflammatory phenotype [11]. Signaling through the vagus nerve has strong anti-inflammatory effects in tissue-specific and systemic inflammation [1]. Efferent potentials from the brainstem inhibit cytokine production in the spleen through a complex pathway that also includes the splanchnic sympathetic nerves [12]. Recent understanding of this pathway suggests a non-neural link between the vagus nerve and the spleen, possibly through vagus-induced mobilization of peripheral lymphocyte pools that subsequently accumulate in the spleen [12].

Autonomic activity can be measured through a range of techniques that include clinical cardiovascular reflex testing and heart rate variability (HRV) [13]. HRV is a noninvasive tool that measures cyclical fluctuations in resting heart rate to indirectly assess cardiac parasympathetic and sympathetic influences. Although vagal tone is considered organ-specific, HRV is often used as a surrogate for systemic vagal output. Decreased HRV is linked to increased cardiovascular morbidity and mortality, as well as an increased prevalence of heart failure and arrhythmias after myocardial infarction [14, 15]. Measures of HRV have also been inversely correlated with serum inflammatory biomarkers (C-reactive protein, interleukin [IL]-6) in healthy individuals as well as in those with cardiovascular diseases [15].

We hypothesized that aberrant autonomic function reflected by HRV is related to disease activity in SLE, and we examined this hypothesis cross-sectionally and longitudinally in a cohort of well-characterized patients with active disease who were receiving standard-of-care treatments. We further explored associations of HRV with a large panel of cytokines, including several pertinent to SLE.

Methods

We conducted a prospective cohort study at the Department of Arthritis and Clinical Immunology of the Oklahoma Medical Research Foundation (OMRF) as a project of the Oklahoma Lupus Cohort, a longitudinal study that includes 610 patients who meet the 1997 SLE classification criteria [16]. The protocol was approved by the OMRF Institutional Review Board (IRB). All patients underwent informed consent procedures consistent with the governance of the OMRF IRB and the Declaration of Helsinki. Fifty-three consecutive patients with SLE from the Oklahoma Lupus Cohort with active disease at baseline were examined at two time points while standard of care for lupus was rendered. Active disease at baseline was defined as Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) of at least 4 points or at least one B score on the British Isles Lupus Assessment Group (BILAG) index [17]. Patients were evaluated using the hybrid SLE-DAI, which is identical to the Safety of Estrogens in Lupus National Assessment (SELENA)-SLEDAI [18, 19] except for the proteinuria definition from the SLEDAI 2000 (SLEDAI-2K) [20, 21]. Presence of flare at each visit was determined by the SELENA-SLEDAI Flare Index (SFI) with the physician global assessment (PGA) [18, 19]. Laboratory tests at each visit included complete blood counts, a comprehensive metabolic panel, urinalysis and C3 and C4

Thanou *et al. Arthritis Research & Therapy* (2016) 18:197

Page 3 of 10

complement levels. In addition to these routine laboratory assessments, serum B lymphocyte stimulator (BLyS) and a proliferation-inducing ligand (APRIL), as well as a panel of plasma cytokines were measured at each visit by enzyme-linked immunosorbent assay and by xMAP® multiplex bead-based immunoassay (eBioscience/Affymetrix, San Diego, California, USA) respectively. The following plasma cytokines were included in the panel: interferon (IFN)-γ, IL-12p70, IL-2, IL-1α, IL-1β, interleukin (IL)-1 receptor antagonist (IL-1RA), IL-15, IL-17A, IL-21, tumor necrosis factor (TNF)-α, soluble tumor necrosis factor receptors I and II (sTNFRI/II), soluble CD40L (sCD40L), BLyS, APRIL, transforming growth factor (TGF)-β, IL-10, IFN-α, interferon gamma-induced protein 10 (IP-10), macrophage inflammatory protein 1 alpha (MIP-1α), monokine induced by interferon gamma (MIG), IL-8, plasminogen activator inhibitor 1 (PAI-1), stem cell factor (SCF), and resistin (see Table S1 in Additional file 1).

Medical records were reviewed and a questionnaire about variables that might affect HRV was completed at each clinic visit. Potentially confounding variables that were evaluated included any heart rhythm other than sinus, cardiomyopathy (left ventricular ejection fraction <40 %), recent (<1 year) myocardial infarction or unstable angina, heart failure (New York Heart Association class III or IV), uncontrolled hypertension (systolic blood pressure >160 mmHg on at least three antihypertensive medications), mitral valve prolapse (other than mild), recurrent vasovagal syncopal episodes, unilateral or bilateral vagotomy, concomitant use of medications potentially affecting autonomic function (e.g., anticholinergic medications and β-blockers), significant neurologic disorder (e.g., Parkinson's disease, multiple sclerosis, Guillain-Barré syndrome), major untreated depression or psychosis, obstructive sleep apnea, asthma or chronic obstructive pulmonary disease not controlled by medications, any other disease causing clinically significant dyspnea at the time of assessment, and uncontrolled insulin- or non-insulin-dependent diabetes. Patients were also questioned about recent exercise and tobacco use (preceding 12 hours), any caffeine consumed in the 6 hours prior to testing, and alcohol consumed within 12 hours of testing.

A 5 minute electrocardiogram (ECG) tracing was recorded at each visit using a modification of the commercially available AliveCor iPhone ECG device (AliveCor, San Francisco, CA, USA). ECG electrode patches were attached to the right and left forearms and connected to the device with a custom-made attachment to enable stable ECG recording (lead I) over 5 minutes. Recordings were transmitted online to a Health Insurance Portability and Accountability Act (HIPAA)-compliant server with validated encryption technology. ECG results were processed blindly, and the following HRV parameters were obtained by time domain analysis: (1) square root of the

mean of the squares of differences between adjacent NN intervals (RMSSD) and (2) percentage of pairs of adjacent NN intervals differing by more than 50 milliseconds (pNN50). The following HRV parameters were obtained by frequency domain analysis: (1) low-frequency power (LF power), (2) high-frequency power (HF power), and (3) the LF/HF ratio [22]. Both short-duration (5 minutes) and long-duration (24 hours) ECG recordings are acceptable options for measuring HRV [22]. Nonetheless, frequency domain analysis is the preferred method when short-duration recordings are examined [22]. Normative values of these HRV measures have yet to be established, and HRV testing in isolation cannot differentiate increased sympathetic from decreased parasympathetic signaling. However, HF power overall reflects activity of the efferent vagus nerve to the heart, and LF power is considered a measure of sympathetic activity, whereas the ratio of low to high frequency (LF/HF) reflects sympathetic to parasympathetic balance [13]. Reduced RMSSD and pNN50 are also indicative of parasympathetic insufficiency.

Statistical analysis

Categorical variables were expressed as percentages and continuous variables as median and interquartile range (IQR). Paired t tests or Wilcoxon signed-rank tests were used to evaluate changes in disease activity and HRV between visits. A chi-square test with the Yates correction was used to compare immune-suppressive medications and parameters affecting HRV between visits. We used linear regression to evaluate cross-sectional associations of HRV and SLE disease activity, cytokines, medications, and tobacco, caffeine, and alcohol exposure. The natural logarithm of each cytokine was employed to achieve normality. Factors significant in univariate analysis ($p < 0.1$) were entered into multivariate models. To avoid overfitting, no more than one parameter for each ten subjects was examined in the multivariate regression models, which allows for a nonbiased calculation of the regression coefficients [23]. Changes in HRV between baseline and follow-up were also examined as predictors of changes in disease activity by linear regression. The same approach was used to evaluate whether changes in HRV between baseline and follow-up could be predicted by changes in cytokines. In these models, only cytokines found to be significantly associated with HRV in the baseline regression analysis were included. Statistical significance was declared at $p < 0.05$. SigmaPlot 12.5 software (Systat Software, San Jose, CA, USA) was used for all statistical analyses.

Results

Characteristics of the study population

Fifty-two female and one male patient were studied. Their median age at baseline was 46 (IQR 36 – 51 years).

Thanou *et al. Arthritis Research & Therapy* (2016) 18:197

Page 4 of 10

The sample comprised 18 Caucasian, 18 African American, 7 Native American, 7 Hispanic, and 3 Asian patients. The median time between visits was 1.8 months (IQR 1.1–3.3 months). Disease activity data are shown in Table 1. Patients with active disease in this routine lupus clinic setting most often had arthritis or mucocutaneous manifestations. Arthritis and rash as defined by the SLE-DAI were present in 41 (77.4 %) and 14 (26.4 %) patients, respectively, at baseline. Thirty (56.6 %) and thirteen (24.5 %) patients, respectively, had A or B scores on the BILAG musculoskeletal and/or mucocutaneous domains at baseline. At baseline, 21 (39.6 %) patients had low C3 and/or C4 complement levels, and 10 (18.8 %) had positive results for anti-double-stranded DNA (anti-dsDNA) antibodies by *Crithidia luciliae* assay. Most patients were receiving hydroxychloroquine (Table 1), and about one-fourth were receiving chronic corticosteroids. Azathioprine, methotrexate, or mycophenolate mofetil was prescribed in 15–28 % of the patients, alone or in combination with hydroxychloroquine and prednisone.

Parameters of HRV did not significantly change between baseline and follow-up (see Table S2 in Additional file 1). Exercise and exposure to tobacco, caffeine, and alcohol were similar between visits. Few patients had comorbidities known to affect HRV, including two patients with heart failure, two with recent seizures, one with untreated depression, one with diabetes, and one with

asthma not controlled while on medications. Similar results were obtained when these seven individuals were omitted from the analysis; therefore, they were included in the final results. Exposure to medications known to increase HRV (β-blockers, calcium channel blockers, and clonidine) or decrease HRV (tricyclic antidepressants, antihistamines, β-agonists) did not differ between baseline and follow-up (Table 1). At baseline, seven patients were taking at least one medication known to increase HRV, eight were taking at least one known to decrease HRV, and five were taking combinations from both groups.

HRV is inversely associated with disease activity and flare at baseline

Relationships of HRV with disease activity were examined at baseline by univariate linear regression (Table 2). Total BILAG disease activity scores were inversely related to HRV parameters [pNN50 ($p = 0.019$), HF power ($p = 0.020$), LF/HF ($p = 0.024$)], and similar associations were observed when we focused on the most commonly involved organ, the musculoskeletal system [pNN50 ($p = 0.043$), HF power ($p = 0.010$)]. Total SLEDAI scores and arthritis by SLEDAI had a similar trend of association with HRV [LF/HF ($p = 0.073$ for total SLEDAI), HF power ($p = 0.088$ for SLEDAI arthritis)]. HRV was not associated with

Table 1 Characteristics of the study population at baseline and follow-up

Number of patients	Baseline ($n = 53$)	Follow-up ($n = 53$)	p Values
Disease activity, median (IQR)			
SLEDAI	6 (4–9)	4 (2–6)	**<0.001**
BILAG	9 (8–16)	2 (1–15)	**<0.001**
PGA	1.5 (1.2–1.8)	0.9 (0.6–1.4)	**<0.001**
Medications, n (%)			
HCQ	35 (66)	36 (68)	1.000
AZA	9 (17)	8 (15)	1.000
MTX	10 (19)	15 (28)	0.360
MMF	11 (21)	10 (19)	1.000
CS	13 (25)	12 (23)	1.000
Parameters affecting HRV, n (%)			
Tobacco exposure	14 (26)	16 (30)	0.829
Caffeine	27 (51)	30 (57)	0.697
Medications ↑HRV	12 (23)	11 (21)	1.000
Medications ↓HRV	13 (25)	11 (21)	0.816

Abbreviations: *SLEDAI* Systemic Lupus Erythematosus Disease Activity Index, *BILAG* British Isles Lupus Assessment Group, *PGA* physician global assessment, *HCQ* hydroxychloroquine, *AZA* azathioprine, *MTX* methotrexate, *MMF* mycophenolate mofetil, *CS* corticosteroids
P values < 0.05 are indicated in bold type

Table 2 Association of disease activity with HRV parameters at baseline by univariate linear regression

Dependent variables	Independent variables			
	RMSSD	pNN50	HF	LF/HF
BILAG	$p = 0.259$ $\beta = -0.024$	**$p = 0.019$** $\beta = -0.385$	**$p = 0.020$** $\beta = -0.108$	**$p = 0.024$** $\beta = 0.630$
BILAG mucocutaneous	$p = 0.870$ $\beta = 0.002$	$p = 0.124$ $\beta = -0.142$	$p = 0.313$ $\beta = -0.027$	$p = 0.695$ $\beta = 0.062$
BILAG musculoskeletal	$p = 0.346$ $\beta = -0.011$	**$p = 0.043$** $\beta = -0.185$	**$p = 0.010$** $\beta = -0.066$	$p = 0.100$ $\beta = 0.257$
SLEDAI	$p = 0.683$ $\beta = 0.005$	$p = 0.312$ $\beta = -0.101$	$p = 0.488$ $\beta = -0.020$	$p = 0.073$ $\beta = 0.301$
SLEDAI rash	$p = 0.977$ $\beta < 0.001$	$p = 0.420$ $\beta = -0.009$	$p = 0.170$ $\beta = -0.004$	$p = 0.206$ $\beta = 0.024$
SLEDAI arthritis	$p = 0.531$ $\beta < 0.001$	$p = 0.309$ $\beta = -0.010$	$p = 0.088$ $\beta = -0.005$	$p = 0.446$ $\beta = 0.013$
PGA	$p = 0.651$ $\beta < -0.001$	**$p = 0.014$** $\beta = -0.026$	$p = 0.154$ $\beta = -0.004$	$p = 0.062$ $\beta = 0.034$
SFI	$p = 0.456$ $\beta = -0.001$	$p = 0.329$ $\beta = -0.012$	**$p = 0.047$** $\beta = -0.007$	**$p = 0.008$** $\beta = 0.054$

Abbreviations: *BILAG* British Isles Lupus Assessment Group, *HF* high-frequency power, *LF/HF* ratio low frequency to high frequency ratio, *PGA* physician global assessment, *pNN50* percentage of pairs of adjacent NN intervals differing by more than 50 milliseconds, *RMSSD* square root of the mean of the squares of differences between adjacent NN intervals, *SFI* Safety of Estrogens in Lupus Erythematosus National Assessment-SLEDAI flare index, *SLEDAI* Systemic Lupus Erythematosus Disease Activity Index
p values <0.05 are in bold type

Thanou *et al. Arthritis Research & Therapy* (2016) 18:197

Page 5 of 10

the presence of classic serological markers such as anti-dsDNA antibodies and low complement levels. The LF/HF ratio was associated with disease activity by BILAG (p = 0.024) and SFI (p = 0.008), although a trend was also seen in SLEDAI (p = 0.073) and PGA (p = 0.062) (Fig. 1). No association of HRV with tobacco or caffeine exposure or medications affecting autonomic nervous system function was demonstrable in this analysis. At the follow-up visits, no association of HRV with disease activity was observed.
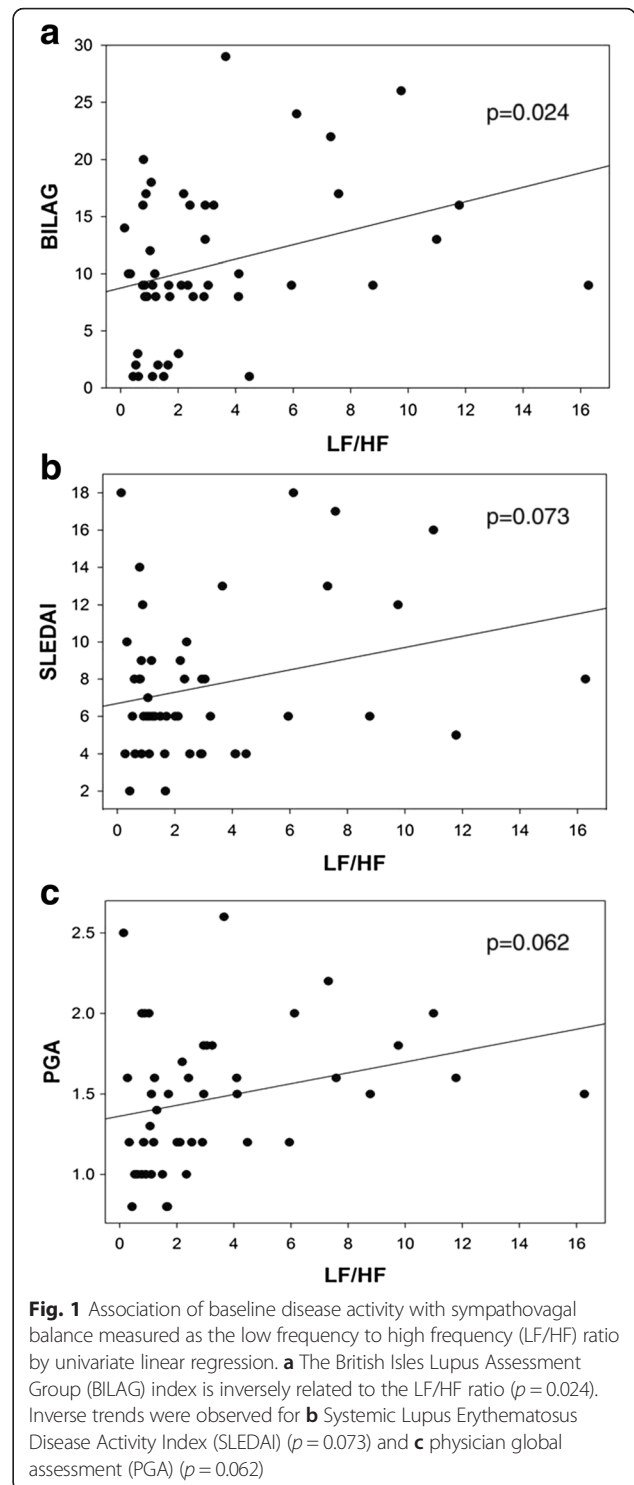
HRV is inversely related to soluble TNFRII and to IFN-induced cytokines

Exploration of associations between HRV and cytokine levels at baseline was performed using linear regression without adjustment for multiple comparisons (Table 3). Elevated plasma sTNFRII and MIG were consistently associated with decreased HRV across all parameters measured (Table 3 and Fig. 2). Inverse associations with one or more parameters of HRV were also observed for plasma IL-1RA, SCF, and IL-15 (Table 3). Trends of similar associations were noted for IFN-α, IP-10 and serum BLyS (p < 0.10). There was no evidence to suggest that plasma IFN-γ, IL-10, IL-12, IL-1β, IL-1α, IL-2, IL-8, IL-17A, IL-21, IL-23, MIP-1α, sCD40L, TNFα, sTNFRI, PAI-1, resistin, TGF-β, or serum APRIL were related to HRV.

Considering the potentially cyclical interplay between HRV and cytokines [10], we further explored which inflammatory pathway is most closely linked as a potential predictor of HRV in a multivariate model including plasma IL-1RA, SCF, IFN-α, IP-10, and serum BLyS. Associations of HRV with plasma MIG or sTNFRII remained significant (MIG: RMSSD p = 0.036, HF power p < 0.001, LF/HF p = 0.003; sTNFRII: RMSSD p = 0.011, HF power p = 0.040) (Table 4). In a correlation matrix of all parameters included in the models, there was a strong relationship between sTNFRII and MIG (Spearman's rank correlation R = 0.751, p < 0.001); thus, these were not combined in the same model. Collinearity was also evident between sTNFRII and SCF (R = 0.508, p < 0.001), between MIG and IP-10 (R = 0.585, p < 0.001), and between MIG and SCF (R = 0.462, p < 0.001), as well as between IP-10 and IL-1RA (R = 0.564, p = 0.001), whereas all other R values were <0.4 (Table S3 in Additional file 1). Plasma sTNFRII was weakly correlated with plasma TNF-α (R = 0.357, p = 0.009) and sTNFRI levels (R = 0.273, p = 0.048).

Some cytokines associated with HRV are also associated with disease activity

We further examined associations of baseline disease activity with plasma and serum cytokines by univariate linear regression (see Table S4 in Additional file 1).

Fig. 1 Association of baseline disease activity with sympathovagal balance measured as the low frequency to high frequency (LF/HF) ratio by univariate linear regression. **a** The British Isles Lupus Assessment Group (BILAG) index is inversely related to the LF/HF ratio (p = 0.024). Inverse trends were observed for **b** Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (p = 0.073) and **c** physician global assessment (PGA) (p = 0.062)

Plasma MIG was related to BILAG (p = 0.017) and PGA (p = 0.024), but no association was observed for sTNFRII. Several other cytokines appeared to be potentially associated with one or more measures of disease activity, stressing the different pathways that might be related to disease activity in different

Thanou *et al. Arthritis Research & Therapy* (2016) 18:197

Page 6 of 10

Table 3 Association of plasma cytokines with HRV parameters at baseline by univariate linear regression

Dependent variables	Independent variables			
	RMSSD	pNN50	HF	LF/HF
IL-1RA	$p = 0.949$ $\beta < -0.001$	$p = 0.286$ $\beta = -0.069$	**$p = 0.011$** $\beta = -0.037$	$p = 0.067$ $\beta = 0.147$
SCF	$p = 0.133$ $\beta = -0.004$	**$p = 0.049$** $\beta = -0.042$	$p = 0.373$ $\beta = -0.005$	**$p = 0.034$** $\beta = 0.076$
IL-15	$p = 0.402$ $\beta = -0.003$	$p = 0.054$ $\beta = -0.054$	**$p = 0.025$** $\beta = -0.018$	$p = 0.148$ $\beta = 0.069$
IFN-α	$p = 0.920$ $\beta < -0.001$	$p = 0.089$ $\beta = -0.079$	$p = 0.063$ $\beta = -0.019$	$p = 0.313$ $\beta = 0.059$
IP-10	$p = 0.202$ $\beta = -0.005$	$p = 0.088$ $\beta = -0.048$	$p = 0.122$ $\beta = -0.012$	$p = 0.189$ $\beta = 0.064$
MIG	**$p = 0.007$** $\beta = -0.011$	**$p = 0.015$** $\beta = -0.077$	**$p = 0.018$** $\beta = -0.021$	**$p = 0.026$** $\beta = 0.119$
sTNFRII	**$p < 0.001$** $\beta = -0.007$	**$p = 0.01$** $\beta = -0.042$	**$p = 0.039$** $\beta = -0.010$	**$p = 0.024$** $\beta = 0.064$
BLyS[a]	$p = 0.556$ $\beta = 0.002$	$p = 0.201$ $\beta = -0.026$	$p = 0.140$ $\beta = -0.008$	$p = 0.098$ $\beta = 0.055$

Abbreviations: BLyS B lymphocyte stimulator, *HF* high-frequency power, *IL* interleukin, *IL-1RA* interleukin 1 receptor antagonist, *IP-10* interferon gamma-induced protein 10, *LF/HF* low frequency to high frequency ratio, *MIG* monokine induced by interferon gamma, *pNN50* percentage of pairs of adjacent NN intervals differing by more than 50 milliseconds, *RMSSD* square root of the mean of the squares of differences between adjacent NN intervals, *SCF* stem cell factor, *sTNFRII* soluble tumor necrosis factor receptor II
[a]Serum BLyS
p values <0.05 are in bold type

subsets of patients with SLE, only some of which might have an impact on HRV.

### Changes in HRV between visits are associated with changes in SLEDAI

Changes in SLEDAI between visits showed a significant inverse association with changes in RMSSD ($p = 0.007$), and there was also a trend of association with changes in pNN50 ($p = 0.094$) (Table 5). Trends of association ($p > 0.05$) of BILAG and PGA with RMSSD and HF power were also observed.

Changes in the LF/HF ratio are related to changes in cytokines

Using linear regression, we examined changes in HRV in relation to changes in those cytokines that had been inversely related to HRV at baseline (Table 5). Changes of the LF/HF ratio were correlated with similar changes across all of those cytokines, including sTNFRII ($p = 0.021$) and MIG ($p = 0.003$). Changes in HF power were inversely related to changes only in serum BLyS ($p = 0.036$), plasma IFN-α ($p = 0.004$), and IL-15 ($p = 0.012$).

Discussion

Although autonomic dysregulation is well documented in SLE, much remains to be learned about its direct or
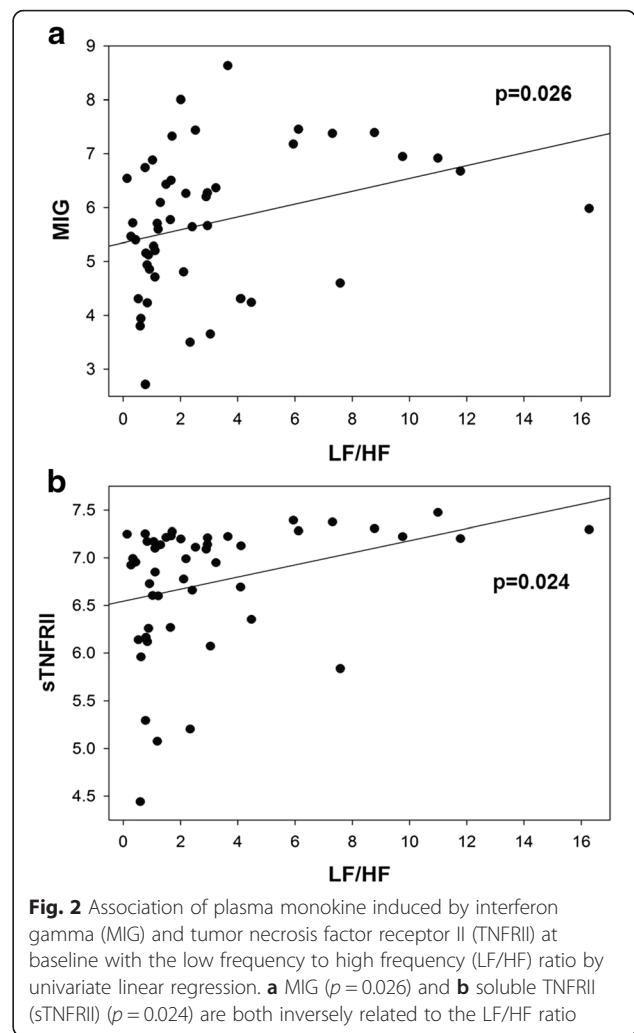
Fig. 2 Association of plasma monokine induced by interferon gamma (MIG) and tumor necrosis factor receptor II (TNFRII) at baseline with the low frequency to high frequency (LF/HF) ratio by univariate linear regression. **a** MIG ($p = 0.026$) and **b** soluble TNFRII (sTNFRII) ($p = 0.024$) are both inversely related to the LF/HF ratio

indirect relationships to disease activity. Although abnormalities in cardiovascular reflex testing and HRV have been described in SLE previously, these did not correlate with disease activity measured by BILAG [3] or SLEDAI [4, 6, 8]. In the present study, we found potential relationships between HRV and disease activity measured by SLEDAI, BILAG, and PGA, as well as the presence or absence of flare (Table 2). Furthermore, HRV changes between visits (time domain analysis and HF power) were correlated with changes in disease activity across all indices (Table 5). At follow-up, disease activity was generally improved, and the range of disease was far narrower in the population (Table 1), deterring the determination of cross-sectional linear relationships at those visits. These results are consistent with the known anti-inflammatory properties of vagus signaling [24] and raise the possibility that HRV might be a noninvasive marker for SLE disease activity and improvement, at least in a relevant subset of patients.

To begin a process by which such patients could be identified, we explored the association of HRV with a large

Thanou *et al. Arthritis Research & Therapy* (2016) 18:197

Page 7 of 10

Table 4 Multivariate linear regression of each heart rate variability parameter (dependent variable) with plasma cytokines (independent variables) at baseline

Independent variables	RMSSD		pNN50		HF		LF/HF	
	p Value	β coefficient	*p* Value	β coefficient	*p* Value	β coefficient	*p* Value	β coefficient
Model I								
IL-1RA	0.523	5.701	0.968	0.030	**<0.001**	−10.414	**0.009**	1.772
SCF	0.635	7.867	0.381	1.262	**0.034**	10.300	0.520	0.730
IFN-α	0.895	1.063	0.392	−0.602	0.739	−0.736	0.854	0.101
IP-10	0.762	5.658	0.725	0.567	**0.001**	19.838	**0.005**	−4.126
MIG	**0.036**	−34.531	0.072	−2.498	**<0.001**	−21.391	**0.003**	3.526
BLyS[a]	0.598	8.028	0.411	−1.087	**0.023**	−10.276	0.110	1.724
Model II								
IL-1RA	0.474	5.922	0.893	0.102	**0.018**	−8.913	**0.039**	1.555
SCF	0.559	9.075	0.384	1.259	0.185	8.802	0.488	0.927
IFN-α	0.867	1.262	0.414	−0.576	0.915	−0.336	0.949	0.041
IP-10	0.530	−8.722	0.618	−0.640	0.249	6.810	0.095	−2.070
sTNFRII	**0.011**	−51.594	0.078	−3.116	**0.040**	−16.762	0.061	3.100
BLyS[a]	0.476	10.003	0.504	−0.867	0.229	−7.168	0.303	1.253

Abbreviations: BLyS B lymphocyte stimulator, *HF* high-frequency power, *IFN* interferon, *IL* interleukin, *IL-1RA* interleukin 1 receptor antagonist, *IP-10* interferon gamma-induced protein 10, *LF/HF* low frequency to high frequency ratio, *MIG* monokine induced by interferon gamma, *pNN50* percentage of pairs of adjacent NN intervals differing by more than 50 milliseconds, *RMSSD* square root of the mean of the squares of differences between adjacent NN intervals, *SCF* stem cell factor, *sTNFRII* soluble tumor necrosis factor receptor II
[a]Serum BLyS
p values <0.05 are in bold type

panel of cytokines that was previously found at our institution to be relevant to SLE [25] (arrayed in Table S1 in Additional file 1). HRV was examined not only as a predictor (Tables 3 and 5) but also as an outcome (Table 4) of peripheral inflammation. Although the exact relationship between these variables is not yet known, evidence in both directions supports the hypothesis of a cyclical relationship [10]. This was an exploratory study, which limits the conclusions that can be drawn. Given the exploratory nature of our study and our small sample size, we elected not to adjust for multiple comparisons, in order to be able to detect associations of modest strength [26]. Notably, several strong preliminary associations were observed that provide useful hypotheses for further testing. In analysis that was not adjusted for multiple comparisons or other covariates, all HRV measurements were predictive of plasma MIG, an IFN type II-related cytokine, and sTNFRII, whose shedding has been associated with lupus flares [27] (Tables 3 and 5). HRV was also predictive of plasma SCF, a major macrophage growth and differentiation factor [28], while consistent inverse relationships with HRV were observed for two other IFN-related cytokines, IP-10 and IFNα, as well as for IL-1RA and serum BLyS (Tables 3 and 5). Although no relationship of HRV with plasma IFN-γ or TNF-α was noted in this analysis, our results support a potential association of vagal signaling with a Th1-mediated myeloid cell pathway, known to be important in subsets of patients with

lupus [29]. The inverse association of HRV with plasma IL-15, a cytokine implicated in intestinal autoimmunity [30], is also intriguing because vagal signaling is known to reach myenteric neurons in close contact with intestinal macrophages [31]. Certain cytokines previously associated with HRV in experimental models of acute inflammation, such as TNF-α and IL-1β [24], were not impacted by HRV in this study of patients with lupus. Larger studies, however, might illuminate whether there is a subset of patients where these relationships could be observed or whether there is some reason why these are not linked to HRV or myeloid pathways in lupus. Cytokines can also differ between the systemic circulation and the tissue microenvironment, and autonomic regulation at the tissue level can be discordant with systemic autonomic effects. These considerations further limit the conclusions that can be drawn from this preliminary study.

Clinically evident arthritis was the most prevalent SLE manifestation in our cohort, and arthritis alone was associated with decreased parasympathetic activity. A direct effect of vagal signaling in the joints is unlikely; nonetheless, this finding might be clinically meaningful, considering the promising role of intermittent electrical vagal nerve stimulation in animal models of arthritis [32] and in patients with rheumatoid arthritis [33].

Multiple potentially confounding factors can affect HRV, including age, cardiovascular disease, diabetes, renal

Thanou *et al. Arthritis Research & Therapy* (2016) 18:197

Page 8 of 10

Table 5 Association of changes in disease activity and plasma cytokines with changes in heart rate variability by univariate linear regression

Dependent variables		Independent variables			
		ΔRMSSD	ΔpNN50	ΔHF	ΔLF/HF
Changes in disease activity	ΔBILAG	$p = 0.081$ $β = −0.036$	$p = 0.605$ $β = −0.109$	$p = 0.168$ $β = −0.075$	$p = 0.119$ $β = 0.542$
	ΔSLEDAI	**$p = 0.007$** $β = −0.023$	$p = 0.094$ $β = −0.144$	$p = 0.326$ $β = −0.023$	$p = 0.431$ $β = 0.116$
	ΔPGA	$p = 0.209$ $β = −0.002$	$p = 0.704$ $β = −0.006$	$p = 0.067$ $β = −0.007$	$p = 0.174$ $β = 0.034$
Cytokine changes	ΔsTNFRII	$p = 0.824$ $β < −0.001$	$p = 0.789$ $β = 0.011$	$p = 0.168$ $β = −0.014$	**$p = 0.021$** $β = 0.150$
	ΔMIG	$p = 0.453$ $β = −0.004$	$p = 0.963$ $β = −0.003$	$p = 0.176$ $β = −0.019$	**$p = 0.003$** $β = 0.253$
	ΔBLyS[a]	$p = 0.298$ $β = −0.002$	$p = 0.994$ $β < −0.001$	**$p = 0.036$** $β = −0.010$	$p = 0.057$ $β = 0.054$
	ΔIFN-α	$p = 0.901$ $β < 0.001$	$p = 0.526$ $β = 0.037$	**$p = 0.004$** $β = −0.050$	**$p = 0.012$** $β = 0.356$
	ΔIL-1RA	$p = 0.840$ $β = −0.003$	$p = 0.794$ $β = −0.044$	$p = 0.199$ $β = −0.055$	**$p = 0.023$** $β = 0.646$
	ΔIP-10	$p = 0.671$ $β = −0.001$	$p = 0.679$ $β = 0.014$	$p = 0.945$ $β < −0.001$	$p = 0.895$ $β = 0.008$
	ΔSCF	$p = 0.085$ $β = −0.004$	$p = 0.603$ $β = −0.011$	$p = 0.154$ $β = −0.008$	**$p = 0.001$** $β = 0.108$
	ΔIL-15	$p = 0.764$ $β = −0.001$	$p = 0.471$ $β = 0.033$	**$p = 0.012$** $β = −0.026$	**$p = 0.010$** $β = 0.154$

Abbreviations: *BILAG* British Isles Lupus Assessment Group, *BLyS* B lymphocyte stimulator, *HF* high-frequency power, *IFN* interferon, *IL* interleukin, *IL-1RA* interleukin 1 receptor antagonist, *LF/HF* low frequency to high frequency ratio, *MIG* monokine induced by interferon gamma, *PGA* physician global assessment, *pNN50* percentage of pairs of adjacent NN intervals differing by more than 50 milliseconds, *RMSSD* square root of the mean of the squares of differences between adjacent NN intervals, *SCF* stem cell factor, *SLEDAI* Systemic Lupus Erythematosus Disease Activity Index, *sTNFR* soluble tumor necrosis factor receptor
[a]Serum BLyS
p values <0.05 are in bold type

failure, obstructive sleep apnea, tobacco, caffeine, and drugs [34–36]. Although few patients in this study had such obvious features, cardiovascular disease and prediabetes are often subclinical in SLE [37, 38], so further evaluation of these potential confounders is in order. No association with smoking or caffeine consumption was evident, but more sensitive measures to record and quantify the degree of these exposures might have captured an effect. It is also possible that HRV is more sensitive to psychosocial factors than lupus disease activity itself, especially in patients with mild to moderate disease. We did not formally assess depression, pain, sleep disturbances, and fatigue, all of which are common in SLE and known to be associated with decreased HRV [39, 40]. A more comprehensive assessment of autonomic nervous system function by additional clinical cardiovascular reflex testing, as well as assessment of baroreflex sensitivity and respiratory sinus arrhythmia, might have also allowed better discernment of these effects.

Frequency domain parameters correlated better than time domain indices with disease activity and/or cytokines in this study. This is somewhat expected, as we used only 5-minute ECG recordings, and time domain analysis has higher variability with shorter durations [22]. In addition to the limitations of HRV analysis [41], those pertinent to the clinical measures of lupus activity should be acknowledged to prevent overinterpretation of these findings [42].

Conclusions

Our results suggest that impaired HRV, particularly the LF/HF ratio, is associated with lupus disease activity and several cytokines related to IFN type II and TNF pathways. The cytokines with the strongest association were MIG and sTNFRII, confirming and expanding previous connections between vagal signaling and immunity. These data support a potentially important interplay between autonomic nervous system function and lupus disease activity, as well as a possible role of vagal modulation as adjunctive therapy in SLE. This can be approached by pharmacological or electrical treatments or through cognitive behavioral therapy approaches [24]. Such therapies are rapidly entering clinical research programs and may be applicable to SLE and other clinical inflammatory disorders.

Thanou *et al. Arthritis Research & Therapy* (2016) 18:197

Page 9 of 10

Additional file

Author details
[1]Arthritis and Clinical Immunology Research Program, Oklahoma Medical Research Foundation, 825 N.E. 13th Street, Oklahoma City, OK 73104, USA. [2]Heart Rhythm Institute, University of Oklahoma Health Sciences Center, 1200 Mark Everett Drive, TCH 6E103, Oklahoma City, OK 73104, USA. [3]Departments of Medicine and Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA.

References
1. Andersson U, Tracey KJ. Neural reflexes in inflammation and immunity. J Exp Med. 2012;209:1057–68.
2. Maule S, Quadri R, Mirante D, Pellerito RA, Marucco E, Marinone C, et al. Autonomic nervous dysfunction in systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA): possible pathogenic role of autoantibodies to autonomic nervous structures. Clin Exp Immunol. 1997;110:423–7.
3. Laversuch CJ, Seo H, Modarres H, Collins DA, McKenna W, Bourke BE. Reduction in heart rate variability in patients with systemic lupus erythematosus. J Rheumatol. 1997;24:1540–4.
4. Louthrenoo W, Ruttanaumpawan P, Aramrattana A, Sukitawut W. Cardiovascular autonomic nervous system dysfunction in patients with rheumatoid arthritis and systemic lupus. QJM. 1999;92:97–102.
5. Harle P, Straub RH, Wiest R, Mayer A, Scholmerich J, Atzeni F, et al. Increase of sympathetic outflow measured by neuropeptide Y and decrease of the hypothalamic-pituitary-adrenal axis tone in patients with systemic lupus erythematosus and rheumatoid arthritis: another example of uncoupling of response systems. Ann Rheum Dis. 2006;65:51–6.
6. Stojanovich L, Milovanovich B, de Luka SR, Popovich-Kuzmanovich D, Bisenich V, Djukanovich B, et al. Cardiovascular autonomic dysfunction in systemic lupus, rheumatoid arthritis, primary Sjogren syndrome and other autoimmune diseases. Lupus. 2007;16:181–5.
7. Capellino S, Lowin T, Angele P, Falk W, Grifka J, Straub RH. Increased chromogranin A levels indicate sympathetic hyperactivity in patients with rheumatoid arthritis and systemic lupus erythematosus. J Rheumatol. 2008; 35:91–9.
8. Aydemir M, Yazisiz V, Basarici I, Avci AB, Erbasan F, Belgi A, et al. Cardiac autonomic profile in rheumatoid arthritis and systemic lupus erythematosus. Lupus. 2010;19:255–61.
9. Milovanović B, Stojanović L, Milićevik N, Vasić K, Bjelaković B, Krotin M. Cardiac autonomic dysfunction in patients with systemic lupus, rheumatoid arthritis and sudden death risk. Srp Arh Celok Lek. 2010;138:26–32.
10. McAllen RM, Cook AD, Khiew HW, Martelli D, Hamilton JA. The interface between cholinergic pathways and the immune system and its relevance to arthritis. Arthritis Res Ther. 2015;17:87.
11. Pongratz G, Straub RH. The sympathetic nervous response in inflammation. Arthritis Res Ther. 2014;16:504.
12. Martelli D, McKinley MJ, McAllen RM. The cholinergic anti-inflammatory pathway: a critical review. Auton Neurosci. 2014;182:65–9.
13. Freeman R. Assessment of cardiovascular autonomic function. Clin Neurophysiol. 2006;117:716–30.
14. Villareal RP, Liu BC, Massumi A. Heart rate variability and cardiovascular mortality. Curr Atheroscler Rep. 2002;4:120–7.
15. Haensel A, Mills PJ, Nelesen RA, Ziegler MG, Dimsdale JE. The relationship between heart rate variability and inflammatory markers in cardiovascular diseases. Psychoneuroendocrinology. 2008;33:1305–12.
16. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997;40(9):1725.
17. Yee CS, Farewell V, Isenberg DA, Griffiths B, Teh LS, Bruce IN, et al. The BILAG-2004 index is sensitive to change for assessment of SLE disease activity. Rheumatology (Oxford). 2009;48:691–5.
18. Buyon JP, Petri MA, Kim MY, Kalunian KC, Grossman J, Hahn BH, et al. The effect of combined estrogen and progesterone hormone replacement therapy on disease activity in systemic lupus erythematosus: a randomized trial. Ann Intern Med. 2005;142:953–62.
19. Petri M, Kim MY, Kalunian KC, Grossman J, Hahn BH, Sammaritano LR, et al. Combined oral contraceptives in women with systemic lupus erythematosus. N Engl J Med. 2005;353:2550–8.
20. Gladman DD, Ibañez D, Urowitz MB. Systemic Lupus Erythematosus Disease Activity Index 2000. J Rheumatol. 2002;29:288–91.
21. Touma Z, Urowitz MB, Gladman DD. SLEDAI-2K for a 30-day window. Lupus. 2010;19:49–51.
22. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Circulation. 1996;93:1043–65.
23. Austin PC, Steyerberg EW. The number of subjects per variable required in linear regression analyses. J Clin Epidemiol. 2015;68:627–36.
24. Tracey KJ. The inflammatory reflex. Nature. 2002;420:853–9.
25. Kaur K, Zheng NY, Smith K, Huang M, Li L, Pauli NT, et al. High Affinity antibodies against influenza characterize the plasmablast response in SLE patients after vaccination. PLoS One. 2015;10:e0125618.
26. Rothman KJ. No adjustments are needed for multiple comparisons. Epidemiology. 1990;1:43–6.
27. Munroe ME, Vista ES, Guthridge JM, Thompson LF, Merrill JT, James JA. Proinflammatory adaptive cytokine and shed tumor necrosis factor receptor levels are elevated preceding systemic lupus erythematosus disease flare. Arthritis Rheumatol. 2014;66:1888–99.
28. Pixley FJ, Stanley ER. CSF-1 regulation of the wandering macrophage: complexity in action. Trends Cell Biol. 2004;14:628–38.
29. Karonitsch T, Feierl E, Steiner CW, Dalwigk K, Korb A, Binder N, et al. Activation of the interferon-gamma signaling pathway in systemic lupus erythematosus peripheral blood mononuclear cells. Arthritis Rheum. 2009;60:1463–71.
30. Abadie V, Jabri B. IL-15: a central regulator of celiac disease immunopathology. Immunol Rev. 2014;260:221–34.
31. Matteoli G, Gomez-Pinilla PJ, Nemethova A, Di Giovangiulio M, Cailotto C, van Bree SH, et al. A distinct vagal anti-inflammatory pathway modulates intestinal muscularis resident macrophages independent of the spleen. Gut. 2014;63:938–48.
32. Levine YA, Koopman FA, Faltys M, Caravaca A, Bendele A, Zitnik R, et al. Neurostimulation of the cholinergic anti-inflammatory pathway ameliorates disease in rat collagen-induced arthritis. PLoS One. 2014;9:e104530.
33. Koopman FA, Schuurman PR, Vervoordeldonk MJ, Tak PP. Vagus nerve stimulation: a new bioelectronics approach to treat rheumatoid arthritis? Best Pract Res Clin Rheumatol. 2014;28:625–35.

Thanou *et al. Arthritis Research & Therapy* (2016) 18:197

Page 10 of 10

34. Dinas PC, Koutedakis Y, Flouris AD. Effects of active and passive tobacco cigarette smoking on heart rate variability. Int J Cardiol. 2013;163:109–15.
35. Rajendra Acharya U, Paul Joseph K, Kannathal N, Lim CM, Suri JS. Heart rate variability: a review. Med Biol Eng Comput. 2006;44:1031–51.
36. Karapetian GK, Engels HJ, Gretebeck KA, Gretebeck RJ. Effect of caffeine on LT, VT and HRVT. Int J Sports Med. 2012;33:507–13.
37. Doria A, Shoenfeld Y, Wu R, Gambari PF, Puato M, Ghirardello A, et al. Risk factors for subclinical atherosclerosis in a prospective cohort of patients with systemic lupus erythematosus. Ann Rheum Dis. 2003;62:1071–7.
38. Parker B, Bruce I. SLE and metabolic syndrome. Lupus. 2013;22:1259–66.
39. Kemp AH, Quintana DS, Felmingham KL, Matthews S, Jelinek HF. Depression, comorbid anxiety disorders, and heart rate variability in physically healthy, unmedicated patients: implications for cardiovascular risk. PLoS One. 2012;7:e30777.
40. Kulshreshtha P, Gupta R, Yadav RK, Bijlani RL, Deepak KK. A comprehensive study of autonomic dysfunction in the fibromyalgia patients. Clin Auton Res. 2012;22:117–22.
41. Kamath MV, Fallen EL. Power spectral analysis of heart rate variability: a noninvasive signature of cardiac autonomic function. Crit Rev Biomed Eng. 1993;21:245–311.
42. Thanou A, Merrill JT. Top 10 things to know about lupus activity measures. Curr Rheumatol Rep. 2013;15:334.