Impact of disease duration on proteomic bioprofile and prognosis in rheumatoid arthritis patients

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Abstract

Objective
Cardiovascular disease worsens the prognosis of rheumatoid arthritis (RA) and vice-versa. Inflammation may be a common pathway for both conditions. It is expected that a longer RA duration leads to a greater inflammatory cumulative exposure burden; however, studies on the association between RA disease duration and outcomes are scarce. Our aim is to compare the characteristics, biomarker expression and outcomes according to the duration of RA.

Methods
Prospective cohort study including 399 RA patients, with detailed clinical, echocardiographic, and proteomic phenotyping that were compared across tertiles of RA disease duration. Cox proportional models were used to study the association of disease duration with cardiovascular outcomes.

Results
RA duration tertiles were: tertile 1 with median of 3.2; tertile 2 with median of 8.8; and tertile 3 with median of 21.8 years. Compared to tertile 1, patients in tertile 3 were older, had more erosive disease, more frequent echocardiographic alterations, lower haemoglobin and walked a shorter distance on the 6MWT. Natriuretic peptides, cathepsin L1, galectin 9, matrix metalloproteinase-12, adrenomedullin and tumour necrosis factor receptor 11A were higher in patients with longer disease duration. Compared to patients in tertile 1, those in tertile 3 had higher risk of a subsequent cardiovascular hospitalisation or cardiovascular death (HR 2.71, 95%C1 1.06-6.92, p=0.04).

Conclusion
RA patients with longer disease duration had more organ damage and worse outcomes than those with shorter disease duration. Biomarker expression suggested that patients with longer RA duration had activation of pathways related to inflammation, extracellular matrix organisation, fibrosis and congestion.

Key words
rheumatoid arthritis, disease duration, prognosis, biomarkers
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Introduction
Cardiovascular disease (CVD) in rheumatoid arthritis (RA) is being better characterised in the last decades,  
although many patients with RA and concomitant CVD remain unidentified and undertreated (1).  
The prognosis of RA and CVD patients is worsened when both conditions are present, and it has  
been hypothesised that inflammation is a common pathway.  
High disease activity, linked to a higher inflammatory status, is often identified as the prominent feature  
of CVD among RA patients (2-6).  
Considerations about the pathophysiology and clinical course of RA are  
important.  
In early disease, autoantibodies and inflammation are predominant (7).  
In the long run, the damage accrual and chronic inflammation, may lead  
to organ fibrosis and a poor prognosis (1-3, 10).  
It is expected that a longer RA duration leads to a greater inflammatory  
cumulative exposure burden,  
but in which way this can affect CVD is still subject of discussion.  
The available data are scarce and contradictory,  
with some authors advocating that the duration of the disease does not affect  
the risk and prognosis of CVD (3, 11, 12).  
However, it is clinically plausible that a longer RA duration is associated  
with a poorer cardiovascular prognosis both because of the longer inflammatory-fibrotic burden and  
the use of older medications including corticosteroids.  
In this regard, circulating biomarkers could be a way to better understand the  
biological differences according to RA duration and the link between RA duration  
and the risk of CVD.  
To help clarify the link between RA disease duration and CVD, in the present  
study, we aim to compare the characteristics, proteomic expression and cardiac  
objective outcomes according to the duration of RA.

Methods
Study population
This prospective single-centre cohort study includes consecutive patients  
with RA aged 18 years or older,  
followed in the Autoimmune Disease Unit of a Portuguese University Hospital  
(Centro Hospitalar Universitário do Porto), prospectively enrolled from  
June 2016 to June 2018 (ClinicalTrials.gov identifier: NCT03960515).  
RA was diagnosed based on the 2010  
ACR/EULAR Classification criteria.  
Patients who presented active neoplasm or had a short life expectancy  
(<6 months), severe dementia, or severe fragility (inability to walk or totally  
dependent on a third person) were excluded.  
The cause of death and cardiovascular hospitalisation (as heart failure, myocardial infarction, stroke,  
or transient ischaemic attack) was prospectively collected and independently adjudicated.  
The main outcome was a composite of time-to-first of myocardial infarction, stroke, heart failure or  
cardiovascular death.  
This study was conducted following the principles of the Declaration of Helsinki and approved by the hospital  
ethics committee under number 2016-023 (020-DEFI/020-CES).  
All patients signed written informed consent prior to entry into the study.  
An independent external data cleaning, consolidation, and verification to ensure  
data accuracy was performed.

Patient evaluation, echocardiogram, and routine laboratory tests
We prospectively collected medical history, physical examination, treatments,  
and a RA-specific questionnaire.  
A six-minute walking test (6MWT) was performed according to the American  
Thoracic Society guidelines (5).  
Echocardiogram was performed by an experienced echocardiographer, blinded  
to clinical data, following international recommendations (6).  
Routine blood laboratory tests were collected.  
C-reactive protein (CRP) was measured by the enzyme-linked immunosorbent assay (Olympus CRP  
Latex Calibrator Normal Set®), high  
sensitivity troponin T (hs-TnT) by the  
Elecsys (Roche Diagnostics®), and  
N-terminal-pro b-type natriuretic peptide (NT-pro BNP) by Gen 5 STAT test  
(Roche Diagnostics®).

Plasma Olink® biomarkers
We measured a protein biomarker panel (Olink® CVDFI panel) that comprised 92 biomarkers from a wide  
range of pathophysiological domains.
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### Table I. Baseline characteristics of the patients by tertiles of disease duration.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Disease duration (tertiles), years</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tertile 1 (3.2, 1.3, 4.5)</td>
<td>Tertile 2 (8.8, 6.9, 11.1)</td>
</tr>
<tr>
<td>n=399</td>
<td>133</td>
<td>133</td>
</tr>
<tr>
<td>Age (years), median (IQR)</td>
<td>56.0 (46.0, 65.0)</td>
<td>61.0 (50.0, 68.0)</td>
</tr>
<tr>
<td>Age at diagnosis, median (IQR)</td>
<td>52.4 (42.6, 62.2)</td>
<td>51.5 (40.6, 59.5)</td>
</tr>
<tr>
<td>Male</td>
<td>32 (24.1%)</td>
<td>37 (27.8%)</td>
</tr>
<tr>
<td>BMI (kg/m²), median (IQR)</td>
<td>26.0 (23.8, 29.5)</td>
<td>26.4 (23.9, 29.7)</td>
</tr>
<tr>
<td>WC (cm), median (IQR)</td>
<td>90.0 (80.0, 99.0)</td>
<td>91.0 (83.0, 103.0)</td>
</tr>
</tbody>
</table>

**Comorbidities and RA history**

**Diabetes mellitus** | 19 (14.3%) | 22 (16.5%) | 12 (9.0%) | 0.18 |
**Dyslipidaemia** | 53 (39.8%) | 65 (48.9%) | 72 (54.1%) | 0.062 |
**Previous cardiovascular disease** | 8 (6.0%) | 16 (12.0%) | 15 (11.3%) | 0.20 |
| **Articular erosions** | 34 (27.0%) | 45 (34.9%) | 55 (41.7%) | 0.046 |

**Symptoms and physical exam**

**Dyspnea or fatigue** | 49 (36.8%) | 39 (29.3%) | 31 (23.3%) | 0.054 |
**SBP (mmHg), median (IQR)** | 133.0 (114.0, 146.0) | 133.0 (123.0, 144.0) | 132.0 (122.0, 144.0) | 0.70 |
**DBP (mmHg), median (IQR)** | 76.0 (68.0, 84.0) | 77.0 (68.0, 82.0) | 76.0 (69.0, 82.0) | 0.93 |
**Heart rate (bpm), median (IQR)** | 77.0 (70.0, 86.0) | 79.0 (72.0, 89.0) | 78.0 (70.0, 89.0) | 0.39 |
**Dyspnea or fatigue** | 2.5 (2.0, 3.8) | 2.6 (2.0, 3.4) | 2.6 (2.1, 3.2) | 0.84 |
**Dyspnea or fatigue** | 21 (16.7%) | 22 (17.6%) | 22 (15.7%) | 0.006 |

**Echocardiogram and laboratorial data**

**LVEF, %** | 62.0 (56.3, 66.0) | 59.5 (56.0, 64.0) | 62.0 (56.0, 66.0) | 0.079 |
**Echocardiogram alterations**<sup>a</sup> | 95 (73.1%) | 94 (79.7%) | 101 (87.8%) | 0.016 |
| **LVH** | 12 (9.3%) | 10 (8.7%) | 14 (12.4%) | 0.61 |
| **LAVi >34 ml/m²** | 41 (31.5%) | 42 (36.2%) | 46 (40.0%) | 0.38 |
| **E/e’ >13** | 15 (11.6%) | 11 (9.4%) | 16 (14.2%) | 0.53 |
| **Lateral or septal e’ >9** | 87 (67.4%) | 83 (70.9%) | 96 (85.0%) | 0.005 |
| **Haemoglobin, g/dL** | 13.2 (12.4, 14.0) | 13.4 (12.4, 14.5) | 12.9 (11.9, 13.9) | 0.014 |
| **Anaemia** | 23 (17.6%) | 20 (15.2%) | 24 (26.0%) | 0.068 |
| **eGFR, ml/min/1.73m²** | 92.6 (77.0, 106.0) | 91.0 (75.0, 102.0) | 89.0 (73.0, 100.4) | 0.21 |
| **eGFR <60 ml/min** | 6 (4.5%) | 11 (8.3%) | 17 (12.8%) | 0.054 |
| **NT-pro BNP, pg/mL** | 67.6 (34.5, 127.2) | 85.6 (43.6, 168.0) | 106.5 (57.7, 203.9) | <0.001 |
| **NT-pro BNP >125 pg/mL** | 35 (26.5%) | 41 (32.3%) | 59 (45.4%) | 0.005 |
| **Troponin T >14 pg/mL** | 9 (7.0%) | 13 (10.2%) | 20 (15.0%) | 0.11 |
| **CRP, mg/dL** | 2.3 (1.2, 7.3) | 3.0 (1.2, 8.3) | 3.9 (1.3, 8.1) | 0.46 |

**Medications**

| ACEi or ARBs | 45 (33.8%) | 43 (32.3%) | 64 (48.1%) | 0.014 |
| Beta-blockers | 10 (7.5%) | 15 (11.3%) | 18 (13.5%) | 0.28 |
| Loop diuretics | 4 (3.0%) | 1 (0.8%) | 5 (3.8%) | 0.26 |
| Statins | 37 (27.8%) | 51 (38.3%) | 62 (46.6%) | 0.007 |
| Corticosteroids | 55 (41.4%) | 57 (42.9%) | 69 (51.9%) | 0.18 |
| Methotrexate | 93 (70.5%) | 78 (58.6%) | 77 (58.3%) | 0.068 |
| NSAIDs | 21 (15.8%) | 35 (26.3%) | 45 (33.8%) | 0.003 |
| “Biologics” | 11 (8.3%) | 37 (27.8%) | 27 (20.3%) | <0.001 |
| 6MWT total distance, m | 405.0 (360.0, 440.0) | 375.0 (330.0, 425.0) | 360.0 (260.0, 390.0) | <0.001 |

**a**Previous cardiovascular disease include: myocardial infarction or angina pectoris or stroke or valvular heart disease or atrial fibrillation; <sup>b</sup> echocardiographic alterations defined by a left atrial volume indexed >34 ml/m² or left ventricular hypertrophy or E/e’ >13 or septal or lateral e’ >9; BMI: body mass index; WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; LVEF: left ventricular ejection fraction; LVH: left ventricular hypertrophy; LAVi: left atrial volume indexed; RA: rheumatoid arthritis; DAS28 with ESR: Disease Activity Score 28 with erythrocyte sedimentation rate; eGFR: estimated glomerular filtration rate; NT-pro BNP: N-terminal pro brain natriuretic peptide; CRP: C-reactive protein; ACEi or ARBs: angiotensin converting enzyme inhibitors or angiotensin receptor blockers; NSAIDs: non-steroidal anti-inflammatory drugs; 6MWT: 6-minute walking test.

The Olink® CVDII panel was purposefully selected because it contains several known human circulating proteins associated with cardiovascular and inflammatory diseases. The overview of the 92 circulating proteins is depicted in Supplementary Table S1, including their full names and Uniprot ID. In Supplementary Table S2, the proteins are described according to their main biological functions. The CVDII Olink® panel displayed a mean intra-assay and inter-assay coefficient of variation between 9.1% and 11.7%.

**Statistical analysis**

Sample description and comparison of patients according disease duration and tertiles was performed using Chi-square and Kruskal-Wallis tests, as appropriate. Continuous variables are presented as mean and standard deviation (if normally distributed) or median and 25-75% quartiles (if non-normally distributed). All variables with a p-value of less than 0.05 in the univariable analysis (Table I) were entered in a stepwise (forward) model with a p-value of less than 0.05 set for a variable to enter and stay in the final model. Ordered logistic
regression was used to study the circulating proteins across tertiles of RA duration. Cox proportional hazard regression models and Kaplan-Meier curves were used to study the association of disease duration with cardiovascular outcomes. All analyses were performed using STATA® (StataCorp. 2019. Stata Statistical Software: Release 16.1. College Station, TX: StataCorp LP). A p-value < 0.05 was considered statistically significant.

**Results**

**Baseline characteristics of the patients by tertiles according to disease duration**

A total of 399 patients with RA were included, with 133 patients included in each RA duration tertile. The median (percentile25–75) RA duration in years for each tertile was: 3.2 (1.3, 4.5) in tertile 1; 8.8 (6.9, 11.1) in tertile 2; and 21.8 (17.5, 29.6) in tertile 3.

The baseline characteristics comparing the RA duration tertiles are shown in Table I. Compared to patients with a shorter disease duration (tertile 1), those with a longer disease duration (tertile 3) were older, had more erosive and active disease, more echocardiographic alterations, lower hemoglobin and walked a shorter distance on the 6MWT. Tertile 3 had a higher proportion of patients with a NT-pro BNP above 125 pg/mL and were treated more frequently with ACEi/ARBs, statins and NSAIDS.

**Association between proteomic biomarkers and disease duration**

Of the 94 proteomic biomarkers studied, 7 increased their concentrations in plasma as disease got longer with a false discovery rate (FDR) correction < 5%: natriuretic peptides, cathepsin L1 (CTSL1), galectin 9; BNP: B-type natriuretic peptide; MMP12: matrix metalloproteinase-12; ADM: adrenomedullin; TNFRSF11A: tumour necrosis factor receptor superfamily member 11A. Table II and Suppl. Table S3).

**Association of circulating proteins with rheumatoid arthritis duration.**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Beta (95%CI)</th>
<th>p-value*</th>
<th>FDR5*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT-pro BNP (log)</td>
<td>0.32 (0.15-0.50)</td>
<td>&lt;0.001</td>
<td>0.026</td>
</tr>
<tr>
<td>CTSI1</td>
<td>0.70 (0.29-1.11)</td>
<td>0.001</td>
<td>0.026</td>
</tr>
<tr>
<td>GaI9</td>
<td>0.93 (0.38-1.49)</td>
<td>0.001</td>
<td>0.026</td>
</tr>
<tr>
<td>BNP</td>
<td>0.27 (0.11-0.43)</td>
<td>0.002</td>
<td>0.040</td>
</tr>
<tr>
<td>MMP12</td>
<td>0.37 (0.13-0.60)</td>
<td>0.002</td>
<td>0.040</td>
</tr>
<tr>
<td>ADM</td>
<td>0.62 (0.22-1.03)</td>
<td>0.003</td>
<td>0.040</td>
</tr>
<tr>
<td>TNFRSF11A</td>
<td>0.58 (0.19-0.98)</td>
<td>0.004</td>
<td>0.048</td>
</tr>
</tbody>
</table>

*All the biomarkers had a lower than 5% false discovery rate (FDR) after adjustment (see the methods section for details).

**Fig. 1. Cardiovascular outcome associations by duration of rheumatoid arthritis.**

Tertiles of rheumatoid arthritis duration: Tertile 1: 3.2 (1.3, 4.5) years; Tertile 2: 8.8 (6.9, 11.1) years; Tertile 3: 21.8 (17.5, 29.6) years.

The outcome was a composite of myocardial infarction, stroke, heart failure or cardiovascular death. Over a maximum follow-up time of 3 years, a total of 41 cardiovascular events (i.e. a composite of myocardial infarction, stroke, heart failure or cardiovascular death) occurred, and patients with longer RA duration had the highest risk of cardiovascular events with 8/133 (6.0%) events in tertile 1, 13/133 (9.8%) events in tertile 2, and 20/133 (15.0%) events in tertile 3: HR (95%CI) 2.71 (1.06–6.92), p=0.037 for tertile 3 vs. tertile 1, and HR (95%CI) 2.07 (0.77–5.51), p=0.15 for tertile 2 vs. tertile 1, adjusted for age, sex, estimated glomerular filtration rate and diabetes.

**Discussion**

In this study we identified groups of RA patients, based on their disease duration, with different clinical and echocardiographic characteristics, proteom-
ic expression, and prognosis. Patients with a longer disease duration (tertile 3) had more signs of cardiac dysfunction (echocardiographic alterations and higher pro-BNP value) and worst functional capacity. The proteomic associations with longer RA duration, suggest that patients with long-standing disease express higher levels of markers related to congestion (e.g., NT-pro BNP and ADM), inflammation (e.g., CTSL1 and TNFRSF11A), and fibrosis (e.g., Gal9 and MMP2); findings that support the hypothesis that longer RA duration is associated with greater cardiovascular and joint damage.

Despite having an average of 20 years of RA diagnosis, the median age of patients with longer RA duration (i.e., tertile 3) was 60 years old, i.e., a relatively young patient group in whom the impact of the disease may be important. Moreover, our data suggest that the duration of RA is an important risk factor for CVD, because RA patients with longer disease duration (tertile 3) had a 2.7-fold higher risk of a subsequent cardiovascular event compared to patients with a shorter RA duration (tertile 1). Our findings suggest a much stronger association for disease duration than the simple consequences of aging, possibly due to the cumulative damage driven by inflammation and fibrosis. This is supported by the proteomic expression, suggesting that patients with longer RA duration had activation of pathways related to congestion, extracellular matrix organisation, fibrosis, and inflammation.

Patients with longer RA duration (i.e., tertile 3) had higher levels of proteins known to be involved in the progression of RA, pannus formation and bone and cartilage erosion, such as ADM (7), MMP12 (8) or CTSL1 (9). Most of these protein-biomarkers have also been associated with an increased risk of all-cause of death and cardiovascular hospitalisation (10-13). The higher expression of NT-pro BNP suggests that cardiac and vascular dysfunction with increased filling pressures in the heart also play a role in disease progression and enhanced cardiovascular risk, as NT-pro BNP is the single strongest prognosticator of adverse cardiovascular outcomes across multiple populations, including RA (11, 14). The higher expression of TNF receptor family proteins with longer RA duration supports a central role of inflammation that ultimately may lead to fibrosis and permanent organ dysfunction.

Together, these findings may pave the way for new studies aiming to develop personalised treatments depending on the duration of the disease, namely drugs with a safer cardiovascular profile for long standing RA patients targeting congestion, inflammation and fibrosis.

Limitations

The design of this study was inherently observational, thus, causality cannot be inferred. Furthermore, our cohort came from a single centre and some of the findings may reflect specific socio-demographic characteristics and local practice patterns which may limit the generalisation of the findings. Finally, in our study, we do not know the evolution and changes of disease activity over the years, which may have had an impact on the results.

Conclusion

RA duration was associated with a higher risk of CVD and a worse prognosis. Compared to patients with shorter RA duration, those with longer disease expressed higher level of proteins related to inflammation, extracellular matrix organisation, fibrosis and congestion.

References