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ORIGINAL ARTICLE

Susceptibility to infections and adaptive immunity in adults with heart failure

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Abstract

Aims Heart failure (HF) is a systemic inflammatory disorder with infections being an important cause of morbidity and mortality. We asked if HF patients have a higher susceptibility to infections compared with the general population and if a subtle secondary immunodeficiency facilitates infectious complications.

Methods and results In a cohort of 92 patients with HF with reduced ejection fraction, we analysed recirculating lymphocyte subpopulations, serum immunoglobulin levels, and specific antibody titres against pneumococcal antigens. We quantified susceptibility to infections of the respiratory tract with a validated questionnaire and compared it to the general population. Susceptibility to infections of the respiratory tract was comparable in HF patients and the general population. Hypogammaglobulinaemia was present in 16% of HF patients, but anti-pneumococcal titres showed no evidence of specific secondary antibody deficiency. Relative lymphopaenia in our HF cohort was due to B lymphocytopenia with a relative reduction in naive B-cells and expansion of memory B-cells while CD4+ and CD8+ T-lymphocytes as well as NK-cell counts were comparable between HF and healthy donors. The intake of the angiotensin receptor neprilysin (CD10) inhibitor (ARNI) sacubitril/valsartan was associated with increased B-lymphocyte counts, possibly by an increased output of CD10+ transitional B lymphocytes from the bone marrow.

Conclusion Despite a reduction of B lymphocytes in HF and mild hypogammaglobulinaemia, patients showed no evidence of secondary immunodeficiency or increased susceptibility to infections. The relevance of B-cell lymphopenia in HF patients and modulation of B-cell counts under ARNI treatment remains to be investigated.

Keywords Heart failure; Respiratory tract infection; B lymphocyte; T lymphocyte; Immunoglobulin; Sacubitril/valsartan

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Introduction

Heart failure (HF) is a disease of great clinical and socio-economic importance worldwide. The prevalence in industrialized countries is 1–2% and continues to rise with age.[1] The aetiology is multifactorial and includes cardiovascular and non-cardiovascular risk factors with coronary artery disease being the main risk factor in industrialized countries. Hospital admission for HF is associated with a poor prognosis with a 1 year mortality after hospitalization of 32–33% and a 5 year mortality of 64–66%.[2] Among the most common factors precipitating hospitalization are infections of the respiratory tract/pneumonias, ischaemia, and arrhythmia.[3,4] Association of infections and hypogammaglobulinaemia in patients on the waiting list for heart transplantation has been reported in a small cohort.[5] Despite the fact that respiratory tract infections are an important cause of death in HF patients,[3,6–8] to our knowledge, the question if HF patients suffer from an increased susceptibility to infections compared with the general population has not been investigated so far.

HF is a chronic inflammatory multi-system disease and there is overwhelming evidence for the role of innate immunity including macrophages in the healthy heart as well as in tissue remodelling after myocardial injury.[9] Various inflammatory markers/cytokines, for example, C-reactive protein, IL-6, Il-1, TNF, ST2, and Gal-3 are elevated in HF and correlate with a worse prognosis.[10] A Dutch epidemiological study has shown a correlation of both increased granulocyte count and atherosclerosis in the general population and increased lymphocyte counts and protection against atherosclerosis.[11] In the HF population, an increased neutrophil/lymphocyte ratio (N/L ratio) has been correlated with acute decompensation and with a poor prognosis for HF hospitalization.[12,13] Indeed, lymphocytes play a crucial role in atherogenesis and stability of atherogenic plaques.[14] Large randomized trials have tested the effect of immunosuppressive therapy in coronary artery disease/myocardial infarction on cardiovascular endpoints.[15,16] Both the anti-IL1β antibody canakinumab and low-dose colchicine treatment reduced cardiovascular events but increased fatal infections and pneumonias, respectively. In summary, the available evidence shows that HF patients display a state of chronic inflammation influencing atherogenesis and cardiac remodelling on the one side, but are at risk of serious, potentially fatal complications after infections one the other side.

In the current study, we aimed to identify immunologic factors predisposing to infections in a cohort of 92 HF patients at our centre. We undertook a detailed analysis of recirculating lymphocyte subpopulations, serum immunoglobulin levels and specific antibody titres against pneumococcal antigens. To assess susceptibility to infections, we used a validated questionnaire and compared it to the general population.

Patients, material, and methods

Patient cohort

The study was approved by the institutional review board in compliance with the Declaration of Helsinki (Nr. 224/17). All patients provided informed consent and were recruited from the University Heart Center Freiburg/Bad Krozingen. Inclusion criteria were HF with an EF $\leq$ 45% and New York Heart Association (NYHA) Stages II–IV. Exclusion criteria were acute infection at inclusion, malignancy, steroid treatment, or other immunosuppressive therapy. We recruited 98 patients with HF, of those six had to be excluded from the analysis: two proved to have incidental finding of monoclonal B-cells on flow cytometry and four due to violation of inclusion criteria. Of 92 analysed patients (*Table 1*), 88 blood samples and 83 questionnaires were available. The mean age of the entire cohort was 64 years [median 62, interquartile range (IQR) 55–76 years]. Men constituted 78% (*n* = 72) and women 22% (*n* = 20) of the cohort. The mean age of female participants was 66 years (median 62, IQR 51–82 years), of male participants 63 years (median 62, IQR 55–76 years) (*Table 1*).

Control cohorts

Control cohort for lymphocyte subsets

Controls for lymphocyte subsets were obtained from anonymized blood donors by the rheumatology diagnostic lab of the University Medical Center Freiburg. Because there was a male preponderance among our HF cohort and because the blood donors were younger compared with our HF cohort, we selected the control subjects form the blood donor pool as follows: (i) We included only every second female proband and (ii) only blood donors aged >28 years were included as the youngest patient in the HF cohort was 29 years old. Thus, reference values were generated from 88 healthy donors (HDs) with a median age of 47 years (IQR 40–54 years). Male blood donors represented 66% (*n* = 58) and female blood donors represented 34% (*n* = 30) of the control cohort (*Table 1*).

Control cohort for susceptibility to infections

Susceptibility to infections in HF patients was assessed with a standardized self-reported questionnaire validated in a cross-sectional, epidemiological study conducted in southern Germany of the general population (AWIS[17]). Questions referred to frequency and severity of respiratory tract infections, underlying chronic diseases, medication, smoking habits, contact to children below the age of 10, data on the highest educational achievement, as well as height and weight. The AWIS study comprised 12 839 participants (57%

Table 1 Demographics of patient and control cohorts

	HF patients	Control cohort infectious susceptibility (general population)	Control cohort lymphocyte counts (blood donors)
Number of probands	92	184	88
Age median (IQR)	62 years (55–76)	64 years (54–70)	47 years (40–54)
Gender, *n* (%)			
Male	72 (78%)	144 (78%)	58 (66%)
Female	20 (22%)	40 (22%)	30 (34%)

HF, heart failure; IQR, interquartile range.

female, 43% male) with a mean age of 46.8 years. The AWIS study population served as a pool for the control group. Controls were selected according to gender and stratified according to age in a ratio of 1:2 to reduce variance (*Table 1*). Patients with HF were divided into six groups based on their year of birth. The more often, a birth cohort was represented, the smaller the age range selected for a group. Group 0 comprised the years 1981–1994, Group 1, the years 1971–1982; Group 2, the years 1961–1976; Group 3, the years 1951–1965; Group 4, the years 1946–1955; Group 5, the years 1941–1949; and Group 6, the years 1939–1942. In the corresponding age groups of the AWIS cohort, the controls were selected appropriately by gender. Because there were too few test subjects in the AWIS cohort in Group 6 for a 1:2 matching, the controls for this age group were also drawn from the somewhat younger Group 5. The average age at the time of data collection in the control group was 61 years (median 64, IQR 54–70 years) (*Table 1*).

Material and methods

After collection, blood samples were stored at room temperature and processed within 24 h. Flow cytometry staining was performed from whole blood for lymphocyte subsets and from frozen peripheral blood mononuclear cells for B-lymphocyte subsets. The following antibodies were used: anti-CD3, BV421, UCHT1 (clone); anti-CD10, BV510, HI10a; anti-CD19, APC/Cy7, HIB19; anti-CD27, BV421, M-T21; anti-CD38, PerCP, HIT2; anti-CD45, PerCP, HI30; Anti-IgD, Pe-Cy7, IA6-2 (all Biolegend, London, UK); anti-CD4, APC, SK3; anti-CD21, PE, B-ly4; Anti-IgG, Alexa Fluor 700, G18-145 (all Becton Dickinson, Heidelberg, Germany); anti-CD8, FITC, B9.11; anti-CD16, PE, 3G8; anti-CD19, Pe-Cy7, J3-119; anti-CD56, PE, N901 (NKH-1) (all Beckman Coulter, Krefeld, Germany); Anti-IgA, FITC, Goat, polyclonal (Southern Biotech, Birmingham, USA); Anti-IgM, Alexa Fluor 647, goat, polyclonal (Jackson ImmunoResearch, Ely, UK). Measurements were performed on a FACS Gallios (Beckman Coulter, Krefeld, Germany). Data were analysed with Kaluza Analysis (Version 2.1, Beckman Coulter, Krefeld, Germany). Serum immunoglobulin levels were measured on a nephelometer (BN Prospec System Siemens, Erlangen, Germany). Serum immunoglobulin (Ig) reference ranges were adapted from the Community Bureau of References.[18] Specific antibodies against pneumococci were assessed with VaccZyme™ Anti-PCP IgG Enzyme Immunoassay Kit (The binding site, Birmingham, UK) according to manufacturer's instructions. Statistical analyses were performed with GraphPad Prism (Version 9, GraphPad, La Jolla, USA) and Stata Version 14 (STATSCorp, USA). Mann–Whitney *U* test was used for comparison between groups. A P value <0.05 was considered statistically significant.

Results

Among 92 patients, clinical severity of HF was assigned to NYHA Stage II in 37% (n = 34), to NYHA III in 53% (n = 49) and to NYHA IV in 9% (n = 8). A NYHA stage could not be established in one patient due to immobility. Causes for HF were ischaemic cardiomyopathy in 48% (n = 44) and dilated cardiomyopathy in 24% (n = 22). Adults with complex congenital heart defects constituted 5% of the cohort (n = 5). Other reasons for HF were hypertension, arrhythmogenic cardiomyopathy, valve disease, myocarditis, post-chemotherapy, or idiopathic. Further patient characteristics are summarized in *Table 2*.

Lymphocyte subsets

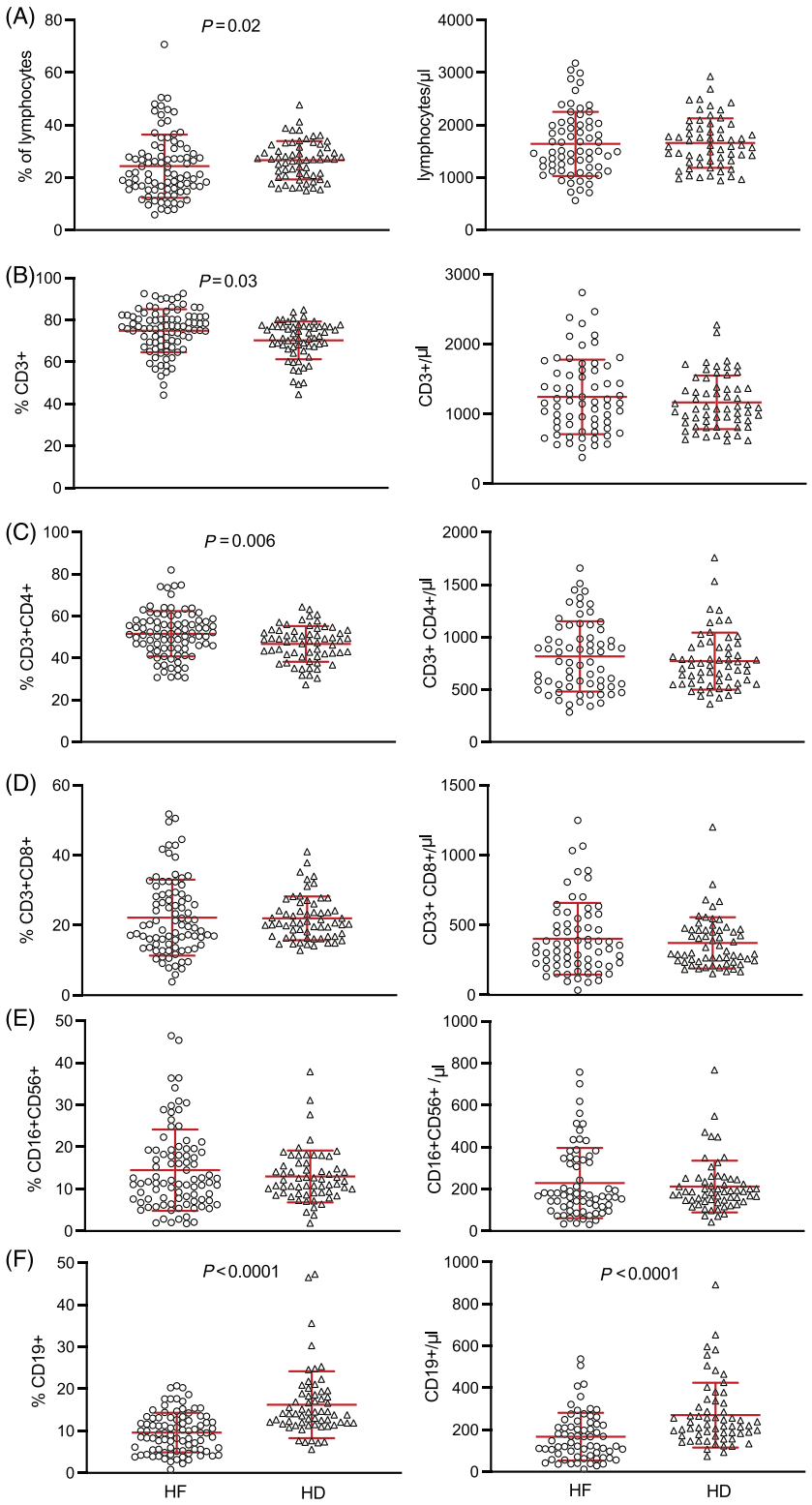
An increased neutrophil/lymphocyte ratio is a biomarker in HF. In our cohort, relative lymphocyte counts were reduced compared with HDs (*Figure 1A*, mean ± standard deviation: HF 24 ± 11%, HD: 27 ± 7%, P = 0.0163), but absolute counts were comparable between patients and controls (HF 1641 ± 612/μL, HD: 1657 ± 471/μL) indicating an expansion of non-lymphocyte leukocytes in the HF cohort, most likely neutrophils. T lymphocytes (CD3+: HF 75 ± 10%, HD: 70 ± 9%, P = 0.0032) and the subset of CD4+ T lymphocytes (CD3 + CD4+: HF 52 ± 11%, HD: 47 ± 8%, P = 0.0056) showed a relative expansion in HF patients. However, absolute whole T lymphocyte (CD3+: HF: 1243 ± 535/μL, HD: 1166 ± 384/μL) and CD4+ T-lymphocyte counts (CD3 + CD4+: HF: 815 ± 336/

Table 2 Characteristics of HF patients

LVEF median (IQR)	27.5% (20–32.5%)
NYHA, *n* (%)	
Stage II	34 (37%)
Stage III	49 (53%)
Stage IV	8 (9%)
Unknown	1 (1%)
Aetiology of HF, *n* (%)	
Ischaemic	44 (48%)
Dilated cardiomyopathy	22 (24%)
Congenital heart defect	5 (5%)
others	21 (23%)
Medication, *n* (%)	
With beta-blocker	88 (96%)
With ARNI	45 (49%)
Comorbidities, *n* (%)	
COPD/emphysema	9 (10%)
Asthma	4 (4%)
Diabetes	23 (25%)
Of those insulin dependant	10
Chronic kidney disease	14 (15%)
BMI median (IQR)	27 (24–30)
Current or former smoker	42 (46%)

HF, heart failure; IQR, interquartile range; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.
Legend: Comorbidities, body mass index, and smoking habits were retrieved from the AWIS questionnaire (self-reported data).

Figure 1 Relative and absolute lymphocyte counts in heart failure (HF) compared with healthy donors (HDs). Relative (left column) as well as absolute (right column) counts of lymphocyte and lymphocyte subsets are shown in HF patients (open circles) compared with HD (open triangle). Relative whole lymphocyte counts are shown as percentage of leukocytes, lymphocyte subpopulations as percentage of lymphocytes. Error bars show mean with standard deviation.

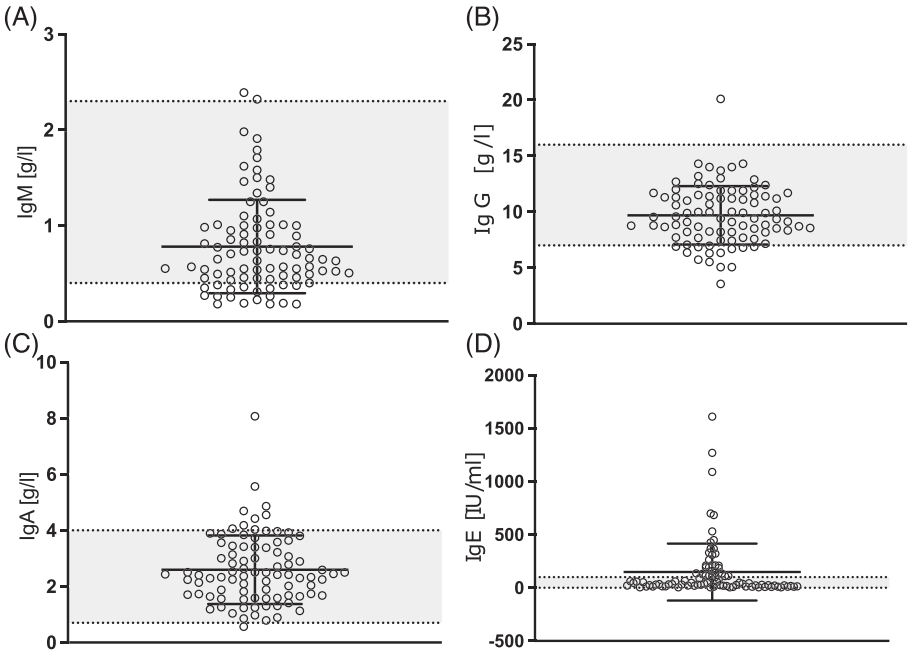


μL, HD 770 ± 272/μL) were comparable (*Figure 1B,C*). Both relative and absolute counts were comparable for cytotoxic T cells (CD3+ CD8+: HF 22 ± 11%, 401 ± 256/μL; HD: 22 ± 6%, 372 ± 183/μL) and natural killer (NK) cells (CD16+ CD56+: HF 14 ± 10%, 229 ± 167/μL; HD: 13 ± 6%, 212 ± 123/μL) (*Figure 1D,E*). In contrast, HF patients showed a highly significant reduction of B-lymphocyte counts, both for relative (HF: 10 ± 5%, HD: 16 ± 8%, $P < 0.0001$) and absolute (HF: 167 ± 114/μL, HD: 270 ± 155/μL, $P < 0.0001$) numbers compared with HD (*Figure 1F*). There was a weak correlation of increasing age and declining B-cell counts (Spearman correlation: percentage of B-cells, $r = -0.3$; absolute B-cells $r = -0.39$, $P = 0.001$). The reduction of B-cell numbers was not related to the duration of HF in patients (data not shown). In summary, HF patients had a marked reduction of both relative and absolute B-lymphocyte counts in the peripheral blood compared with HD while absolute T-cell and NK-cell counts were comparable. Duration of HF did not influence B-cell numbers.

Immunoglobulin levels and specific antibody titres in HF patients

B lymphocytes differentiate into immunoglobulin producing plasma cells. Data on serum immunoglobulins were available in 88 patients (*Figure 2*). IgM levels were below the reference range in 20% (n = 18/88), IgG levels were reduced in 16% (n = 14/88). A reduction of both IgG and IgM serum levels was noted in 6% (n = 5). IgA serum levels were reduced in only one patient and above the reference range in 7% (n = 6/88). Serum IgE levels were above the reference range in 35% (n = 31/88). There was no correlation of IgM, IgG, IgA, or IgE levels and B-cell counts (data not shown). To assess if the reduced IgG and IgM levels were associated with reduced functionality of immunoglobulins, we assessed specific antibody titres against pneumococcal antigens (anti-PCP). Because guidelines recommend seasonal influenza and pneumococcal vaccination, HF patients should have protective anti-PCP titres. Anti-PCP titres below 10 mg/L are considered non-protective, titres above 43.8 mg/L protective.[19] Among our cohort 59% (n = 51/87) had protective, 6% (n = 5/87) had non-protective anti-PCP titres and 37% (n = 31/87) patients exhibited titres in the grey zone between 10–43.8 mg/L. Vaccination status in our HF cohort was available for influenza but not for pneumococci. Anti-PCP titres were significantly higher in patients who had received an influenza vaccination (anti-PCP titre in patients with influenza vaccination: median 77 mg/L, IQR 35–171 mg/L vs. no influenza vaccination: 39 mg/l, IQR 15–72 mg/L, P = 0.0003). In summary, our data confirm the literature on the prevalence of hypogammaglobulinaemia in HF patients; however, we could not find evidence for specific antibody deficiency in our cohort.

Figure 2 Serum immunoglobulin values in HF. IgM (A), IgG (B), IgA (C), and IgE (D) serum values in patients with HF. The reference range is marked in grey. Error bars show mean with standard deviation.



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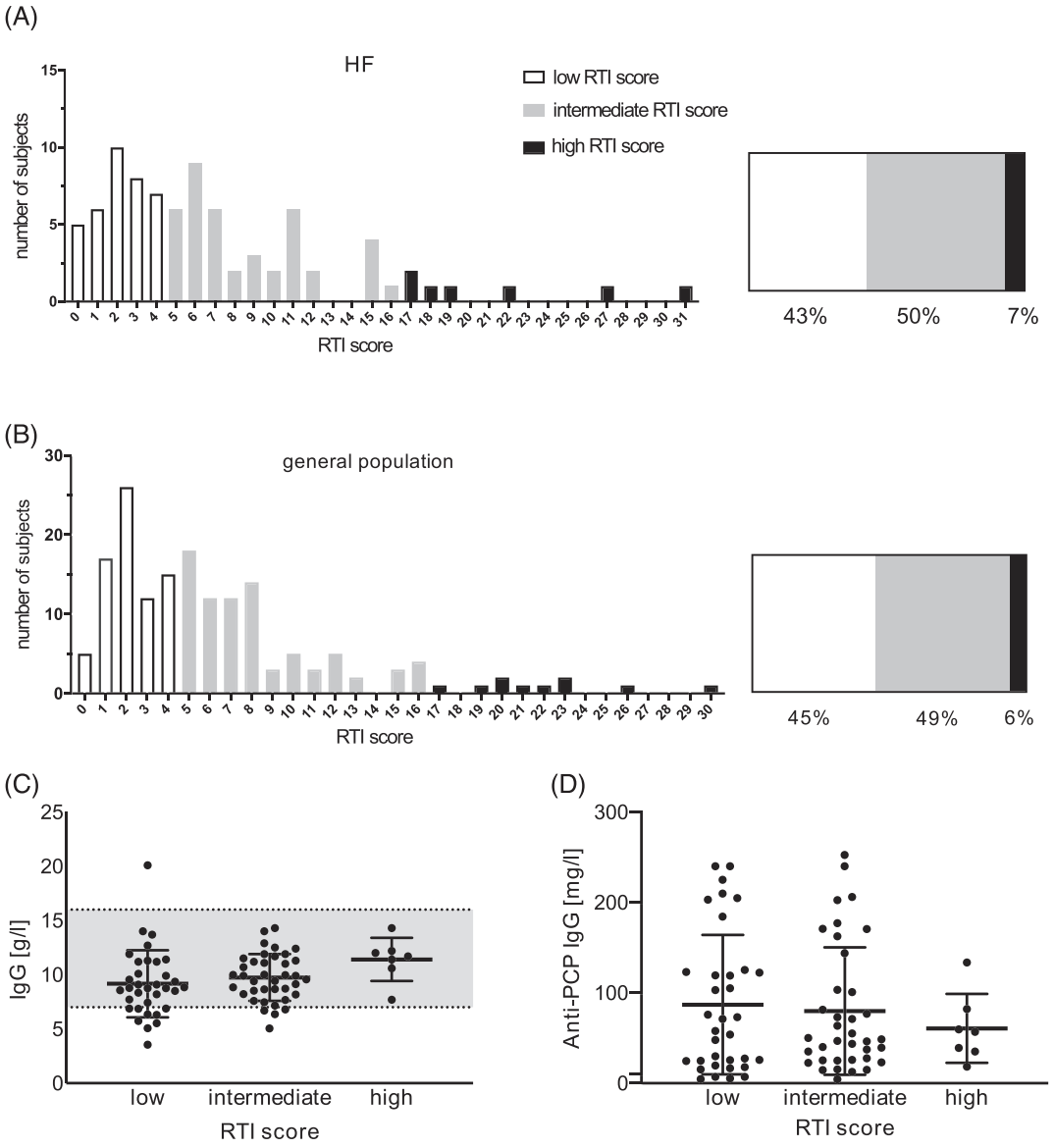
The self-reported susceptibility to infections is comparable between heart failure patients and the general population

We assessed the susceptibility to infections in HF patients with a standardized questionnaire validated in the general population.[17] From the questionnaire, a respiratory tract infection score (RTI score) is calculated. The RTI score ranges from 0 to 50, with an RTI score of up to 4 reflecting a low susceptibility to infections and a RTI score of 17 or more corresponding to a high susceptibility to infections. Intermediate RTI scores are defined as 5–16. We found a low RTI score in 42% (*n* = 35/83), an intermediate RTI score in 51% (*n* = 42/83) and a high RTI score in 7% (*n* = 6/83) of patients (*Figure 3A*). This distribution corresponded to the distribution of the RTI score in the

Figure 3 Susceptibility to infections. (A, B) Panels on the left column show the number of subjects (*y* axis) with the respective respiratory tract infection (RTI) score (*x* axis) for HF patients (A) and a control cohort from the general population (B). Low RTI score (≤4) is symbolized as a clear rectangle, intermediate RTI score (5–16) as a grey rectangle and high RTI score (≥17) as a black rectangle. Percentage of subjects with low, intermediate or high RTI score within the respective cohort (A: HF, B: general population) are shown in the right column. (C,D) Serum IgG (C) and anti-pneumococcal titres (D) are shown stratified according to RTI score. Error bars show mean with standard deviation.



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general population: RTI low 45% (*n* = 75/166), RTI intermediate 49% (*n* = 81/166) and high RTI score 6% (*n* = 10/166) of probands (*Figure 3B*). Thus, in our cohort of HF patients we could not detect evidence for an augmented susceptibility to infections. Moreover, both hypogammaglobulinaemia and low anti-PCP titters did not correlate with susceptibility to infections (*Figure 3C,D*). This further supports the finding that low B-cell counts as well as hypogammaglobulinaemia do not lead to secondary immunodeficiency in HF patients.

Skewing of B-lymphocyte subpopulations in heart failure patients with low B-lymphocyte counts

To characterize the marked reduction of B-cells in the peripheral blood of patients with HF we conducted a detailed analysis of peripheral B-lymphocyte subpopulations. Early B-cell progenitors develop in the bone marrow, transitional B-cells egress from the bone marrow and migrate to the spleen to mature into naive B-cells. Naive B-cells recirculate in the peripheral blood and secondary lymphocyte organs. Upon antigen encounter, naive B-cells (CD27-IgD+) differentiate into memory B-cells in germinal centres of secondary lymphoid organs or in the marginal zone. In the peripheral blood we characterized marginal zone memory B-cells (CD27+ IgM+ IgD+), 'IgM only' memory B-cells (CD27+ IgM + IgD−), class switched memory B-cells (IgD− IgG+ and IgD− IgA+) as well as CD21low B-cells. CD21low B-cells are B-cells with antigen-presenting capacities enriched in many autoimmune and infectious diseases. HF patients with low B-cell counts ('HF low') had a relative reduction in naive B-cells compared with HF patients with B-cell counts within the reference range ('HF normal') (HF low: 61 ± 18% vs. HF normal: 73 ± 13% *P* = 0.0074, *Figure 4*). This was accompanied by a relative expansion of IgM memory (HF low: 9.1 ± 9% vs. HF normal: 4.7 ± 2.7%, *P* = 0.0122), IgG memory (HF low: 6.3 ± 4.1% vs. HF normal: 4.3 ± 3.3%, *P* = 0.0183), and IgA memory B-cells (HF low: 7.7 ± 5.2% vs. HF normal: 4.5 ± 2.7%, *P* = 0.0009, *Figure 4*) indicating a skewing towards more differentiated B-cell subsets. Also CD21low B-cells were expanded in HF patients with low B-cell counts compared with HF patients with B-cell counts within the reference range (HF low: 6 ± 4.7% vs. HF normal: 2.9 ± 3.1%, *P* = <0.0001). Frequencies of transitional B-cells (HF low: 0.7 ± 0.6% vs. HF normal: 0.9 ± 0.6%, *P* = ns) and marginal zone memory B-cells (HF low: 9 ± 7% vs. HF normal: 9 ± 7%, *P* = ns) were comparable between HF patients with low and normal B-cell counts. In summary, the reduction of B-cell numbers in HF patients was correlated with a relative reduction of naive B-cells and an expansion of germinal centre derived memory B-cells as well as CD21low B-cells.

Sacubitril/valsartan influences B-lymphocyte counts and subsets

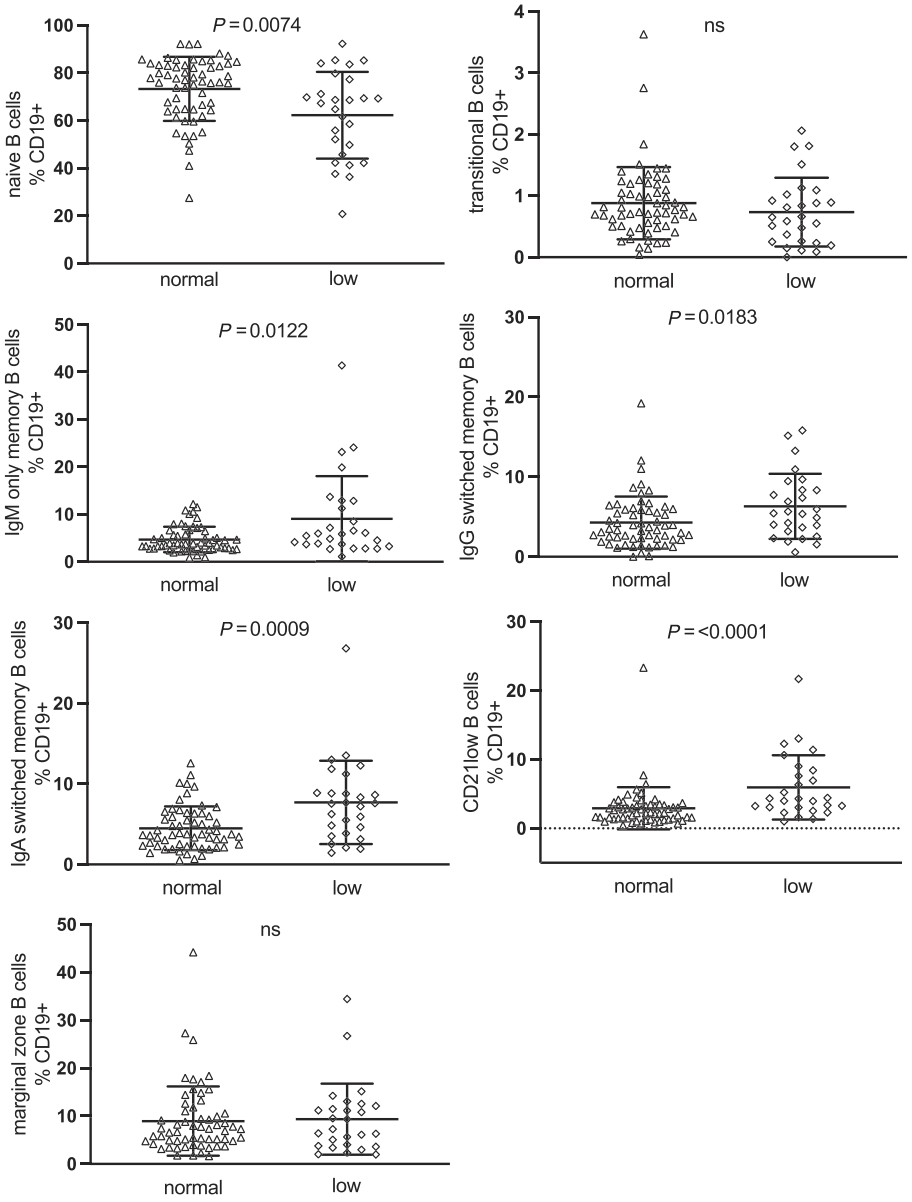
The angiotensin receptor neprilysin inhibitor (ARNI) sacubitril/valsartan is approved for the treatment of symptomatic patients with HF.[20] Sacubitrilate inhibits neprilysin—a molecule also known as CD10. The enzyme CD10 is widely expressed in the body, regulates the cytokine environment, and is involved in intracellular signal transmission. CD10 plays a role in early B-cell development and is expressed on immature B lymphocytes in the bone marrow until the stage of transitional B-cells. In the further course of B-cell development CD10 is downregulated and re-expressed in germinal centre B-cells. In our cohort about one-half of HF patients with available B-cell subpopulations received sacubitril/valsartan (*n* = 44/87) while the other half of the cohort did not (*n* = 43/87). When comparing the two subgroups we found significantly more B-cells in the sacubitril/valsartan group (+sacubitril/valsartan: 187 ± 89 CD19+/μ vs. −sacubitril/valsartan: 145 ± 136 CD19+/μL *P* = 0.0062, *Figure 5A*). The increase in B lymphocytes was distributed over all subsets except CD21low B-cells which were expanded with low B-cell counts (*Figure 5A*). However, the increase was most pronounced in CD10 expressing, transitional B-cells showing an increase in both relative (+sacubitril/valsartan: 1.05 ± 0.65% vs. −sacubitril/valsartan: 0.62 ± 0.41% transitional B-cells, *P* = 0.0015, *Figure 5A*) and absolute numbers (+sacubitril/valsartan: 1.0 ± 1.3 transitional B-cells/μL vs. −sacubitril/valsartan: 2.1 ± 1.9 transitional B-cells/μL *P* = 0.0009, *Figure 5B*). This suggests that besides cardiovascular effects the treatment with sacubitril/valsartan might influence B-cell development.

Discussion

Heart failure is a multisystem disorder and infections in HF patients significantly contribute to morbidity and mortality. In our cohort, susceptibility to infections in HF patients was surprisingly identical to age-matched controls among the general population suggesting that complications of respiratory tract infections but not the frequency are higher. A possible shortcoming of our study is the relatively low number of included patients and the fact that patients with acute infections were excluded from the study. This exclusion criterion was introduced to assess lymphocyte subpopulations in a steady-state and to avoid acute infection-related changes. It, however, might have introduced a potential bias towards patients with less susceptibility to infections. Thus, larger studies in HF are warranted to confirm our findings.

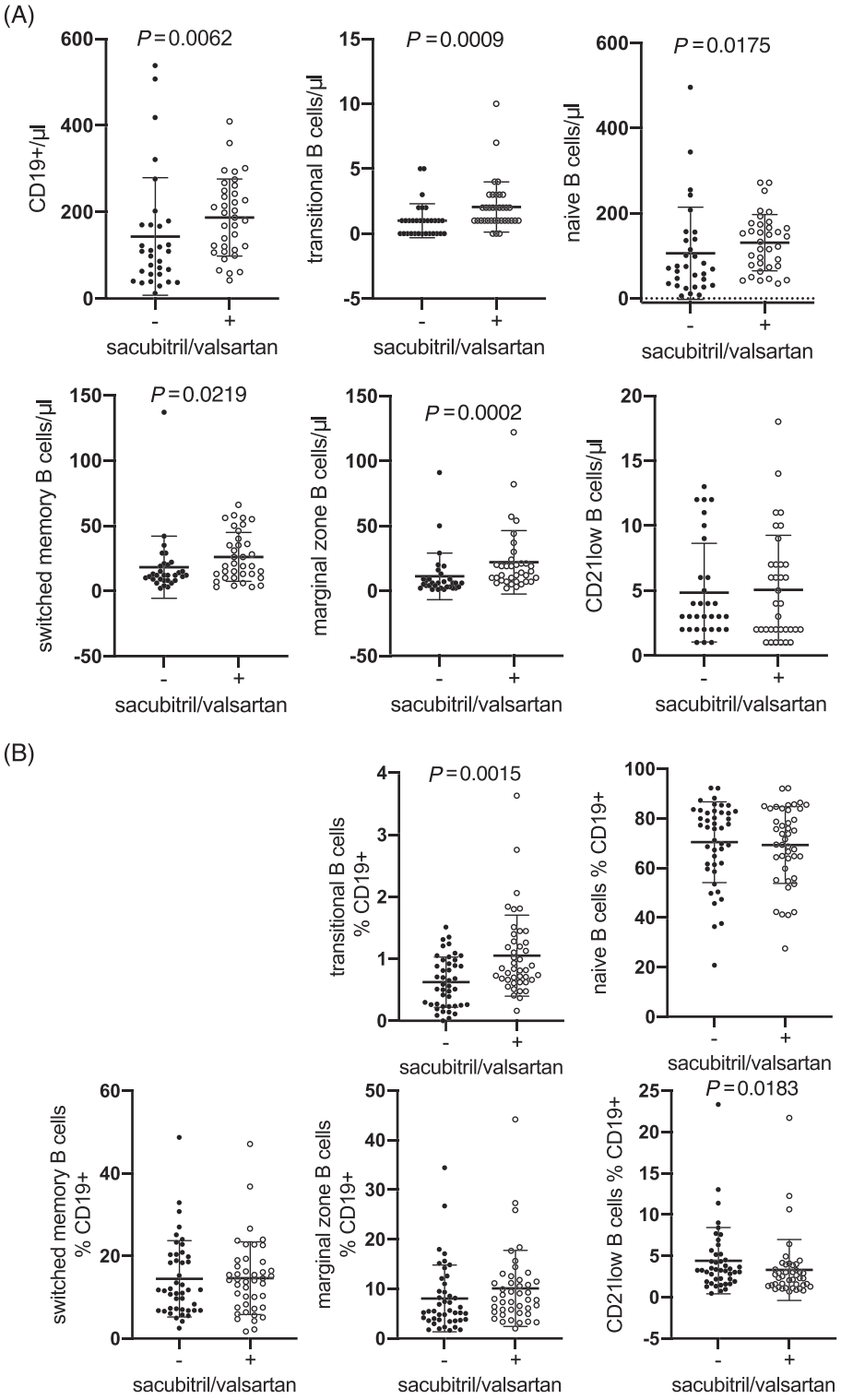
Corresponding to the comparable susceptibility to infections in HF patients, we were not able to identify warning signs of secondary antibody deficiency: While we

Figure 4 Heart failure (HF) patients with low B-cell counts show skewing towards more differentiated B-lymphocyte subsets. B-lymphocyte subsets (% of B-cells) of HF patients with normal B-cell counts (open triangle) are shown compared with HF patients with low B-cell counts (open rectangle). Error bars show mean with standard deviation.



confirmed previous findings on hypogammaglobulinaemia[5] with a prevalence of 16% of reduced IgG and 20% reduced IgM levels, most of the patients had only mild hypogammaglobulinaemia, that is, only one patient had IgG levels below 4 g/L. Specific antibody levels, examined as anti-PCP titres, were preserved in the majority of patients, and we found higher anti-PCP titres in influenza-vaccinated patients. Because PCP vaccination status was not directly recorded in our patient cohort, we hypothesize that patients not being vaccinated against influenza are more likely also not vaccinated against pneumococci. In consequence we believe that low anti-PCP titres observed in some HF patients resulted more likely from lack of vaccination and not from secondary antibody deficiency. The preservation of specific antibody titres in our cohort is also supported by antibody titre data after influenza vaccination in HF.[21] Nonetheless, immunoglobulin homeostasis is altered in HF. Besides reduced IgG and IgM levels in some patients, we found elevated IgE levels in 35% of patients. IgE is a mediator of mast cell activation and elevated in many disorders associated with immune dysregulation. Elevated serum IgE levels have been reported previously in ischaemic heart disease[22] and

Figure 5 Increased B lymphocyte count in patients treated with sacubitril/valsartan. Absolute (A) and relative (B) B-lymphocyte counts are stratified according to intake of sacubitril/valsartan. Patients without intake of sacubitril/valsartan are symbolized as closed circles, patients with sacubitril/valsartan intake as open circles. Error bars show mean with standard deviation.



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IgE-mediated mast cell degranulation is involved in atherosclerotic plaque development.[23] In our cohort, IgE levels were elevated in ischaemic and non-ischaemic HF also in the absence of apparent atherosclerotic disease, suggesting a more general immune activation. Higher IgG and IgM levels were shown to protect from cardiovascular events in patients with hypertension[24]; thus, lower IgG/IgM levels in HF might be a consequence of this observation. Elevated cortisol levels or reduced albumin levels have been implicated in the pathophysiologic mechanism of hypogammaglobulinaemia; however, the exact mechanism remains elusive.

Lymphopenia and an elevated neutrophil/lymphocyte ratio is a well-known phenomenon in HF. In a small cohort, lymphocytopenia has been attributed to a relative decrease in circulating CD4+, CD8+ T, and B lymphocytes.[25] In contrast our cohort showed a relative increase in T cells and no difference upon investigation of absolute T-cell counts. We found a marked reduction of both absolute and relative B-cell counts with a skewing towards more differentiated B-cell subsets among HF patients. One limitation of our study is the higher mean age of the HF compared with the control cohort. With increasing age, a slight reduction of CD4, CD8, and B-cells has been described with a more pronounced reduction of naive and an increase in memory B-cells and T-cells.[26] However, the marked reduction of B-cells in our HF cohort widely exceeds the slow decline of lymphocyte counts described in ageing.[26] B-cells have been shown to be involved in monocyte mobilization after myocardial injury in mice[27] and cardiomyopathy is partly dependent on B-cells in experimental mice.[28] B-cells in the heart have been located intravascularly in close contact with the intimia both in mice and humans.[29] In humans, lower total, naive, and memory B-cells were associated with a higher risk for cardiovascular events in atherosclerosis.[30] If the reduced recirculating B-cell counts in HF result from decreased generation, higher decay or from mobilization of B-cells into the tissue and if reduced recirculating B lymphocytes play a role in the pathophysiology of HF remains an open question.

Medication with sacubitril/valsartan was associated with higher B-cell counts in our cohort, especially transitional B-cells were expanded. Neprilysin/CD10 is a membrane bound zinc-dependent endopeptidase widely expressed in the body. Via its exopeptidase activity CD10 modulates the extracellular cytokine milieu.[31] In mice, the inhibition of CD10 promotes B-cell reconstitution,[32] and *in vitro* its inhibition increases pro-/pre-B-cells upon stromal co-culture.[33] Thus, CD10 is thought to modulate human B-cell development by affecting the cytokine milieu of the bone marrow and spleen. Our data provide associative evidence that CD10 inhibition also increases B-cell counts in humans. Whether this change is due to a direct effect on B-cells or their precursors, a bystander effect on other CD10 expressing cell types and if it is related to the therapeutic effect of sacubitril/valsartan itself in HF is speculative. In the future, ARNI might also facilitate delayed B-cell reconstitution after B-cell depleting therapies or other states of B-cell deficiency in the future.

In summary, our data suggest that HF patients do not have an increased susceptibility to infections or secondary antibody deficiency. Our study describes a reduction of circulating B lymphocytes in HF patients, and for the first time shows an association of B-cell counts and ARNI. Further research is warranted to elucidate the pathophysiologic relationship between B lymphocytes, ARNI, and HF.

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Conflict of interest

The authors declare no conflict of interest.

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