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Initial white blood cell count is an independent risk factor for survival in patients with dilated cardiomyopathy

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ABSTRACT

Background: The impact of white blood cell count (WBCc) on the outcome of patients with non-ischemic left ventricular (LV) dysfunction is unknown. In the present study we investigated the influence of WBCc on mortality and cardiac inflammation in patients with reduced LV systolic function in the absence of ischemic or valvular etiology.

Methods and results: We included 381 patients with reduced left ventricular (LV) ejection fraction (LVEF ≤45%) quantified by two-dimensional echocardiography. Coronary artery disease and valvular diseases were excluded by angiography and echo, respectively, in all patients. WBCc was quantified routinely upon first hospital admission. In 291 patients, endomyocardial biopsies from the right ventricle were performed upon first hospital admission for assessment of cardiac inflammation. Follow-up was up to 5.5 years (median 2.93 [1.7;4.0]). Information on vital status of patients was obtained from official resident data files. WBCc >11 Gpt/l was associated with significantly increased mortality in patients with severe LV dilation (end-diastolic diameter (LVEDD) >70 mm quantified by echocardiography) in comparison to patients showing WBCc ≤11 Gpt/l (41.7% vs 13.6%, p=0.02). Multivariable Cox regression analysis showed that WBCc predicts mortality independently of other cardiovascular risk factors and LVEF (hazard ratio 1.14; p=0.04). Doses of heart failure medication did not differ significantly in patients with LVEDD >70 mm and WBCc >11 Gpt/l when compared to LVEDD >70 mm and WBCc ≤11 Gpt/l (percent of maximum doses: ß-blockers p=0.51, ACE inhibitors p=0.56, AT1 antagonists p=0.77, aldosterone antagonists p=0.35). WBCc including its subpopulations (monocytes, lymphocytes and granulocytes) did not show a significant correlation with cardiac amounts of $CD3^+$-lymphocytes (r=0.02, p=0.78) or $CD68^+$-macrophages (r=1.0, p=0.09) (n=291).

Conclusion: WBCc at first hospital admission predicts long term-mortality in patients with dilated cardiomyopathy independently of cardiovascular risk factors.

1. Introduction

Dilated cardiomyopathy (DCM) is characterized by ventricular chamber enlargement and systolic dysfunction [1]. It has an estimated prevalence of 1:2500, constitutes the third most common type of heart failure and is the most frequent cause of heart transplantation [1]. The clinical course of DCM ranges from sufficient myocardial recovery to end-stage heart failure leading to increased mortality. As recently published, the overall outcome of patients with DCM is favorable [2]. However, approximately 10% of DCM patients died within four years in this study. The most predictive factor for cardiac recovery in these patients was the left ventricular end-diastolic diameter derived from two dimensional echocardiography. Etiological aspects involving the great variation in clinical causes are largely unknown. Autoimmune processes, including systemic immune activation and cardiac infiltration of immune cells, have been the focus of intense investigation in recent years. For example, cardiodepressant autoantibodies have been determined to play a possible role in cardiac dysfunction [3]. Viral infection of the myocardium, which can be present in DCM, is strongly regulated by innate immunity at least in experimental models [4,5]. In patients with suspected myocarditis that could lead to DCM, it has been found from analyses of endomyocardial biopsies that cardiac inflammation (>14 $CD3^+$ T lymphocytes and/or $CD68^+$ macrophages per 1 mm^2 in the myocardium in addition to enhanced expression of HLA class II molecules) is an independent risk factor for mortality [6]. Knowledge about the potential role of systemic activation of immune cells in cardiomyopathies has been developed

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from experimental models that primarily investigate cellular immunity in autoimmune and viral myocarditis [7]. For example, a TH17 subset, that was IL-6 stimulated, was essential in developing autoimmune myocarditis in mice [8]. In the case of viral myocarditis, adoptive transfer of CD4 + CD25 + regulatory T cells led to decreased virus load in mice after infection with coxsackievirus B3 [9]. Allografted anti-inflammatory (M2) macrophages resulted in an attenuation of viral myocarditis in mice, to which increased contents of T regulatory cells also contributed [10]. However, data on the prognostic role of systemic immune activation in patients with DCM are rare.

In a clinical setting, WBCc was an independent predictor for one-year mortality in patients with acute ischemic heart failure due to ST-segment elevation myocardial infarction [11]. As recently published, WBCc is associated with the incidence of heart failure. However, the prognostic role of WBCc in patients with non-ischemic and non valvular LV dysfunction has not yet been studied [12].

In the present study we investigated the impact of WBCc on mortality and cardiac inflammation in patients with non-ischemic and non-valvular left ventricular (LV) systolic dysfunction.

2. Methods

2.1. Study population

A total of 381 patients were included in this study in 2005 and 2011. They fulfilled the following inclusion criteria: 1. left ventricular (LV) systolic dysfunction evaluated by two-dimensional echocardiography (LV ejection fraction ≤45%), 2. exclusion of ischemic or valvular etiology of LV dysfunction by coronary angiography and two dimensional echocardiography, respectively, 3. WBCc measurement at first hospital admission and 4. known vital status (with a minimum follow-up time of three months for living patients). Patients presenting with cardiogenic shock were not included in this study. In 291 patients myocardial biopsies from the right ventricular septum were performed at first hospital admission [13]. Biopsies were evaluated histologically and immunohistochemically as described previously [6]. All patients gave informed and written consent. The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

2.2. Echocardiography

As described previously [14], echocardiographic parameters were performed in all patients by 2-dimensional echocardiography at first hospital admission according to the American College of Cardiology/American Heart Association guidelines [15]. Left ventricular ejection fraction was quantified according to the biplane Simpson methodology [14]. Left ventricular dilation was assessed by measurement of the LV end-diastolic diameter (LVEDD) [14].

2.3. Study design and end points

The study was designed in a prospective longitudinal manner. Patients received heart failure medical treatment leaned to the current guidelines of the European Society of Cardiology [16]. Median follow-up was 2.93 years [1.7;4.0]. The primary end point of this study was all-cause mortality. Information on vital status of patients was obtained from official resident data files in May 2011. Subjects were censored either at last known date of contact (either a clinic visit or date of information from the population registry) or at death. Correlation of the amount of systemic immune cells and the amount of cardiac immune cells was investigated as secondary end points.

2.4. White blood cell counts, C-reactive protein and nt-pro brain natriuretic peptide

Blood samples were obtained during the first admission in our hospital and recorded in the electronic case report form. Total WBCc, neutrophil, basophil and eosinophil granulocytes and monocytes were measured in an EDTA-anticoagulated whole-blood specimen according to a standard protocol for clinical routine in our hospital [17]. C-reactive protein (CRP) and nt-pro brain natriuretic peptide (nt-proBNP) level were quantified according to routine diagnostic methods in our clinic [35].

2.5. Analyses of endomyocardial biopsies

We performed histopathological and immunohistochemical analyses of endomyocardial biopsies as previously described [6]. Immunohistological analyses were used to investigate cardiac inflammation by treating the paraffin embedded tissue sections with an avidin-biotin-immunoperoxidase method according to the manufacturer's protocol (Vectastatin Elite ABC kit, Vectastatin®, USA) [6]. Monoclonal antibodies were used to evaluate cardiac cell infiltration of $CD3^+$ T-lymphocytes (Novocastra laboratories, UK), and $CD68^+$ macrophages according to the current guidelines for the Definition and Classification of Cardiomyopathies [1].

2.6. Statistical analyses

For descriptive statistics the study population was divided into two groups according to the patients' WBCc measurement (≤11 Gpt/l, >11 Gpt/l). Continuous data are expressed as median with 1st and 3rd quartiles; categorical data are expressed as percentage.

For the two-group comparison we used Mann–Whitney-U-Test for continuous data or Fisher Exact Test for categorical data. Spearman correlation analyses were furthermore performed to evaluate the association between clinical laboratory parameters. Survival analysis was done calculating Kaplan–Meier curves and log-rank test was used for curve comparison. Multivariable Cox regression models were applied to assess the association between WBCc and all-cause mortality, with adjustment for age and cardiovascular risk factors. A value of $p<0.05$ was considered statistically significant. Analysis was stratified according to patients with a WBCc>11 and ≤11 Gpt/l as a cutoff for normal according to previous studies [11,18]. Stratification according to LVEDD was performed as previously published (LVEDD <60 mm, 60–70 mm, >70 mm) [2].

The software packages SAS 9.1 (SAS Institute Inc., Cary, NC, USA) and STATA, Intercooled Stata/SE 10.1 (StataCorp, College Station, Texas, USA) were used for the statistical analyses.

3. Results

3.1. Baseline characteristics according to white blood cell count

In total, 381 patients with non-ischemic and non-valvular LV dysfunction were included in our study. In all patients, WBCc was quantified at first hospital admission. Fig. 1 shows, that WBCc was normally distributed. Table 1 shows the baseline characteristics of the whole patients cohort distributed by WBCc (≤11 Gpt/l and >11 Gpt/l). We observed 344 patients with a WBCc ≤11 Gpt/l and 37 patients with a WBCc >11 Gpt/l. These two groups did not differ significantly regarding age, gender, LV ejection fraction and cardiovascular risk factors at first admission. In addition, heart-failure medication did not differ indexed by similar percent of the maximum dose of ß-blockers, ACE inhibitors, AT1 antagonists and aldosterone antagonists evaluated at first admission. The number of days between baseline examination and censoring is called observation time, which was not significantly different between the two WBCc groups (Table 1).

3.2. Baseline characteristics of patients with severe dilated cardiomyopathy according to left ventricular dimension

In all patients of our study, the LVEDD was quantified by two-dimensional echocardiography at first admission. As shown in Table 2, 130 patients displayed severe LV dilation indexed by a LVEDD >70 mm. In this population, patients with WBCc >11 Gpt/l were significantly younger when compared to patients with WBCc ≤11 Gpt/l. Cardiovascular risk factors, gender and percent of maximum heart failure medication doses did not differ significantly among this two groups.

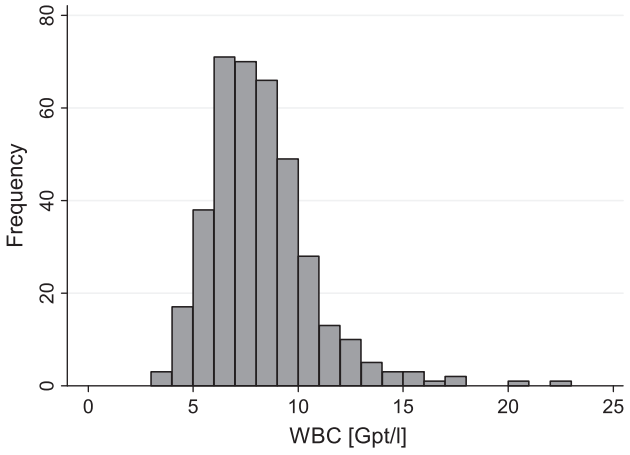

Fig. 1. Distribution of leucocytes. Shows the distribution of white blood cell count (WBC) in our patients group.