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ORIGINAL ARTICLE

# Disease-modifying anti-rheumatic drugs improve autonomic neuropathy in arthritis: DIANA study

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**Abstract** Autonomic neuropathy (AN) is a risk predictor for sudden cardiac death in rheumatoid arthritis (RA) and ankylosing spondylitis (AS). However, the impact of most commonly employed disease-modifying anti-rheumatic drug (DMARD) therapy on autonomic neuropathy in rheumatic diseases is not known. Hence, we investigated the efficacy of DMARDs on autonomic neuropathy in RA and AS. We performed autonomic function assessment in 60 patients in this open-label, 12-week pilot study including 42 patients with RA, 18 with AS, and 30 aged-matched healthy subjects. The methodology included assessment of cardiovascular autonomic reflex tests according to Ewing. Parasympathetic dysfunction was established by performing three tests: heart rate response to deep breathing, standing, and Valsalva tests. Sympathetic dysfunction was examined by applying two tests: blood pressure response to standing and handgrip tests. Sudomotor function was assessed by Sudoscan. Cardiovascular reflex tests were impaired significantly among the patients as compared to healthy subjects ($p<0.05$). Autonomic neuropathy was more pronounced in biologic-naive RA and AS patients. After treatment with combination synthetic DMARDs, parasympathetic, and sudomotor dysfunction significantly ($p<0.05$) improved in RA and AS. Biologic DMARDs significantly improved parasympathetic, sympathetic and peripheral sympathetic autonomic neuropathy ($p<0.05$) in biologic-naive RA and AS patients. In conclusion, synthetic DMARDs improved parasympathetic and sudomotor dysfunction in both DMARD-naive RA and AS patients. However, biologic DMARDs improved parasympathetic, sympathetic and sudomotor dysfunction to a greater extent than synthetic DMARDs in both RA and AS patients.

**Keywords** Ankylosing spondylitis · Autonomic neuropathy · Disease-modifying anti-rheumatic drugs · Rheumatoid arthritis · Sudoscan

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## Introduction

Cardiovascular autonomic dysfunction is the most common type of autonomic nervous system dysfunction in rheumatic diseases occurring in 24–100 % of rheumatic patients [1]. Autonomic neuropathy is a significant risk predictor for sudden cardiac death in rheumatoid arthritis (RA), ankylosing spondylitis (AS), and systemic lupus erythematosus (SLE) [2, 3]. There is an increased risk for sudden cardiac death in RA and SLE related to severe autonomic neuropathy (AN) with sympathetic predominance leading to fatal arrhythmias [2]. Most rheumatic diseases are characterized by excess cardiovascular (CV) morbidity and mortality [4].

Autonomic neuropathy in rheumatic diseases was first reported by Bennett and Scott in 1965 [5] and Toussirot et al. in 1999 in ankylosing spondylitis (AS) [6]. However, even after half a century, we do not have any therapeutic treatment strategy for cardiovascular autonomic dysfunction in rheumatic diseases. We do not even know as to how commonly employed therapy (disease-modifying anti-rheumatic drugs [DMARDs]) in the treatment of rheumatic diseases impacts the autonomic neuropathy associated with these disorders.

Against this background, we aimed to determine the efficacy of synthetic and biologic DMARDs on autonomic neuropathy in RA and AS, and we also examined the relations between the indices of disease variables and indicators of autonomic neuropathy in RA and AS patients.

## Material and methods

### Study participants

The study population consisted of 42 RA and 18 AS patients and 30 unrelated, aged-matched healthy control subjects. Seventeen (17/30) DMARD-naive and five (5/12) biologic-naive RA patients were positive for rheumatoid factor (RF). The baseline demographic and clinical features of RA and AS patients and healthy control subjects are summarized in Table 1. The diagnoses of RA and AS were according to the ACR 2010 classification criteria and modified New York criteria, respectively [7, 8]. Inflammatory disease activity was assessed using the 28-joint-count disease activity score (DAS28) and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) in RA and AS respectively at 0, 6, and 12 weeks. Erythrocyte sedimentation rate (ESR) was measured by Westergreen method and C-reactive protein (CRP) level was determined using standard commercial kits. Autonomic measures were double-blinded to the physician and patients for the study duration. Therefore, the patients were treated without influence of the autonomic function data.

Patients were excluded from the study if they had any of the following conditions: presence of skin lesions that could affect the ability to assess their neuropathic pain, renal or liver insufficiency, thyroid disorder, diabetes mellitus, pregnancy, under neuroprotective drugs, β-blockers, antihypertensive drugs, vitamin B12 deficiency, anemia, cardiac failure, cardiac arrhythmia, acute thrombosis, pericarditis or nephritis, paraneoplastic neuropathy, alcoholism, smoking, and under steroid therapy. Patients with disorders responsible for neuropathy and neurological disorders other than rheumatic disorders were also excluded. Patients with RA and AS who were scheduled to start treatment with synthetic DMARDs and biologic DMARDs for active disease were referred to the Cardio Rheuma Division for the assessment of autonomic function. The researcher involved in assessing the autonomic function was blinded to the treatment protocol. The project was approved by the regional institutional clinical ethics committee of Punjabi University Patiala, India, and was performed in accordance with the declaration of Helsinki and the code of Good Clinical Practice.

### Treatment administration

All DMARD-naive RA patients were initiated on methotrexate (10–15 mg/week) along with hydroxychloroquine (400 mg/day). All biologic-naive RA patients were on a continuing regimen of methotrexate (10–15 mg/week)+ hydroxychloroquine (400 mg/day)+sulfasalazine (2 g/day) or leflunomide (10–20 mg/day) and were treated with three infusions of infliximab (3 mg/kg/infusion) and tocilizumab (8 mg/kg/infusion) or two infusions of rituximab (1 g/infusion) as an add-on therapy. DMARD-naive AS patients were initiated on sulfasalazine 1–3 g/day. biologic-naive AS patients were on sulfasalazine in a dose of 2–3 g/day and were treated with TNFi infliximab (5 mg/kg/infusion) or etanercept (50 mg/week) as add-on therapy. Stable low

**Table 1** Baseline demographic and clinical characteristics of RA, AS, and healthy subjects

| | Rheumatoid synthetic DMARD naive ($n$=30) | Arthritis biologic DMARD naive ($n$=12) | Ankylosing synthetic DMARD naive ($n$=10) | Spondylitis biologic DMARD naive ($n$=08) | Controls ($n$=30) |
|---|---|---|---|---|---|
| Age (years) | 42.5±8.2 | 53.3±7.2 | 32.0±14.2 | 39.5±10.8 | 40±7.5 |
| Disease duration | 5.4±6.8 | 11.5±8.1 | 6.6±5.8 | 5.7±3.7 | – |
| Sex, F/M | 19/11 | 8/4 | 3/7 | 3/5 | 18/12 |
| Height (cm) | 158±5.2 | 162±11.9 | 164±5.7 | 166±11.3 | 167±9.5 |
| Weight (kg) | 65.4±20.5 | 65.2±11.9 | 70.6±14.3 | 74.7±12.9 | 64.6±13.1 |
| BMI (kg/m$^2$) | 24.6±4.4 | 24.7±4.5 | 25.2±5.0 | 25.1±4.3 | 22.6±2.9 |
| ESR (mm, 1st h) | 34.06±6.9 | 58.66±30.2 | 36.50±26.8 | 38.37±21.7 | – |
| CRP (mg/dl) | 18.68±16.6 | 10.73±11.2 | 30.92±26.6 | 19.72±21.3 | – |
| DAS28 | 5.28±0.89 | 5.97±0.78 | – | – | – |
| BASDAI | – | – | 3.43±2.64 | 4.41±0.92 | – |
| BASFI | – | – | 4.16±2.83 | 4.12±1.26 | – |

Values are shown as mean±SD

*F* female, *M* male, *BMI* body mass index, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *DAS28* disease activity score in 28 joints, *BASDAI* bath ankylosing spondylitis disease activity index, *BASFI* bath ankylosing spondylitis functional index, *DMARD* disease-modifying anti-rheumatic drug

dose of oral prednisone (≤7.5 mg/day) or equivalent was also allowed.

### Assessment of autonomic neuropathy

Cardiovascular autonomic function assessment was done using noninvasive cardiovascular reflex tests, the current gold standard for the diagnosis of cardiovascular autonomic neuropathies. Peripheral sympathetic autonomic function was assessed by FDA-approved Sudoscan (Impeto Medical Device, EZS 01750010193 Paris, France) [9]. All tests were performed under standardized conditions, in climate-controlled rooms (temperature 23 °C), in the morning. Autonomic function tests were carried out at 0, 6, and 12 weeks in case of synthetic DMARD-naive patients and before each administration of biologic DMARDs for three consecutive doses (two doses in case of rituximab).

Clinical measurements of cardiovascular autonomic neuropathy were carried out using the battery of five cardiovascular reflex tests, described by Ewing and Clark [10]. Cardiovascular autonomic neuropathy (CAN) was considered to exist if at least two tests were positive [11].

- *Parasympathetic dysfunction* was diagnosed by applying three tests: heart rate response to deep breathing (HRD), heart rate response to standing (HRS), and heart rate response to Valsalva (HRV) tests.
- *Sympathetic dysfunction* was assessed with two tests: blood pressure response to standing (BPS) and blood pressure response to handgrip (BPH) tests.

Results of each cardiovascular reflex test are expressed as normal (0), borderline (1), or abnormal (2), as in reference values according to Ewing [10]. Maximal possible cumulative score is 10 (i.e., if all five tests had abnormal). A cumulative score of 0 or 1 was considered normal, while a score of 2 or 3 was interpreted as moderate autonomic dysfunction. Patients with scores between 4 and 10 were considered with severe dysfunction [10].

### Statistical analysis

Test values are reported as mean±standard deviation. For parametrically distributed data, the analysis of variance (ANOVA) test was used, followed by Dunnett test to identify differences in all groups in comparison to healthy controls. Comparisons between two categories were made using Student's *t* test for continuous variables. Multiple linear regression analysis was performed between sudoscan, both CAN and disease variables in RA and AS patients. A value of $p \leq 0.05$ was considered statistically significant. Statistical analysis was done using the Prism GraphPad program version 6 for Windows 7.0.

## Results

### Rheumatoid arthritis

DMARD- or biologic-naive RA patients had significantly impaired heart rate response to deep breath (HRD), heart rate response to standing (HRS), heart rate response to Valsalva (HRV), and sudomotor function as compared to healthy controls (Table 2). Blood pressure response to standing (BPH) was found significantly lower in biologic-naive RA patients. However, BPS was not impaired as compared to the healthy controls in DMARD or biologic-naive RA patients (Table 2). Many RA patients had abnormal autonomic neuropathy score (Table 3); 47 % (14/30) of DMARD-naive and 83 % (10/12) of biologic-naive RA patients had severe autonomic neuropathy score. Thirty-three percent (10/30) of DMARD-naive and 41 % (5/12) of biologic-naive RA patients had sudomotor dysfunction. None of the healthy population had autonomic neuropathy.

Changes in the cardiovascular autonomic function and sudomotor function after treatment with combination synthetic DMARDs (csDMARDs) and biologic DMARDs in RA patients are shown in Table 4. After treatment, 28 % (4/14) and 60 % (6/10) improvement was seen in DMARD-naive and biologic-naive RA patients with severe autonomic neuropathy, respectively (Table 3). After treatment with biologic DMARDs, 80 % (4/5) of RA patients improved sudomotor dysfunction. However, there was no improvement of sudomotor function in DMARD-naive patients (Table 3). Patients with normal autonomic function had no change in their autonomic function after treatment with synthetic or biologic DMARDs (Table 3).

The study RA patients were also analyzed with respect to seropositivity. A total of 53 % (9/17) of DMARD-naive RF-positive and 38 % (5/13) of DMARD-naive RF-negative patients had severe cardiovascular autonomic neuropathy score. A total of 100 % (5/5) of biologic-naive RF-positive and 71 % (5/7) of biologic-naive RF-negative RA patients had severe CAN score. A total of 29.4 % (5/17) of DMARD-naive RF-positive RA and 38.4 % (5/13) of DMARD-naive RF-negative RA patients had sudomotor dysfunction. A total of 60 % (3/5) of biologic-naive RF-positive and 28 % (2/7) of biologic-naive RF-negative patients had sudomotor dysfunction. After 12 weeks of treatment with csDMARDs, 33 % (3/9) of DMARD-naive RF-positive patients and 20 % (1/5) of DMARD-naive RF-negative patients exhibited improved CAN. After treatment with biologic DMARDs, 80 % (4/5) of RF-positive patients and 60 % (3/5) of RF-negative patients

**Table 2** Results of autonomic function tests in patients with RA and AS and in a healthy control group at baseline

| | DMARD-naive RA ($n$=30) | Biologic-naive RA ($n$=12) | DMARD-naive AS ($n$=10) | Biologic-naive AS ($n$=08) | Healthy controls ($n$=30) |
|---|---|---|---|---|---|
| HRD (bpm) | 13.00±5.67* | 6.16±2.32* | 12.10±6.80* | 11.88±5.41* | 17.43±2.17 |
| HRS (R-R ratio) | 0.96±0.08* | 0.98±0.10* | 1.08±0.24* | 1.02±0.02* | 1.16±0.22 |
| HRV (R-R ratio) | 1.23±0.09* | 1.28±0.12* | 1.28±0.18* | 1.28±0.10* | 1.41±0.09 |
| BPS (mmHg) | 5.26±3.08 | 5.0±2.48 | 4.40±2.33 | 3.62±1.68 | 3.90±1.53 |
| BPH (mmHg) | 15.20±4.28 | 12.83±5.28* | 16.40±3.23 | 12.50±4.75* | 17.40±2.02 |
| Sudoscan (μS) | 62.60±13.5** | 58.92±11.9** | 65.65±7.2** | 65.19±12.8** | 76.23±6.6** |

Values are shown as mean±SD

*HRD* heart rate (HR) response to deep breathing, *HRS* HR response to standing, *HRV* heart rate response to Valsalva, *BPS* blood pressure (BP) response to standing, *BPH* BP response to handgrip, *DMARD* disease-modifying anti-rheumatic drug, *RA* rheumatoid arthritis, *AS* Ankylosing spondylitis

*$p$<0.05 versus controls, $p$<0.001 versus controls

had improved CAN. A total of 66 % (2/3) and 100 % (2/2) of biologic RF-positive and RF-negative patients respectively improved sudomotor dysfunction. Multiple linear regression analysis of autonomic function with disease duration, disease severity, and biomarkers of inflammation (ESR and CRP) is shown in Table 5.

Ankylosing spondylitis

DMARD- and biologic-naive AS patients had significantly impaired HRD, HRS, HRV, and sudomotor function as compared to healthy controls (Table 2). BPH was found significantly impaired in biologic-naive AS patients. BPS was not impaired as compared to the healthy controls in DMARD- or biologic-naive AS patients (Table 2). None of the healthy population had CAN and sudomotor dysfunction (Table 2). A total of 50 % (5/10) of DMARD-naive and 50 % (4/8) of biologic-naive AS patients had severe autonomic neuropathy score. A total of 20 % (2/10) of DMARD-naive and 37 % (3/8) of biologic-naive AS patients had sudomotor dysfunction (Table 3).

Effects of csDMARDs and biologic DMARDs on cardiovascular autonomic function and sudomotor function in AS patient are shown in Table 4. After treatment, 20 % (1/5) and 100 % (4/4) improvement was seen in severe autonomic neuropathy in DMARD- and biologic-naive patients, respectively (Table 3). All biologic DMARD-treated patients [100 % (3/3)] improved sudomotor dysfunction. There was no improvement in sudomotor dysfunction in AS patients treated with csDMARDs (Table 3). Patients with normal autonomic function had no change in their autonomic function after treatment with synthetic or biologic DMARDs (Table 3). Multiple linear regression analysis of autonomic function with disease duration, diseases severity, and biomarkers of inflammation (ESR and CRP) in AS is shown in Table 6.

## Discussion

This is the first study to assess the clinical impact of synthetic and biologic DMARDs on autonomic neuropathy in RA and AS patients. We also assessed the association between the indices of disease variables and indicators of autonomic neuropathy.

**Table 3** The results of the cardiovascular autonomic tests according to original Ewing autonomic neuropathy score and sudomotor function

| Characteristics of autonomic neuropathy | DMARD-naive RA $n$=30 BT AT | Biologic-naive RA $n$=12 BT AT | DMARD-naive AS $n$=10 BT AT | Biologic-naive AS $n$=8 BT AT |
|---|---|---|---|---|
| Normal (1) | 8 16 | 1 8 | 4 6 | 0 4 |
| Moderate (2-3) | 8 4 | 1 0 | 1 0 | 4 2 |
| Severe (≥4) | 14 10 | 10 4 | 5 4 | 4 0 |
| Sudomotor dysfunction (<60 μs) | 10 10 | 5 1 | 2 2 | 3 0 |

*BT* before treatment, *AT* after treatment, *DMARD* disease-modifying anti-rheumatic drug, *RA* rheumatoid arthritis, *AS* Ankylosing spondylitis

**Table 4** Changes in the cardiovascular autonomic function and peripheral sympathetic function indices after 6 and 12 weeks of therapy versus at baseline

| | | Rheumatoid arthritis ($n$=42) | | Ankylosing spondylitis ($n$=18) | |
|---|---|---|---|---|---|
| | | DMARD naive ($n$=30) | Biologic naive ($n$=12) | DMARD naive ($n$=10) | Biologic naive ($n$=18) |
| HRD (bpm) | Week 0 | 13.00±5.67 | 6.16±2.32 | 12.10±6.80 | 11.88±5.41 |
| | Week 6 | 13.37±5.37 | 10.25±2.22* | 12.8±5.94 | 12.75±5.23 |
| | Week 12 | 14.20±5.46 | 15.42±3.60* | 14.20±5.5* | 17.75±4.02* |
| HRS (R-R ratio) | Week 0 | 0.96±0.08 | 0.98±0.10 | 1.08±0.24 | 1.02±0.02 |
| | Week 6 | 0.97±0.07 | 1.03±0.10* | 1.12±0.22 | 1.07±0.05 |
| | Week 12 | 1.06±0.17* | 1.09±0.10* | 1.15±0.21* | 1.13±0.08* |
| HRV (R-R ratio) | Week 0 | 1.23±0.09 | 1.28±0.12 | 1.28±0.18 | 1.28±0.10 |
| | Week 6 | 1.22±0.10 | 1.30±0.07 | 1.37±0.18 | 1.28±0.08 |
| | Week 12 | 1.24±0.10* | 1.34±0.06* | 1.36±0.17 | 1.31±0.10 |
| BPS (mmHg) | Week 0 | 5.26±3.08 | 5.0±2.48 | 4.40±2.33 | 3.62±1.68 |
| | Week 6 | 5.90±3.58 | 3.7±2.1 | 5.2±2.90 | 4.0±2.39 |
| | Week 12 | 6.0±2.87 | 3.0±1.0 | 4.80±2.70 | 3.75±1.66 |
| BPH (mmHg) | Week 0 | 15.20±4.28 | 12.83±5.28 | 16.40±3.23 | 12.50±4.75 |
| | Week 6 | 15.43±3.52 | 15. .17±3.4 | 16.60±3.27 | 15.25±2.81 |
| | Week 12 | 15.90±3.86 | 16.50±2.3* | 17.40±2.50 | 17.75±1.66* |
| Sudoscan (μS) | Week 0 | 62.60±13.5 | 58.9±12 | 65.65±7.2 | 65.19±12.8 |
| | Week 6 | 62.77±13.2 | 62.6±8.5* | 72.7±9.5* | 73.25±7.66* |
| | Week 12 | 63.68±13.0* | 67.5±6.6* | 75.45±8.78* | 77.7±5.48* |

Values are shown as mean±SD. All treatment efficacy comparison was compared after specified intervals

*HRD* heart rate (HR) response to deep breathing, *HRS* HR response to standing, *HRV* HR response to Valsalva, *BPS* blood pressure (BP) response to standing, *BPH* BP response to hand grip, *DMARD* disease-modifying anti-rheumatic drug

*$p<0.05$ versus week 0

Rheumatoid arthritis

In this study, DMARD- or biologic-naive RA patients had significantly impaired cardiovascular autonomic and sudomotor function as compared to healthy controls. A total of 78.5 % of DMARD- or biologic-naive RA patients had impaired cardiovascular autonomic function, and sudomotor function was impaired in 35 % of DMARD- or biologic-naive

**Table 5** Multiple linear regression analysis at baseline and after treatment with csDMARDs and biologic DMARDs in RA patients

| | HRD RAD RAB | HRS RAD RAB | HRV RAD RAB | BPS RAD RAB | BPH RAD RAB | Sudoscan RAD RAB |
|---|---|---|---|---|---|---|
| DD | −0.29* 0.14* | −0.002 0.005 | −0.001 0.007 | 0.009−0.16 | −0.06 0.38 | −0.21−0.49 |
| | −0.31 0.07 | −0.002−0.002 | −0.02 0.006 | −0.003 0.10* | −0.03−0.02 | −0.26 0.70 |
| DS | 0.24 1.97* | 0.01−0.02 | −0.02 0.06* | 0.16 0.48 | 0.54−0.39 | −3.91 9.79* |
| | −1.29−1.03 | 0.03−0.01 | 0.11 0.013 | −0.08 0.15 | −0.51−0.64 | 0.89 5.83 |
| ESR | −0.19 0.04* | −0.001−0.002* | 0.003−0.002* | 0.16* 0.009 | −0.17−0.03 | 0.35 0.02 |
| | −0.05 0.01 | −0.007−0.001 | 0.03 0.008* | −0.01 0.022* | 0.14−0.03 | 0.26−0.02 |
| CRP | 0.16* −0.02 | 0.001 0.006 | 0.001−0.007* | −0.004−0.17* | 0.02 0.37* | −0.32* −0.11 |
| | 0.15 0.06 | 0.003 0.003 | 0.04−0.005 | 0.01−0.06* | −0.04 0.18 | −0.41 0.03 |
| Sudoscan | −0.57 4.01* | 39.28−79.8 | 33.99−31.1 | −0.60−8.30* | −0.14−4.53 | – |
| | −0.60 0.06 | 12.47 0.44 | 37.71 32.3 | −0.31−3.23 | −0.47−0.63 | – |

Values are shown as regression coefficient. Multiple linear regression analysis in csDMARDs and biologic DMARDs: autonomic function variables with disease duration (DD), disease severity (DS), ESR and CRP in DMARD-naive and biologic-naive RA patients. First row of each parameter refers to baseline and second row refers to after treatment

*HRD* heart rate (HR) response to deep breathing, *HRS* HR response to standing, *HRV* heart rate response to Valsalva, *BPS* blood pressure (BP) response to standing, *BPH* BP response to handgrip, *RAD* DMARD-naive RA, *RAB* biologic-naive RA

*$p\leq0.05$

**Table 6** Multiple linear regression analysis at baseline and after treatment with csDMARDs and biologic DMARDs in AS patients

| | HRD<br>ASD ASB | HRS<br>ASD ASB | HRV<br>ASD ASB | BPS<br>ASD ASB | BPH<br>ASD ASB | Sudoscan<br>ASD ASB |
|---|---|---|---|---|---|---|
| DD | 0.51−1.30*<br>0.34 -0.96* | −0.01 0.002<br>−0.01−0.009* | 0.002−0.03*<br>−0.002−0.02* | 0.12−0.17<br>0.12−0.10 | 0.02 0.95<br>0.02−0.36 | −0.31−1.91<br>−0.46−0.04 |
| DS | 1.62* −3.04<br>1.46 0.51 | 0.02−0.008<br>0.01−0.01 | −0.001−0.02<br>−0.002−0.02 | −0.22 1.61*<br>0.01 0.22 | −0.14−2.73<br>0.01−0.79 | −0.93−6.49<br>−0.04 0.56 |
| ESR | −0.18 0.12<br>−0.18 0.15* | −0.005−0.001<br>−0.006−0.002* | −0.003 0.003*<br>−0.004 0.004 | 0.01 0.02<br>0.005−0.09 | −0.07−0.04<br>−0.03−0.06 | 0.02 0.17<br>−0.02−0.21 |
| CRP | −0.09−0.03<br>−0.11−0.04* | −0.003−0.007<br>−0.004−0.002* | −0.002 0.001<br>−0.001 0.005 | −0.03 0.03*<br>−0.001 0.07 | 0.01−0.16<br>0.05−0.03 | −0.11 0.22<br>0.03 0.08 |
| Sudoscan | −0.56−0.45<br>−0.25−0.16 | −11.53−19.5<br>16.31−8.73 | 13.57 32.4<br>−1.33−12.16 | −1.73−6.39<br>−3.19−1.99 | 0.86 1.71<br>1.14 0.30 | – – |

Values are shown as regression coefficient. Multiple linear regression analysis in csDMARDs and biologic DMARDs: autonomic function variables with disease duration (DD), disease severity (DS), ESR and CRP in DMARDs naive and biologic-naive AS patients. First row of each parameter refers to baseline and second row refers to after treatment

*HRD* heart rate (HR) response to deep breathing, *HRS* HR response to standing, *HRV* heart rate response to Valsalva, *BPS* blood pressure (BP) response to standing, *BPH* BP response to handgrip, *ASD* DMARD-naive AS patient, *ASB* biologic-naive AS patient

*$p \leq 0.05$

RA patients. Previous studies have demonstrated impaired cardiovascular autonomic tests in 61–85 % of RA patients [2, 11, 12]. In the present study, biologic-naive RA patients had greater prevalence of autonomic neuropathy as compared to the DMARD-naive RA patients. This may be because biologic-naive RA patients had a longer disease duration and a more severe disease since they had inadequate response to synthetic DMARDs. Consistent with these observations, CAN correlates with disease duration, disease severity, and inflammatory markers.

Treatment with synthetic DMARDs over 12 weeks is associated with a significant, albeit small, improvement in HRD, HRS, and sudomotor function, suggesting improvement in parasympathetic and peripheral sympathetic dysfunction. After treatment with biologic DMARDs for 12 weeks, there was significant improvements in HRD, HRS, HRV, BPH, and sudomotor function, suggesting improvement in parasympathetic, sympathetic, and peripheral sympathetic dysfunction. TNFi infliximab (3 mg/kg/infusion) has recently been shown to improve both sympathetic and parasympathetic cardiovascular autonomic dysfunction in a patient with seropositive RA after 6 weeks of treatment [13]. In another case study, a 61-year-old seropositive RA female patient with severe disease activity and parasympathetic autonomic dysfunction, there was a rapid improvement with normalization of autonomic dysfunction after 12-week treatment with interleukin (IL)-6 receptor inhibitor, tocilizumab (8 mg/kg/infusion) [14].

We assume that the improvement in autonomic function in the biologic-naive RA patients may have resulted from the anti-inflammatory and immunological effects of biologic DMARDs on the cholinergic anti-inflammatory pathway via the vagus nerve. A recent study has shown that autonomic dysfunction in RA reflected in low vagus activity related to increased level of IL-1β [15]. Importantly, there is also a negative correlation between depressed levels of vagus nerve activity and elevated levels of the proinflammatory cytokines in patients with RA [16] with reduced control of systemic inflammation which may further impair the disease [15]. This proposal is also supported by the observation that heart rate variability, a marker of vagus nerve tone, is inversely related to levels of inflammatory markers (IL-6 and CRP) in the Coronary Artery Risk Development in Young Adults (CARDIA) study of the evolution of risk factors in young adults [17]. A recent study by David et al. also showed that cardiac autonomic imbalance correlates with IL-6 concentrations in newly diagnosed diabetic patients [18]. Circulating level of TNF has been described as an independent predictor of depressed heart rate variability [19]. Furthermore, a recent study has demonstrated that heart rate variability predicts anti-TNF therapy response in RA patients [20].

The improvement in autonomic dysfunction in synthetic DMARD-naive RA patients is likely to be related to inhibition of proinflammatory cytokines and the anti-inflammatory effects of these drugs. Methotrexate has been shown to decrease secretion of IL-1 and IL-6 in RA [21]. Hydroxychloroquine decreases the IL-6 levels in inflammatory arthritis [22]. Leflunomide also exerts anticytokine (TNF-α, IL-1β, IL-6) properties through its active metabolite A77 1726 in human RA synoviocytes [23].

The findings of the present prospective pilot study in biologic- and DMARD-naive RA patients have revealed that parasympathetic autonomic dysfunction is more prominent than sympathetic autonomic dysfunction. After treatment, the improvement was also greater in parasympathetic dysfunction. Results from earlier research also suggest predominance of parasympathetic dysfunction in RA, PsA, and

diabetes [6, 24, 25]. This may be due to reduced vagus nerve activity because reduced vagus nerve activity has been demonstrated in chronic inflammatory diseases including SLE and RA [15, 26]. The vagus nerve is the major parasympathetic division of the autonomic nervous system, and 75 % of all parasympathetic activity is regulated by the vagus nerve [27].

In the present study, seropositive RA patients appear to have greater likelihood of parasympathetic, sympathetic, and peripheral sympathetic dysfunction than seronegative patients. Sandhu et al., earlier, have shown that seropositive RA patients have greater tendency to impairment of parasympathetic autonomic cardiovascular reflexes than the seronegative patients [28]. The influence of seropositive status on peripheral sympathetic dysfunction in RA has not yet been reported. The response of CAN to treatment with DMARDs was also better in seropositive patients.

In RA patients, disease duration and disease severity (DAS28) correlated with a number of variables of autonomic neuropathy before treatment in the present study. This suggests that a longer and more severe disease was more likely to result in impaired autonomic function. Biomarkers of inflammation (ESR and CRP) correlated with variables of autonomic neuropathy before and after biologic treatment, suggesting that these may predict the occurrence of autonomic neuropathy and response to treatment especially with biologics. Sudomotor function correlates with both sympathetic and parasympathetic dysfunction in biologic-naive RA patients. The situation in DMARD-naive RA patients is in contrast to that in biologic-naive RA patients. There is an association between sudomotor function and autonomic function in the latter but not in the former. It is possible that this may be due to association of sudomotor dysfunction with a more severe disease.

## Ankylosing spondylitis

Our study results demonstrated significant differences in values of cardiovascular reflexes and sudomotor function between patients with AS and healthy control subjects. biologic-naive AS patients had both sympathetic and parasympathetic neuropathy. However, csDMARD-naive patients had predominance of parasympathetic dysfunction. Autonomic neuropathy was identified in 77.7 % of biologic- and treatment-naive patients, and sudomotor dysfunction was identified in 27 % of biologic- and treatment-naive patients. Earlier research has shown 60 % cardiovascular autonomic neuropathy in AS patients [29]. In the present study, biologic-naive AS patients had greater impairment of autonomic neuropathy as compared to the DMARD-naive AS patients (Tables 1 and 3). This may be because biologic-naive AS patients had a more active disease (BASDAI score ≥4.0). An earlier study by Toussirot and colleagues has shown decreased parasympathetic activity in AS patients with more active disease (BASDAI score >5) [6]. Another recent study has shown that parasympathetic system is affected in AS whereas sympathetic system is affected when the disease activity increases [3].

Treatments with synthetic DMARDs over 12 weeks have shown significant improvement in HRD, HRS, and sudomotor function, suggesting improvement in parasympathetic dysfunction and peripheral sympathetic dysfunction. After treatment with biologic DMARDs, there was significant improvements in HRD, HRS, BPH, and sudomotor function, suggesting improvement in parasympathetic, sympathetic, and peripheral sympathetic dysfunction. TNFi infliximab (5 mg/kg/infusion) has been shown to improve both CAN (parasympathetic and sympathetic) and sudomotor dysfunction after 6-week treatment in an AS patient with severe autonomic dysfunction [30].

We assume that the improvement in autonomic neuropathy in biologic-naive AS patients has been modulated through cholinergic anti-inflammatory pathway via the vagus nerve. Experimental stimulation of the vagus nerve caused decreased TNF synthesis in the liver, spleen, and heart. After vagotomy, animals showed an exaggerated TNF response to an inflammatory stimulus [31–33]. These results suggest that individuals may develop exaggerated immune response due to dysfunction of cholinergic anti-inflammatory pathway. This concept has been demonstrated by the observation that HRV, a marker of vagus nerve tone, is inversely related to levels of inflammatory markers (IL-6 and CRP) in the CARDIA study [17]. This concept is also supported in the literature by demonstrating impaired autonomic function in inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus, and diabetes [15, 16, 25–27].

Sulfasalazine in AS has anti-inflammatory and immunomodulatory effects through inhibition of the production of TNF-α, IL-1, and IL-6 [34, 35]. The anticytokine and anti-inflammatory effects of DMARDs possibly influence the autonomic nervous system predominantly through the vagus nerve which improves the autonomic neuropathy and modulates autonomic disease expression through the cholinergic anti-inflammatory pathway.

In our study, we found that parasympathetic dysfunction is more pronounced in both biologic- and treatment-naive AS patients. This indicates that sympathetic dysfunction is only apparent when the disease is more active. However, sympathetic autonomic dysfunction was also observed in biologic-naive AS patients. After treatment, the improvement was also greater in parasympathetic dysfunction in naive AS patients. Similar to our study, Borman et al. demonstrated presence of parasympathetic dysfunction (heart rate variation with deep breathing, HRS, and R-R interval variation) in AS patients with high disease activity [36].

Disease duration and disease severity correlate with parasympathetic and sympathetic dysfunction before and after

treatment in biologic-naive AS patients. Disease severity (BASDAI) also correlates with parasympathetic dysfunction before treatment in naive AS patients. Biomarkers of inflammation (ESR and CRP) correlate with a number of CAN variables in biologic-naive AS patients before and after treatment, suggesting that in AS, also ESR and CRP these may predict the occurrence of CAN and response to treatment especially with biologics. Earlier studies on autonomic neuropathy in AS patients have shown a positive correlation between parasympathetic indicators and disease activity (BASDAI) and biomarkers of inflammation (ESR and CRP) [6, 36].

### Study strength and limitation

The strength of this study is that it is the first study to assess impact of both synthetic and biologic DMARDs on indicators of autonomic neuropathy. This study also highlights the risk factors for autonomic neuropathy and predictors of response to therapy. All consecutive naive patients initiating a DMARD for RA and AS were included to avoid selection bias. As with any exploratory effort, this pilot study had its limitations. Inflammatory cytokines were not estimated in this study. Nevertheless, this study does provide important information on autonomic neuropathy in RA and AS. This study was not designed to identify which elements of intensive DMARD therapy contributed most to the reduction in autonomic neuropathy.

In conclusion, parasympathetic autonomic dysfunction is more prominent than sympathetic dysfunction in both DMARD- and biologic-naive RA and AS patients. Sympathetic dysfunction is apparent when the disease is more active. Autonomic function is impaired to a greater extent in seropositive RA patients, and they had better response to synthetic as well as biologic DMARDs as compared to the seronegative RA patients. Biologic DMARDs significantly improved parasympathetic, sympathetic, and sudomotor function, while synthetic DMARDs improved parasympathetic and sudomotor function in both RA and AS. Disease duration, disease severity, and biomarkers of inflammation are potential risk factors for autonomic neuropathy in RA and AS. ESR, CRP, and seropositivity may help in predicting response to treatment.

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**Conflict of interest** None.

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