International Journal of Cardiology 209 (2016) 74–76

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Correspondence

Comparing inflammatory cell density in the myocardium and coronary arteries in rheumatoid arthritis patients versus controls with myocardial infarction: A post-mortem case–control study

Inge A.M. van den Oever [a,b,e,*,1], Alper M. van Sijl [a,b,1], Umit Baylan [c,1], Marieke M. ter Wee [b,1], Casper G. Schalkwijk [d,1], Paul A.J. Krijnen [c,1], Michael T. Nurmohamed [a,b,1], Alexandre E. Voskuyl [b,1], Hans W.M. Niessen [c,1], Suat Simsek [e,1]

[a] Department of Rheumatology, Amsterdam Rheumatology and Immunology Centre | Reade, Dokter Jan van Breemenstraat 2, 1056, AB, Amsterdam, The Netherlands
[b] Department of Rheumatology, Amsterdam Rheumatology and Immunology Centre | VU University Medical Centre, De Boelelaan 1117, 1081, HV, Amsterdam, The Netherlands
[c] Department of Pathology and Cardiac Surgery, ICaR-VU, VU University Medical Centre, De Boelelaan 1117, 1081, HV, Amsterdam, The Netherlands
[d] Department of Internal Medicine, UMCM, P. Debyelaan 25, 6229, HX, Maastricht, The Netherlands
[e] Department of Internal Medicine, Medical Centre Alkmaar, Wilhelminalaan 12, 1815, JD, Alkmaar, The Netherlands

ARTICLE INFO

Article history:
Received 23 January 2016
Accepted 2 February 2016
Available online 3 February 2016

Keywords:
Rheumatoid arthritis
Coronary disease
Myocardial infarction
Inflammation
Post-mortem

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease and associated with an increased risk of developing an unrecognized but often fatal myocardial infarction (MI) [1,2]. Atherosclerosis is considered an immune-mediated inflammatory disease, whereby large numbers of active immune cells, including macrophages and lymphocytes, are found in the atherosclerotic plaque that can produce inflammatory cytokines [3]. Increasing evidence suggests that the intramyocardial vasculature also plays an important role in MI induction [4]. We have shown depositions of the advanced glycation endproduct (AGE) N-epsilon carboxy-methyl lysine (CML) in the intramyocardial vasculature in advance of and subsequent to MI [5]. To date, only one study has actually compared atherosclerotic plaques of coronary arteries in deceased RA patients with age- and sex-matched controls. This study revealed less multivessel disease, less severe grade of stenosis, but increased medial and adventitial inflammation and an increased frequency of vulnerable plaques in coronary arteries of RA patients [6].

We hypothesized that the systemic inflammatory process in RA, not only leads to inflamed epicardial coronary arteries resulting in vulnerable plaques, but also negatively affects the myocardial tissue and the intramyocardial arteries. The present study analyzed the effect of RA in MI patients at different levels: 1) the inflammatory status of epicardial coronary arteries; 2) the inflammatory status of intramyocardial arteries; 3) inflammation of the heart post MI.

This is a retrospective case–control autopsy study. From a post-mortem tissue database of subjects who underwent autopsy within 24 h after death at the Department of Pathology, VU University Medical Centre (VUmc), Amsterdam, The Netherlands between January 1990 and December 2010, tissue-slides of the infarct related coronary artery and myocardium of all subjects who were diagnosed with RA and an acute MI at autopsy were selected and matched for age, sex, year of death, grade of stenosis and infarct phase to control subjects without RA, who were also diagnosed with MI at autopsy. The present study was conducted in accordance with the Declaration of Helsinki and according to the guidelines of the ethics committee of the VUmc. The use of autopsy material after completion of the diagnostic process is part of the patient informed consent in the VUmc.

The myocardial tissue was taken from the infarct area (left ventricle, LV), its microscopically determined border area (also LV) and the non-infarcted area (right ventricle, RV) of the heart of the same patient. Based on pre-defined criteria the myocardial infarct age was microscopically categorized into three phases: 1) early MI phase (death 3–12 h after MI), 2) neutrophilic granulocyte MI phase (12 h–5 days after MI) and 3) chronic MI phase (5–14 days after MI) [5]. Of each of the infarct-related coronary arteries, the grade of stenosis was calculated (lumen surface/lumen + intima surface, in percentage) and categorized into 4 groups: 0–25%, 25–50%, 50–75% and 75–100%.

Tissue-slides were stained with CD45 (lymphocytes), CD68 (macrophages), tryptase (mast cells), myeloperoxidase (neutrophilic granulocytes) and the advanced glycation end product N-epsilon-(carboxymethyl)lysine (CML) and subsequently quantified by two

* Corresponding author at: Department of Internal Medicine, Medical Centre Alkmaar, Wilhelminalaan 12, 1815, JD, Alkmaar, The Netherlands.
E-mail address: I.A.M.vanden.Oever@mca.nl (I.A.M. van den Oever).
[1] This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

http://dx.doi.org/10.1016/j.ijcard.2016.02.065

Table 1
Study population characteristics.

Characteristics	Rheumatoid arthritis (n = 5)	controls (n = 5)
Demographics		
Age, years	78 ± 6	76 ± 8
Male gender	2 (40)	2 (40)
Stay at ICU/CCU before death	1 (20)	1 (20)
Cardiovascular risk factors		
Hypertension	2 (40)	2 (40)
Dyslipidemia	1 (20)	1 (20)
Diabetes mellitus	0	0
Prior myocardial infarction	3 (60)	2 (40)
Prior other vascular disease	1 (20)	1 (20)
Inflammatory disease characteristics		
Duration of rheumatoid arthritis (years)	6.6 (1.3–24.5)	–
Number of infarct related epicardial coronary artery slides, n	12	11
Left artery descending	6	5
Right coronary artery	0	2
Ramus circumflexus	6	4
Grade of stenosis, 0–100%, median (IQR)	30 (21–44)	21 (20–33)
Coronary stenosis grade in percentage, n		
0–25	5	7
25–50	5	3
50–75	1	1
75–100	1	0
Total amount of myocardial slides, n	65	66
Amount of slides infarct area (LV)	21	23
Amount of slides border area (LV)	25	22
Amount of slides of non-infarct area (RV)	19	21
Infarction phase per patient, n		
Phase 1 (3–12 h)	0	0
Phase 2 (12 h-5 days)	2	2
Phase 3 (5–14 days)	3	3
Infarct location per patient, n		
Anterior	2	2
Posterior	3	3
Inferior	0	1
Lateral	1	1
Septal	2	1

Results presented as means ± standard deviations, number (percentages) or median (interquartile range) n number, IQR interquartile range, LV left ventricle, RV right ventricle.

investigators (AMS and IAMO) [7]. In the myocardial slides, the intramyocardial coronary arteries positive for CML were given an intensity score: 1 = weak positivity; 2 = moderate positivity; 3 = strong positivity. The CML intensity score [1–3] was multiplied by the amount of intramyocardial coronary arteries positive for that score. The surfaces of the myocardial tissue slides and of the coronary slides were measured, using a video overlay system, the Q-PRODIT 5.2 (Leica, Cambridge, UK), whereby a computer was linked to a camera placed on a microscope. Finally, cell density (cells/mm^2) and CML positivity (immunohistochemical score/mm^2) were calculated by dividing the number of cells or the CML intensity score respectively, by the surface of the tissue component of interest. Mann–Whitney tests were used for statistical analyses. Subject characteristics are presented as number and percentage, mean ± standard deviation or median + interquartile range (IQR) in case of a skewed distribution. Two sided p-values less than 0.05 were considered statistically significant. All analyses were performed by SPSS 19.0 (Chicago, IL, USA).

In total, five subjects with RA and MI and five controls with MI but without RA were included in this study. The patient and tissue slide characteristics are presented in Table 1. The characteristics were not significantly different between RA patients and controls.

In the adventitial layer of the infarct related epicardial coronary artery of RA patients significantly more lymphocytes (9.1 (3.8–29.7) vs 3.6 (1.8–5.5), p = 0.01) and mast cells (5.0 (1.7–7.4) vs. 1.3 (1.0–2.7), p = 0.02) were found compared to controls. Except for a significantly lower cell density for neutrophilic granulocytes (1.7 (0.0–4.0) vs 5.7 (5.1–15.7), p = 0.02) in the intima of RA patients, no other significant differences in inflammatory cell count in the intima, media and adventitia were found in RA patients compared to controls.

The results of the analyses of the CML intensity score and inflammatory cells within the heart tissue slides are depicted in Fig. 1. In the infarct area, CML intensity score (1.3 IQR 0.8–1.8 vs. 0.1 IQR 0.1–0.3 immunohistochemical score/mm^2) and mast cell count (3.4 IQR 2.4–4.1 vs. 2.1 IQR 1.4–2.7 cells/mm^2) were significantly higher in RA patients compared to controls. In the border area, CML intensity scores and the numbers of lymphocytes and macrophages were significantly higher in the RA versus control group (0.7 IQR 0.4–1.3 vs. 0.3 IQR 0.2–0.5 immunohistochemical score/mm^2, 15.6 IQR 9.9–20.8 vs. 8.7 IQR 4.9–13.2 cells/mm^{2}and 14.6 IQR 6.7–23.9 vs. 8.5 IQR 4.4–13.5 cells/mm^2, respectively).

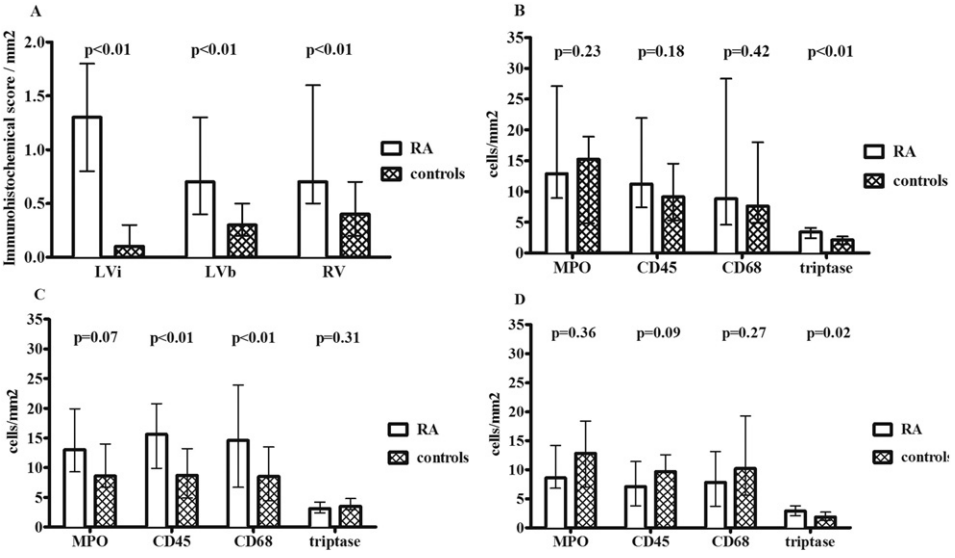

Fig. 1. Immunohistochemical analysis of N-epsilon-(carboxymethyl)lysine (CML) and Inflammatory cell densities in the heart tissue of rheumatoid arthritis (RA) patients versus controls. A. N-epsilon-(carboxymethyl)lysine in heart tissue. B. Inflammatory cell densities in left ventricle infarct area. C. Inflammatory cell densities in left ventricle border area. D. Inflammatory cell densities in right ventricle non-infarcted area. MPO = neutrophilic granulocytes, CD45 = lymphocytes, CD68 = macrophages, tryptase = mast cells. Results are shown as median and interquartile range (IQR), p < 0.05 was considered significant.

Also in the non-infarcted RV CML intensity scores (0.7 IQR 0.5–1.6 vs. 0.4 IQR 0.2–0.7 immunohistochemical score/mm^2, $p = 0.005$) and the number of mast cells (2.9 IQR 2.1–3.8 vs. 1.9 IQR 1.3–2.7 cells/$mm^{2)}$ were significantly higher in RA patients compared to controls.

In conclusion, this study shows that the inflammatory status is increased in RA patients with MI at different levels, namely 1) in the adventitia of infarct related epicardial coronary arteries; 2) in the intramyocardial vasculature and 3) in the border area of the infarcted heart, compared with controls. These findings support the hypothesis that RA patients are prone to develop more vulnerable plaques due to more inflammation in the coronary arteries, but also develop increased intramyocardial inflammation indicative for a higher risk on myocardial tissue damage post MI. This might not only explain the higher incidence of cardiovascular events in this population, but also the higher fatality rate after myocardial infarction.

Funding

This research was partly funded by the Rheumatology Grant awarded by the Dutch Society for Rheumatology.

Conflict of interest

The authors have no conflict of interests to declare.

References

[1] H. Maradit-Kremers, C.S. Crowson, P.J. Nicola, K.V. Ballman, V.L. Roger, S.J. Jacobsen, et al., Increased unrecognized coronary heart disease and sudden deaths in rheumatoid arthritis: a population-based cohort study, Arthritis Rheum. 52 (2) (2005) 402–411.
[2] S. Van Doornum, C. Brand, B. King, V. Sundararajan, Increased case fatality rates following a first acute cardiovascular event in patients with rheumatoid arthritis, Arthritis Rheum. 54 (7) (2006) 2061–2068.
[3] R. Virmani, A.P. Burke, A. Farb, F.D. Kolodgie, Pathology of the vulnerable plaque, J. Am. Coll. Cardiol. 47 (8 Suppl.) (2006) C13–C18.
[4] J. Herrmann, J.C. Kaski, A. Lerman, Coronary microvascular dysfunction in the clinical setting: from mystery to reality, Eur. Heart J. 33 (22) (2012) 2771–2782b.
[5] A. Baidoshvili, P.A. Krijnen, K. Kupreishvili, C. Ciurana, W. Bleeker, R. Nijmeijer, et al., N(epsilon)-(carboxymethyl)lysine depositions in intramyocardial blood vessels in human and rat acute myocardial infarction: a predictor or reflection of infarction? Arterioscler. Thromb. Vasc. Biol. 26 (11) (2006) 2497–2503.
[6] M.C. Aubry, H. Maradit-Kremers, M.S. Reinalda, C.S. Crowson, W.D. Edwards, S.E. Gabriel, Differences in atherosclerotic coronary heart disease between subjects with and without rheumatoid arthritis, J. Rheumatol. 34 (5) (2007) 937–942.
[7] J. Fronczek, R. Lulf, H.I. Korkmaz, B.I. Witte, F.R. van de Goot, M.P. Begieneman, et al., Analysis of inflammatory cells and mediators in skin wound biopsies to determine wound age in living subjects in forensic medicine, Forensic Sci. Int. 247 (2015) 7–13.