A complex pattern of autonomic dysfunction in familial Mediterranean fever. Results from a controlled cross-sectional study

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ABSTRACT

Objective. *Autonomic dysfunction (AD) has been described in various chronic inflammatory diseases. Studies of AD in patients with familial Mediterranean fever (FMF) are inconclusive. We aimed to assess AD in a cohort of FMF patients.*

Methods. *Signs and symptoms of AD were investigated in patients with FMF and compared to age and gender matched healthy controls. Symptoms of AD were assessed by COMPASS-31, a validated questionnaire to evaluate orthostatic, vasomotor, secretomotor, gastrointestinal, pupillomotor and bladder function domains. Assessment of objective AD comprised heart rate variability during deep breathing, skin conductance changes during mental arithmetic, blood pressure response to pain and dynamic infrared pupillometry.*

Results. *25 patients and 25 healthy controls were included and evaluated by COMPASS-31 and objective testing of AD. FMF patients had higher median COMPASS-31 total scores than controls (23.7 vs. 1.6, p=0.024). Significant differences were also found in the secretomotor and gastrointestinal subdomains (4.2 vs. 0.0; p<0.001 and 8.0 vs. 0.0; p=0.004, respectively). Symptoms of autonomic dysfunction were correlated with patient reported global disease activity (r=0.71; p<0.001) and pain level (r=0.68; p<0.001). There were no differences in heart rate variability (HRV), skin conductance, blood pressure response to pain or sympathetic pupillomotor function between patients and controls. FMF patients revealed impaired parasympathetic pupillomotor function that was not associated with clinical parameters. However, patients that were on IL-1-blocking therapy had better parasympathetic pupillary function than patients on conventional treatment.*

Conclusion. *FMF patients have AD in terms of symptoms and parasympathetic pupillomotor function. Dynamic pupillometry can provide additional information on autonomic regulation in patients with FMF.*

Introduction

Familial Mediterranean fever (FMF) is the most common hereditary autoinflammatory disorder, affecting about 150,000 patients worldwide (1). Characteristic disease features include periodic inflammatory flares with fever, peritonitis and pleuritis (2). Dysregulation of IL-1β secretion plays a key role in the pathophysiology of the disease and IL-1β also serves as therapeutic target (3). Recently, IL-1-blocking therapy with canakinumab has been approved for colchicine-refractory disease in various countries.

Chronic inflammation has been linked to premature atherosclerotic and cardiovascular disease in various diseases. For example, a disease-activity related increased risk for cardiovascular events has been described for rheumatoid arthritis, psoriatic arthritis and systemic lupus erythematosus (4-6). The increased cardiovascular (CV) risk is supposed to be orchestrated by traditional risk factors, upregulation of adhesion molecules, proinflammatory pathways, dysfunctional cellular immune responses and endothelial dysfunction (7). In addition, autonomic dysfunction (AD) may contribute to an increased CV risk in patients with inflammatory disorders (8-10). For instance, reduced heart rate variability is a major feature of cardiac autonomic dysfunction and represents an isolated risk factor for cardiovascular events (11-14).

Concerning FMF, studies of AD resulted in conflicting results (15-20). Heart rate variability (HRV) measurement may not be sensitive enough to detect early autonomic impairment. In addition, involvement of autonomic nerves other than the vagus nerve may be missed by HRV assessment.

Infrared pupillometry is another validated tool to measure autonomic dysfunction (21). It has been used in various autoimmune diseases such as rheumatoid arthritis, small vessel vasculitis, systemic sclerosis and systemic lupus erythematosus and showed only weak or no correlation with HRV (22-24). It thus may serve as additional tool for a more comprehensive approach of autonomic testing. Data on pupillometric findings in FMF is still lacking.

Several parts of the central nervous system that are involved in autonomic regulation express IL1 receptors (25). We therefore assumed that autonomic findings may differ between patients that are treated only with colchicine and patients on additional anakinra therapy.

Hence, the aim of the study was to assess symptoms and objective signs of autonomic dysfunction and to investigate associations with disease specific parameters in a cohort of FMF patients living in Germany.

Methods

Subjects

Patients with established diagnosis of FMF were included in the study. The diagnosis of FMF was based on clinical classification criteria as described previously (26). In addition, diagnosis of FMF was confirmed by moleculargenetic testing. Epidemiological, clinical and laboratory features of the FMF patients were collected. Patients with comorbidities or comedications which may affect the autonomous nervous system (*e.g.* diabetes mellitus, beta blockers, antidepressants) were excluded. The clinical phenotype was characterised by symptoms during flares, average flare frequency and duration, moleculargenetic findings, risk factors for or presence of AA-amyloidosis and pharmacotherapy (colchicine monotherapy *vs.* additional IL1-blocking agent). The patients had to speak and understand German.

Patients were compared to age- and gender-matched healthy controls. Written consent was obtained from patients and controls. Controls had to be entirely free of any medical or psychiatric disease. The study complies with the declaration of Helsinki and was approved by the local ethic's commitee of the university of Munich (study no. 22-14).

COMPASS-31 Questionnaire

The COMPASS-31 questionnaire is a validated instrument for the assessment of symptoms of autonomic dysfunction. With 31 questions, the questionnaire addresses the six subdomains of orthostatic, vasomotor, secretomotor, gastrointestinal, pupillomotor and bladder dysfunction symptoms (27). The raw scores of each subdomain are multiplied by a weighting factor according to the subdomain's clinical significance. The total score is obtained by adding up the scores of each subdomain, resulting in a final score between 0 to 100. Higher scores correspond to more severe symptoms. For this study, we used a German version of the questionnaire, which was already used in a previous study (28).

Autonomic function testing

Four standardised methods of autonomic testing were applied to patients and healthy controls. Heart rate variability during deep breathing was performed as parasympathetic cardiovascular reflex test, skin conductance changes during mental arithmetics and blood pressure response to pain were performed for sympathetic response. Finally, infrared dynamic pupillometry assessed the parasympathetic and sympathetic axis of the pupillary light reflex.

Heart rate variability (HRV) during deep breathing

After a recovery phase of five minutes in a supine position, the patient was asked to breathe with a frequency of six breaths per minute over a period of two minutes under continuous ECG registration. HRV was assessed by dividing the longest by the shortest R-R interval of each breathing cycle, which resulted in the so-called E/I ratio. Median E/I ratios of each patient were calculated and compared to controls.

Skin conductance measurement (SCM)

After a recovery phase of five minutes, SCM was measured at two fingers. To apply mental stress, the patient was then asked to sequentially subtract two-digit from four-digit numbers. The mean difference of SCM before and after performing mental arithmetic of patients was compared to controls.

Blood pressure response to pain (cold pressor test)

Systolic and diastolic blood pressure was measured non-invasively by sphygmomanometry at rest in a sitting position. After immersion of one hand into ice water, the patient was asked to keep his respiratory frequency throughout the test to avoid bias from Valsalva effects. Blood pressure was measured again immediately after, then one and two minutes after immersion of the hand into ice water. A rise of the diastolic blood pressure of less than 10 mmHg was defined as a pathological result. In addition, the pain level at the various time points was assessed by a visual analogue scale ranging from 0 (no pain) to 10 (most severe pain).

Dynamic infrared pupillometry

Dynamic infrared pupillometry was performed in a dark room after an accommodation time of 10 minutes (compact integrated pupillograph; AMTech Pupilknowlogy GmbH, Heidelberg, Germany). Pupillary light reflex was assessed for both eyes by using a light stimulus of 10.000 cd m-2 and a duration of 200 ms. The following parameters were measured: maximum constriction velocity (mm/s); amplitude of pupillary constriction (mm); relative amplitude of pupillary constriction (amplitude divided by pupil diameter before light stimulus); late dilatation velocity (mm/s). Amplitude, relative amplitude and maximum constriction velocity reflect the active parasympathetic part of the light reflex, whereas the late dilatation velocity reflects the active sympathetic part (21). At baseline, light reflex of both eyes was compared in order to rule out side differ-

ences. Pupillometry was performed at afternoon, between 3 p.m. and 6 p.m., in order to rule out circadian changes of sympathetic and parasympathetic tone. Medical professionals that served in part as healthy controls may have a lower sympathetic tone in the hospital as they are more accustomed to the setting than patients. Both patients and controls were therefore stimulated sympathetically by pain (immersion of one hand into ice water), and the pupillometric measurements were repeated after 1, 2, 3, 4 and 5 minutes thereafter.

Statistical analysis

SPSS version 22 was used for statistical analysis. Comparison between patients and controls was performed by Mann-Whitney U-tests for not normally distributed variables and by Student's *t*-test for normally distributed variables. Fisher's exact test was used to detect differences in categorial variables between patients, controls and patient subgroups. Pearson's or Spearman's correlation analysis were used as appropriate to investigate correlations between COMPASS-31 scores, clinical and autonomic function parameters. For comparison of COMPASS-31 subdomains, Bonferroni correction was used ($p<0.008$) for adjusting for multiple testing, as COMPASS-31 is validated as composite score.

Results

Characteristics of the study population

25 FMF patients and 25 healthy controls have been enrolled into the study and completed the COMPASS-31 questionnaire and autonomic testing.

Mean age of the patients was 37.4±8.5 years (male to female ratio 12/13). 72% of the patients were of Turkish origin, other nationalities included Greek, Armenian and Iranian. Molecular genetic testing was positive in 24 of 25 cases, with 42% homozygotic and 58% compound heterozygotic findings. All patients received colchicine in a mean daily dose of 1.4 mg ± 0.8. 57% of the patients responded well to colchicine whereas 32% were treated with add-on anakinra due to ongoing disease activity under colchicine. Patients with add-on anakinra were more likely to be homozygote than patients on colchicine monotherapy. No patient had symptoms of congestive heart failure or arrhythmias. Four patients had elevated nt-proBNP levels (Table I). Only three patients received echocardiography at baseline showing normal left ventricular function and normal wall thickness. The remaining clinical and demographic characteristics were not different between these two subgroups (Table I).

The control patients were of Caucasian ethnicity with male to female ratio of 10/15 and a mean age of 34±10.8 years.

Table I. Demographic data.

Parameter	All patients (n=25)	Anakinra (n=8)	Conventional (n=17)	*p*-value (Anakinra *vs*. conventional)
Gender male/female	12/13	5/3	7/10	0.411
Age	37 (± 8.7)	37.5 (± 11.7)	37.6 (± 7.6)	0.982
Disease duration (years)	19.4 (± 12.7)	24.3 (± 10.8)	18.2 (± 12.9)	0.200
Molecular genetics				
heterozygote	6 (24%)	2	4	1.000
homozygote	10 (40%)	6	4	**0.028**
compound	8 (32%)	0	8	**0.026**
negative	1 (4%)	0	1	1.000
Disease activity (patient reported)				
VAS global	20.2 (± 32.1)	36.3 (± 36.7)	12.2 (± 27.3)	0.083
VAS pain	23.3 (± 35.0)	40.6 (± 41.1)	14.7 (± 29.1)	0.087
Flares per month				
<2	11 (44%)	3	11	0.389
>2	14 (56%)	5	6	
Flare duration				
0-4 days	15 (60%)	3	12	0.194
>4 days	10 (40%)	5	5	
Symptoms during flare				
fever	20 (80%)	8	12	0.140
constitutional	6 (24%)	2	4	1.000
peritonitis	19 (76%)	7	12	0.624
nausea/vomiting	4 (16%)	2	2	1.000
cephalgia	4 (16%)	2	2	0.429
pleurisy	20 (80%)	6	14	1.000
lymphadenopathy	1 (4%)	0	1	1.000
arthralgia/myalgia	14 (56%)	4	10	1.000
arthritis	13 (52%)	4	9	1.000
palpitations	6 (24%)	1	5	0.624
eye inflammation	15 (60%)	6	9	0.402
rash	7 (28%)	1	6	0.362
aphtous ulcers	2 (8%)	1	1	0.426
Therapy				
analgesics during flare	13 (52%)	6	7	0.237
colchicine	21 (84%)	7	14	1.000
colchicine dose	0.5 mg: 3 (12%) 1 mg: 8 (32%) 1.5 mg: 7 (28%) 2-3 mg: 3 (12%)	0.5 mg: 2 1 mg: 1 1.5 mg: 2 2-3 mg: 2	0.5 mg: 1 1 mg: 7 1,5 mg: 5 2-3 mg: 1	n/a
Colchicine responders	12 (48%)	3	9	0.118
Anakinra	8 (32%)	8	0	n/a
Amyloid related parameters				
confirmed amyloidosis	2 (8%)	2	0	0.093
nt-proBNP-elevation	4 (16%)	1	3	1.000
mean nt-proBNP pg/ml	62.8 (± 51.7)	68.2 (± 66.7)	60.3 (± 45.6)	0.745
SAA elevated under current therapy	12 (48%)	3	9	0.673
mean Serum Amyloid A mg/l	28.2 (± 79.1)	10.0 (± 15.9)	38.5 (± 97.8)	0.441
mean CRP mg/dl	1.7 (± 2.8)	2.3 (± 2.9)	1.4 (± 2.8)	0.486
mikroalbuminuria	5 (20%)	3	2	0.283
hepatosplenomegaly	10 (40%)	4	6	0.588

VAS: visual analogue scale; SAA: serum amyloid A.

Symptoms of autonomic dysfunction

FMF-patients had significantly higher median COMPASS-31 total scores than controls (23.7 *vs*. 1.6, p=0.024; Fig. 1). The difference remained significant after exclusion of the gastrointestinal subdomain of the questionnaire. We checked because patients often have gastrointestinal symptoms due to serositis and colchicine therapy. Subdomain analysis showed that patients had higher median scores in the secretomotor and the gastrointestinal domain than healthy controls (4.2 *vs*. 0.0; p<0.001 and 8.0 *vs*. 0.0; p=0.004, respectively; Table II, Fig. 1). Median COMPASS-31 scores were not associated with gender, molecular genetics, flare frequency, anakinra therapy or albuminuria. Neither did COMPASS-31 total scores correlate with disease duration, age or serum amyloid A concentrations. However, there was a significant correlation with patient reported disease activity regarding global VAS and pain VAS (Fig. 1).

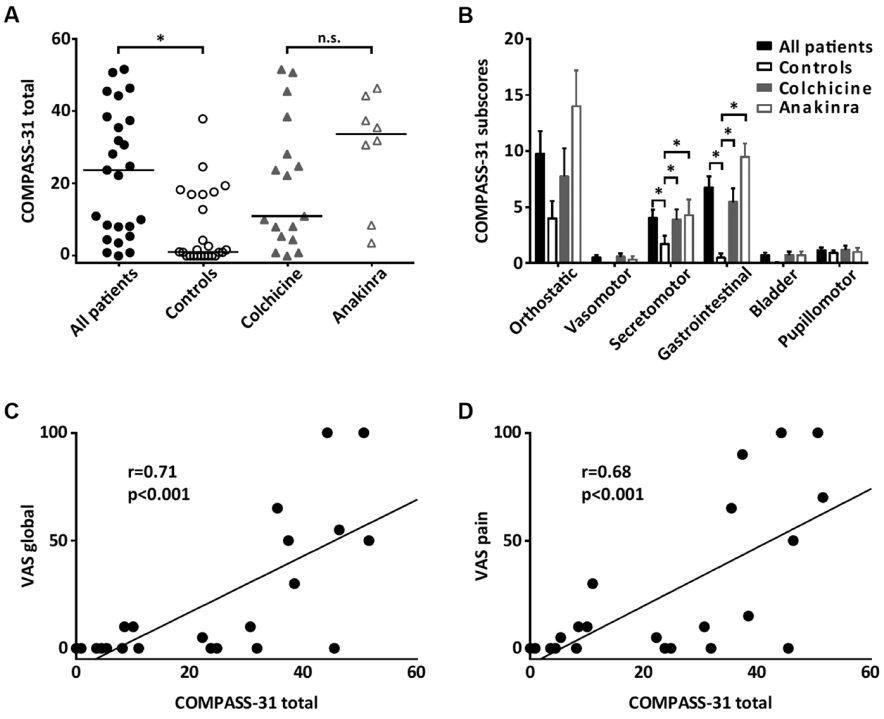

Fig. 1. A. Comparison of COMPASS-31 total scores between groups; bars: median score; *p<0.05; **B.** COMPASS-31 subdomain scores; *p<0.008; **C** and **D.** Correlation of COMPASS-31 total score with visual analogue scales (VAS; global and pain) at the time of assessment.

Heart rate variability

Between patients and controls, no differences of HRV were found as assessed by median E/I ratios during deep breathing (1.24±0.08 *vs*. 1.29±0.15, p=0.434, Table II). E/I ratios did not correlate with demographic or disease specific parameters. There was no correlation with COMPASS-31 scores and no correlation with other autonomic testing parameters.

Skin conductance

Compared to healthy controls, patients had trend to lower mean skin conductance measurements at baseline and during calculating, but the differences did not reach significance (2.7 *vs*. 4.7 µS, p=0.06 and 4.5 *vs*. 5.4 µS, p=0.36, respectively; Table II). Skin conductance measurements at baseline and during mental arithmetics showed significant inverse correlations with age (r=-0.43, p=0.03 and r=-0.42, p=0.02, respectively). After adjusting for age, differences between patients and controls still did not reach significance.

Cold pressor test

Mean deltas of systolic and diastolic blood pressures did not differ between patients and controls (25 *vs*. 19 mmHg, p=0.21; and 16 *vs*. 14 mmHg, p=0.43, respectively; Table II). The number of patients with a pathologic diastolic blood pressure increase of less than 10 mmHg did not differ significantly from healthy controls (p=0.52).

No associations with demographic or clinical disease parameters were found. Skin conductance did not correlate with COMPASS-31 scores or other autonomic testing parameters.

Dynamic infrared pupillometry

At baseline, there was only a non-significant trend towards a reduced parasympathetic response in patients (amplitude and maximum constriction velocity; Table II). After sympathetic stimulation however, the difference became significant at minute 2 to 4 for amplitude and at min 1 to 5 for constriction velocity. Relative amplitude was only different at min 4. Late dilatation velocity as sympathetic parameter did not differ at any point in time.

Pupillary function differed between patients on colchicine monotherapy and patients with add-on anakinra (Table II; Fig. 2). Patients on anakinra had higher baseline values for amplitude and maximum constriction velocity compared to patients on colchicine only (1.78 mm *vs*. 1.51 mm; p=0.007 and 5.14 mm/s *vs*. 4.33 mm/s; p=0.009, respectively), but not for relative amplitude or late dilatation velocity. The pupillometric parameters did not differ between anakinra treated patients and controls. Hence, anakinra treatment seems to have an effect of the pupillary light reflex and can be a confounder. When we compared only the colchicine treated patients with the controls, the differences in parasympathetic parameters amplitude and constriction velocity were higher and even significant at baseline.

Discussion

The key findings of our study can be summarised as follows: (a) Patients with FMF suffer from symptoms of autonomic function that may contribute to disease burden as they are correlated with patient reported disease activity. (b) Dynamic infrared pupillometry is a valuable tool to assess central autonomic dysfunction in patients with FMF which might be exemplary for other inflammatory conditions. (c)

Table II. Results of COMPASS-31 and autonomic testing.

Parameter	FMF patients	Controls	*p*-value (FMF *vs.* control)	Anakinra	Conventional treatment	*p*-value (anakinra *vs.* conventional)	*p*-value (conventional *vs.* control)
Gender m/f	12 / 13	10 / 15	0.776	5/3	7/10		
Age	37 ± 8.7	34 ± 10.8	0.227	37.5 (± 11.7)	37.6 (± 7.6)	0.982	0.266
COMPASS-31 total	23.7 [± 15.5]	1.7 [± 8.5]	**0.024**	33.7 [± 14.0]	10.5 [± 15.0]	0.194	**0.006**
COMPASS-31 subdomains:							
Orthostatic	12.0 [± 10.0]	0.0 [± 6.0]	0.080	16.0 [± 8.0]	0.0 [± 9.5]	0.194	0.194
Vasomotor	0.0 [± 0.0]	0.0 [± 0.0]	0.059	0.0 [± 0.0]	0.0 [± 0.3]	0.669	0.012
Secretomotor	4.3 [± 3.8]	0.0 [± 0.0]	**0.004**	3.2 [± 3.8]	3.2 [± 3.2]	0.754	0.027
Gastrointestinal	8.0 [± 4.7]	0.0 [± 0.0]	**<0.001**	10.7 [± 3.2]	3.1 [± 3.5]	**0.049**	**<0.001**
Bladder	0.0 [± 0.5]	0.0 [± 0.0]	0.027	0.0 [± 1.0]	0.0 [± 0.4]	0.887	0.022
Pupillomotor	1.0 [± 1.1]	1.0 [± 0.9]	0.396	1.0 [± 1.0]	0.5 [± 1.3]	0.842	0.618
Median E/I ratio	1.24 [± 0.08]	1.29 [± 0.15]	0.68	1.30 [± 0.08]	1.23 [± 0.08]	0.110	0.434
Δ skin conductance (μS)	4.47 (± 4.69)	5.81 (± 4.66)	0.498	6.83 (± 5.29)	3.53 (± 4.17)	0.084	0.159
Δ RR sys mmHg	25 (± 14)	19 (19)	0.205	26 (± 19)	24 (± 12)	0.913	0.267
Δ RR dia mmHg	16 (± 7)	14 (± 9)	0.430	13 (± 8)	17 (± 7)	0.252	0.253
Ampl baseline	1.58 (± 0.30)	1.74 (± 0.31)	0.102	1.78 (± 4.66)	1.51 (± 0.29)	**0.007**	**0.016**
1 min	1.49 (± 0.31)	1.71 (± 0.40)	0.054	1.64 (± 0.31)	1.44 (± 0.30)	0.118	**0.023**
2 min	1.58 (± 0.31)	1.79 (± 0.35)	**0.037**	1.73 (± 0.28)	1.52 (± 0.32)	**0.091**	**0.013**
3 min	1.61 (± 0.32)	1.82 (± 0.34)	**0.048**	1.82 (± 0.26)	1.53 (± 0.31)	**0.023**	**0.008**
4 min	1.60 (± 0.28)	1.83 (± 0.32)	**0.013**	1.77 (± 0.20)	1.52 (± 0.29)	**0.016**	**0.003**
5 min	1.68 (± 0.36)	1.83 (± 0.29)	0.111	1.92 (± 0.35)	1.57 (± 0.33)	**0.018**	**0.007**
V constriction baseline	4.56 (± 0.79)	5.02 (± 0.80)	0.062	5.14 (± 0.70)	4.33 (± 0.70)	**0.009**	**0.005**
1 min	4.40 (± 0.87)	4.99 (± 0.94)	**0.027**	4.79 (± 1.03)	4.25 (± 0.76)	0.124	**0.007**
2 min	4.71 (± 0.90)	5.30 (± 0.86)	**0.023**	5.17 (± 0.95)	4.52 (± 0.84)	0.079	**0.004**
3 min	4.81 (± 0.84)	5.41 (± 0.89)	**0.021**	5.29 (± 0.75)	4.61 (± 0.83)	**0.049**	**0.004**
4 min	4.88 (± 0.86)	5.49 (± 0.81)	**0.013**	5.42 (± 0.80)	4.63 (± 0.80)	**0.016**	**0.001**
5 min	5.01 (± 0.87)	5.58 (± 0.72)	**0.014**	5.51 (± 0.74)	4.79 (± 0.87)	**0.043**	**0.002**
V dilatation baseline	0.57 (± 0.20)	0.61 (± 0.16)	0.529	0.60 (± 0.19)	0.56 (± 0.21)	0.576	0.399
1 min	0.61 (± 0.16)	0.59 (± 0.21)	0.706	0.65 (± 0.14)	0.60 (± 0.18)	0.407	0.988
2 min	0.62 (± 0.17)	0.69 (± 0.22)	0.284	0.69 (± 0.10)	0.60 (± 0.19)	0.184	0.165
3 min	0.63 (± 0.22)	0.69 (± 0.25)	0.405	0.73 (± 0.23)	0.59 (± 0.21)	0.125	0.160
4 min	0.60 (± 0.19)	0.64 (± 0.18)	0.433	0.60 (± 0.15)	0.60 (± 0.21)	0.998	0.499
5 min	0.66 (± 0.26)	0.70 (± 0.22)	0.330	0.79 (± 0.33)	0.59 (± 0.21)	0.085	0.124
Relative ampl baseline	24% (± 5.6)	27% (± 6.4)	0.217	27% (± 4)	24% (± 6)	0.077	0.081
1 min	22% (± 5.2)	26% (± 7.8)	0.062	24% (± 5)	22% (± 5)	0.299	0.053
2 min	24% (± 5.5)	28% (± 7.2)	0.054	26% (± 5)	23% (± 6)	0.222	**0.037**
3 min	25% (± 5.3)	29% (± 7.2)	0.066	27% (± 4)	24% (± 5)	0.125	**0.032**
4 min	25% (± 4.7)	30% (± 7.3)	**0.019**	27% (± 3)	24% (± 5)	0.117	**0.014**
5 min	27% (± 5.9)	29% (± 9.2)	0.330	29% (± 5)	26% (± 6)	0.128	0.186

COMPASS-31: composite autonomic symptom score; E/I: exspiratory/inspiratory; RR sys: systolic blood pressure; RR dia: diastolic blood pressure; Ampl: amplitude; v: velocity; [ ]: interquartile range; ( ): standard deviation.

Anakinra treatment seems to alter the parasympathetic portion of the pupillary light reflex. This may indicate an influence of IL1-blocking therapy on central autonomic regulation.

This is the first study that assessed symptoms of autonomic dysfunction and dynamic infrared pupillometry in FMF patients. Symptoms of dysautonomia can be missed during the routine examination, as they are not assigned to the disease either by the physician or by the patient. However, they can contribute greatly to disease burden, as indicated by the strong correlation with patient reported disease activity. On the other hand, high disease activity could also lead to increased introspection, so that the autonomic symptoms could in part be explained by an exaggerated description of symptoms. This was observed in fibromyalgia patients that had high COMPASS-31 scores but no evidence for objective autonomic dysfunction assessed by autonomic reflex screen (29). However, observations of autonomic symptoms in other inflammatory diseases suggest that these symptoms may indeed be caused by acute or chronic inflammation. Acute viral diseases such as Zika-Virus infection have been linked with increased autonomic symptoms (30). Autonomic symptoms are correlated with patient-reported and physician-reported disease activity in Sjögren's syndrome (31). Furthermore,

autonomic symptoms and fatigue are correlated in MS patients and both are thought to partly result from inflammatory afferent vagus activation (32). Obviously cardiovascular autonomic tests and sweat secretion tests only cover part of the autonomic nervous system and do not necessarily correlate with the involvement of other organ systems. Based on our data, we therefore consider it probable that chronic inflammation is causally responsible for the perceived symptoms. A longitudinal application of COMPASS-31 with intraindividual correlation with inflammatory activity could provide further information.

Regarding heart rate variability, previous studies in FMF patients revealed conflicting results. Delayed heart rate recoveries and abnormal heart rate variability parameters have been found in one study (16). In contrast, other authors found normal heart rate variabilities in the supine and upright position (19). It has been argued that successful therapy with colchicine may explain the normal findings. Hence, in effort to investigate the patients with the highest inflammatory burden, HRV was studied in colchicine refractory patients, and again, no impairment was found (20). Sample sizes of available studies may be too small to find discrete but significant differences, but according to the majority of the available evidence, the cardiac branch of the vagus nerve is seldomly affected in FMF cohorts. This is in line with our findings of normal E/I ratio. At least in our study, normal responses were also observed in one sympathetic cardiovascular reflex test (cold pressor test) and in skin conductance during sympathetic stimulation.

Classical autonomic function tests may be insufficient to allow conclusions to be drawn about the involvement of the autonomic nervous system in FMF patients. A possible explanation would be a speckled pattern of involvement in which other organ systems, such as the gastrointestinal tract, urinary bladder or central areas of autonomous regulation, are more affected than the cardiovascular system. Objective measurements of gastrointestinal motility and bladder function are time-consuming

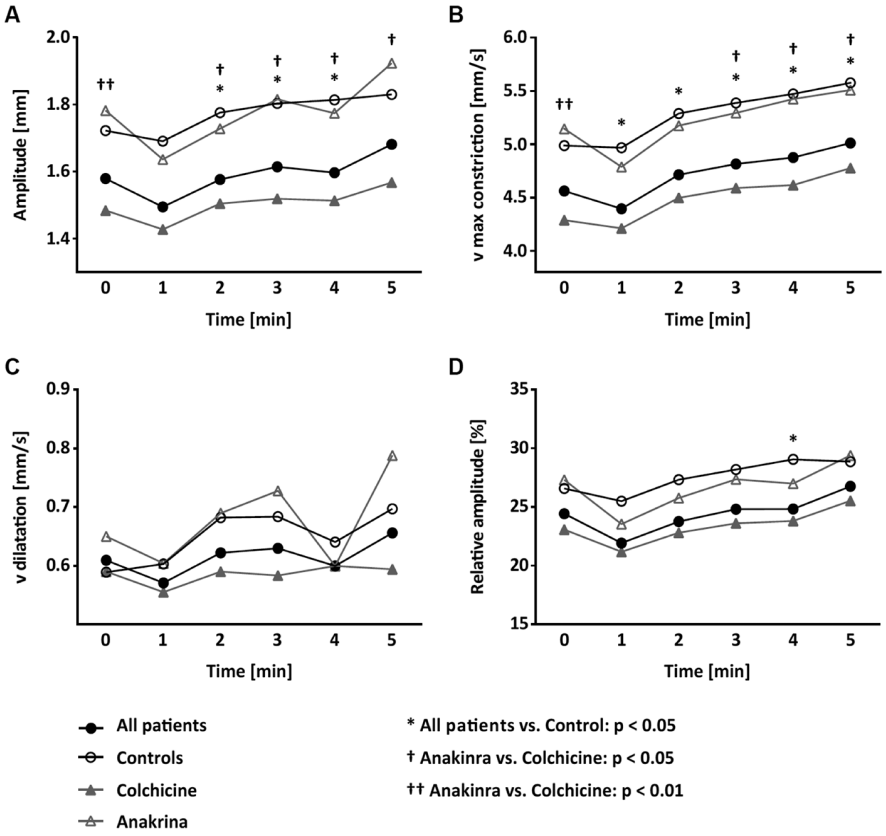

Fig. 2. Comparison of mean parasympathetic (**A, B, D**) and sympathetic (**C**) pupillometry parameters; unstimulated (0 min) and after immersion of one hand into ice water.

and unpleasant for the patient. In contrast, dynamic infrared pupillometry is a highly standardised quick and non-invasive method to assess pupillary autonomic function (21).

FMF patients of our cohort revealed impaired parasympathetic but normal sympathetic responses to light stimulus. Of note, this pupillary autonomic dysfunction did neither correlate with patient reported outcomes (VAS global, VAS pain, COMPASS-31) nor with the other autonomic tests or markers of inflammation. This could indicate that there is either an isolated dysfunction of the pupil innervation or that central areas of autonomous regulation are affected more generally in this condition. The latter hypothesis could be supported by the fact that this parasympathetic dysfunction was not present in patients treated with anakinra. However, this is not certain to be concluded since the study had no interventional longitudinal design. In theory, IL-1 blocking therapy may have central anti-inflammatory properties that exceed the effect of colchicine alone. This could be of particular interest in regard to neuroinflammatory disease sequelae.

IL-1β can act on the central nervous system by afferent vagus stimulation as well as by direct binding to neuronal receptors (33). This can lead to neuropsychiatric symptoms such as anxiety and depression (34). FMF patients can suffer a whole range of central neurological symptoms and comorbidities, including fatigue, depression and possibly demyelinating disease (35-38), which may be favourably affected by IL-1 blocking therapy. Hence, the impact of IL1-blocking therapy on neuroinflammation in FMF deserves further evaluation and the pupillary light reflex might serve as surrogate marker for neuroinflammation in future prospective studies.

Our study has several limitations. Symptoms of autonomic dysfunction were gathered by a questionnaire. Therefore, a possible recall bias cannot be excluded but this would equally apply for both patients and healthy controls. For some patients, German was not their mother tongue, which is why

some questions may not have been optimally understood. However, all patients who spoke broken German completed the questionnaire in the presence of a German-speaking physician and a German-speaking accompanying person in order to minimise comprehension problems. Another weakness was that autonomic testing with a better validated autonomic reflex testing device such as the composite autonomic assessment score (CASS) was not available at our institution. In addition, we used only short-time measurements of the autonomous parameters in an artificially generated situation. Therefore, in contrast to 24-hour HRV measurements, fluctuations in autonomic function in everyday situations and circadian changes could not be detected. However, there is some evidence that short-term cardiovascular reflex tests correlate with 24-hour HRV (39) and other advantages of the deep breathing test are the reproducibility and its robustness (40). Pupillometry and cold pressor test also have good reproducibility (41, 42), while the sympathetic skin response is subject to greater intra-individual fluctuations and interpretation is therefore difficult (43).

Another problem for the interpretation of the data is the ethnic difference between patients and controls. While the patients were predominantly of Turkish origin, the controls were almost all Caucasian. We cannot rule out the possibility that ethnic differences had an influence on the measurement results. Finally, it cannot be excluded, that the cohort was not large enough to detect small yet significant differences of autonomic dysfunction regarding HRV, cold pressor test and skin conductance.

Conclusion

Our study covers for the first time a wide range of autonomic symptoms and autonomic tests in patients with FMF. It shows that the investigation of autonomous function is very complex and probably cannot be sufficiently assessed by a test alone. In addition, the study provides evidence that drug therapy approaches may differ with regard to their effect on the autonomic nervous system. In particular, IL-1 blocking therapies seem to have a positive effect on central switching points of autonomous regulation. Further prospective studies would help to define the importance of pupillometry as a possible tool for therapy stratification with regard to neuroinflammatory aspects of the disease. The inclusion of the COMPASS-31 questionnaire in the diagnostic armamentarium could also provide valuable insights into the extent of the symptoms of the disease and thus also the suffering of patients. In this way, the effect of different therapies on autonomous perception could also be examined.

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