ORIGINAL RESEARCH ARTICLE

Peripheral endothelial dysfunction in myalgic encephalomyelitis/chronic fatigue syndrome

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Abstract

Aims Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a complex multisystem disease. Evidence for disturbed vascular regulation comes from various studies showing cerebral hypoperfusion and orthostatic intolerance. The peripheral endothelial dysfunction (ED) has not been sufficiently investigated in patients with ME/CFS. The aim of the present study was to examine peripheral endothelial function in patients with ME/CFS.

Methods and results Thirty-five patients [median age 40 (range 18–70) years, mean body mass index 23.8 ± 4.2 kg/m^2, 31% male] with ME/CFS were studied for peripheral endothelial function assessed by peripheral arterial tonometry (EndoPAT2000). Clinical diagnosis of ME/CFS was based on Canadian Criteria. Nine of these patients with elevated antibodies against β2-adrenergic receptor underwent immunoadsorption, and endothelial function was measured at baseline and 3, 6, and 12 months follow-up. ED was defined by reactive hyperaemia index ≤1.81. Twenty healthy subjects of similar age and body mass index were used as a control group. Peripheral ED was found in 18 of 35 patients (51%) with ME/CFS and in 4 healthy subjects (20%, $P < 0.05$). Patients with ED, in contrast to patients with normal endothelial function, reported more severe disease according to Bell score (31 ± 12 vs. 40 ± 16, $P = 0.04$), as well as more severe fatigue-related symptoms (8.62 ± 0.87 vs. 7.75 ± 1.40, $P = 0.04$) including a higher demand for breaks [9.0 (interquartile range 7.0–10.0) vs. 7.5 (interquartile range 6.0–9.25), $P = 0.04$]. Peripheral ED showed correlations with more severe immune-associated symptoms ($r = -0.41$, $P = 0.026$), such as sore throat ($r = -0.38$, $P = 0.038$) and painful lymph nodes ($r = -0.37$, $P = 0.042$), as well as more severe disease according to Bell score ($r = 0.41$, $P = 0.008$) and symptom score ($r = -0.59$, $P = 0.005$). There were no differences between the patient group with ED and the patient group with normal endothelial function regarding demographic, metabolic, and laboratory parameters. Further, there was no difference in soluble vascular cell adhesion molecule and soluble intercellular adhesion molecule levels. At baseline, peripheral ED was observed in six patients who underwent immunoadsorption. After 12 months, endothelial function had improved in five of these six patients (reactive hyperaemia index 1.58 ± 0.15 vs. 2.02 ± 0.46, $P = 0.06$).

Conclusions Peripheral ED is frequent in patients with ME/CFS and associated with disease severity and severity of immune symptoms. As ED is a risk factor for cardiovascular disease, it is important to elucidate if peripheral ED is associated with increased cardiovascular morbidity and mortality in ME/CFS.

Introduction

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a multisystem disorder characterized by severe fatigue with the hallmark symptom of exertion intolerance. This condition of symptom worsening called post-exertion malaise typically shows a delayed recovery over more than 24 h.[1] The pathophysiological mechanism of ME/CFS is not resolved yet, but there is ample evidence of dysregulation of immune and autonomic nervous systems.[2,3] The difficulties of diagnosing of ME/CFS are related to the complexity of symptoms and an absence of validated diagnostic tests available to date.

Endothelial dysfunction (ED) is an established cardiovascular (CV) risk factor that contributes to the development of atherosclerosis and predicts CV events.[4] ED describes abnormal function and alteration of endothelial cells leading to reduced vasodilatative response. Diminished availability of nitric oxide and other endothelium relaxing and contracting factors are considered as a pathophysiological mechanism.[5,6] ED is found in various conditions including diabetes mellitus, arterial hypertension, chronic heart failure, and stroke.[5,7–9] CV symptoms such as autonomic dysregulation, postural hypotension, postural tachycardia syndrome, and reduced cardiac and blood volume have been observed in patients with ME/CFS.[2,10,11] There is growing evidence that ED is frequent in autoimmune disease and is associated with chronic inflammation.[12,13]

There are consistent reports of vascular dysfunction in ME/CFS. Cerebral hypoperfusion was diagnosed by using single photon emission computed tomography and other techniques by various groups.[14,15] Cerebral hypoperfusion has been shown to correlate with fatigue and orthostatic intolerance.[16,17] Previously, ED of large and small arteries was shown using flow-mediated dilatation.[18] A diminished post-occlusive hyperaemic response of the brachial artery and of forearm skin microcirculation was observed. In contrast, a recent study comparing cytokine levels with various CV parameters including endothelial function assessed by EndoPAT reported no difference in patients with ME/CFS and controls.[19] However, another study reported an enhanced acetylcholine-induced vasodilatory response of the peripheral microvasculature in ME/CFS.[20]

The aim of the present study was to investigate peripheral endothelial function in patients with ME/CFS and to correlate it with symptom severity and biomarkers. Endothelial function was assessed by non-invasive finger plethysmograph EndoPAT2000. Using this method, changes of peripheral arterial tone in the forefinger are assessed as a response to reactive hyperaemia induced by forearm ischaemia.[21,22]

Materials and methods

Study participants

Thirty-five patients with post-infectious ME/CFS diagnosed at the Institute of Medical Immunology at the Charité Berlin between 2014 and 2016 were included. Diagnosis of ME/CFS was based on Canadian Criteria[1] and exclusion of other medical or neurological diseases, which may cause fatigue. Patients had chronic disease ranging from <1 to 40 years. Nine patients with elevated β2-adrenergic receptor antibodies participated in a prospective clinical study of immunoadsorption performed at five consecutive days to

Table 1 Baseline characteristics and biochemical parameters of the study groups

Parameter	Controls $N = 20$	All patients $N = 35$	P-value	RHI > 1.81 $N = 17$	RHI ≤ 1.81 $N = 18$	P-value
Age, mean ± SD (years)	48 ± 14	42 ± 11	0.9	43 ± 14	41 ± 9	0.8
Female sex, N (%)	10 (50)	24 (69)	0.2	13 (77)	11 (61)	0.3
Body mass index, mean ± SD (kg/m^2)	24.4 ± 3.3	23.8 ± 4.2	0.6	24.4 ± 4.2	23.2 ± 4.3	0.4
Heart rate, mean ± SD (b.p.m.)	61 ± 8	68 ± 13	0.03	68 ± 15	68 ± 10	0.9
Systolic blood pressure, mean ± SD (mmHg)	123 ± 14	126 ± 15	0.5	128 ± 19	124 ± 11	0.4
Diastolic blood pressure, mean ± SD (mmHg)	74 ± 8	79 ± 12	0.1	79 ± 14	79 ± 9	1.0
Biochemical parameters						
C-reactive protein, mean ± SD (mg/dL)	1.5 (0.7–2.2)	0.6 (0.3–1.2)	0.9	0.6 (0.3–3.3)	0.7 (0.3–1.1)	1.0
Albumin, mean ± SD (g/L)	38.6 ± 3.1	45.2 ± 3.0	<0.001	45.0 ± 3.3	45.3 ± 2.8	0.8
Haemoglobin, mean ± SD (g/dL)	13.6 ± 0.8	14.2 ± 1.3	0.2	14.2 ± 1.1	14.2 ± 1.4	1.0
Creatinine, mean ± SD (mg/dL)	0.9 ± 0.1	0.8 ± 0.1	0.01	0.8 ± 0.1	0.79 ± 0.11	0.8
Aspartate transaminase, mean ± SD (U/L)	26.6 ± 8.2	25.4 ± 7.3	0.5	25.4 ± 8.0	24.4 ± 6.9	1.0
Alanine transaminase, mean ± SD (U/L)	21.2 ± 10.9	26.7 ± 17.1	0.3	24.8 ± 19.7	28.4 ± 14.8	0.6
Gamma-glutamyltransferase, mean ± SD (U/L)	23.9 ± 13.8	21.5 ± 21.1	0.3	23.5 ± 25.4	19.5 ± 15.0	0.6
Haemoglobin A1c, median (IQR) [ln (%)]	5.3 ± 0.4	5.4 ± 1.0	0.9	5.4 ± 0.6	5.4 ± 1.3	1.0

IQR, interquartile range; RHI, reactive hyperaemia index; SD, standard deviation.

remove IgG from plasma as described previously.[23] Twenty healthy subjects of similar age (range 24–75 years) and body mass index were used as a control group. The study was approved by the Ethics Committee of Charité - Universitätsmedizin Berlin in accordance with the 1964 Declaration of Helsinki and its later amendments. All study participants gave written informed consent.

Assessment of symptoms

The presence and severity of symptoms was assessed using a questionnaire developed by Fluge *et al.*[24] based on the Canadian Criteria. Symptoms were classified quantitatively according to a scale ranging from '1' to '10', there '1' indicates no symptoms and '10' indicates very severe symptoms. The fatigue score was calculated as the mean of fatigue, malaise after exertion, demand for breaks in daily life and daily functioning, cognitive score as mean of memory disturbance, concentration ability and mental tiredness and immune score as mean of painful lymph nodes, sore throat, and flu-like symptoms. Furthermore, the questionnaire assessed muscle pain and the cumulative severity of reported symptoms (disease severity). Symptom classification was based on patient's self-report. Symptoms of autonomic dysfunction were assessed by the Composite Autonomic Symptom Score (COMPASS-31) questionnaire[25] examining autonomic functions in the domains of orthostatic, vasomotor, secretomotor, gastrointestinal, bladder, and pupillomotor regulation. Based on these domains, a weighted total score ranging from '0' indicating no to '100' indicating severe symptoms of autonomic dysfunction was calculated. In addition, disease severity was examined by Bell score focusing on the level of restriction in daily functioning.[26] Completely bedridden patients are classified as '0', and patients with unrestricted daily functioning as '100'.

Assessment of endothelial function

Peripheral endothelial function was evaluated by the peripheral arterial tonometry (PAT) device (EndoPAT2000, Itamar, Israel). Assessments were performed at baseline under standardized conditions after at least 15 min of supine rest in a quiet, air-conditioned room. EndoPAT quantifies the endothelium-mediated changes in vascular tone, elicited by a 5 min occlusion of the brachial artery using a standard blood pressure cuff. When the cuff is released, the surge of blood flow causes a dilatation. The post-occlusion dilatation in relation to pre-occlusion is calculated as reactive hyperaemia index (RHI). ED was defined as RHI ≤1.81 based on previous cohort studies.[5,27] In patients who were treated by immunoadsorption,[23] peripheral endothelial function was additionally assessed at 12 months follow-up.
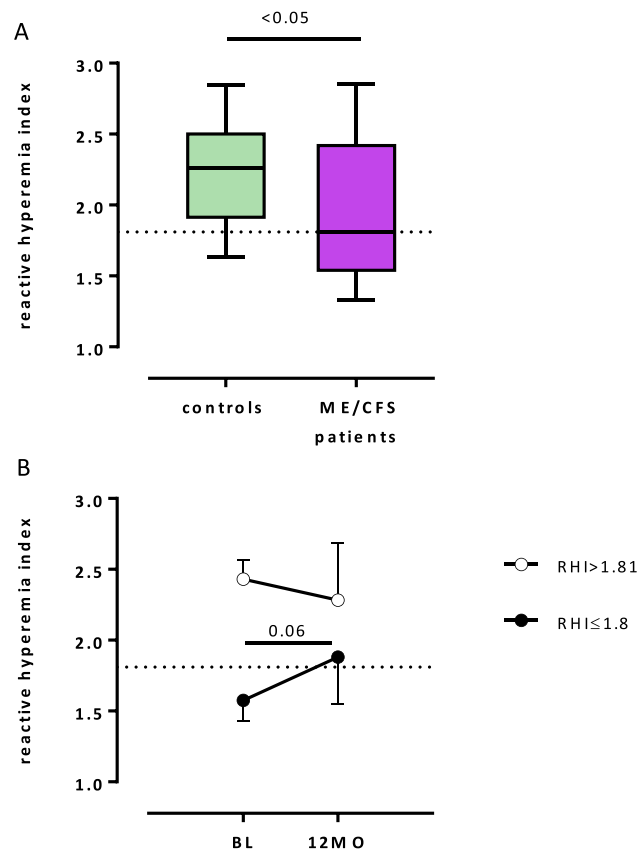
Blood sampling

Venous blood samples were obtained in all patients at a morning visit to the outpatient clinic. Standard biochemical parameters were assessed by routine laboratory measurement. Soluble vascular cell adhesion molecule (sVCAM) and soluble intercellular adhesion molecule (sICAM) were assessed using ELISA technology (R&D Systems, Minneapolis).

Statistical analysis

Statistical data analyses were performed using IBM SPSS Statistics 22.0 and the software GraphPad Prism 6.0. For variables with $n > 30$, parametric tests were used according to central limit theorem. Variables with $n < 30$ were tested for

Figure 1 (A) Reactive hyperaemia index (RHI) was assessed in 35 patients with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and 20 healthy subjects by peripheral arterial tonometry (EndoPAT2000). Significantly reduced RHI was observed in patients compared with the healthy controls. (B) RHI was assessed in nine patients with ME/CFS at baseline (BL) before treatment with one cycle of immunoadsorption and at 12 months. Endothelial dysfunction defined as a diminished RHI ≤1.81 was found in six patients at BL (black circle). Patients with endothelial dysfunction showed improvement of RHI at 12 months as a trend ($P = 0.06$).

normal distribution using Shapiro–Wilk test. All data were presented as mean ± standard deviation, median (interquartile range), median (range), or percentage as appropriate. For comparative analysis, unpaired t-test was used. Not normally distributed parameters were logarithmically transformed (ln). Correlation analysis was performed using parametric Pearson coefficient and non-parametric Spearman coefficient as appropriate to distribution. Analysis of bivariate parameters was performed using χ^2-test. A one-tailed P-value of <0.05 was considered statistically significant for clinical scores, and a two-tailed P-value of <0.05 for laboratory parameters. Due to multiple testing, P-values are descriptive.

Results

Clinical characteristics of the study groups are presented in *Table 1*. There was no difference between the ME/CFS patients and control subjects regarding demographic and clinical characteristics except heart rate, albumin, and creatinine (*Table 1*). However, RHI was significantly reduced in patients compared with healthy controls (1.95 ± 0.47 vs. 2.21 ± 0.37, P < 0.05, *Figure 1A*).

Disease-specific characteristics of the patient cohort are presented in *Table 2*. Median disease duration was 5 years (range <1–40), and median disease severity according to the Bell score was 35 (range 10–70). Peripheral ED as defined by RHI ≤1.81 was observed in 18 patients and in 4 healthy subjects (51% vs. 20%, P < 0.05, respectively). Accordingly, patients were divided into two subgroups. Patients with ED showed more severe disease as assessed by Bell score (median 30 vs. 40) and by symptom score (mean 8.6 vs. 7.0) compared with patients with normal endothelial function (both P < 0.05, *Table 2*). In addition, patients with ED reported more severe fatigue-related symptoms (mean 8.62 vs. 7.75, P < 0.05) compared with patients with normal endothelial function. None of the biochemical parameters and immunological markers except basal thyrotropin were significantly different in patients with normal peripheral endothelial function and patients with ED (*Tables 1* and *2*).

Correlation analyses

In the whole patient cohort, a correlation was found between lower RHI and more severe disease according to symptom score (r = −0.59, P = 0.005) and Bell score (r = −0.41, P = 0.008), more severe immune symptoms (r = −0.41, P =

Table 2 Symptom scores and disease-specific and immunological characteristics of the myalgic encephalomyelitis/chronic fatigue syndrome patients

Parameter	All patients	N	RHI > 1.81	N	RHI ≤ 1.81	N	P-value
Infectious disease onset, N (%)	25 (71.4)	35	12 (70.6)	17	13 (72.2)	18	0.9
Disease duration, mean ± SD (years)	8a ± 8	35	7a ± 5	17	7a ± 5	18	0.6
Recurrent respiratory tract infections, N (%)	22 (64.7)	34	9 (52.9)	17	13 (76.5)	17	0.2
Bell score, median (IQR)	35 (20–41)	34	40 (30–52)	17	30 (20–40)	17	0.04
COMPASS-31 score, mean ± SD	47.24 ± 13.83	24	46.84 ± 12.03	10	47.53 ± 15.43	14	0.5
Symptom scores							
Fatigue score, mean ± SD	8.24 ± 1.19	23	7.75 ± 1.40	10	8.62 ± 0.87	13	0.04
Fatigue, mean ± SD	7.83 ± 1.53	23	7.40 ± 1.43	10	8.15 ± 1.57	13	0.1
Post-exertion malaise, median (IQR) (ln)	9.0 (8.0–10.0)	23	9.00 (6.75–9.25)	10	9.0 (8.0–10.0)	13	0.1
Impaired daily functioning, median (IQR) (ln)	9.0 (8.0–9.0)	23	8.5 (7.75–9.25)	10	9.0 (8.0–9.0)	13	0.1
Demand for breaks, median (IQR) (ln)	8.0 (7.0–10.0)	23	7.5 (6.0–9.25)	10	9.0 (7.0–10.0)	13	0.04
Immune score, mean ± SD	5.43 ± 2.71	23	4.43 ± 2.69	10	6.2 ± 2.6	13	0.06
Flu-like symptoms, median (IQR) (ln)	7.0 (6.0–8.0)	23	7.0 (5.25–7.25)	10	8.0 (6.0–9.0)	13	0.3
Sore throat, median (IQR) (ln)	5.0 (1.0–7.0)	23	3.0 (0.75–6.5)	10	6.0 (3.5–8.5)	13	0.2
Painful lymph nodes, mean ± SD	4.7 ± 3.1	23	3.8 ± 3.1	10	5.5 ± 2.9	13	0.1
Cognitive score, median (IQR) (ln)	7.66 (6.0–8.0)	23	7.33 (5.75–8.0)	10	7.66 (6.33–8.17)	13	0.4
Muscle pain, median (IQR) (ln)	7.5 (5.0–9.0)	22	8.00 (5.00–8.5)	9	6.0 (4.0–9.5)	13	0.2
Disease severity, mean ± SD	8.2 ± 1.4	18	7.0 ± 1.7	5	8.6 ± 1.0	13	0.02
Immunological markers							
Basal thyrotropin, mean ± SD (mU/L)	1.70 ± 0.87	26	1.3 ± 0.8	12	2.04 ± 0.81	14	0.03
Immunoglobulin G, mean ± SD (g/L)	9.75 ± 2.22	34	9.95 ± 2.75	17	9.95 ± 2.75	17	0.6
Immunoglobulin M, mean ± SD (g/L)	1.32 ± 1.01	34	1.56 ± 1.19	17	1.56 ± 1.19	17	0.2
Immunoglobulin A, mean ± SD (g/L)	1.93 ± 0.74	34	1.89 ± 0.65	17	1.89 ± 0.65	17	0.8
Interleukin-8, mean ± SD (pg/mL)	117.8 ± 48.0	32	109.1 ± 48.4	15	125.4 ± 47.8	17	0.3
Soluble interleukin-2 receptor, median (IQR) [ln (IU/mL)]	357 (274–466)	24	422 (293–478)	12	325 (261–382)	12	0.9
Soluble VCAM, mean ± SD (ng/mL)	132.9 ± 35.0	35	137.3 ± 28.5	17	137.3 ± 28.5	18	0.5
Soluble ICAM, mean ± SD (ng/mL)	54.3 ± 14.3	35	55.0 ± 12.4	17	55.0 ± 12.4	18	0.8
β2-adrenergic receptor antibody, mean ± SD (U/mL)	9.21 ± 9.57	34	9.6 ± 10.3	17	9.6 ± 10.3	17	0.8
M3 acetylcholine receptor antibody, mean ± SD (U/mL)	7.04 ± 10.41	34	8.4 ± 13.1	17	8.4 ± 13.1	17	0.5

ICAM, intercellular adhesion molecule; IQR, interquartile range; RHI, reactive hyperaemia index; SD, standard deviation; VCAM, vascular cell adhesion molecule.

0.026) including sore throat (*r* = −0.38, *P* = 0.038), painful lymph nodes (*r* = −0.39, *P* = 0.042), and flu-like symptoms (*r* = −0.31, *P* = 0.073) as a trend (*Figure 2*). No significant correlation was found neither with cognitive scores, muscle pain, nor with COMPASS-31 score assessing autonomous dysfunction (*not shown*). Further, age, sex, infectious disease onset, and disease duration did not correlate with RHI.

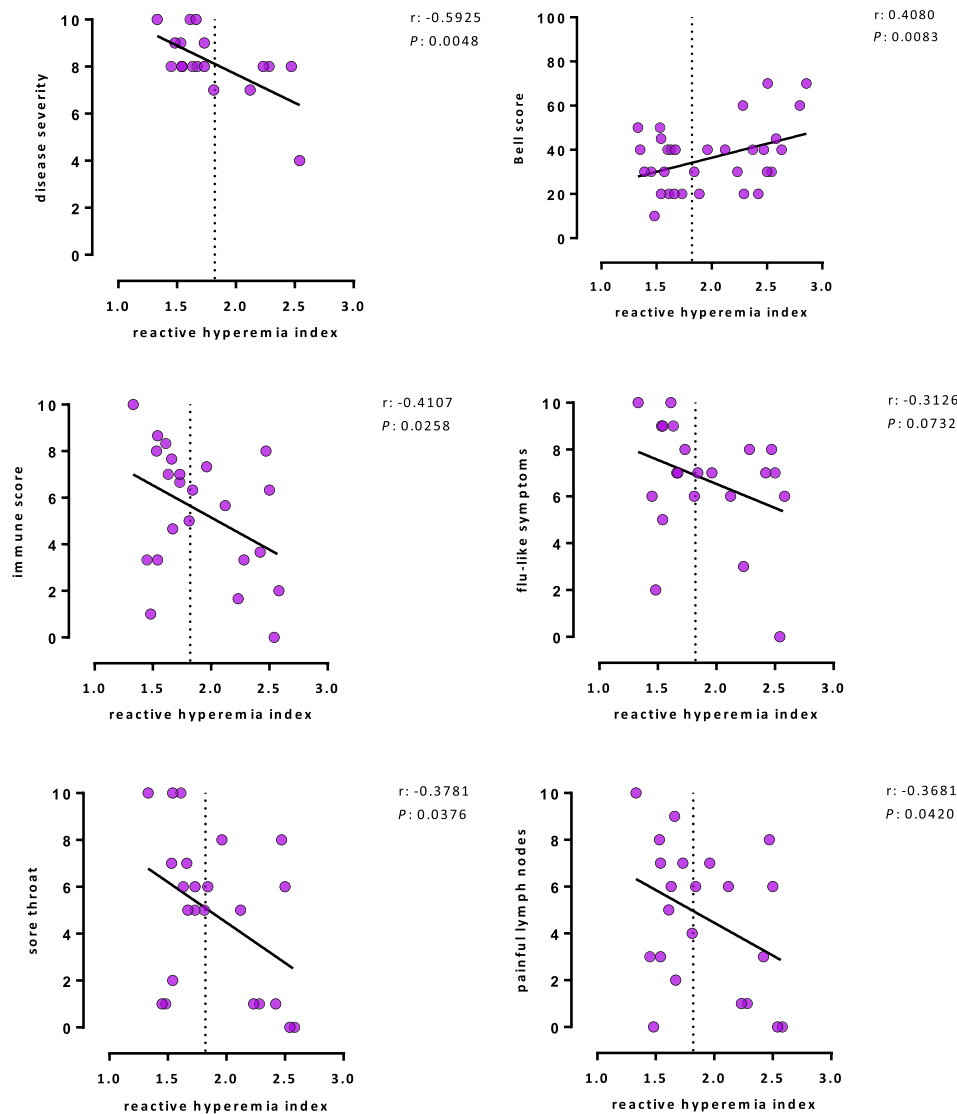## Soluble intercellular adhesion molecule and soluble vascular cell adhesion molecule

No significant differences for sVCAM levels and sICAM levels between ME/CFS patients and healthy controls were observed (*not shown*). Further, levels of both adhesion molecules were not different between patients with normal and diminished RHI of ≤1.81 (*Table 2*).

Peripheral endothelial function after immunoadsorption

Nine patients with elevated levels of autoantibodies against β2-adrenergic receptor underwent immunoadsorption. In five of six patients with peripheral ED at baseline, endothelial function was normalized at 12 months follow-up (RHI 1.58 ± 0.15 vs. 2.02 ± 0.46, *P* = 0.06), while in three patients with normal endothelial function before treatment, no significant change was observed over 12 months (*Figure 1B*).

Figure 2 Patients with endothelial dysfunction reported more severe disease assessed by a disease severity score and Bell score and a significantly higher immune symptom score including sore throat and painful lymph nodes, as well as a tendency for more severe flu-like symptoms.

Discussion

Our study showed that half of the patients with ME/CFS had diminished RHI indicating the presence of peripheral ED. We found a correlation between the peripheral ED and disease severity as well as severity of immune symptoms. Further, we have first evidence that peripheral ED in patients, who had elevated levels of autoantibodies against β2-adrenergic receptor, can improve following immunoadsorption.

Our results of ED in ME/CFS shown by finger plethysmography are in line with a previous study by Newton et al.[18] They had shown a significantly diminished post-occlusive hyperaemic response of the brachial artery and of forearm skin microcirculation compared with healthy controls. In a recent study by Moneghetti and colleagues comparing cytokine levels with various CV parameters, a trend of diminished endothelial function assessed by EndoPAT in patients with ME/CFS compared with controls was observed.[19]

Endothelial dysfunction is known as an important risk factor for CV events. Several studies have shown peripheral ED in patients with various diseases including chronic heart failure, stroke, and pulmonary arterial hypertension based on EndoPAT measurements of RHI.[5,28–30]

ED is characterized by the incapability of endothelial cells in the regulation of vascular tone. Besides traditional CV risk factors inflammatory cytokines, reactive oxygen species, and anti-endothelial autoantibodies can activate endothelial cells, leading to ED.[31] No evidence for an association of ED with pro-inflammatory or metabolic biomarker was found in the present study. Further, no association between soluble endothelial adhesion molecules sVCAM and sICAM with ED was observed. sICAM was shown to correlate with atherosclerosis and sVCAM with endothelial dysregulation in aged persons.[32] Previously, we showed that levels of the long non-coding RNA myocardial infarction-associated transcript were significantly elevated in ME/CFS patients as compared with healthy controls.[33] Myocardial infarction-associated transcript regulates ED and can be up-regulated by inflammatory stimuli.[34]

We observed, however, a relation between immune symptoms and endothelial function in our study. Indeed, ME/CFS patients with lower RHI reported more severe immune-associated symptoms and more severe disease. Although the pathophysiological mechanism of the immune symptoms in ME/CFS is not well understood yet, there is evidence that it is a hallmark symptom of the disease. A recent study by Ohanian et al. showed that immune symptoms were the best discriminating symptoms of ME/CFS and MS-related fatigue.[35] In many patients, immune symptoms occur or increase after exertion. Our findings suggest that the pathomechanism of immune symptoms is related to ED. This is in line with studies showing increased oxidative stress, nitric oxide, and a pro-inflammatory phenotype in ED.[36] It has

been shown that exercise can amplify pre-existing immune abnormalities in ME/CFS. Studies have reported exercise in ME/CFS patients resulting in enhanced production of cytokines, C4 complement activation, and enhanced oxidative stress.[37,38] Further, there may be a direct link between fatigue and ED. In a study in apparently healthy subjects without risk factors for CV disease, it was shown that ED is associated with the level of subjective fatigue.[39]

Finally, we observed a normalization of endothelial function in patients with elevated levels of β2-adrenergic antibodies who underwent immunoadsorption. There is evidence from experimental studies indicating a role of β-adrenergic autoantibodies in the development of ED.[40,41]

In conclusion, the presence of ED in ME/CFS as a major risk factor for CV disease is in accordance with reports of an earlier CV mortality reported in these patients.[42] Therefore, ED may be a prognostic marker to assess risk of CV morbidity and mortality in ME/CFS, which needs to be further analysed in clinical trials. Furthermore, it is important to elucidate the mechanisms leading to ED in order to understand its role in the pathomechanism of ME/CFS.

Limitations

The present study is limited by a relatively small sample size: 35 patients and 20 controls of similar age and body mass index were included. However, this observational clinical trial has a sample size similar to other comparable studies described in the literature.[18,20] In our study, the presence of ED was shown in half of the patients. Our results clearly showed a significantly higher RHI in the control group compared with the patients. In addition, the presence of ED was observed more frequently in patients with ME/CFS as in the control group (51% vs. 20%, $P < 0.05$, respectively). The results of our clinical trial should be confirmed in future studies with a larger sample size.

Conflict of interest

None declared.

Funding

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