EBioMedicine 6 (2016) 231–237

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Autonomic Dysfunction Precedes Development of Rheumatoid Arthritis: A Prospective Cohort Study

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ARTICLE INFO

Article history:
Received 16 December 2015
Received in revised form 2 February 2016
Accepted 16 February 2016
Available online 19 February 2016

Keywords:
Heart rate variability
RA
Autonomic nervous system
Norepinephrine
Heart rate
Preclinical

ABSTRACT

Background: Heart rate variability (HRV) is a validated method to establish autonomic nervous system (ANS) activity. Rheumatoid arthritis (RA) is accompanied by ANS imbalance. We hypothesized that ANS dysfunction may precede the development of RA, which would suggest that it plays a role in its etiopathogenesis.
Methods: First, we assessed HRV parameters in supine (resting) and upright (active) position in healthy subjects (HS, n = 20), individuals at risk of developing arthritis (AR subjects, n = 50) and RA patients (RA, n = 20). Next, we measured resting heart rate (RHR), a parasympathetic HRV parameter, in an independent prospective cohort of AR subjects (n = 45). We also evaluated expression levels of the parasympathetic nicotinic acetylcholine receptor type 7 (α7nAChR) on circulating monocytes.
Findings: Both AR subjects (68 beats per minute (bpm), interquartile range (IQR) 68–73) and RA patients (68 bpm, IQR 62–76) had a significantly higher RHR compared to HS (60 bpm, IQR 56–63). RHR was significantly higher at baseline in individuals who subsequently developed arthritis. Expression levels of α7nAChR were lower in AR subjects with RHR ≥ 70 bpm compared to those with RHR < 70 bpm, consistent with reduced activity of the parasympathetic cholinergic anti-inflammatory pathway.
Interpretation: These data support the notion that autonomic dysfunction precedes the development of RA.

1. Introduction

Autonomic imbalance, defined by increased heart rate (HR) and decreased heart rate variability (HRV), is associated with increased morbidity and mortality in patients with various diseases (Inoue et al., 2013). Autonomic imbalance has been demonstrated in autoimmune diseases like rheumatoid arthritis (RA), systemic lupus erythematosus, ankylosing spondylitis and inflammatory bowel disease (Aydemir et al., 2010; Borman et al., 2008; Sharma et al., 2009), where it has traditionally been considered a result of chronic inflammation. In RA, a prototypic immune-mediated inflammatory disease, several studies demonstrated reduced activity of the parasympathetic nervous system (PNS), whereas others found an overactive sympathetic nervous system in RA patients (Adlan et al., 2014; Koopman et al., 2011). HRV measurements were also shown to predict response to biological treatment in RA patients (Holman and Ng, 2008). These observations are relevant because activation of the PNS via stimulation of the vagus nerve and the nicotinic acetylcholine receptor type 7 (α7nAChR) can reduce inflammation, which has been termed as the cholinergic anti-inflammatory pathway (Borovikova et al., 2000; Van Maanen et al., 2009). In addition to measurement of HRV, assessment of sympathetic nervous system (SNS)-related hormones, such as epinephrine and norepinephrine, is informative about autonomic dysfunction (Igari et al., 1977; Vlcek et al., 2008).

We postulated that autonomic dysfunction may precede the development of RA, which would suggest that it plays a role in the etiopathogenesis of this disease. Previous studies in healthy individuals have shown that decreased HRV parameters are associated with development of disease, such as hypertension and diabetes, as an independent risk factor (Thayer and Lane, 2007). Individuals at risk of developing autoantibody positive RA can be identified by detection of the autoantibodies IgM-rheumatoid factor (IgM-RF) and anti-citrullinated protein antibodies (ACPA); the risk of developing arthritis is further increased by smoking, overweight and genetic factors (Klareskog et al., 2009; Gerlag et al., 2015). Not all identified individuals will develop RA, but the presence of

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http://dx.doi.org/10.1016/j.ebiom.2016.02.029

multiple serum autoantibodies increases the risk of developing RA in two years up to ~40% (Bos et al., 2010). Identification of additional risk factors is necessary to provide insight into pathogenic mechanisms contributing to the development of clinically manifest disease, and might also lead to development of preventive strategies.

To evaluate whether autonomic dysfunction might contribute to the development of RA rather than being the result of chronic inflammation, we measured HRV as well as plasma levels of (nor)epinephrine, and the expression of α7nAChR on peripheral blood monocytes in individuals at risk of developing RA, healthy subjects and RA patients. In a validation cohort, we examined the relationship between heart rate at baseline and development of RA after follow-up.

2. Materials & Methods

2.1. Study Subjects

RA patients (n = 20), healthy subjects (HS, n = 20) and individuals at risk of developing RA (AR subjects, n = 50) were included in a prospective observational study (study cohort). A sample size calculation was performed, whereby 18 subjects in the RA and HS groups would have 80% power to detect a difference in mean high frequency (HF) of 14.6 assuming a standard deviation (SD) of 15 (Evrengul et al., 2004) using a two group t-test with 0.05 two-sided significance level. We expected a smaller difference between HS and AR subjects, and RA and AR subjects, and have therefore included more AR subjects. AR subjects were defined as being positive for IgM-RF, ACPA or both, having either arthralgia, or a positive family history for RA (phase (c) + (d) or (c) + (a), but without any evidence of arthritis upon standardized physical examination according to European League Against Rheumatism (EULAR) recommendations (Gerlag et al., 2012; Scott et al., 1996). RA patients were classified according to the 2010 American College of Rheumatology and EULAR classification criteria (Aletaha et al., 2010) and had arthritis in at least one joint, despite a stable dose of methotrexate and in some patients prednisone (n = 4, median dose 6.25 mg per day, interquartile range (IQR) 5–9.4 mg per day) for at least one month. HS were negative for IgM-RF and ACPA. Individuals with a history of cardiovascular or neurological disease, diabetes, anti-hypertensive treatment, active infection or use of antibiotics in the 7 days preceding study assessments were excluded. The study was performed according to the principles of the Declaration of Helsinki, approved by the institutional review board of the Academic Medical Center (AMC), the Netherlands (reference NL34802.018.10), and all study subjects gave written informed consent.

A second, independent at risk (AR) cohort (validation cohort) was used to validate the results. This cohort was started at the Department of Clinical Immunology and Rheumatology AMC in 2005 and has been described before (de Hair et al., 2013, 2014). Similar in- and exclusion criteria were used as described above.

2.2. Study Design

In the study cohort, during one single visit demographics, anthropometric data, smoking and alcohol status, medical history, RA medication history and current medication were obtained between 8:30 and 10 am, after patients had fasted for 8 to 10 h. Continuous heart rate (HR) and blood pressure (BP) measurements were obtained non-invasively using finger arterial pressure waveform recording of the left hand by Nexfin (Edwards Lifesciences, BMEYE B.V. Amsterdam, the Netherlands), a validated method to assess HRV parameters (Rang et al., 2004; Prevoo et al., 1995). The recording session contained two parts: ten-minute period in supine (resting) position, and a ten-minute period of orthostatic stress in upright (active) position. Individuals were allowed to equilibrate to the positional change before data were collected, followed by assessment of RA disease activity (Disease Activity Score of 28 joints (DAS28) with erythrocyte sedimentation rate (ESR) (Prevoo et al., 1995)) and a blood draw.

In the validation cohort we determined vital signs at yearly visits, including resting heart rate (RHR) in sitting position, using an automated monitor system. This measurement took place in non-fasting state either in the morning or the afternoon.

2.3. Analysis of Heart Rate Variability Parameters

HRV recordings were analyzed as described before (Rang et al., 2004) according to Task Force Guidelines (Anon., 1996). A period of ≥250 consecutive heart beats without interfering signals was selected for both positions. HRV parameter analysis is categorized into time and frequency domain (TD, FD) analysis. TD parameters are: HR, BP, respiratory rate (RR), standard deviation of all beat-to-beat intervals (SDNN) and root mean square of successive differences (RMSSD). RHR and SDNN are mainly influenced by the PNS, while in upright position the SNS becomes more active. RMSSD reflects PNS activity in both positions. FD parameters of the pulse interval were determined as described before (Zhang et al., 2013) by in-house developed digital Fourier transform (DFT, Matlab, courtesy W.J. Stok, MSc). The FD parameter for PNS is HF (0.15–0.40 Hz) and of both SNS and PNS are low frequency (LF: 0.04–0.15 Hz) and LF/HF ratio. LF expressed in normalized units (nu) instead of ms^2 shows the influence of the SNS more distinctly, as it is controlled for total power and very low frequency (TP and VLF) (Anon., 1996). Furthermore, gain of the baroreceptor reflex sensitivity (BRS) was calculated from the transfer from systolic BP to heart period in the LF-band, representing ability of the cardiovascular system to counteract beat-to-beat changes in BP. Overall, lowered vagal efferent results in lower HRV parameters, except for HR, RR and LF/HF ratio which increase.

2.4. Measurement of Norepinephrine, Epinephrine and α7nAChR Expression in Peripheral Blood

Norepinephrine and epinephrine in peripheral blood could be measured in 16/20 (80%) HS, 43/50 (86%) AR subjects and 19/20 (95%) RA patients from the study cohort. Fourteen AR subjects gave informed consent for additional peripheral blood samples to measure α7nAChR expression on monocytes. Assays are described in the Methods Appendix.

2.5. Statistics

Data are presented as median with IQR. Demographic data from individuals enrolled in the study and validation cohorts were analyzed with non-parametric tests (Kruskal–Wallis test, Mann–Whitney U test) if not normally distributed or by parametric tests (analysis of variance (ANOVA), Student's t-test) if normally distributed. Categorical demographic variables were tested using Pearson's Chi-Square test. HRV parameters and SNS-related hormones were analyzed using multivariate linear regression analysis with following confounding factors: age, gender, body mass index (BMI), smoking pack years and plasma triglycerides. Confounders were chosen based on the literature (Valentini and Parati, 2009) and differences between the groups in the study cohort. The study cohort was designed to evaluate changes in AR subjects compared to RA patients and HS rather than to compare RA and HS. In the validation cohort Cox's proportional hazard regression analysis was performed to evaluate the association of RHR with arthritis development, taking follow-up time and the following potentially confounding factors into account: age, gender, BMI and smoking pack years. Plasma triglycerides were available in a subset of individuals from the validation cohort and these were not included as confounder. Medication was considered as confounding factor as well. There is no known influence of disease-modifying antirheumatic drugs (DMARDs), or prednisone on HRV parameters. Nonsteroidal anti-inflammatory drugs (NSAIDs) are known to influence the cardiovascular system (Bhala et al., 2013; Antman et al., 2007) and could therefore influence HRV. Adding NSAIDs as a confounder did not alter results of the study in either cohort, and