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

M. Brandão[1,2], T. Fonseca[2], R. Quelhas Costa[3], J.C. Oliveira[1,4], H. Cyrne Carvalho[1,5,6], P. Rodrigues[1,5], F. Zannad[7], P. Rossignol[7,8], M.B. Ferreira[1,9], J.P. Ferreira[7,10]

[1]Unidade Multidisciplinar de Investigação Biomédica, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal; [2]Unidade de Imunologia Clínica, Centro Hospitalar Universitário do Porto, Porto, Portugal; [3]Department of Internal Medicine, Centro Hospitalar de Entre o Douro e Vouga, Aveiro, Portugal; [4]Clinical Chemistry Service, Centro Hospitalar de Entre o Douro e Vouga, Aveiro, Portugal; [5]Cardiology Department Centro Hospitalar Universitário do Porto, Portugal; [6]Instituto de Ciências Biomédicas Abel Salazar, Porto, Portugal; [7]Université de Lorraine, INSERM, Centre d'Investigations Cliniques Plurithématique 1433, CHRU de Nancy, Inserm U1116 F-CRIN INI-CRCT (Cardiovascular and Renal Clinical Trialists), Nancy, France; [8]Department of Medical Specialties and Nephrology-Haemodialysis, Princess Grace Hospital, Monaco, and Centre d'Hémodialyse Privé de Monaco; [9]Hospital da Luz Arrábida, Porto, Portugal; [10]UnIC@RISE, Department of Surgery and Physiology, Faculty of Medicine of the University of Porto, Portugal.

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%CI 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

*Mariana Brandão, MD**
*Tomás Fonseca, MD**
Rita Quelhas Costa, MD
José Carlos Oliveira, MD
Henrique Cyrne Carvalho, MD, PhD
Patrícia Rodrigues, MD, PhD
Faiez Zannad, MD, PhD
Patrick Rossignol, MD, PhD
*Maria Betânia Ferreira, MD, PhD***
*João Pedro Ferreira, MD, PhD***

**Co-first authors*
***Co-last authors*

Please address correspondence to:
Mariana Brandão
Centro Hospitalar Universitário do Porto,
Largo do Prof. Abel Salazar,
4099-001 Porto, Portugal.
E-mail:
marianabrandao.uic@chporto.min-saude.pt

Received on November 24, 2022; accepted in revised form on February 20, 2023.

© Copyright CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2023.

Funding: this research was funded by Fundação para a Ciência e a Tecnologia (FCT) to UMIB (UIDB/00215/2020, and UIDP/00215/2020), ITR - Laboratory for Integrative and Translational Research in Population Health (LA/P/0064/2020).

Competing interests: F. Zannad reports personal fees from Applied Therapeutics, Bayer, Boehringer, BMS, CVRx, Cardior, Cereno pharmaceutical, Cellprothera, CEVA, KBP, Merck, Novartis, NovoNordisk, Owkin, Pfizer, Servier; having stock options at G3 Pharmaceutical and equities at Cereno Pharmaceutical, Cardiorenal, Eshmoun Clinical Research and being the founder of Cardiovascular Clinical Trialists. P. Rossignol reports grants and personal fees from Boehringer Ingelheim, grants and personal fees from Novartis, Vifor/Relypsa, AstraZeneca and Bayer, personal fees from Cincor and KBP, grants and personal fees from Novonordisk, personal fees from Roche and Fresenius, personal fees and other from CardioRenal, Idorsia and Sequana. The other authors have declared no competing interests.

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 cardiovascular 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).[2] RA was diagnosed based on the 2010 ACR/EULAR Classification criteria.[4] 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® CVDII panel) that comprised 92 biomarkers from a wide range of pathophysiological domains.

Table I. Baseline characteristics of the patients by tertiles of disease duration.

Characteristics	Disease duration (tertiles), years			*p*-value
	Tertile 1 3.2 (1.3, 4.5)	Tertile 2 8.8 (6.9, 11.1)	Tertile 3 21.8 (17.5, 29.6)	
n=399	133	133	133	-
Age (years), median (IQR)	56.0 (46.0, 65.0)	61.0 (50.0, 68.0)	65.0 (56.0, 72.0)	<0.001
Age at diagnosis, median (IQR)	52.4 (42.6, 62.2)	51.5 (40.6, 59.5)	39.1 (29.2, 48.6)	<0.001
Male	32 (24.1%)	37 (27.8%)	24 (18.0%)	0.16
BMI (kg/m^2), median (IQR)	26.0 (23.8, 29.5)	26.4 (23.9, 29.7)	25.6 (23.2, 29.2)	0.77
WC (cm), median (IQR)	90.0 (80.0, 99.0)	91.0 (83.0, 103.0)	92.0 (82.0, 100.0)	0.41
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
DAS28 with ESR	2.5 (2.0, 3.8)	2.6 (2.0, 3.4)	2.6 (2.1, 3.2)	0.84
DAS28 with ESR >3.2	21 (16.7%)	29 (22.5%)	44 (33.3%)	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*[1]	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/m^2	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%)	34 (26.0%)	0.068
eGFR, ml/min/1.73m^2	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
"Biologicals"	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, 435.0)	360.0 (260.0, 390.0)	<0.001

*Previous cardiovascular disease include: myocardial infarction or angina pectoris or stroke or valvular heart disease or atrial fibrillation); *[1] echocardiographic alterations defined by a left atrial volume indexed >34 ml/m^2 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 (percentile$_{25\text{-}75}$) 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 (GAL9), matrix metalloproteinase-12 (MMP12), adrenomedullin (ADM) and tumour necrosis factor receptor superfamily member 11A (TNFRSF11A) (Table II and Suppl. Table S3).

Association of disease duration and cardiovascular outcomes

Over a median follow-up period of 1.5 (percentile$_{25\text{-}75}$ 0.7–2.3) years (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 (Fig. 1).

Table II. Association of circulating proteins with rheumatoid arthritics disease duration.

Biomarker	Beta (95%CI)	*p*-value*	FDR5*
NT-pro BNP (log)	0.32 (0.15-0.50)	<0.001	0.026
CTSL1	0.70 (0.29-1.11)	0.001	0.026
Gal9	0.93 (0.38-1.49)	0.001	0.026
BNP	0.27 (0.11-0.43)	0.001	0.026
MMP12	0.37 (0.13-0.60)	0.002	0.040
ADM	0.62 (0.22-1.03)	0.003	0.040
TNFRSF11A	0.58 (0.19-0.98)	0.004	0.048

*All the biomarkers had a lower than 5% false discovery rate (FDR) after adjustment (see the methods section for details).
The biomarkers are ordered by strength of the association (*p*-value).
A positive beta coefficient means that the expression of the biomarker is increased with longer rheumatoid arthritis duration in an ordered logistic regression model.
Multivariable model was adjusted for age, sex, estimated glomerular filtration rate and diabetes.
NT-pro BNP (log): logarithm N-terminal pro b-type natriuretic peptide; CTSL1: cathepsin L1; GAL9: galectin 9; BNP: B-type natriuretic peptide; MMP12: matrix metalloproteinase-12; ADM: adrenomedullin; TNFRSF11A: tumour necrosis factor receptor superfamily member 11A.

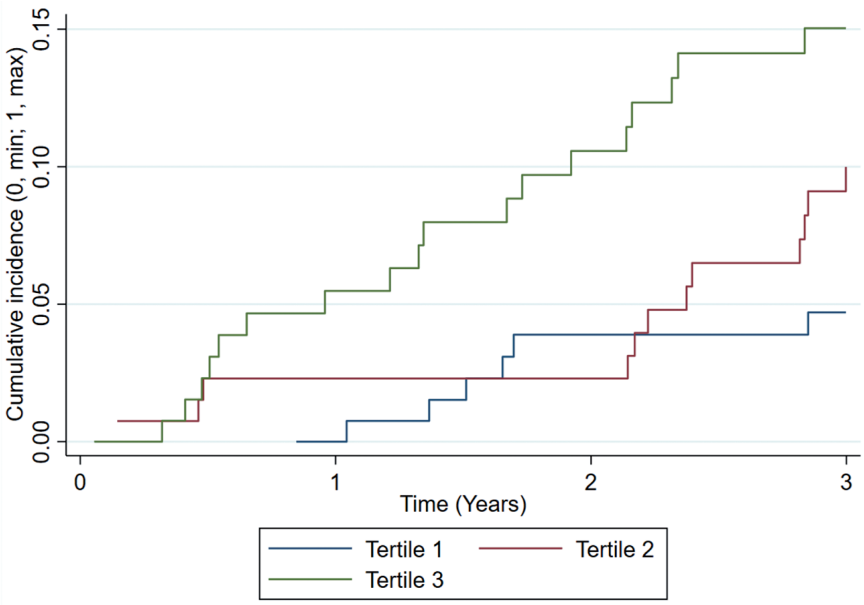

Fig. 1. Cardiovascular outcome associations by duration of rheumatoid arthritis.
Tertiles of rheumatoid arthritis duration: Tertile 1: 3.2 (1.3, 4.5) years; Tertile 2: 8.8 (6.9, 11.1) years; Tertile 3: 21.8 (17.5, 29.6) years.
The outcome was a composite of myocardial infarction, stroke, heart failure 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 sociodemographic 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

1. WEYAND CM, GORONZY JJ: Immunometabolism in early and late stages of rheumatoid arthritis. *Nat Rev Rheumatol* 2017; 13(5): 291-301. https://doi.org/10.1038/nrrheum.2017.49
2. CHENG Q, CHEN X, WU H, DU Y: Three hematologic/immune system-specific expressed genes are considered as the potential biomarkers for the diagnosis of early rheumatoid arthritis through bioinformatics analysis. *J Transl Med* 2021; 19(1): 18. https://doi.org/10.1186/s12967-020-02689-y
3. COUTANT F, MIOSSEC P: Evolving concepts of the pathogenesis of rheumatoid arthritis with focus on the early and late stages. *Curr Opin Rheumatol* 2020; 32(1): 57-63. https://doi.org/10.1097/bor.0000000000000664
4. VILLENEUVE E, NAM J, EMERY P: 2010 ACR-EULAR classification criteria for rheumatoid arthritis. *Rev Bras Reumatol* 2010; 50(5): 481-3.
5. LABORATORIES ATSCOPSFCPF: ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med* 2002; 166(1): 111-7. https://doi.org/10.1164/ajrccm.166.1.at1102
6. LANG RM, BADANO LP, MOR-AVI V *et al.*: Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Eur Heart J Cardiovasc Imaging* 2015; 16(3): 233-70. https://doi.org/10.1093/ehjci/jev014
7. GONZALEZ-REY E, CHORNY A, O'VALLE F, DELGADO M: Adrenomedullin protects from experimental arthritis by down-regulating inflammation and Th1 response and inducing regulatory T cells. *Am J Pathol* 2007; 170(1): 263-71. https://doi.org/10.2353/ajpath.2007.060596
8. LIU M, SUN H, WANG X *et al.*: Association of increased expression of macrophage elastase (matrix metalloproteinase 12) with rheumatoid arthritis. *Arthritis Rheum* 2004; 50(10): 3112-7. https://doi.org/10.1002/art.20567
9. BEHL T, CHADHA S, SEHGAL A *et al.*: Exploring the role of cathepsin in rheumatoid arthritis. *Saudi J Biol Sci* 2022; 29(1): 402-10. https://doi.org/10.1016/j.sjbs.2021.09.014
10. TER MAATEN JM, KREMER D, DEMISSEI BG *et al.*: Bio-adrenomedullin as a marker of congestion in patients with new-onset and worsening heart failure. *Eur J Heart Fail* 2019; 21(6): 732-43. https://doi.org/10.1002/ejhf.1437
11. FERREIRA MB, FONSECA T, COSTA R *et al.*: Prevalence, risk factors and proteomic bioprofiles associated with heart failure in rheumatoid arthritis: The RA-HF study. *Eur J Intern Med* 2021; 85: 41-49. https://doi.org/10.1016/j.ejim.2020.11.002
12. MOLVIN J, JUJIC A, BACHUS E *et al.*: Cardiovascular biomarkers predict post-discharge re-hospitalization risk and mortality among Swedish heart failure patients. *ESC Heart Fail* 2019; 6(5): 992-99. https://doi.org/10.1002/ehf2.12486
13. CHANG CK, CHANG KH, CHENG WC *et al.*: Lipid metabolomic signature might predict subclinical atherosclerosis in patients with active rheumatoid arthritis. *Clin Exp Rheumatol* 2022 Oct 4. https://doi.org/10.55563/clinexprheumatol/int08c
14. MANTEL A, HOLMQVIST M, ANDERSSON DC, LUND LH, ASKLING J: Association between rheumatoid arthritis and risk of ischemic and nonischemic heart failure. *J Am Coll Cardiol* 2017; 69(10): 1275-85. https://doi.org/10.1016/j.jacc.2016.12.033