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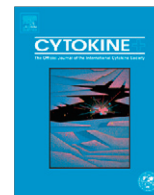

Systemic inflammation and cell activation reflects morbidity in chronic heart failure

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ABSTRACT

Chronic heart failure (CHF) leads to complex effects distant from the heart. As these changes may be reflected in the balance of systemic inflammatory and fibrotic immunomodulators we measured these potential biomarkers in ambulatory CHF patients. Using the New York Heart Association (NYHA; levels II–IV) functional classification, 30 CHF patients were compared with 21 age and gender matched controls. Peripheral blood levels of regulatory cytokines (TNF-α, TGF-β, KGF, IL-8, IL-10 and IL-12) and markers of cellular activation (CD11b, CD16, CD18, CD34, HLADR, CXCR1 and CCR5) were analysed by ELISA and flow cytometry, respectively. NYHA classification, which reflected increasing pulmonary microvascular pressure (E:E') but not ejection fraction, was positively associated with TGF-β and IL-10 ($p \leqslant 0.03$). Similarly, monocytes, as well as cell surface expression of the neutrophil adhesion molecule CD11b, and the macrophage complement receptor complex (CD11b/CD18), were increased in CHF patients ($p \leqslant 0.03$), while the chemokine receptor CXCR1 was decreased on cells of CHF patients. Twenty month follow-up of CHF subjects identified monocyte number as a powerful prognostic factor for cardio-pulmonary adverse events ($p = 0.001$); however, no concurrent relationship with cellular activation marker expression was found. In subjects with CHF, monocytes, TGF-β, IL-10, CD11b/CD18 and CXCR1 expression in peripheral blood may act as novel biomarkers of immune activation and remodelling. Given the importance of dyspnea and the relationship of pulmonary microvascular pressure to the NYHA classification, we suggest these findings may reflect a contribution by the lung.

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1. Introduction

Chronic heart failure (CHF) results in distant systemic organ dysfunction. While plasma b-type natriuretic peptide (BNP) is a reliable correlate with disease severity and outcome in CHF, it is primarily a hemodynamic cardiac marker and may not reflect this dysfunction, or the generalized inflammation and subsequent tissue repair that occur distant to the heart [1,2].

As dyspnea is the cardinal manifestation of CHF we have previously focused on remodelling of the lung including establishment of the unique biomarker surfactant protein-B. Surfactant protein-B is a lung epithelium specific protein that leaks into the circulation in CHF correlating better than BNP with hospitalisation due to CHF [3]. Further, using a rat CHF model we have reported an increase in alveolar type II cells and surfactant content which allows for homeostatic maintenance of respiratory mechanics despite fibrotic pulmonary remodelling in a rodent model of CHF [4]. In conditions of chronic injury the balance of tissue growth factors, predominantly transforming growth factor (TGF)-β and keratinocyte growth factor (KGF), regulate tissue remodelling. The dominance of each respectively resulting in fibrosis, through activation and proliferation of myofibroblasts, or healthy epithelial regeneration [5,6].

In addition, in this rodent model of CHF we found that disruption of the endothelial and epithelial barriers in response to elevated pulmonary microvascular pressure increases systemic chemotactic and inflammatory cytokines, such as interleukin (IL)-8, which mediate a rapid influx of leukocytes into the lung [4]. In CHF patients, production of these mediators as well as leukocyte activation is quantifiable through determination of plasma concentrations and cell surface expression of binding proteins such as CD11b/CD18 and CD16 and chemokine receptors such as CXCR1 and CCR5.

Abbreviations: CHF, chronic heart failure; NYHA, New York Heart Association; IL, interleukin; BNP, b-type natriuretic peptide; TGF, transforming growth factor; KGF, keratinocyte growth factor; TNF, tumour necrosis factor.

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As circulating biomarkers are logistically simple to measure and potentially provide valuable mechanistic and clinical information, to gain further insights, we studied inflammatory and fibrotic mediators as well as leukocyte activation in the peripheral blood of ambulatory CHF patients compared with age and gender matched controls. We hypothesized that the balance of these mediators and cells in peripheral blood would reflect CHF symptoms.

2. Materials and methods

2.1. Participants

Sequential ambulatory subjects attending the Heart Failure Clinic of a tertiary level hospital, Flinders Medical Centre, Adelaide, Australia, with a minimum 12 month history of systolic CHF and a left ventricular ejection fraction of less than 40%, were approached to enter the study over a 2 year period. Patients were excluded on the basis of a history of acute or chronic lung disease. Current diagnostic details and medications were recorded and CHF symptoms were classified according to the criteria of the New York Heart Association (NYHA; levels II–IV). Age and gender matched controls without history of significant cardiovascular diagnoses or exclusions, as above, were recruited via regional advertising. The study complies with the Declaration of Helsinki, the protocol was approved by the Flinders Clinical Research Ethics Committee, Flinders Medical Centre, and all participants provided written informed consent.

A single venous blood sample was obtained from all participants for measurement of soluble mediators, determination of cellular differentials and expression of cell surface molecules, as below.

2.2. Biomarker measurements

Blood samples were divided into sodium citrate tubes for cytokine and cell surface marker analysis, and lithium heparin tubes for N-terminal pro-B-type natriuretic peptide (NT-proBNP), before being centrifuged and plasma aliquoted and stored at −80 °C for batched analysis. TGF-β, KGF, tumour necrosis factor (TNF)-α, IL-8, IL-10 and IL-12 concentrations were quantified by enzyme linked immunosorbent assay using matched antibodies, as previously described (R&D Systems, Minneapolis, MN) [4]. NT-proBNP levels were quantified by electrochemiluminescence immunoassay kit (Roche Ltd., Basel, Switzerland). Limits of detection were 20, 40, 2, 1, 2 and 4 pg/ml for cytokines and 20 pg/ml for NT-proBNP, respectively.

An aliquot of each blood sample was used to determine the cellular differential using a Beckman Coulter Counter Model HmXAL (Beckman Coulter Inc., Brea, CA). Leukocyte surface markers were analysed by six colour flow cytometry. Briefly, packed blood cells were incubated with fluorochrome labelled antibodies (CD11b, PE; CD16, PECy7; CD18, FITC; CD34, PECY7; CD40, FITC; CD45, APCCy7; CCR5, PECy5; CXCR1, PECy5; HLADR, APC; BD Biosciences, NJ USA) for 30 min before erythrocyte lysis (Lysis Solution, BD Biosciences), followed by a single wash in PBS/0.1 M Azide and fixation (FACS Fix, BD Bioscience). Mean fluorescence intensity of each fluorochrome was determined on the CD45^{+} leukocytes using a BD FACSCanto flow cytometer (BD Biosciences) and data were analysed using BD FACSDiva software. Expression analysis was performed on both total CD45^{+} population and following differentiation of leukocyte populations into lymphocytes, monocytes/macrophages, total polymorphonuclear cells, CD45^{+Lo} and CD45^{+Hi} polymorphonuclear cells, by CD45^{+} versus side scatter parameters (Fig. 1).

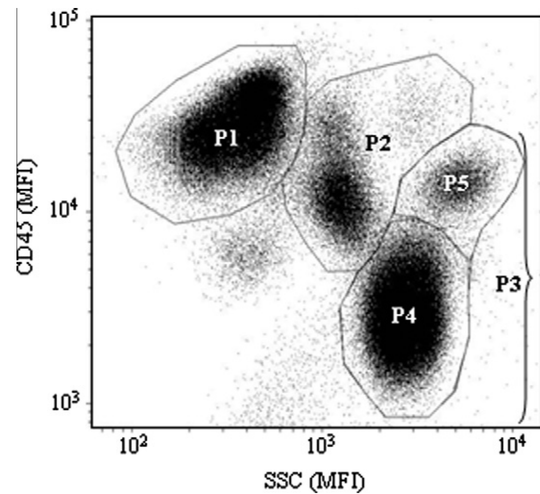

Fig. 1. Gating of leukocyte subpopulations by CD45^{+} and side scatter parameters. P1 – lymphocytes; P2 – monocytes/macrophages; P3 – total polymorphonuclear cells (P4 + P5); P4 – CD45^{+Lo} polymorphonuclear cells; P5 – CD45^{+Hi} polymorphonuclear cells.

2.3. Follow-up

CHF patients were examined 20 months following sample collection for determination of current NYHA classification, hospital admission due to cardio-pulmonary complications or mortality. Control subjects were contacted via surface mail and asked to return a questionnaire relating to hospital admission due to cardio-pulmonary conditions.

2.4. Statistical analyses

Statistical analyses were performed using PASW 18.0 software (SPSS Inc., Chicago, IL). Comparisons of CHF patient versus control subjects were made by Mann–Whitney *U* statistics and between NYHA groups by Jonckheere–Terpstra non-parametric statistics due to the skewed nature of the data, which could not be normalized by log transformation. Continuous variables are expressed as median and percentiles and categorical variables as number and percentage. A p-value $\leqslant 0.05$ was used as the level of reportable significance.

The effect of monocyte number on time to adverse events was assessed with a Kaplan–Meier survival curve and log rank test used for initial comparison. A step-wise multivariate Cox regression model was developed to determine relative predictive value of the significant predictors (NT pro-BNP, monocyte number, NYHA class).

3. Results

3.1. Subject populations

Thirty patients with CHF (NYHA II–IV) and 21 age and gender matched control subjects were enrolled. Baseline characteristics are detailed in Table 1. Diabetes and the intake of a range of standard pharmacological treatments were more common in CHF patients.

Disease characteristics between NYHA classes are given in Table 2. Mitral valve inflow *E* wave velocity to mitral annular velocity (E:E') ratio, a marker of increased left sided cardiac filling pressure and pulmonary microvascular pressure, increased with increased NYHA score. However, ejection fraction was similarly low in all three NYHA groups. Ischemic injury was the predominant cause

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