



HAL
open science

Clinical and prognostic associations of autoantibodies recognizing adrenergic/muscarinic receptors in patients with heart failure

George Markousis-Mavrogenis, Waldemar B Minich, Ali A Al-Mubarak, Stefan D Anker, John G F Cleland, Kenneth Dickstein, Chim Lang, Leong Ng, Nilesh J Samani, Faiez Zannad, et al.

► To cite this version:

George Markousis-Mavrogenis, Waldemar B Minich, Ali A Al-Mubarak, Stefan D Anker, John G F Cleland, et al.. Clinical and prognostic associations of autoantibodies recognizing adrenergic/muscarinic receptors in patients with heart failure. *Cardiovascular Research*, 2023, 119 (8), pp.1690-1705. 10.1093/cvr/cvad042 . hal-04023791

HAL Id: hal-04023791

<https://hal.univ-lorraine.fr/hal-04023791v1>

Submitted on 10 Mar 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NoDerivatives 4.0 International License

Clinical and prognostic associations of autoantibodies recognizing adrenergic/muscarinic receptors in patients with heart failure

George Markousis-Mavrogenis^a, MD (Hons); Waldemar B. Minich^{b,c}, MSc, PhD; Ali A. Al-Mubarak^a, MD; Stefan D. Anker^{d,e}, MD, PhD; John G.F. Cleland^f, MD, PhD; Kenneth Dickstein^g, MD, PhD; Chim C. Lang^h, MD; Leong L. Ngⁱ, MD; Nilesh J. Samaniⁱ, MD; Faiez Zannad^j, MD, PhD; Marco Metra^k, MD, PhD; Petra Seemann^{b,c}, MSc, PhD; Antonia Hoeg^b, MSc, PhD; Patricio Lopez^{b,c}, MSc, PhD; The BIostat-CHF Consortium; Dirk J. van Veldhuisen^a, MD, PhD; Rudolf A. de Boer^a, MD, PhD; Adriaan A. Voors^a, MD, PhD; Peter van der Meer^a, MD, PhD; Lutz Schomburg^{*†}, MSc, PhD; Nils Bomer^{*a}, MSc, PhD.

- a. Department of Cardiology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands
- b. Institute for Experimental Endocrinology, Charité – Universitätsmedizin Berlin, Hessische Straße 4A, CCM, D-10115 Berlin, Germany
- c. ImmunometriX GmbH i.L. Berlin, Germany
- d. Division of Cardiology and Metabolism – Heart Failure, Cachexia & Sarcopenia; Department of Cardiology (CVK); and Berlin-Brandenburg Center for Regenerative Therapies (BCRT), at Charité University Medicine, Berlin, Germany
- e. Department of Cardiology and Pneumology, University Medicine Göttingen (UMG), Göttingen, Germany & DZHK (German Center for Cardiovascular Research)
- f. Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow G12 8TA; National Heart & Lung Institute, Imperial College London SW3 6LY. UK.
- g. University of Bergen, Stavanger University Hospital, Stavanger, Norway
- h. Division of Molecular & Clinical Medicine, University of Dundee, Dundee DD1 9SY, UK
- i. Department of Cardiovascular Sciences, University of Leicester, Glenfield Hospital, Leicester, UK and NIHR Leicester Biomedical Research Centre, Glenfield Hospital, Leicester, LE3 9QP, UK
- j. Université de Lorraine, Inserm CIC 1403, CHRU, Nancy, France
- k. Cardiology, ASST Spedali Civili, Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, University of Brescia, Brescia, Italy

*These authors have contributed equally to the manuscript.

†Corresponding author

Short Title: Autoantibodies in Heart Failure

Keywords: beta 1, beta 2, beta 3, M2, immune system, autoimmunity

1 **Address for correspondence:** Prof. Lutz Schomburg, Charité – Universitätsmedizin Berlin, 10115 Berlin,
2 Germany. Email address: lutz.schomburg@charite.de

3 **Abstract**

4 **Aims.** The importance of autoantibodies (AABs) against adrenergic/muscarinic receptors in heart failure
5 (HF) is not well-understood. We investigated the prevalence and clinical/prognostic associations of four
6 AABs recognizing the M2-muscarinic receptor or the β 1-, β 2-, or β 3-adrenergic receptor in a large and well-
7 characterized cohort of patients with HF.

8 **Methods and Results.** Serum samples from 2256 patients with HF from the BIOSTAT-CHF cohort and
9 299 healthy controls were analyzed using newly established chemiluminescence immunoassays. The
10 primary outcome was a composite of all-cause mortality and HF-rehospitalization at 2-year follow-up, and
11 each outcome was also separately investigated. Collectively, 382 (16.9%) patients and 37 (12.4%) controls
12 were seropositive for ≥ 1 AAB ($p=0.045$). Seropositivity occurred more frequently only for anti-M2 AABs
13 ($p=0.025$). Amongst patients with HF, seropositivity was associated with the presence of comorbidities
14 (renal disease, chronic obstructive pulmonary disease, stroke, atrial fibrillation), and with medication use.
15 Only anti- β 1 AAB seropositivity was associated with the primary outcome [hazard ratio (95% confidence
16 interval): 1.37 (1.04-1.81), $p=0.024$] and HF-rehospitalization [1.57 (1.13-2.19), $p=0.010$] in univariable
17 analyses, but remained associated only with HF-rehospitalization after multivariable adjustment for the
18 BIOSTAT-CHF risk model [1.47 (1.05-2.07), $p=0.030$]. Principal component analyses showed considerable
19 overlap in B-lymphocyte activity between seropositive and seronegative patients, based on 31 circulating
20 biomarkers related to B-lymphocyte function.

21 **Conclusions.** AAB seropositivity was not strongly associated with adverse outcomes in HF and was mostly
22 related to the presence of comorbidities and medication use. Only anti- β 1 AABs were independently
23 associated with HF-rehospitalization. The exact clinical value of AABs remains to be elucidated.w

1 **Translational Perspective.**

2 Currently, little is known regarding the clinical/prognostic associations of ANS-AABs in patients with HF.
 3 We show that out of four investigated ANS-AABs, only anti- β 1 AAB seropositivity was independently
 4 associated with HF-rehospitalization. Seropositivity for any of the investigated AAB was not associated
 5 with changes in circulating B-lymphocyte-related markers. Seropositivity was highly similar when
 6 compared to healthy controls. These findings support a limited prognostic role for ANS-AABs in the general
 7 HF population and suggest that ANS-AAB generation may be constitute a previous event unrelated to HF.
 8 Future studies should investigate specific target groups such as Chagas cardiomyopathy, non-hereditary
 9 DCM and PPCM.

11 **Abbreviation list**

ABBREVIATION	DEFINITION
HF	Heart failure
AAB	Autoantibody
ANS-AAB	Autoantibodies against autonomic nervous system receptors
DCM	Dilated cardiomyopathy
BIOSTAT-CHF	A systems BIOlogy Study to Tailored Treatment in Chronic Heart Failure
LVEF	Left ventricular ejection fraction
BNP	Brain natriuretic peptide
NT-PROBNP	N-terminal pro-brain natriuretic peptide
ACEI/ARB	Angiotensin converting enzyme inhibitor/angiotensin receptor blocker
BB	β -adrenoreceptor blocker
CRP	C-reactive protein
HS-CTNT	High-sensitivity cardiac troponin-T
EGFR	Estimated glomerular filtration rate
MDRD	Modification of Diet in Renal Disease
HR	Hazard ratio
95% CI	95% confidence interval
IL-6	Interleukin-6
ANOVA	Analysis of variance
PCA	Principal component analysis
COPD	Chronic obstructive pulmonary disease
AF	Atrial fibrillation
MRA	Mineralocorticoid receptor antagonist

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

Introduction

Many studies have demonstrated the intricate relationship between immune activation and pathophysiological mechanisms in HF.¹ Nevertheless, direct immunomodulatory interventions have as of yet no established role in the clinical management of HF.

Antibodies are soluble forms of the B-cell receptor that are secreted by activated plasma cells and constitute an important part of the adaptive immune response.² The generation of an effective immune response requires a broad antibody repertoire, which is achieved with impressive efficiency by recombination of immunoglobulin genes.³ This permits the generation of antibodies with potentially infinite specificities. Unavoidably, this process also leads to the generation of antibodies against self-antigens. Specialized regulatory mechanisms have evolved to eliminate self-reactive lymphocytes⁴; nevertheless, these do not always function optimally. Accordingly, self-reactive lymphocytes and autoantibodies (AABs) feature prominently in numerous autoimmune rheumatic diseases⁵ and other disease states.⁶

A relationship between AABs against autonomic nervous system receptors (ANS-AABs) and HF has been described previously, particularly in patients with non-hereditary dilated cardiomyopathy (DCM)⁷ and Chagas cardiomyopathy.⁸ However, to our knowledge, most reports are limited to small cohorts and no data from large, well-characterized, and diverse populations currently exist in the literature. We thus aimed to investigate the prevalence of ANS-AAB seropositivity in patients with HF, and to determine its clinical associations and potential relationships with outcomes.

1 **Methods**

2 Patients

3 We performed a post-hoc analysis of the BIOSTAT-CHF index cohort (n=2516), which has been
4 described previously.⁹ Briefly, BIOSTAT-CHF was a multi-center study enrolling patients from 11
5 European countries. Participants were aged ≥ 18 years, had symptoms of new-onset or worsening HF,
6 combined with a left ventricular ejection fraction (LVEF) $\leq 40\%$ or brain-type natriuretic peptide (BNP)
7 and/or N-terminal pro-BNP (NT-proBNP) plasma levels >400 pg/mL or $>2,000$ pg/mL, respectively.
8 Participants were either not previously treated with angiotensin converting enzyme inhibitors/angiotensin
9 receptor blockers (ACEi/ARB) and/or β -adrenoreceptor blockers (BB) or were receiving $\leq 50\%$ of guideline-
10 recommended target doses and anticipated their initiation or up-titration. All patients were treated with loop
11 diuretics. Participants could be enrolled as inpatients or outpatients. The primary outcome was a composite
12 of all-cause mortality and rehospitalization for HF censored at 2-year follow-up, with each component
13 separately constituting a secondary outcome. The study protocol was approved by local and national ethics
14 committees (EudraCT 2010-020808-29; R&D Ref Number 2008-CA03; MREC Number 10/S1402/39) and
15 all participants provided written informed consent. The study was performed according to the principles
16 outlined in the Declaration of Helsinki.

17 Autoantibody Measurements

18 Measurements of autoantibody titers against $\beta 1$ -, $\beta 2$ - and $\beta 3$ -adrenergic receptors, as well as M2-
19 muscarinic receptors were performed using chemiluminescence immunoassays (ImmunometriX, Berlin,
20 Germany) on blood serum samples from 2256/2516 (89.7%) patients from the BIOSTAT-CHF index cohort,
21 and in 299 controls that self-identified as healthy [acquired commercially from in.vent Diagnostica (Berlin,
22 Germany)]. Assay development and calculation of binding indices are described in the **Supplementary**
23 **Methods**. For quality control of the anti- $\beta 1$ AAB assay, one negative and one positive sample from the

1 control group (binding index below the median and higher than 10 respectively) was measured in duplicate
2 in all microtiter plates during analysis (n=26 duplicates each). As there is no prior knowledge on the
3 threshold for relevant AABs concentrations, the 99th percentile value of negative controls (1.3883) was used
4 to define a cut-off under which an assay would be considered definitively negative (henceforth
5 “seronegative”), while the 1st percentile value of positive controls (7.3818) was similarly used to define a
6 cut-off above which, an assay would be considered definitively positive (henceforth “seropositive”)
7 (**Supplementary Figure 1**). Patients with assay results in-between the two cut-off values were labelled
8 “intermediate”. The same cut-offs were also applied for defining subgroups in the remaining AAB assays.

9 Laboratory Indices

10 Estimated glomerular filtration rate (eGFR) was calculated using the modification of diet in renal
11 disease (MDRD) formula. NT-proBNP and hs-cTnT were measured using sandwich immunoassays (Roche
12 Inc.), and CRP was measured using competitive immunoassays on a Luminex platform (Alere Inc.). Anemia
13 was defined according to the World Health Organization definition.

14 Biomarker Measurements

15 Plasma biomarkers were determined using the proximity extension assay technology (Olink
16 proteomics Inc.), as part of four biomarker panels involving 92 biomarker measurements each
17 (Cardiovascular-II, Cardiovascular-III, Immune Response and Oncology II panels), thus 368 biomarkers in
18 total. The complete list of available biomarkers has been reported previously.¹⁰ Overlapping biomarkers
19 between the four panels were amphiregulin, c-kit ligand and tissue factor pathway inhibitor-2 (measured
20 twice), as well as interleukin-6 (IL-6) (measured three times). For overlapping biomarkers, the mean of all
21 measurements was used, leaving a total of 363 unique biomarkers. Additionally, eight biomarkers were
22 excluded from analyses because >10% of measurements were outside the assay’s limit of detection. Thus,
23 in total, 355 biomarkers were available for further analysis.

1 Pathway Over-representation Analysis

2 Pathway over-representation analysis of the 355 biomarkers was performed using the “GProfiler”
3 pathway analyzer (version e104_eg51_p15_3922dba).¹¹ The results of the analysis were classified based on
4 the gene ontology classification of biological processes (annotation 2022-01-13).¹² Corrections for multiple
5 testing were performed using the built-in g:SCS algorithm, using a false discovery rate threshold of 5%.
6 Only significantly over-represented processes that included ≥ 5 of their constituents, were included in the
7 final selection. The biomarker corneodesmosin could not be analyzed by GProfiler, and since the biomarkers
8 BNP and NT-proBNP share the same protein designation, the effective number of analyzed biomarkers was
9 eventually 353.

10 Selection of GO Biological Processes Related to B-lymphocytes and Antibodies

11 The pathway over-representation analysis of the 353 biomarkers yielded 768 over-represented
12 biological processes. Of these, 13 contained the terms “B cell” and “immunoglobulin”. To eliminate
13 potential data redundancies due to member overlap between processes¹⁰, the 13 selected processes were
14 visualized in a directed acyclic graph, and the most distal non-redundant processes were selected (**Figure**
15 **1A, Figure 1B, Supplementary Figure 2**). This yielded five processes related to B-lymphocytes and
16 antibodies, represented by different combinations of 31 biomarkers (**Figure 1C**).

17 Statistical Analysis

18 Statistical analyses were conducted using R-studio (R version 4.2.0). Normality of continuous
19 variables was determined by visual inspection of histograms and Q-Q plots. Baseline characteristics were
20 compared for each of the four investigated AABs between seronegative, intermediate, and seropositive
21 patients. Normally distributed variables were compared using one-way analyses of variance (ANOVA), not-
22 normally distributed continuous variables using Kruskal-Wallis tests, and categorical/binary variables using
23 Chi-square tests. In case of a significant ANOVA result, pairwise post-hoc testing with independent t-tests

1 using Bonferroni correction was performed. Chemiluminescence assay results for each AAB were compared
2 between patients with HF and healthy controls when considering the corresponding binding index as
3 continuous variable using the Mann-Whitney U test, and when classified based on the previously defined
4 groups by using Chi-square tests. Associations between autoantibody status and the primary outcome and
5 all-cause mortality were investigated using Kaplan-Meier curves, while associations with hospitalization
6 were investigated using cumulative incidence function curves while considering all-cause mortality a
7 competing risk. Univariable associations were investigated with log-rank tests, while multivariable
8 corrections for previously published risk models for this cohort ⁹ were performed using Cox regression,
9 when statistical assumptions were met. These included, among others, age, comorbidities, measures of
10 congestion, NT-proBNP, and renal function ⁹. Statistical testing in survival analyses was performed using
11 the log-rank test. Statistical significance was considered as $p \leq 0.05$, considering the exploratory character of
12 this study.

13 Significant findings from survival analyses were further verified with propensity-score matching
14 (MatchIt package v. 4.5.0) based on the aforementioned variables of BIOSTAT-CHF risk model.
15 Seropositive patients were matched 1:1 to seronegative patients. A nearest-neighbor matching algorithm
16 with a caliper of 0.2 and without replacement, taking into account the region of common support, was used
17 for this purpose.¹³ Unmatched observations were discarded. Statistical as well as graphical balance
18 diagnostics tools were performed in order to evaluate the matching process.

19 To investigate the relative state of B-lymphocyte function in seronegative and seropositive patients,
20 principal component analyses (PCA) were conducted for each AAB, by excluding patients that were
21 categorized as intermediate. Subsequently, the results were plotted on biplots with concentration ellipses for
22 each group.

23

1 **Results**

2 Baseline characteristics for the entire cohort are presented in **Table 1**. Mean age was 69 (12) years,
3 and 602 (26.7%) were women. Ischemia was the most prevalent primary etiology of HF (46%) and median
4 NT-proBNP was 2721.0 pg/mL [1204.3, 5741.8]. Median LVEF was 30% [25-37] and 216 (10.7%) patients
5 had an LVEF>40%. Healthy controls had a median age of 32 years [24, 40] (range: 18-63 years) and 134
6 (44.8%) were women.

7 Comparison of Assay Binding Indices in Healthy Controls and Patients with Heart Failure

8 Assay binding indices for each AAB are presented as continuous variables using half-violin plots
9 with superimposed boxplots and individual data points in **Figure 2A**. There were no statistically significant
10 differences between healthy controls and patients with HF in median binding index for anti- β 1 AABs [1.22
11 (1.00, 1.59) vs. 1.23 (1.01, 1.73), $p=0.238$], anti- β 2 AABs [1.24 (0.97, 2.01) vs. 1.25 (1.01, 1.88), $p=0.516$]
12 and anti-M2 AABs [1.32 (1.05, 2.02) vs. 1.31 (1.00, 2.25), $p=0.722$]. Patients with HF had a higher median
13 binding index for anti- β 3 AABs [1.10 (0.89, 1.39) vs. 1.19 (1.01, 1.46), $p<0.001$].

14 Classification of Autoantibody Status

15 The relative prevalence of seronegative, intermediate, and seropositive status amongst healthy
16 controls and patients with HF are presented in **Figure 2B**. The prevalence of seropositivity amongst patients
17 with HF in increasing order was 1.8% ($n=41$) for anti- β 3, 5.1% ($n=114$) for anti- β 1, 5.9% ($n=133$) for anti-
18 β 2, and 7.6% ($n=172$) for anti-M2 AABs. The proportion of groups was significantly different between
19 patients with HF compared with healthy controls only for anti-M2 AABs, driven by a higher proportion of
20 seropositive patients ($p=0.025$). In total, 382 (16.9%) patients with HF were classified as seropositive for at
21 least one AAB, compared with 37 (12.4%) of the healthy controls ($p=0.045$). Summary statistics for assay
22 binding indices in total and stratified by AAB status are presented in **Supplementary Tables 1 and 2**,
23 respectively.

1 The co-occurrence of either intermediate or seropositive status between the different AABs is
2 presented in **Figure 2C**, while the co-occurrence of seropositive status only is presented in **Figure 2D**.
3 Among the 382 patients that were seropositive for at least one AABs, co-occurrence of seropositivity for
4 any of the remaining AABs had a low prevalence, with the highest value being observed for seropositivity
5 for all four AABs in 15/382 (3.9%) patients. Collectively, 340/382 (89.0%) patients were seropositive for
6 only one of the studied AABs.

7 Clinical Associations of Autoantibody Seropositivity

8 Baseline characteristics stratified by seronegative, intermediate, and seropositive groupings for each
9 AAB are presented in **Tables 1-4** for anti- β 1, anti- β 2, anti- β 3, and anti-M2 AABs, respectively.

10 *Anti- β 1-Autoantibodies*

11 Anti- β 1 AAB seropositivity was associated with a lower prevalence of a primary cardiomyopathy
12 etiology [seronegative vs. intermediate vs. seropositive: 372 (27.1%) vs. 169 (23.2%) vs. 15 (13.4%),
13 $p=0.002$], a higher prevalence of a history of renal disease [366 (26.2%) vs. 216 (29.1%) vs. 46 (40.1%),
14 $p=0.004$], anemia [446 (35.1%) vs. 250 (37.0%) vs. 50 (47.2%), $p=0.043$], and chronic obstructive
15 pulmonary disease (COPD) [232 (16.6%) vs. 133 (17.9%) vs. 30 (26.3%), $p=0.030$]. These were also
16 mirrored by corresponding changes in serum creatinine and hemoglobin ($p=0.027$ and 0.047 respectively).
17 Lastly, seropositivity was associated with a lower prevalence of mineralocorticoid receptor antagonist
18 (MRA) use [766 (54.8%) vs. 379 (51.0%) vs. 51 (44.7%), $p=0.045$]. Amongst patients with a primary
19 ischemic HF etiology, β 1-seropositivity did not differ significantly amongst those with and without a history
20 of MI [40 (5.2%) vs. 17 (7.0%), $p=0.514$].

21

1 ***Anti-β2-Autoantibodies***

2 Anti-β2 AAB seropositivity was associated with history of stroke [129 (9.7%) vs. 63 (7.9%) vs. 20
3 (15.0%), p=0.027], and current smoking (p=0.021). The proportion of seropositive patients with an
4 LVEF \geq 40% was modestly lower compared to seronegative patients [145 (12.2%) vs. 60 (8.5%) vs. 11
5 (9.2%), p=0.034]. Seropositive patients were more likely to have been recruited as outpatients rather than
6 inpatients [458 (34.6%) vs. 223 (28.0%) vs. 53 (39.8%), p=0.001]. Similar to anti-β1 seropositive patients,
7 anti-β2 seropositivity was associated with a lower prevalence of MRA use [677 (51.1) vs. 458 (57.5%) vs.
8 61 (45.9%), p=0.004]. Seropositive diabetics were also less likely to be using oral hypoglycemic agents
9 (p=0.042).

10 ***Anti-β3-Autoantibodies***

11 Anti-β3 AAB seropositivity was associated with a higher prevalence of atrial fibrillation (AF) and
12 COPD [687 (43.7%) vs. 316 (49.4%) vs. 21 (51.2%), p=0.038, and 260 (16.5%) vs. 123 (19.2%) vs. 12
13 (29.3%), p=0.044, respectively]. In addition, mean eGFR was lower in seropositive patients [65.92 (26.57)
14 vs. 62.98 (24.91) vs. 60.50 (31.33), p=0.032], with corresponding increases in serum creatinine (p=0.027).
15 Lastly, a greater proportion of seropositive patients was at the guideline-recommended target dose for BB
16 [84 (5.3%) vs. 36 (5.6%) vs. 7 (17.1%), p=0.006].

17 ***Anti-M2-Autoantibodies***

18 Seropositivity for anti-M2 AABs was associated with a higher probability of being at the guideline-
19 recommended target dose for ACEi [157 (13.0%) vs. 105 (12.1%) vs. 33 (19.2%), p=0.040], as well as
20 higher probability of MRA use [615 (50.8%) vs. 477 (54.8%) vs. 104 (60.5%), p=0.026].

21

1 Survival Analysis

2 Kaplan-Meier survival curves are presented for each AAB in **Figure 3A** for the combined outcome,
3 and all-cause mortality alone (**Figure 3B**) at 2-year follow-up. Cumulative incidence function curves for
4 the competing risk analysis of HF rehospitalization at 2-year follow-up with all-cause mortality as a
5 competing risk are presented in **Figure 3C**. Regarding the primary outcome, anti- β 1 seropositive patients
6 trended towards a poorer prognosis, without reaching statistical significance ($p=0.068$). Anti- β 3 status was
7 associated with the primary outcome, with seropositive patients having an overall better prognosis
8 ($p=0.016$). Anti- β 2 and anti-M2 status were not significantly associated with the primary outcome. There
9 were no significant associations between AAB status and all-cause mortality. Competing risks analysis
10 revealed only a significant association between anti- β 1 seropositivity and a higher probability of HF
11 rehospitalization ($p=0.029$). A sensitivity analysis was performed after merging seronegative and
12 intermediate patients because these groups showed comparable results in previous survival analyses. This
13 revealed that the primary outcome and HF rehospitalization alone were more frequent only in patients who
14 were seropositive for anti- β 1 AABs ($p=0.023$ and 0.008 , respectively) (**Figure 4**).

15 Since the two significant comparisons for anti- β 1 AABs in the sensitivity analysis also met the
16 criteria for the proportionality of hazards assumption, additional multivariable analyses were performed
17 (Cox regression for the combined outcome and competing risks regression for rehospitalization). Compared
18 with seronegative/intermediate status, anti- β 1 seropositivity was associated with the combined outcome
19 [hazard ratio (HR), 95% confidence interval (95% CI): 1.37 (1.04-1.81), $p=0.024$] but ceased to be
20 significant when corrected for the previously published BIOSTAT-CHF risk model [HR (95% CI): 1.27
21 (0.97-1.68), $p=0.087$]. Anti- β 1 seropositivity was associated with HF rehospitalization both in univariable
22 analysis [HR (95% CI): 1.57 (1.13-2.19), $p=0.010$], and when adjusted for the BIOSTAT-CHF risk model
23 [HR (95% CI): 1.47 (1.05-2.07), $p=0.030$]. Additional validation was performed by means of propensity
24 score matching as described in the methods section. All patients that were anti- β 1 seropositive except one

1 (n=113), were matched 1:1 to seronegative patients, forming well-balanced groups (standardized mean
2 differences <0.1, variance ratios <2). Seropositive patients had significantly higher risk for HF-
3 rehospitalization at 2-year follow-up (standardized HR (95% CI) 1.83 (1.08-3.10), p=0.024).

4 Because patients with intermediate AAB status seemed to have a different prognosis, particularly
5 in the case of anti- β 3 AABs (**Figure 3**), additional analyses were performed in patients with intermediate
6 AAB status for all AABs, in order to investigate whether the AAB titer as a continuous variable was
7 associated with any of the examined outcomes (**Supplementary Table 3**). Intermediate patients for anti- β 3
8 AABs showed an inverse association between anti- β 3 AAB titer and the combined outcome [HR (95% CI):
9 0.82 (0.71-0.94) for each 1-unit increase, p=0.006], which remained significant after adjustment for the
10 corresponding risk model [HR (95% CI): 0.83 (0.72-0.95), p=0.009]. In patients with intermediate anti- β 2
11 status, AAB titers were associated with reduced all-cause mortality [HR (95% CI): 0.83 (0.72-0.95),
12 p=0.008]. However, this was no longer significant after multivariable correction for the corresponding risk
13 model (p=0.196). Patients with intermediate status for anti- β 1/anti-M2 AABs did not show associations
14 between their corresponding AAB titers and any examined outcomes.

15 Analysis of Circulating B-lymphocyte-Associated Markers in Relation to Autoantibody Seropositivity

16 PCA was used on the 31 B-lymphocyte-related biomarkers (**Figure 5**). Concentration ellipses were
17 plotted for seropositive and seronegative individuals per AAB. All sub-analyses demonstrated considerable
18 overlap between seropositive and seronegative patients. The relative contribution of the 31 biomarkers to
19 the 1st and 2nd principal components is presented in **Supplementary Figure 3**.

20 **Discussion**

21 In this post-hoc analysis of a large and heterogeneous cohort of patients with HF, 16.9% of patients
22 were seropositive for at least one ANS-AAB. Overall, the prevalence of seropositivity did not significantly
23 differ from that of healthy controls, with the exception of a significantly higher prevalence of seropositivity

1 for anti-M2 AABs. Seropositivity for ANS-AABs was associated with the presence of comorbidities (renal
2 disease, COPD, AF, stroke), or ARB/MRA use. Only seropositivity for anti- β 1 AABs was significantly
3 associated with a higher probability of HF rehospitalization at 2-year follow-up. PCA revealed considerable
4 overlap of B-lymphocyte activity between seropositive and seronegative patients with HF, based on 31
5 circulating biomarkers.

6 Most studies evaluating ANS-AABs in patients with HF have been conducted in patients with
7 Chagas cardiomyopathy and non-hereditary DCM, mostly focusing on anti- β 1 and anti-M2 AABs.
8 Approximately a third of patients with asymptomatic Chagas disease have detectable anti- β 1, anti- β 2 and
9 anti-M2 AABs, while almost all who develop cardiomyopathy, are seropositive for all aforementioned
10 AABs.⁸ Among patients with non-hereditary DCM, anti- β 1 and anti-M2 AABs were detected in 31% and
11 36-39% of patients, respectively.¹⁴ Interestingly, immunoglobulin subclasses may also be relevant, as in
12 patients with DCM the presence of IgG3-anti- β 1 AABs was associated with a better LVEF during follow-
13 up compared with seronegative patients, or those with non-IgG3-anti- β 1 AABs.¹⁵ Furthermore, an increased
14 prevalence of ANS-AAB seropositivity has been reported in women with peripartum cardiomyopathy
15 (PPCM) compared with healthy pregnant controls.¹⁶ In patients with non-hereditary DCM, anti- β 1 AABs
16 are also associated with an increased risk of ventricular tachycardia and sudden cardiac death¹⁷, while anti-
17 M2 AABs predict rehospitalization but not all-cause mortality in PPCM.¹⁸ In our study, we only identified
18 an independent association between seropositivity for anti- β 1 AABs with HF rehospitalization at 2-year
19 follow-up, similar to a previous study in a smaller cohort.¹⁹ This might be explained by counterbalance of
20 anti- β 1 AAB actions by anti- β 2 AABs,²⁰ although their co-occurrence was limited. Conversely,
21 seropositivity for anti- β 3 AABs trended towards an association with better prognosis. However, the low
22 seroprevalence of anti- β 3 AABs made additional analyses difficult.

23 ANS-AABs can potentially influence cardiac function by causing inappropriate positive or negative
24 chronotropic or inotropic effects, by inducing cardiomyocyte apoptosis, or by activating the complement

1 cascade.^{14,21–23} Other authors reported that anti- β 1 AABs from patients with DCM enhanced T-cell
2 proliferation via PKA/MAPK-signaling, which was prevented by metoprolol.²⁴ They also reduced
3 interferon- γ production, while increasing IL-4 production by T-cells.²⁴ Anti- β 1 AABs also impaired
4 endothelial function in In Wistar rats by negatively affecting NO signaling.²⁵ In contrast, anti- β 3 AABs from
5 patients with HF protected rats with abdominal aortic banding from developing LV dysfunction and
6 dilation.²⁶ Anti- β 1 and anti-M2 AABs have been found to induce HF in rodents, which could be reversed
7 upon their removal.^{27,28} This suggests a drug-like function on their corresponding target receptors.²⁸
8 However, the pharmacology governing these interactions is as of yet unclear²⁸ and the agonist or antagonist
9 characteristics of anti- β 1 AABs can also be of interest with regard to whether their removal would lead to
10 clinical benefit.²⁷

11 Extensive studies in the setting of autoimmunity have led to the identification of numerous
12 mechanisms underlying the generation of AABs. One such mechanism has been termed “molecular
13 mimicry”, which describes the process of antibody generation against microbial proteins, which are also
14 structurally homologous to self-peptides.²⁹ A search of the literature did not reveal the presence of any
15 structural homologues of ANS receptors; still, structural similarities between microbial peptides and ANS
16 receptors cannot be ruled out. A different mechanism involves the generation of AABs against strictly
17 intracellular antigens, as often occurs in autoimmune diseases (e.g. anti-nuclear antibodies, anti-cytoplasmic
18 antibodies, etc.).³⁰ The latter is less likely in this case, considering that ANS receptors are transported to the
19 cell membrane. An additional explanation could be that B-lymphocytes expressing B-cell receptors that
20 recognize self-antigens with high affinity are efficiently depleted or functionally silenced, while B-
21 lymphocytes with medium- or low-affinity receptors may escape tolerance mechanisms and give rise to
22 antibody-producing plasma cells.³¹ Collectively, circulating markers of B-lymphocyte function did not
23 differ significantly between seropositive and seronegative patients in our PCA analyses, suggesting that
24 ANS-AAB generation was not an acute process.

1 Autoreactive B-lymphocytes and plasma cells also occur in the general population and
2 seropositivity for AABs associated with thyroid disorders or autoimmune diseases does not per se lead to
3 clinical manifestations.^{14,32,33} Yet, both within the field of cardiovascular disease, as well as in other disease
4 states, circulating ANS-AABs have been implicated in the pathophysiology of various diseases. The reasons
5 behind the differential effects of circulating AABs in the general population and in specific disease processes
6 remain unknown. Natural autoreactive immunoglobulin-M (IgM) antibodies may protect from autoimmune
7 disease³⁴, while the subtype of circulating IgG antibodies as well as the glycosylation and sialylation status
8 of their Fc-segments regulate higher affinity binding to inhibitory Fcγ receptors (FcγRIIA, FcγRIIB).³¹
9 Interestingly, animal studies have shown that the generation of IgG antibodies with pro-inflammatory
10 profiles requires a T-lymphocyte-dependent immune response³⁵, which is in agreement with previous
11 findings by our group underlying the important prognostic role of T-lymphocyte activation in HF.¹⁰
12 Furthermore, anti-β1 AABs from patients with HF lead to cardiac fibrosis, cardiomyocyte apoptosis, LV
13 dilatation, and increases in LV mass in wild-type mice, which was not observed in mice lacking T-
14 lymphocytes.³⁶ The same study showed that blockade of IL-6 production using small interfering RNA in
15 wild-type mice ameliorated cardiomyocyte apoptosis, and that anti-β1 AABs upregulated IL-6 production
16 in T-lymphocytes from patients with HF.³⁶ These findings illustrate the interplay between T-lymphocytes
17 and ANS-AABs and are further supported by the prognostic value of IL-6 in HF.³⁷

18 Limitations

19 The post-hoc character of this study and the relatively low prevalence of AAB seropositivity suggest
20 that some analyses may have been underpowered, particularly in the case of anti-β3 AABs. Additional
21 characteristics for the healthy control population were not available and could thus not be accounted for in
22 statistical analyses. Additionally, the control population was significantly different from the examined
23 patients (younger, more women) and our findings could suggest potential age-dependency of AAB
24 formation. Extensive antibody repertoire mapping was not performed, which might have led to the

1 identification of AABs with greater prognostic implications. In addition, our study did not include any
2 patients with Chagas cardiomyopathy or PPCM and lacked the necessary data to determine the presence of
3 non-hereditary DCM. The BIOSTAT-CHF cohort included patients that were suboptimally treated for HF
4 and might thus not be representative of the general HF population. Furthermore, it could be hypothesized
5 that the up-titration of HF treatments after initial blood sampling could have masked or attenuated the effects
6 of AABs on outcomes. Lastly, we did not evaluate the functional characteristics of measured AABs (e.g.,
7 inhibitory, stimulatory) and the specific isotype/class, subclass and allotype of AABs were not determined.

8 **Conclusion**

9 In a large and heterogeneous population of patients with HF, seropositivity for ANS-AABs was not
10 strongly associated with adverse outcomes and was mostly related to existing comorbidities and medication
11 use, while only seropositivity for anti- β 1 AABs was independently associated with rehospitalization for HF.
12 Further studies are necessary to better elucidate the potential prognostic significance and clinical
13 associations of ANS-AABs in patients with non-hereditary DCM, Chagas cardiomyopathy or PPCM.

14

15 **Acknowledgements:** No acknowledgements to disclose.

16 **Funding:** BIOSTAT-CHF was funded by the European Commission [FP7-242209-BIOSTAT-CHF;
17 EudraCT 2010-020808-29]. The analytical work was supported by the German Federal Ministry for
18 Economic Affairs and Energy (BMWi) and the European Social Fund (ESF); EXIST-Project “Bridge-
19 ART”, FKZ: 03EFLBE114.

20 **Statement of contribution:** GMM (design, analysis, interpretation, drafting, revision), WBM (analysis,
21 revision), AAaM (design, analysis, interpretation, revision), SDA (acquisition, revision), JGFC (acquisition,
22 revision), KD (acquisition, revision), CCL (acquisition, revision), LLN (acquisition, revision), NJS

1 (acquisition, revision), FZ (acquisition, revision), MM (acquisition, revision), PS (design, acquisition,
2 analysis, revision), AH (design, acquisition, analysis, revision), PL (design, acquisition, analysis, revision),
3 DJvV (acquisition, revision), RAdB (acquisition, revision), AAV (acquisition, revision), PvdM (acquisition,
4 revision), LS (conception, design, analysis, interpretation, revision), NB (conception, acquisition, analysis,
5 interpretation, revision)

6 **Disclosures:** AAV received consultancy fees and/or research grants from AstraZeneca, Bayer, Boehringer
7 Ingelheim, BMS, Cytokinetics, Merck, NovoNordisk, Novartis, and Roche diagnostics. PvdM received
8 consultancy fees and/or grants from Novartis, Corvidia, Singulex, Servier, Vifor Pharma, Astra Zeneca,
9 Pfizer and Ionis. R.A.d.B. received grants from AstraZeneca, Abbott, Boehringer Ingelheim, Cardior
10 Pharmaceuticals GmbH, Ionis Pharmaceuticals, Inc., Novo Nordisk, and Roche and speaker fees from
11 Abbott, AstraZeneca, Bayer, Novartis, and Roche. F.Z. received personal fees for trial oversight
12 committees, advisory boards, or speakers' bureau from Acceleron, Amgen, Applied Therapeutics,
13 AstraZeneca, Bayer, Boehringer, Cardior, Cereno Scientific, Cellprothera, CVRx, G3 Pharmaceuticals,
14 Merck, Merck AG, Novartis, NovoNordisk, Pfizer, Servier, Vifor Fresenius, and is Co-Founder of
15 CardioRenal, CVCT. JGFC received consultancy fees and/or research grants from Abbott, Amgen, Astra-
16 Zeneca, Bayer, Boehringer-Ingelheim, Bristol Myers Squibb, Cytokinetics, Idorsia, Johnson & Johnson,
17 Medtronic, Myokardia, Novartis, NI Medical, Pharmacosmos, Pharma Nord, Philips, Respicardia, Servier,
18 Torrent, Vifor, Viscardia. M.M. reports fees from Actelion, Amgen, Astra-Zeneca, Livanova, Servier, Vifor
19 pharma and WindTree Therapeutics as member of clinical trials committees or advisory boards and from
20 Abbott vascular, Bayer, Boehring Ingelhelm and Edwards Therapeutics for speeches at sponsored
21 meetings in the last three years. W.B.M., P.S. and P.L. are founders of ImmunometriX. W.B.M. and L.S.
22 are listed as inventors on a patent application. All other authors have no relationships to disclose that could
23 be construed as a conflict of interest with regard to this manuscript.

1 References

- 2 1. Linthout S Van, Tschöpe C. Inflammation – Cause or Consequence of Heart Failure or Both? *Curr Heart*
3 *Fail Rep* *Curr Heart Fail Rep*; 2017;**14**:251–265.
- 4 2. Shapiro-Shelef M, Calame KC. Regulation of plasma-cell development. *Nat Rev Immunol* Nature Publishing
5 Group; 2005;**5**:230–242.
- 6 3. Schatz DG, Ji Y. Recombination centres and the orchestration of V(D)J recombination. *Nat Rev Immunol*
7 Nature Publishing Group; 2011;**11**:251–263.
- 8 4. Basten A, Silveira PA. B-cell tolerance: Mechanisms and implications. *Curr Opin Immunol* Elsevier Current
9 Trends; 2010;**22**:566–574.
- 10 5. Lleo A, Invernizzi P, Gao B, Podda M, Gershwin ME. Definition of human autoimmunity - autoantibodies
11 versus autoimmune disease. *Autoimmun Rev* Elsevier; 2010;**9**:A259–A266.
- 12 6. Siloși I, Siloși CA, Boldeanu MV, Cojocaru M, Biciușcă V, Avrănescu CS, Cojocaru IM, Bogdan M,
13 Folcuți RM. The role of autoantibodies in health and disease. *Rom J Morphol Embryol* Editura Academiei
14 Romane; 2016;**57**:633–638.
- 15 7. Jahns R, Boivin V, Hein L, Triebel S, Angermann CE, Ertl G, Lohse MJ. Direct evidence for a β 1-adrenergic
16 receptor–directed autoimmune attack as a cause of idiopathic dilated cardiomyopathy. *J Clin Invest*
17 American Society for Clinical Investigation; 2004;**113**:1419–1429.
- 18 8. Wallukat G, Muñoz Saravia SG, Haberland A, Bartel S, Araujo R, Valda G, Duchon D, Diaz Ramirez I,
19 Borges AC, Schimke I. Distinct Patterns of Autoantibodies Against G-Protein-Coupled Receptors in Chagas’
20 Cardiomyopathy and Megacolon. Their Potential Impact for Early Risk Assessment in Asymptomatic
21 Chagas’ Patients. *J Am Coll Cardiol* *J Am Coll Cardiol*; 2010;**55**:463–468.
- 22 9. Voors AA, Ouwerkerk W, Zannad F, Veldhuisen DJ van, Samani NJ, Ponikowski P, Ng LL, Metra M,
23 Maaten JM ter, Lang CC, Hillege HL, Harst P van der, Filippatos G, Dickstein K, Cleland JG, Anker SD,

- 1 Zwinderman AH. Development and validation of multivariable models to predict mortality and
2 hospitalization in patients with heart failure. *Eur J Heart Fail* Eur J Heart Fail; 2017;**19**:627–634.
- 3 10. Markousis-Mavrogenis G, Tromp J, Ouwerkerk W, Ferreira JP, Anker SD, Cleland JG, Dickstein K,
4 Filippatos G, Lang CC, Metra M, Samani NJ, Boer RA de, Veldhuisen DJ van, Voors AA, Meer P van der.
5 Multimarker profiling identifies protective and harmful immune processes in heart failure: findings from
6 BIOSTAT-CHF. *Cardiovasc Res* Cardiovasc Res; 2021;
- 7 11. Raudvere U, Kolberg L, Kuzmin I, Arak T, Adler P, Peterson H, Vilo J. g:Profiler: A web server for
8 functional enrichment analysis and conversions of gene lists (2019 update). *Nucleic Acids Res* Oxford
9 Academic; 2019;**47**:W191–W198.
- 10 12. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS,
11 Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald
12 M, Rubin GM, Sherlock G. Gene ontology: Tool for the unification of biology. *Nat. Genet.* NIH Public
13 Access; 2000. p. 25–29.
- 14 13. Zhao Q-Y, Luo J-C, Su Y, Zhang Y-J, Tu G-W, Luo Z. Propensity score matching with R: conventional
15 methods and new features. *Ann Transl Med* AME Publications; 2021;**9**:812–812.
- 16 14. Kaya Z, Leib C, Katus HA. Autoantibodies in heart failure and cardiac dysfunction. *Circ Res* Lippincott
17 Williams & Wilkins Hagerstown, MD; 2012;**110**:145–158.
- 18 15. Nagatomo Y, McNamara DM, Alexis JD, Cooper LT, Dec GW, Pauly DF, Sheppard R, Starling RC, Tang
19 WHW, Janosko K, McTiernan C, London B, Hanley-Yanez K, Gorcsan J, Tanaka H, Suffoletto M, Oblak C,
20 McNallan A, Koenig LA, Mather P, Pierson N, Rubin S, Bell Y, Ervin A, Boehmer J, Frey P, Alexis J,
21 Schrack J, LaDuke P, Torre-Amione G, et al. Myocardial Recovery in Patients With Systolic Heart Failure
22 and Autoantibodies Against β 1-Adrenergic Receptors. *J Am Coll Cardiol* J Am Coll Cardiol; 2017;**69**:968–
23 977.
- 24 16. Liu J, Wang Y, Chen M, Zhao W, Wang X, Wang H, Zhang Z, Zhang J, Xu L, Chen J, Yang X, Zhang L.

- 1 The correlation between peripartum cardiomyopathy and autoantibodies against cardiovascular receptors.
2 *PLoS One* PLoS One; 2014;**9**.
- 3 17. Iwata M, Yoshikawa T, Baba A, Anzai T, Mitamura H, Ogawa S. Autoantibodies against the second
4 extracellular loop of beta1-adrenergic receptors predict ventricular tachycardia and sudden death in patients
5 with idiopathic dilated cardiomyopathy. *J Am Coll Cardiol* American College of Cardiology
6 Foundation Washington, D.C.; 2001;**37**:418–424.
- 7 18. Ma G, Wang Y, Hou D, Liu J, Zhang J, Xu L, Wang H, Zhao W, Zhang Y, Zhang L. Association of
8 autoantibodies against the M2-muscarinic receptor with long-term outcomes in peripartum cardiomyopathy
9 patients: A 5-year prospective study. *J Cardiol* Elsevier; 2019;**74**:251–257.
- 10 19. Nagatomo Y, Li D, Kirsop J, Borowski A, Thakur A, Tang WHW. Autoantibodies Specifically Against β 1
11 Adrenergic Receptors and Adverse Clinical Outcome in Patients With Chronic Systolic Heart Failure in the
12 β -Blocker Era: The Importance of Immunoglobulin G3 Subclass. *J Card Fail* NIH Public Access;
13 2016;**22**:417–422.
- 14 20. Cao N, Chen H, Bai Y, Yang X, Xu W, Hao W, Zhou Y, Chai J, Wu Y, Wang Z, Yin X, Wang L, Wang W,
15 Liu H, Fu MLX. β 2-adrenergic receptor autoantibodies alleviated myocardial damage induced by β 1-
16 adrenergic receptor autoantibodies in heart failure. *Cardiovasc Res* Oxford Academic; 2018;**114**:1487–1498.
- 17 21. Zhang Y, Liu J, Shi L, Chen M, Liu J. β 1-Adrenoceptor antibodies induce PPCM via inhibition of PGC-1 α
18 related pathway. *Scand Cardiovasc J* Taylor and Francis Ltd.; 2021;**55**:160–167.
- 19 22. Staudt A, Mobini R, Fu M, Große Y, Stangl V, Stangl K, Thiele A, Baumann G, Felix SB. β 1-adrenoceptor
20 antibodies induce positive inotropic response in isolated cardiomyocytes. *Eur J Pharmacol* Eur J Pharmacol;
21 2001;**423**:115–119.
- 22 23. Shi L, Liu J, Zhang Y, Chen M, Liu J, Shi L. β 1 adrenoceptor antibodies induce myocardial apoptosis via
23 inhibiting PGC-1 α -related pathway. *BMC Cardiovasc Disord* BioMed Central; 2020;**20**.
- 24 24. Du Y, Yan L, Wang J, Zhan W, Song K, Han X, Li X, Cao J, Liu H. β 1-Adrenoceptor Autoantibodies from

- 1 DCM Patients Enhance the Proliferation of T Lymphocytes through the β 1-AR/cAMP/PKA and p38 MAPK
2 Pathways. *PLoS One* Public Library of Science; 2012;**7**:e52911.
- 3 25. Abdelkrim MA, Leonetti D, Montaudon E, Chatagnon G, Gogny M, Desfontis JC, Noireaud J, Mallem MY.
4 Antibodies against the second extracellular loop of β 1- adrenergic receptors induce endothelial dysfunction
5 in conductance and resistance arteries of the Wistar rat. *Int Immunopharmacol* Int Immunopharmacol;
6 2014;**19**:308–316.
- 7 26. Wang J, Li M, Ma X, Bai K, Wang L, Yan Z, Lv T, Zhao Z, Zhao R, Liu H. Autoantibodies against the β 3-
8 Adrenoceptor Protect from Cardiac Dysfunction in a Rat Model of Pressure Overload. *PLoS One* PLOS;
9 2013;**8**:78207.
- 10 27. Bornholz B, Roggenbuck D, Jahns R, Boege F. Diagnostic and therapeutic aspects of β 1-adrenergic receptor
11 autoantibodies in human heart disease. *Autoimmun. Rev.* Autoimmun Rev; 2014. p. 954–962.
- 12 28. Herda LR, Felix SB, Boege F. Drug-like actions of autoantibodies against receptors of the autonomous
13 nervous system and their impact on human heart function. *Br. J. Pharmacol.* Br J Pharmacol; 2012. p. 847–
14 857.
- 15 29. Cusick MF, Libbey JE, Fujinami RS. Molecular mimicry as a mechanism of autoimmune disease. *Clin Rev*
16 *Allergy Immunol* Nature Publishing Group; 2012;**42**:102–111.
- 17 30. Burbelo PD, Iadarola MJ, Keller JM, Warner BM. Autoantibodies Targeting Intracellular and Extracellular
18 Proteins in Autoimmunity. *Front Immunol* Frontiers Media S.A.; 2021;**12**:5.
- 19 31. Ludwig RJ, Vanhoorelbeke K, Leyboldt F, Kaya Z, Bieber K, McLachlan SM, Komorowski L, Luo J,
20 Cabral-Marques O, Hammers CM, Lindstrom JM, Lamprecht P, Fischer A, Riemekasten G, Tersteeg C,
21 Sondermann P, Rapoport B, Wandinger KP, Probst C, Beidaq A El, Schmidt E, Verkman A, Manz RA,
22 Nimmerjahn F. Mechanisms of autoantibody-induced pathology. *Front Immunol* Frontiers Media S.A.;
23 2017;**8**:603.
- 24 32. Haller-Kikkatalo K, Alnek K, Metspalu A, Mihailov E, Metsküla K, Kisand K, Pisarev H, Salumets A, Uibo

- 1 R. Demographic associations for autoantibodies in disease-free individuals of a European population. *Sci*
2 *Rep* Nature Publishing Group; 2017;7.
- 3 33. Akmatov MK, Röber N, Ahrens W, Flesch-Janys D, Fricke J, Greiser H, Günther K, Kaaks R, Kemmling Y,
4 Krone B, Linseisen J, Meisinger C, Moebus S, Obi N, Guzman CA, Conrad K, Pessler F. Anti-nuclear
5 autoantibodies in the general German population: Prevalence and lack of association with selected
6 cardiovascular and metabolic disorders-findings of a multicenter population-based study. *Arthritis Res Ther*
7 *BioMed Central Ltd.*; 2017;**19**:1–9.
- 8 34. Mannoer K, Xu Y, Chen C. Natural autoantibodies and associated B cells in immunity and autoimmunity.
9 *Autoimmunity* Taylor & Francis; 2013;**46**:138–147.
- 10 35. Hess C, Winkler A, Lorenz AK, Holeciska V, Blanchard V, Eiglmeier S, Schoen AL, Bitterling J, Stoehr AD,
11 Petzold D, Schommartz T, Mertes MMM, Schoen CT, Tiburzy B, Herrmann A, Köhl J, Manz RA, Madaio
12 MP, Berger M, Wardemann H, Ehlers M. T cell-independent B cell activation induces immunosuppressive
13 sialylated IgG antibodies. *J Clin Invest* *J Clin Invest*; 2013;**123**:3788–3796.
- 14 36. Du Y, Li X, Yu H, Yan L, Lau WB, Zhang S, Qin Y, Wang W, Ma X, Liu H, Fu M. Activation of T
15 Lymphocytes as a Novel Mechanism in Beta1-Adrenergic Receptor Autoantibody-Induced Cardiac
16 Remodeling. *Cardiovasc Drugs Ther* Springer New York LLC; 2019;**33**:149–161.
- 17 37. Markousis-Mavrogenis G, Tromp J, Ouwerkerk W, Devalaraja M, Anker SD, Cleland JG, Dickstein K,
18 Filippatos GS, Harst P van der, Lang CC, Metra M, Ng LL, Ponikowski P, Samani NJ, Zannad F,
19 Zwinderman AH, Hillege HL, Veldhuisen DJ van, Kakkar R, Voors AA, Meer P van der. The clinical
20 significance of interleukin-6 in heart failure: results from the BIOSTAT-CHF study. *Eur J Heart Fail* *Eur J*
21 *Heart Fail*; 2019;**21**:965–973.

22

23

24

1 **Figure legends**

2 **Figure 1. A.** Directed acyclic graph displaying the 13 over-represented GO biological processes containing
 3 the terms “B cell” and “immunoglobulin”. Non-redundant children terms selected for further analysis are
 4 highlighted in blue. A higher resolution version of this image is presented in **Supplementary Figure 2. B.**
 5 Detailed results of pathway over-representation analysis for the 13 selected biological processes, including
 6 the p-value, intersection size and term size. **C.** Network denoting biomarker membership to the 5 GO
 7 biological processes that were used for further analyses. GO gene ontology; ID identifier; HGNC HUGO
 8 Gene Nomenclature Committee.

9
 10 **Figure 2. A.** Half-violin plots and boxplots with individual data points of assay binding indices for all
 11 investigated autoantibodies, presented separately for healthy controls and patients with HF. **B.** Relative
 12 prevalence of seronegative, intermediate and seropositive status for each autoantibody in healthy controls
 13 and patients with HF. **C.** Venn diagram illustrating the co-occurrence of intermediate and/or seropositive
 14 status for each autoantibody in patients with HF. **D.** Venn diagram illustrating overlapping seropositivity
 15 for each autoantibody in patients with HF. HF heart failure

16
 17 **Figure 3.** Kaplan-Meier curves for the combined outcome (all-cause mortality and rehospitalization for HF)
 18 censored at 2 years (**row A**), and for all-cause mortality alone (**row B**), as well as cumulative incidence
 19 function curves for rehospitalization for HF only with all-cause mortality as a competing risk (**row C**). From
 20 left to right, results of univariable analyses for anti- β 1, anti- β 2, anti- β 3, and anti-M2 autoantibodies. HF
 21 heart failure

22
 23 **Figure 4.** Sensitivity analyses of the results presented in **Figure 3**, by stratifying categories as
 24 seronegative/intermediate and seropositive. Kaplan-Meier curves for the combined outcome (all-cause
 25 mortality and rehospitalization for HF) censored at 2 years (**row A**), and for all-cause mortality alone (**row**
 26 **B**), as well as cumulative incidence function curves for rehospitalization for HF only with all-cause mortality
 27 as a competing risk (**row C**). For anti- β 1 autoantibodies, Cox regression analyses for the combined outcome
 28 and competing risks analyses for HF-related rehospitalization (both univariable and multivariable) are
 29 presented in the results section. HF heart failure

30
 31 **Figure 5.** Biplot of principal component analysis of the 31 biomarkers involved in B-lymphocyte-associated
 32 biological processes. The intermediate group for each AAB was excluded to accentuate potential
 33 differences. Considerable overlap is seen between groups in all comparisons. For optimal visualization, only
 34 the top fifteen biomarkers that contributed the most to the first principal component are shown. The relative
 35 contribution of all examined biomarkers is shown in **Supplementary Figure 3**). AAB autoantibody

36

1

Table 1. Baseline characteristics for the entire patient cohort and stratified according to anti-β1-adrenergic receptor autoantibody status.

Variable	Whole Cohort	Anti-β1 Seronegative	Anti-β1 Intermediate	Anti-β1 Seropositive	p-value
Group Size	2256	1399	743	114	N/A
Demographics					
Age (Years)	69 (12)	69 (12)	69 (11)	70 (12)	0.568
Female sex	602 (26.7%)	401 (28.7%)	167 (22.5%)	34 (29.8%)	0.006*
Recruited as outpatient	822 (32.6%)	455 (32.6%)	242 (32.6%)	37 (32.5%)	0.999
Primary HF Etiology					
Cardiomyopathy	556 (25.1%)	372 (27.1%)	169 (23.2%)	15 (13.4%)	0.002*
Hypertensive	233 (10.5%)	137 (10.0%)	85 (11.7%)	11 (9.8%)	0.468
Ischemic	1018 (46.0%)	619 (45.1%)	342 (47.0%)	57 (50.9%)	0.402
Valvular Disease	167 (7.5%)	104 (7.6%)	49 (6.7%)	14 (12.5%)	0.099
Previous History and Comorbidities					
AF	1024 (45.4%)	636 (45.5%)	331 (44.5%)	57 (50.0%)	0.549
DM	732 (32.5%)	459 (32.9%)	237 (31.9%)	36 (31.6%)	0.884
HT	1398 (62.0%)	868 (62.1%)	458 (61.6%)	72 (63.2%)	0.944
RD	628 (27.9%)	366 (26.2%)	216 (29.1%)	46 (40.4%)	0.004*
Anemia	746 (36.4%)	446 (35.1%)	250 (37.0%)	50 (47.2%)	0.043*
COPD	395 (17.5%)	232 (16.6%)	133 (17.9%)	30 (26.3%)	0.030*
MI	864 (38.3%)	525 (37.6%)	297 (40.0%)	42 (36.8%)	0.525
Stroke	212 (9.4%)	133 (9.5%)	70 (9.4%)	9 (7.9%)	0.849
HF Hospitalization during previous year	711 (31.5%)	439 (31.4%)	242 (32.6%)	30 (26.3%)	0.404
Smoking					
Never	824 (36.6%)	534 (38.3%)	252 (34.0%)	38 (33.6%)	0.16
Past	1105 (49.1%)	656 (47.0%)	390 (52.6%)	59 (52.2%)	
Current	322 (14.3%)	206 (14.8%)	100 (13.5%)	16 (14.2%)	
Clinical Characteristics					
NYHA Functional Class					0.226
I	209 (10.6%)	132 (10.9%)	66 (10.1%)	11 (10.8%)	
II	1014 (51.6%)	644 (53.2%)	323 (49.6%)	47 (46.1%)	
III	663 (33.8%)	391 (32.3%)	236 (36.3%)	36 (35.3%)	
IV	78 (4.0%)	44 (3.6%)	26 (4.0%)	8 (7.8%)	
NYHA Class III or IV	741 (37.7%)	435 (35.9%)	262 (40.2%)	44 (43.1%)	0.095
BMI (kg/m ²)	27.1 [24.1, 30.5]	27.2 [24.2, 30.8]	27.2 [24.0, 30.5]	25.9 [23.6, 29.1]	0.089
HR (beats/min)	76 [67, 90]	76 [67, 88]	76 [67, 90]	75 [67, 85]	0.813
SBP (mm Hg)	125 (22)	125 (22)	125 (23)	126 (21)	0.907
DBP (mm Hg)	75 (13)	75 (13)	75 (14)	73 (14)	0.179
Pulmonary Crackles	1156 (52.8%)	716 (52.7%)	381 (53.1%)	59 (53.2%)	0.984
6MWT Successfully Completed	1413 (64.8%)	888 (65.7%)	459 (64.2%)	66 (58.4%)	0.266
6MWT Distance (m)	220 [0, 350]	222 [0, 356]	216 [0, 350]	160 [0, 328]	0.685
LVEF (%)	30 [25, 36]	30 [25, 35]	30 [25, 37]	33 [25, 40]	0.052
LVEF > 40%	216 (10.7%)	122 (9.8%)	77 (11.7%)	17 (16.7%)	0.062
Laboratory Indices					
NT-proBNP (pg/mL)	2721.0 [1204.3, 5741.8]	2608.5 [1204.3, 5337.5]	2798.0 [1170.0, 6460.0]	3144.0 [1513.0, 6073.0]	0.289
CRP (mg/L)	13.0 [5.8, 26.4]	12.5 [5.8, 26.4]	13.5 [5.8, 26.6]	15.1 [4.8, 26.6]	0.711
eGFR (mL/min/1.73 m ²)	65.0 (26.2)	65.5 (25.7)	64.8 (26.6)	60.1 (29.2)	0.108
Creatinine (μmol/L)	101.0 [82.1, 128.2]	100.0 [81.0, 125.0]	102.0 [84.0, 132.6]	106.1 [88.1, 151.5]	0.027*
Hb (g/dL)	13.2 (1.9)	13.2 (1.9)	13.2 (1.9)	12.8 (1.9) †	0.047*

Downloaded from https://academic.oup.com/advances/advance-article/doi/10.1093/cvr/cvrad042/7072938 by SCD NANCY I user on 10 March 2023

Medications					
BB	1880 (83.4%)	1159 (83.0%)	624 (84.0%)	97 (85.1%)	0.737
BB at target dose	127 (5.6%)	74 (5.3%)	43 (5.8%)	10 (8.8%)	0.295
BB Fraction of Target Dose	0.25 [0.06, 0.50]	0.25 [0.06, 0.48]	0.25 [0.06, 0.50]	0.25 [0.06, 0.50]	0.527
ACEi/ARB	1615 (71.7%)	1011 (72.4%)	531 (71.5%)	73 (64.0%)	0.163
ACEi/ARB at target dose	295 (13.1%)	181 (13.0%)	99 (13.3%)	15 (13.2%)	0.971
ACEi/ARB Fraction of Target Dose	0.25 [0.00, 0.50]	0.25 [0.00, 0.50]	0.25 [0.00, 0.50]	0.25 [0.00, 0.50]	0.267
MRA	1196 (53.1%)	766 (54.8%)	379 (51.0%)	51 (44.7%)	0.045*
Digoxin	424 (18.8%)	259 (18.5%)	149 (20.1%)	16 (14.0%)	0.283
Oral hypoglycemic agents**	457 (62.4%)	290 (63.2%)	150 (63.3%)	17 (47.2%)	0.155

HF heart failure; AF atrial fibrillation; DM diabetes mellitus; HT hypertension; RD renal disease; COPD chronic obstructive pulmonary disease; MI myocardial infarction; NYHA New York heart association; BMI body mass index; HR heart rate; SBP/DBP; systolic/diastolic blood pressure; 6MWT 6-minute walk test; LVEF left ventricular ejection fraction; NT-proBNP N-Terminal pro-Brain Natriuretic Peptide; CRP C-reactive protein; Hb hemoglobin; BB β -adrenoreceptor antagonist; ACEi angiotensin converting enzyme inhibitor; ARB angiotensin receptor blocker; MRA mineralocorticoid receptor antagonist. * $p < 0.05$; **only for patients with diabetes mellitus; †significantly different from seronegative patients in post-hoc testing.

1

Table 2. Baseline characteristics stratified according to anti- β 2-adrenergic receptor autoantibody status.

Variable	Anti- β 2 Seronegative	Anti- β 2 Intermediate	Anti- β 2 Seropositive	p-value
Group Size	1326	797	133	N/A
Demographics				
Age (Years)	69 (12) †	68 (13)	69 (12)	0.046*
Female sex	373 (28.2%)	190 (23.9%)	39 (29.3%)	0.076
Recruited as outpatient	458 (34.6%)	223 (28.0%)	53 (39.8%)	0.001*
Primary HF Etiology				
Cardiomyopathy	325 (24.9%)	197 (25.3%)	34 (26.0%)	0.96
Hypertensive	132 (10.1%)	80 (10.3%)	21 (16.0%)	0.106
Ischemic	606 (46.5%)	359 (46.1%)	53 (40.5%)	0.415
Valvular Disease	96 (7.4%)	62 (8.0%)	9 (6.9%)	0.846
Previous History and Comorbidities				
AF	608 (45.9%)	355 (44.6%)	61 (45.9%)	0.842
DM	451 (34.0%)	242 (30.4%)	39 (29.3%)	0.162
HT	828 (62.5%)	485 (60.9%)	85 (63.9%)	0.695
RD	375 (28.3%)	218 (27.4%)	35 (26.3%)	0.829
Anemia	438 (36.4%)	269 (36.8%)	39 (32.8%)	0.691
COPD	235 (17.7%)	130 (16.3%)	30 (22.6%)	0.207
MI	525 (39.6%)	299 (37.6%)	40 (30.1%)	0.083
Stroke	129 (9.7%)	63 (7.9%)	20 (15.0%)	0.027*
HF Hospitalization during previous year	419 (31.6%)	252 (31.7%)	40 (30.1%)	0.932
Smoking				
Never	494 (37.4%)	284 (35.7%)	46 (34.6%)	0.021*
Past	665 (50.3%)	380 (47.7%)	60 (45.1%)	
Current	163 (12.3%)	132 (16.6%)	27 (20.3%)	
Clinical Characteristics				
NYHA Functional Class				0.32
I	121 (10.5%)	79 (11.4%)	9 (7.8%)	
II	602 (52.2%)	348 (50.1%)	64 (55.7%)	
III	377 (32.7%)	246 (35.4%)	40 (34.8%)	
IV	54 (4.7%)	22 (3.2%)	2 (1.7%)	
NYHA Class III or IV	431 (37.3%)	268 (38.6%)	42 (36.5%)	0.841
BMI (kg/m ²)	27.2 [24.2, 30.9]	27.1 [24.2, 30.4]	26.6 [23.5, 29.5]	0.104
HR (beats/min)	76 [66, 89]	77 [68, 90]	75 [65, 87]	0.158
SBP (mm Hg)	125 (22)	124 (22)	127 (23)	0.221
DBP (mm Hg)	75 (13)	75 (13)	75 (14)	0.95
Pulmonary Crackles	669 (52.2%)	417 (53.6%)	70 (54.3%)	0.787
6MWT Successfully Completed	837 (65.5%)	501 (64.9%)	75 (57.7%)	0.203
6MWT Distance (m)	220 [0, 350]	224 [0, 356]	161 [0, 326]	0.237
LVEF (%)	30 [25, 37]	30 [24, 35]	30 [25, 35]	0.036*
LVEF > 40%	145 (12.2%)	60 (8.5%)	11 (9.2%)	0.034*

Laboratory Indices				
NT-proBNP (pg/mL)	2798.0 [1226.5, 5738.0]	2678.5 [1200.5, 5803.5]	2542.0 [905.5, 5606.0]	0.885
CRP (mg/L)	13.4 [5.8, 26.7]	12.5 [5.9, 24.9]	11.9 [3.7, 26.6]	0.379
eGFR (mL/min/1.73 m ²)	64.2 (25.4)	66.0 (27.3)	66.2 (28.0)	0.275
Creatinine (μmol/L)	101.7 [82.0, 128.0]	101.3 [83.1, 131.9]	96.8 [80.0, 118.8]	0.638
Hb (g/dL)	13.2 (1.9)	13.2 (1.9)	13.4 (1.8)	0.512
Medications				
BB	1107 (83.5%)	660 (82.9%)	113 (85.0%)	0.823
BB at target dose	79 (6.0%)	39 (4.9%)	9 (6.8%)	0.497
BB Fraction of Target Dose	0.25 [0.06, 0.50]	0.25 [0.06, 0.38]	0.25 [0.08, 0.38]	0.617
ACEi/ARB	941 (71.0%)	569 (71.5%)	105 (78.9%)	0.153
ACEi/ARB at target dose	170 (12.8%)	106 (13.3%)	19 (14.3%)	0.869
ACEi/ARB Fraction of Target Dose	0.25 [0.00, 0.50]	0.25 [0.00, 0.50]	0.25 [0.12, 0.50]	0.099
MRA	677 (51.1%)	458 (57.5%)	61 (45.9%)	0.004*
Digoxin	252 (19.0%)	149 (18.7%)	23 (17.3%)	0.886
Oral hypoglycemic agents**	272 (60.3%)	165 (68.2%)	20 (51.3%)	0.042*

HF heart failure; AF atrial fibrillation; DM diabetes mellitus; HT hypertension; RD renal disease; COPD chronic obstructive pulmonary disease; MI myocardial infarction; NYHA New York heart association; BMI body mass index; HR heart rate; SBP/DBP; systolic/diastolic blood pressure; 6MWT 6-minute walk test; LVEF left ventricular ejection fraction; NT-proBNP N-Terminal pro-Brain Natriuretic Peptide; CRP C-reactive protein; Hb hemoglobin; BB β-adrenoreceptor antagonist; ACEi angiotensin converting enzyme inhibitor; ARB angiotensin receptor blocker; MRA mineralocorticoid receptor antagonist. *p<0.05; **only for patients with diabetes mellitus; †significantly different from intermediate patients in post-hoc testing.

1

Table 3. Baseline characteristics stratified according to anti-β3-adrenergic receptor autoantibody status.

Variable	Anti-β3 Seronegative	Anti-β3 Intermediate	Anti-β3 Seropositive	p-value
Group Size	1575	640	41	N/A
Demographics				
Age (Years)	69 (12)	69 (12)	71 (11)	0.346
Female sex	430 (27.3%)	160 (25.0%)	12 (29.3%)	0.495
Recruited as outpatient	504 (32.0%)	213 (33.3%)	17 (41.5%)	0.402
Primary HF Etiology				
Cardiomyopathy	393 (25.5%)	156 (24.6%)	7 (17.9%)	0.532
Hypertensive	154 (10.0%)	76 (12.0%)	3 (7.7%)	0.322
Ischemic	716 (46.5%)	284 (44.9%)	18 (46.2%)	0.794
Valvular Disease	113 (7.3%)	50 (7.9%)	4 (10.3%)	0.732
Previous History and Comorbidities				
AF	687 (43.7%)	316 (49.4%)	21 (51.2%)	0.038*
DM	516 (32.8%)	202 (31.6%)	14 (34.1%)	0.83
HT	988 (62.8%)	385 (60.2%)	25 (61.0%)	0.502
RD	419 (26.6%)	196 (30.6%)	13 (31.7%)	0.142
Anemia	513 (35.5%)	222 (38.9%)	11 (29.7%)	0.248
COPD	260 (16.5%)	123 (19.2%)	12 (29.3%)	0.044*
MI	611 (38.8%)	241 (37.7%)	12 (29.3%)	0.423
Stroke	151 (9.6%)	55 (8.6%)	6 (14.6%)	0.391
HF Hospitalization during previous year	482 (30.6%)	215 (33.6%)	14 (34.1%)	0.374
Smoking				
Never	575 (36.6%)	236 (37.0%)	13 (31.7%)	0.453
Past	765 (48.7%)	321 (50.3%)	19 (46.3%)	
Current	232 (14.8%)	81 (12.7%)	9 (22.0%)	

Clinical Characteristics				
NYHA Functional Class				
I	142 (10.4%)	64 (11.3%)	3 (8.3%)	0.599
II	717 (52.6%)	276 (48.9%)	21 (58.3%)	
III	448 (32.8%)	205 (36.3%)	10 (27.8%)	
IV	57 (4.2%)	19 (3.4%)	2 (5.6%)	
NYHA Class III or IV	505 (37.0%)	224 (39.7%)	12 (33.3%)	0.465
BMI (kg/m ²)	27.2 [24.2, 30.8]	26.9 [23.8, 30.2]	26.6 [24.2, 29.1]	0.224
HR (beats/min)	76 [67, 89]	76 [67, 90]	72 [63, 87]	0.494
SBP (mm Hg)	125 (22)	124 (22)	123 (19)	0.609
DBP (mm Hg)	75 (13)	75 (13)	73 (11)	0.432
Pulmonary Crackles	789 (51.5%)	344 (55.8%)	23 (56.1%)	0.178
6MWT Successfully Completed	997 (65.4%)	394 (64.1%)	22 (56.4%)	0.456
6MWT Distance (m)	225 [0, 360]	215 [0, 344]	197 [0, 314]	0.39
LVEF (%)	30 [25, 36]	30 [24, 36]	30 [25, 35]	0.553
LVEF > 40%	147 (10.5%)	64 (11.2%)	5 (13.9%)	0.737
Laboratory Indices				
NT-proBNP (pg/mL)	2600.0 [1158.0, 5581.0]	2950.0 [1296.5, 5995.5]	2685.5 [1356.5, 5958.8]	0.153
CRP (mg/L)	12.8 [5.7, 26.2]	13.2 [6.0, 26.8]	19.2 [6.9, 32.6]	0.23
eGFR (mL/min/1.73 m ²) †	65.9 (26.6)	63.0 (24.9)	60.5 (31.3)	0.032*
Creatinine (μmol/L)	100.0 [80.9, 126.0]	102.8 [86.0, 133.0]	112.0 [84.0, 140.6]	0.027*
Hb (g/dL)	13.2 (1.9)	13.1 (2.0)	13.4 (1.8)	0.401
Medications				
BB	1307 (83.1%)	538 (84.1%)	35 (85.4%)	0.808
BB at target dose	84 (5.3%)	36 (5.6%)	7 (17.1%)	0.006*
BB Fraction of Target Dose	0.25 [0.06, 0.48]	0.25 [0.08, 0.50]	0.25 [0.05, 0.50]	0.351
ACEi/ARB	1137 (72.3%)	446 (69.7%)	32 (78.0%)	0.309
ACEi/ARB at target dose	201 (12.8%)	87 (13.6%)	7 (17.1%)	0.654
ACEi/ARB Fraction of Target Dose	0.25 [0.00, 0.50]	0.25 [0.00, 0.50]	0.25 [0.12, 0.50]	0.324
MRA	835 (53.1%)	339 (53.0%)	22 (53.7%)	0.996
Digoxin	293 (18.6%)	122 (19.1%)	9 (22.0%)	0.85
Oral hypoglycemic agents**	322 (62.4%)	127 (62.9%)	8 (57.1%)	0.912

HF heart failure; AF atrial fibrillation; DM diabetes mellitus; HT hypertension; RD renal disease; COPD chronic obstructive pulmonary disease; MI myocardial infarction; NYHA New York heart association; BMI body mass index; HR heart rate; SBP/DBP; systolic/diastolic blood pressure; 6MWT 6-minute walk test; LVEF left ventricular ejection fraction; NT-proBNP N-Terminal pro-Brain Natriuretic Peptide; CRP C-reactive protein; Hb hemoglobin; BB β-adrenoreceptor antagonist; ACEi angiotensin converting enzyme inhibitor; ARB angiotensin receptor blocker; MRA mineralocorticoid receptor antagonist. *p≤0.05; **only for patients with diabetes mellitus; †no significant between-group differences in post-hoc testing.

1

Table 4. Baseline characteristics stratified according to anti-M2-muscarinic receptor autoantibody status.

Variable	Anti-M2 Seronegative	Anti-M2 Intermediate	Anti-M2 Seropositive	p-value
Group Size	1213	871	172	N/A
Demographics				
Age (Years)	69 (12)	69 (13)	70 (11)	0.260
Female sex	338 (27.9%)	215 (24.7%)	49 (28.5%)	0.224
Recruited as outpatient	403 (33.1%)	276 (32.0%)	55 (32.0%)	0.862
Primary HF Etiology				
Cardiomyopathy	301 (25.3%)	214 (25.1%)	41 (24.0%)	0.935
Hypertensive	116 (9.7%)	102 (12.0%)	15 (8.8%)	0.195
Ischemic	539 (45.3%)	399 (46.9%)	80 (46.8%)	0.750
Valvular Disease	87 (7.3%)	66 (7.8%)	14 (8.2%)	0.881

Previous History and Comorbidities				
AF	540 (44.6%)	400 (45.9%)	84 (48.8%)	0.539
DM	376 (31.0%)	296 (34.0%)	60 (34.9%)	0.289
HT	733 (60.5%)	555 (63.7%)	110 (64.0%)	0.289
RD	329 (27.2%)	255 (29.3%)	44 (25.6%)	0.449
Anemia	409 (36.9%)	286 (36.1%)	51 (33.6%)	0.704
COPD	204 (16.8%)	152 (17.5%)	39 (22.7%)	0.170
MI	454 (37.5%)	345 (39.6%)	65 (37.8%)	0.611
Stroke	122 (10.1%)	79 (9.1%)	11 (6.4%)	0.275
HF Hospitalization during previous year	383 (31.4%)	276 (32.0%)	52 (30.2%)	0.895
Smoking				
Never	436 (36.0%)	332 (38.2%)	56 (32.6%)	0.308
Past	606 (50.1%)	405 (46.6%)	94 (54.7%)	
Current	168 (13.9%)	132 (15.2%)	22 (12.8%)	
Clinical Characteristics				
NYHA Functional Class				
I	109 (10.4%)	84 (11.0%)	16 (10.5%)	0.518
II	561 (53.4%)	370 (48.6%)	83 (54.2%)	
III	339 (32.3%)	277 (36.4%)	47 (30.7%)	
IV	41 (3.9%)	30 (3.9%)	7 (4.6%)	
NYHA Class III or IV	380 (36.2%)	307 (40.3%)	54 (35.3%)	0.161
BMI (kg/m ²)	27.0 [24.0, 30.5]	27.2 [24.2, 30.8]	27.3 [24.5, 30.5]	0.382
HR (beats/min)	76 [66, 90]	76 [67, 90]	75 [69, 88]	0.898
SBP (mm Hg)	125 (23)	124 (21)	123 (20)	0.131
DBP (mm Hg)	75 (14)	75 (13)	74 (12.2)	0.407
Pulmonary Crackles	615 (52.3%)	454 (54.0%)	87 (50.6%)	0.634
6MWT Successfully Completed	765 (65.3%)	552 (65.6%)	96 (57.8%)	0.142
6MWT Distance (m)	230 [0, 350]	217 [0, 350]	167 [0, 360]	0.539
LVEF (%)	30 [25, 37]	30 [25, 36]	30 [25, 35]	0.832
LVEF > 40%	119 (11.1%)	82 (10.6%)	15 (9.3%)	0.775
Laboratory Indices				
NT-proBNP (pg/mL)	2711.0 [1157.5, 5460.0]	2879.0 [1268.0, 6066.0]	2356.0 [1231.8, 5396.8]	0.253
CRP (mg/L)	13.1 [5.7, 25.0]	13.0 [5.9, 27.1]	13.2 [5.5, 27.1]	0.847
eGFR (mL/min/1.73 m ²)	65.4 (25.8)	64.4 (26.8)	65.0 (26.3)	0.664
Creatinine (μmol/L)	100.0 [82.0, 126.4]	104.2 [83.0, 132.6]	99.5 [83.7, 132.2]	0.167
Hb (g/dL)	13.2 (1.9)	13.2 (1.9)	13.3 (2.0)	0.826
Medications				
BB	1020 (84.2%)	715 (82.1%)	145 (84.3%)	0.410
BB at target dose	69 (5.7%)	45 (5.2%)	13 (7.6%)	0.457
BB Fraction of Target Dose	0.25 [0.06, 0.50]	0.24 [0.06, 0.48]	0.25 [0.06, 0.50]	0.347
ACEi/ARB	866 (71.5%)	625 (71.8%)	124 (72.1%)	0.984
ACEi/ARB at target dose	157 (13.0%)	105 (12.1%)	33 (19.2%)	0.040*
ACEi/ARB Fraction of Target Dose	0.25 [0.00, 0.50]	0.25 [0.00, 0.50]	0.25 [0.00, 0.50]	0.956
MRA	615 (50.8%)	477 (54.8%)	104 (60.5%)	0.026*
Digoxin	223 (18.4%)	169 (19.4%)	32 (18.6%)	0.848
Oral hypoglycemic agents**	227 (60.4%)	191 (64.5%)	39 (65.0%)	0.496

HF heart failure; AF atrial fibrillation; DM diabetes mellitus; HT hypertension; RD renal disease; COPD chronic obstructive pulmonary disease; MI myocardial infarction; NYHA New York heart association; BMI body mass index; HR heart rate; SBP/DBP; systolic/diastolic blood pressure; 6MWT 6-minute walk test; LVEF left ventricular ejection fraction; NT-proBNP N-Terminal pro-Brain Natriuretic Peptide; CRP C-reactive protein; Hb hemoglobin; BB β-adrenoreceptor antagonist; ACEi angiotensin converting enzyme inhibitor; ARB angiotensin receptor blocker; MRA mineralocorticoid receptor antagonist. *p<0.05; **only for patients with diabetes mellitus.

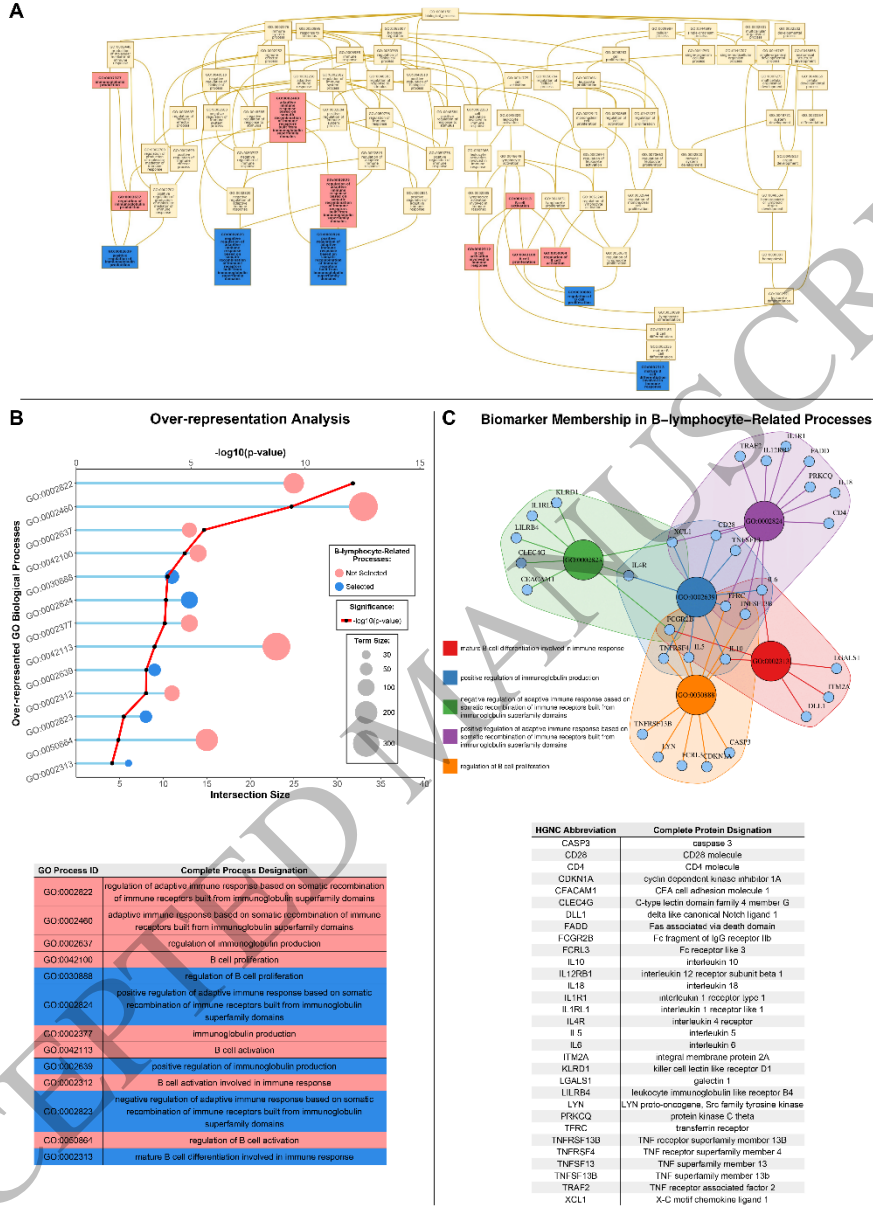


Figure 1
121x160 mm (x DPI)

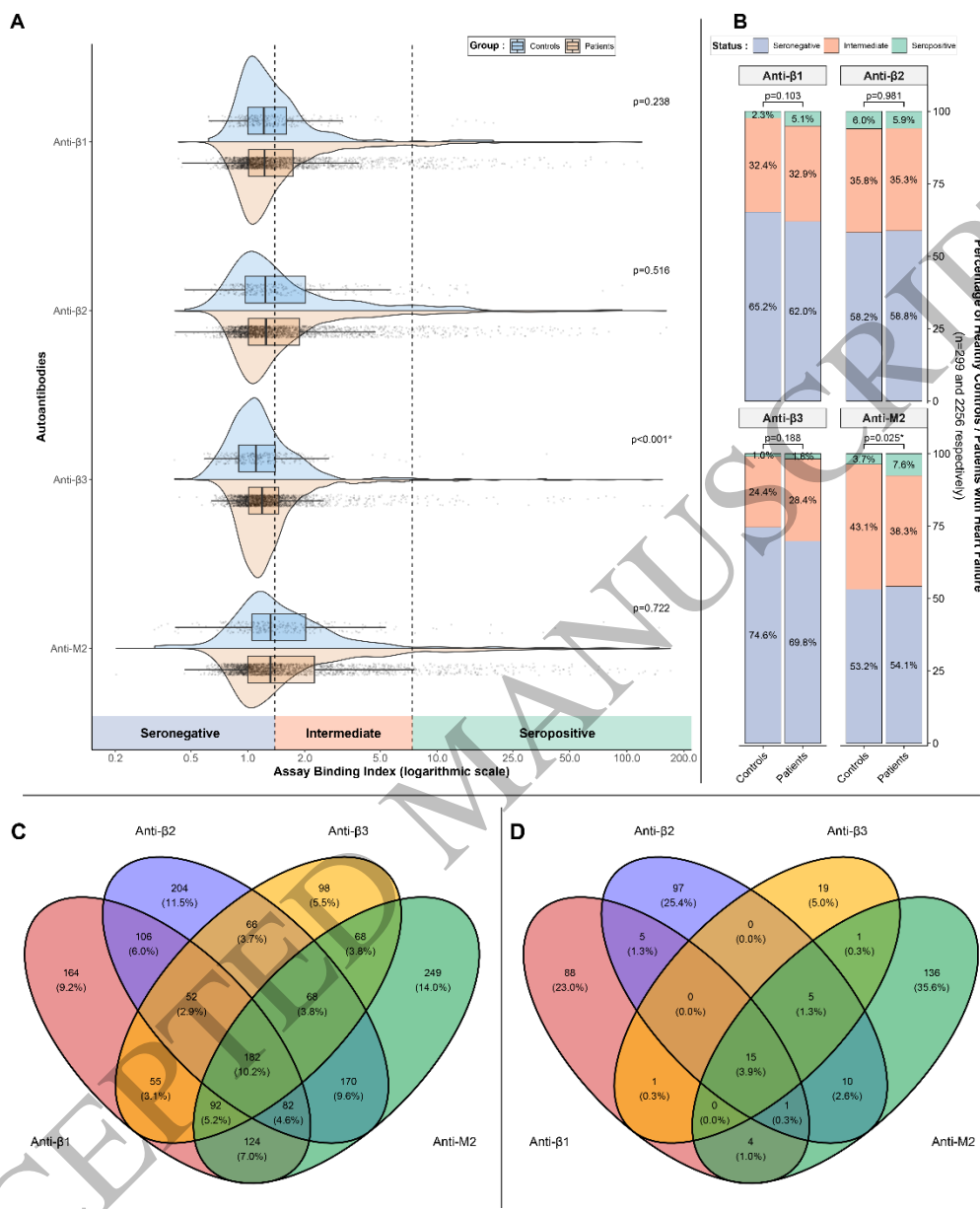


Figure 2
131x160 mm (x DPI)

1
2
3
4

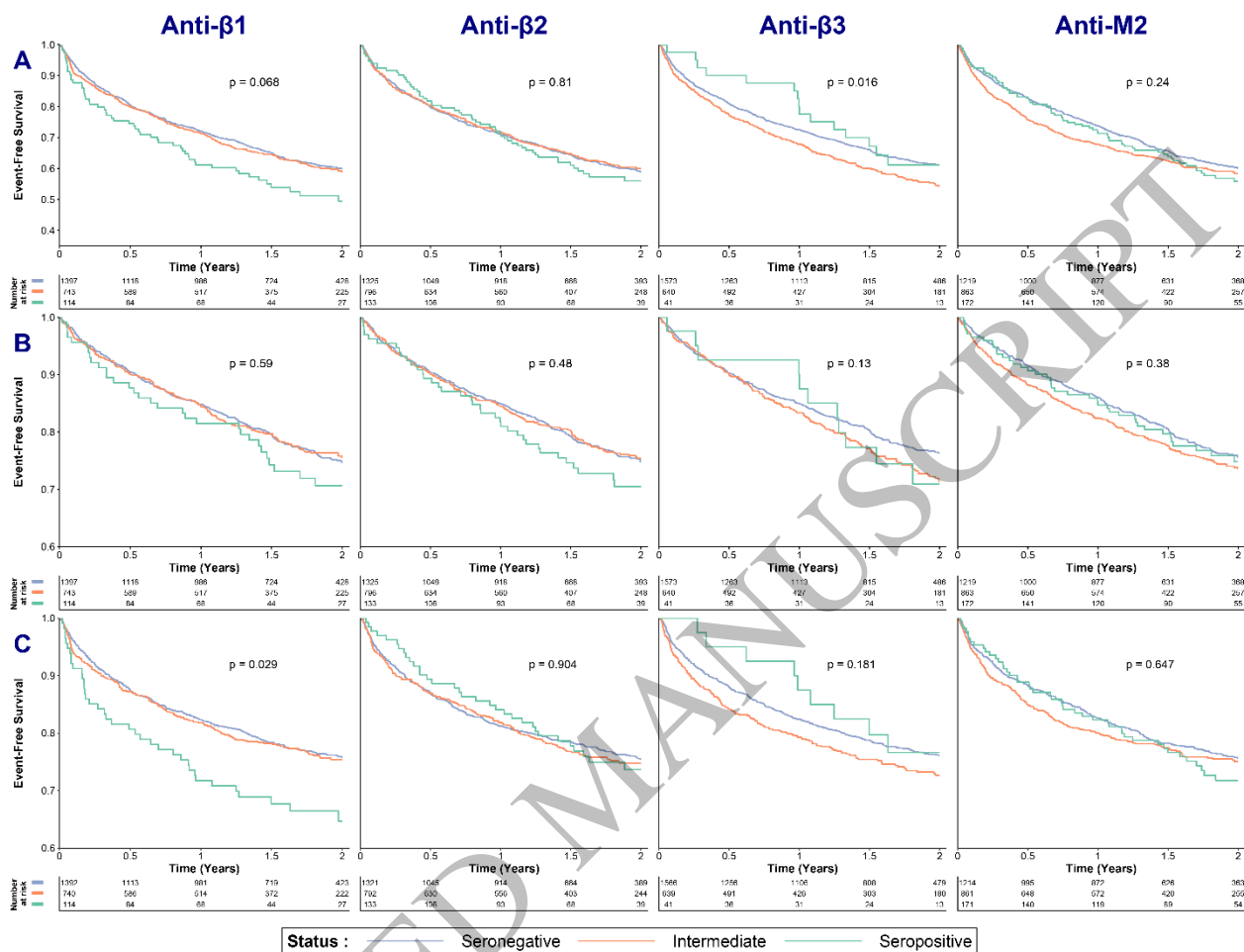


Figure 3
211x160 mm (x DPI)

1
2
3
4

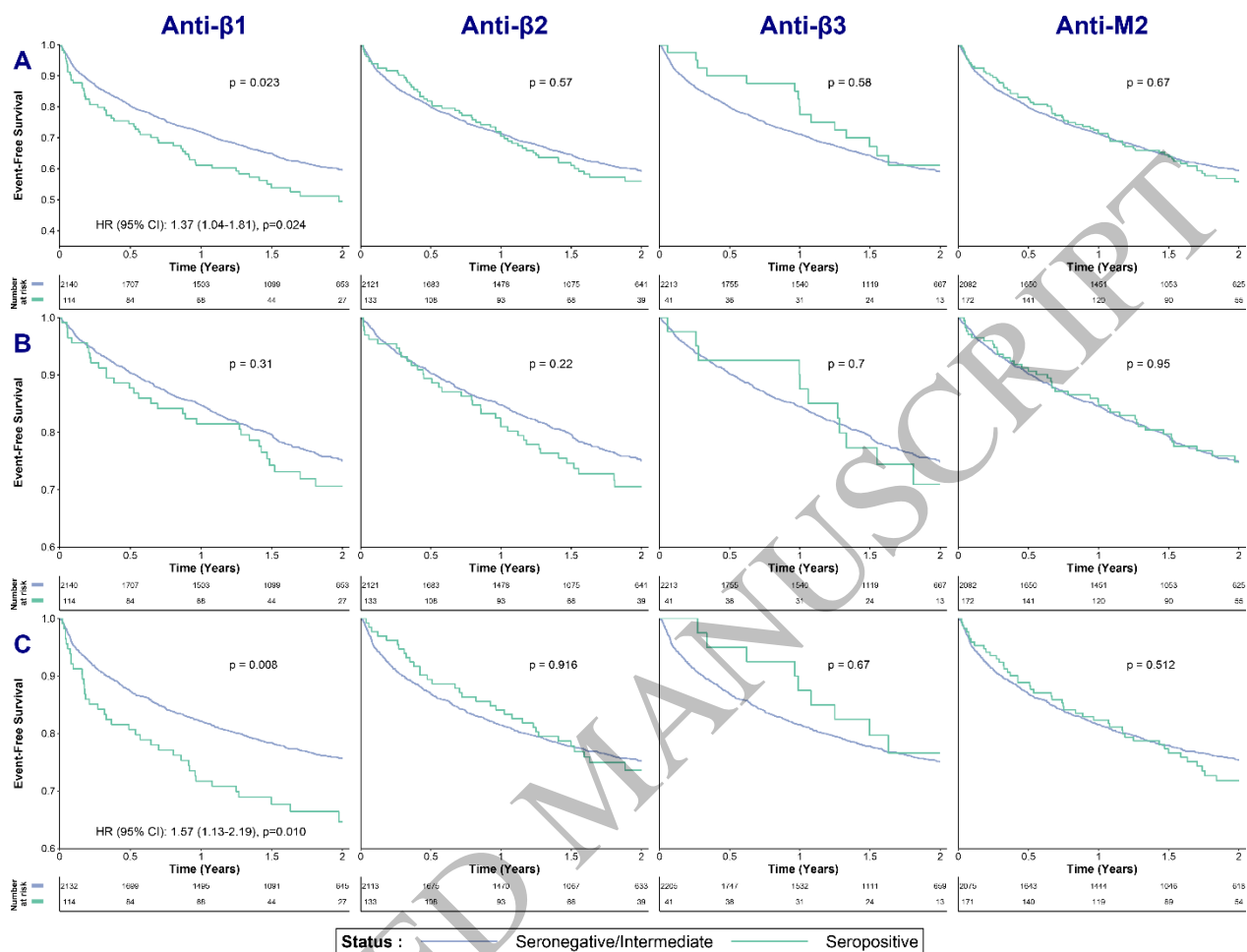


Figure 4
210x160 mm (x DPI)

1
2
3
4

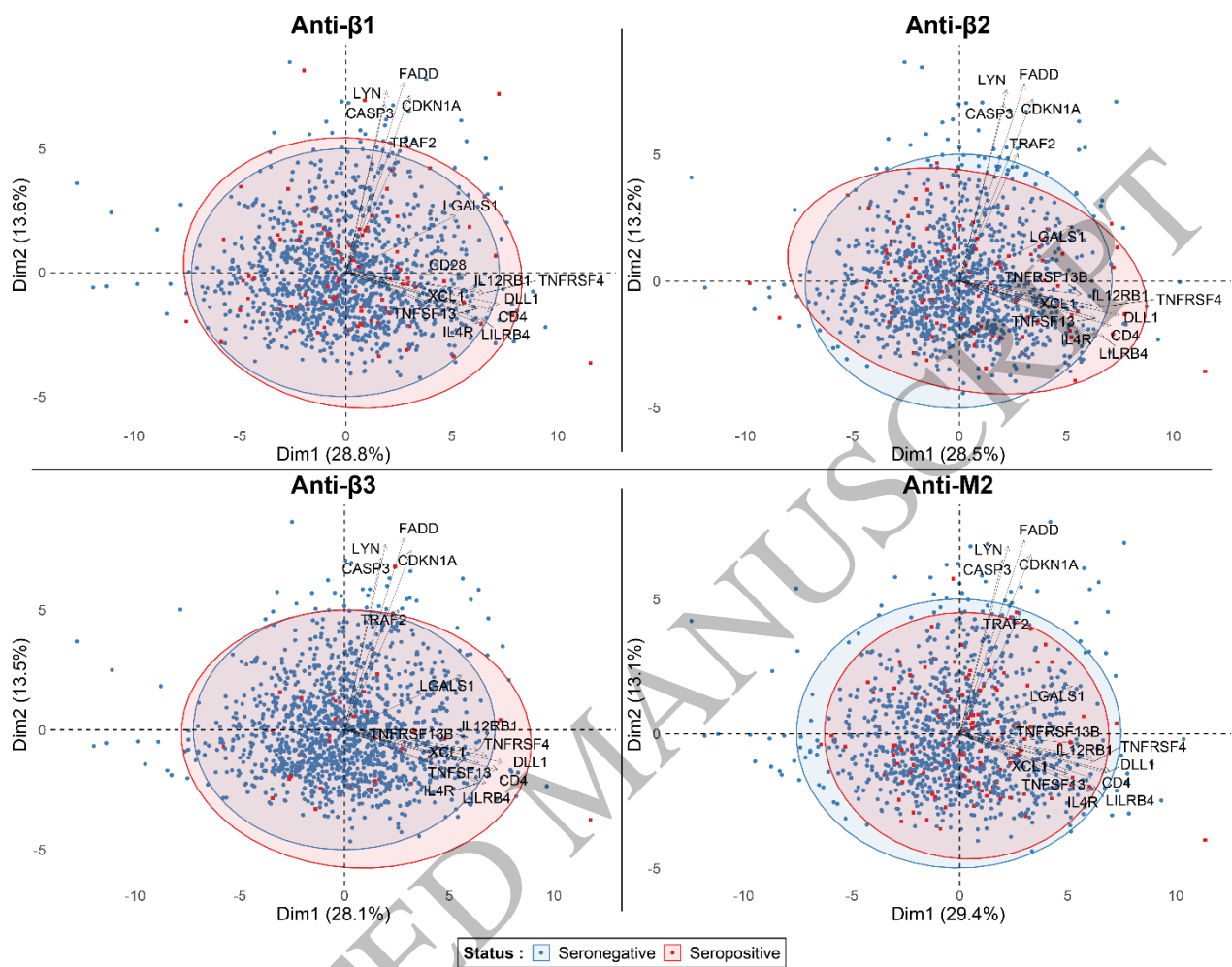
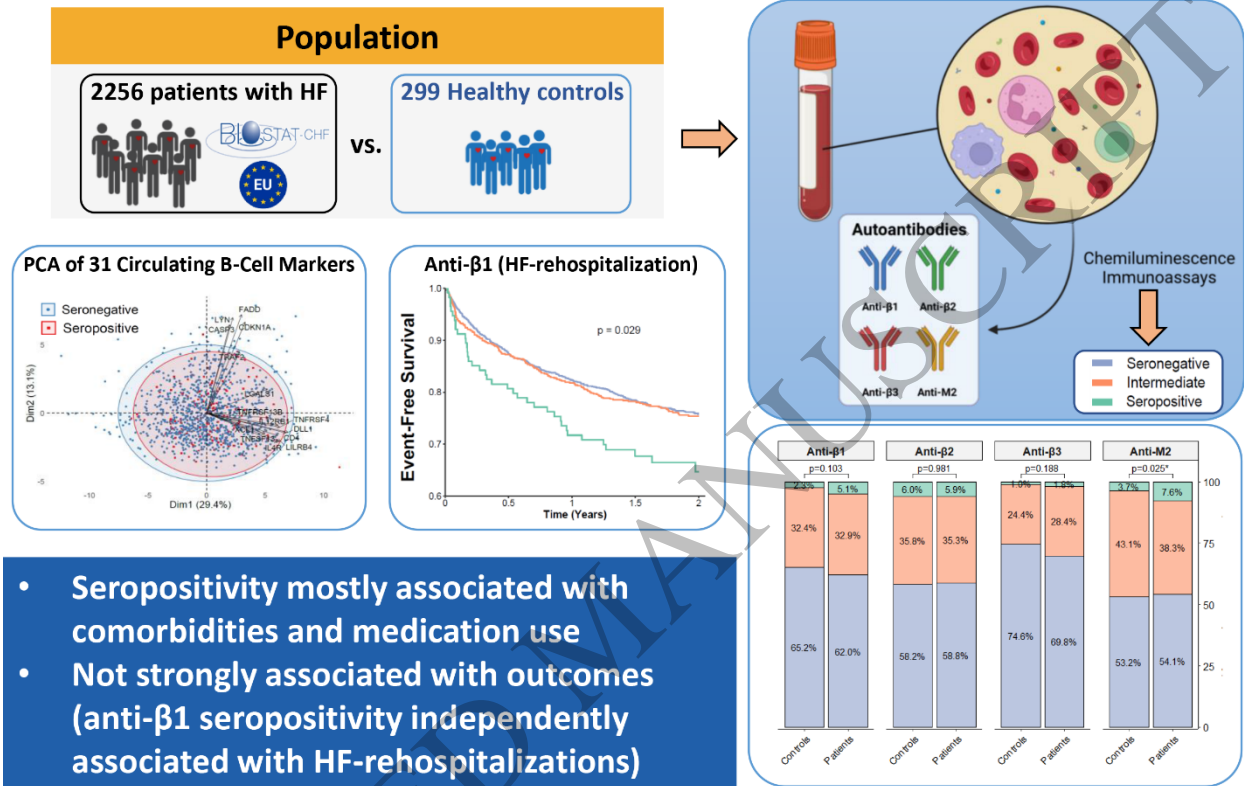


Figure 5
205x160 mm (x DPI)

1
2
3
4

Clinical and Prognostic Associations of Autoantibodies Recognizing Adrenergic/Muscarinic Receptors in Patients with Heart Failure



Graphical Abstract
213x160 mm (x DPI)

1
2
3