Biomarker and transcriptomics profiles of serum selenium concentrations in patients with heart failure are associated with immunoregulatory processes

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Background: Low selenium concentrations are associated with worse outcomes in heart failure (HF). However, the underlying pathophysiologic mechanisms remain incompletely understood. Therefore, we aimed to contrast serum selenium concentrations to blood biomarker and transcriptomic profiles in patients with HF.

Methods: Circulating biomarkers, whole blood transcriptomics and serum selenium measurements in a cohort of 2328 patients with HF were utilized. Penalized linear regression and gene expression analysis were used to assess biomarker and transcriptomics profiles, respectively. As a proof-of-principle, potential causal effects of selenium on excreted cytokines concentrations were investigated using human peripheral blood mononuclear cells (PBMCs).

Results: Mean selenium levels were 60.6 μg/L in Q1 and 122.0 μg/L in Q4. From 356 biomarkers and 20 clinical features, the penalized linear regression model yielded 44 variables with <5 % marginal false discovery rate as predictors of serum selenium. Biomarkers associated positively with selenium concentrations included: epidermal growth factor receptor (EGFR), IFN-gamma-R1, CD4, GDF15, and IL10. Biomarkers associated negatively with selenium concentrations included: PCSK9, TNFSF13, FGF21 and PAI. Additionally, 148 RNA transcripts were found differentially expressed between high and low selenium status ($P_{adj.}<0.05$; log-fold-change$<|0.25|$). Enrichment analyses of the selected biomarkers and RNA transcripts identified similar enriched processes, including regulation processes of leukocyte differentiation and activation, as well as cytokines production. The mRNA expression of two selenoproteins (MSRB1 and GPX4) were strongly correlated with serum selenium, while GPX4, SELENOK, and SELENOS were associated with prognosis. In the *in-vitro* setting, PBMCs supplemented with selenium showed significantly lower abundance of several (pro-)inflammatory cytokines.

Conclusion: These data suggest that immunoregulation is an important mechanism through which selenium might have beneficial roles in HF. The beneficial effects of higher serum selenium concentrations are likely because of global immunomodulatory effects on the abundance of cytokines. MSRB1 and GPX4 are potential modulators of and should be pursued in future research.

1. Introduction

Daily (micro-) nutritional elements intake is an essential environmental factor that is often underestimated. Especially in patients with chronic and complex conditions like heart failure (HF), micronutrients deficiencies may have detrimental prognostic effects [1].

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Micronutrients such as iron, selenium (Se), zinc, copper and several vitamins are integrated with many proteins, which have essential cellular functions [1].

Selenium (Se) deficiency and sub-optimal Se levels are common among patients with HF, as well as in community-based cohorts in several parts of the world [2–5].Se deficiency was associated with new-onset HF in a community-based cohort as well as with worse prognosis in patients with HF as compared to those without deficiency [2,6]. Patients with HF and Se deficiency had higher risk for all-cause mortality and HF hospitalization [6]. Incorporation of Se in the form of selenocysteine is essential for the enzymatic function of 25 proteins, the so-called selenoproteins [7]. These selenoproteins have a broad range of functions, but the majority of them act on the antioxidant defence, redox signalling and thyroid hormone metabolism [8](10). Lethal or detrimental (cardiac) adverse effects were observed in knockout mice models that lack a specific selenoprotein (e.g., GPX4, Txnrd1) [7,9–11]. In addition, inducing Se deficiency in human cardiomyocytes led to lower mitochondrial respiratory capacity and lower ATP production [6], potentially as a consequence of impaired activity of the selenoprotein DIO2 [12]. However, it is still unknown which selenium-related mechanisms may underly outcome in patients with HF and [8](10) how systemic Se deficiency could affect the heart. In this study, we aim to elucidate potential pathophysiological mechanisms that are linked to Se deficiency in patients with HF using whole blood transcriptomics and 363 biomarkers with diverse biologic functions. We also aimed to explore physiological phenomena linked to genes encoding selenoproteins, including the hierarchical regulation of their expressions, their expression changes in relation to Se concentrations as well as their associations with all-cause mortality in HF context.

2. Methods

2.1. Patient population

We performed a post-hoc analysis of the BIOSTAT-CHF (a systems BIOlogy Study to TAilored Treatment in Chronic Heart Failure) index cohort [13,14]. In short, BIOSTAT-CHF was a multi-center, multinational, prospective, observational study that included 2516 patients from 11 European countries with a median follow-up of 21 months [interquartile range (IQR) 15–27 months]. Patients included were older than 18 years old with symptoms of HF that were sub-optimally treated by guideline-based treatment with either left ventricular ejection fraction of ≤40 %, BNP levels >400 pg/mL, or N-terminal pro-brain natriuretic peptide (NT-proBNP) levels >2000 pg/mL. Patients with symptoms of new-onset or worsening HF were included. Patients with septicaemia, acute myocarditis, hypertrophic obstructive, restrictive, or constrictive cardiomyopathy, or who received a heart transplant were excluded. The study protocol was approved by local and national medical ethics committees (EudraCT 2010-020808-29; R&D Ref Number 2008-CA03; MREC Number 10/S1402/39) and all participants provided written informed consent. Further details were described elsewhere [13].

2.2. Laboratory measurements and related definitions

Blood samples were collected at the inclusion timepoint; either during the index hospitalization or at the outpatient clinic and stored subsequently at −80 °C. We used the validated inductively coupled plasma mass spectrometry (ICP-MS) method to measure serum Se levels as described previously [6]. Estimated glomerular filtration rate (eGFR) was calculated based on the Modification of Diet in Renal Disease (MDRD) equation, while iron deficiency was defined as a transferrin saturation (TSAT) < 20 % [15,16] and HF with reduced ejection fraction (HFrEF) as a left ventricular ejection fraction <40 %. An immunoassay based on electrochemiluminescence (Elecsys, Roche Diagnostics, Mannheim, Germany) was used to measure NT-proBNP.

2.3. Biomarker measurements

Plasma biomarkers were determined using the proximity extension assay technology (Olink proteomics Inc.), as part of four biomarker panels involving 92 biomarker measurements each (Cardiovascular-II, Cardiovascular-III, Immune Response and Oncology II panels), thus 368 unique biomarkers in total. The complete list of available biomarkers has been reported previously [11]. Eight biomarkers were excluded from analyses because >10 % of measurements were outside the assay's limit of detection. Two biomarkers and one biomarker were measured twice and three times, respectively, and of these biomarkers, the variables with the lower number of measurements were excluded. The NTproBNP measurement from the central lab was chosen (instead of those measured by OLINK). Thus, in total, 356 biomarkers were available for further analysis.

2.4. Biomarker selection

The ncvreg package in R was used [17] to run a Minimax concave penalty (MCP) linear penalized regression analysis to select the determinants of Se levels. Three categories of potential predictors were included. First, all measured biomarkers except those with a low limit of detection >10 %. Secondly, clinical characteristics associated with cardiovascular risk, including age, sex, body mass index (BMI), alcohol consumption and a history of myocardial infarction, chronic obstructive pulmonary disease (COPD), stroke, peripheral arterial disease, atrial fibrillation (AF), and hypertension, as well as the use of RAAS inhibitors, aldosterone antagonist and beta-blockers next to chronic kidney disease (CKD) (defined by estimated eGFR <60 ml/min/1.73 m^2 according to MDRD). Thirdly, the remained clinical predictors of Se status we determined previously [18], including albumin levels and iron deficiency and the presence of orthopnoea. A complete case analysis was performed. The exact value of the penalization term was determined as the value that minimizes the cross-validation error rate derived from k-fold cross-validation. Only selected features with a marginal false discovery rate (mFDR) < 0.05 were selected for further analysis [19,20]. Penalized regression analysis can overcome the drawbacks of traditional approaches for feature selection (e.g. stepwise multivariable linear regression) [21] and has the statistical properties to select the most relevant predictors by optimizing the variance-bias tradeoff [22]. The applied type of penalization in this study (MCP) has less bias towards features with larger coefficients and has superior performance in the context of highly correlated variables than other penalization methods like least absolute shrinkage and selection operator (LASSO), and was therefore preferred [17,21,23].

2.5. RNA transcripts selection

The BIOSTAT-CHF transcriptomics dataset contains whole blood expression of 944 patients divided in two comparable groups in terms of age and sex. Whole blood transcriptomic profiling was performed in the selected participants using the Affymetrix Human Transcriptomic Array 2.0 (HTA, Thermo Fisher Scientific), as described in detail previously [24]. A differential expression analysis using the LIMMA package from R was used to identify transcript profile in patients with high Se status vs. those with low Se status [25]. To identify the potential distinct profiles, only patients (n = 582) with the highest and lowest tertiles of Se levels were included in the model with Se status as a binary outcome. A total of 17,900 protein-coding transcripts were included in the model and adjusted for age, sex and BMI. Only variables with fold-change >0.25 and adjusted P-value <0.05 were selected for further analysis.

2.6. Protein-protein interaction (PPI) analysis and pathway enrichment analysis

A PPI analysis for the selected biomarkers was performed using the

default settings of STRING database v.11.5 [26] and corrected for the whole genome as a background. The STRING database was selected because of its superiority compared to other databases [27]. For the enrichment analyses of the selected biomarkers and the selected RNA transcripts, the 25 top processes with the lowest FDR as well as with a strength value of higher than 1.0 were constructed in figures. The strength value is defined as the ratio between (a) the number of proteins in the network that are annotated with a term and (b) the number of proteins that is expected by the STRING database to be annotated with this term in a random network of the same size. The whole list of enriched pathways was included in the supplement. The biological processes of the Gene Ontology database and Reactome were reported. Since high Se levels were associated mainly with processes related to adaptive immunity, we investigated the expression of the selected RNA transcripts in various immune cells, using publicly available data obtained from the Human Protein Atlas [28]. These results were visualized using a heatmap.

2.7. In-vitro selenium supplementation in PBMCs and cytokine expression experiment

Since cytokines play important roles in cells signaling and differentiation, we investigated the effects of selenium supplementation on their expressions using human peripheral blood mononuclear cells (PBMCs). PBMCs were obtained from healthy individuals and were isolated and frozen until the day of the assay as described before [29]. The experiment was conducted in two settings: A) PBMCs as a basal state supplemented with and without 100 nM sodium selenite, and as B) PBMCs with anti-CD3/CD28 beads to activate T cells supplemented with and without 100 nM sodium selenite. Each group was treated for 5 days. After the 5th day, the samples were collected and sent to the central laboratory at the UMC Groningen to measure cytokines concentrations in the medium using Human Th9/Th17/Th22 Luminex® Performance Assay 18-plex Fixed Panel.

2.8. Statistical analysis

All statistical analyses were performed using STATA v.16 SE and R v.3.2.3. Patients of the whole cohort were divided into quartiles based on Se concentration. Variables with normal distribution were reported as mean (standard deviation), continuous variables with non-normal distribution as a median (interquartile range), and categorical variables as number (percentage). Baseline characteristics across Se quartiles were analyzed using one-way analysis of variance (ANOVA) for continuous variables with normal distribution, the Kruskal–Wallis test for continuous variables with skewed data and the Chi-squared test for categorical variables. $P < 0.05$ was considered statistically significant.

Since transcriptomics data were measured in a sub-cohort of 944 patients (as described above), a subsequent baseline characteristics analysis was performed. Patients were divided into tertiles based on their Se levels and a comparison was made between patients with the lowest tertile and those with the highest tertile. A *t*-test was used to analyze continuous variables with a normal distribution, the Mann–Whitney *U* test for continuous variables with skewed data, and the Chi-square test for categorical variables.

Furthermore, a multivariable linear regression was performed to investigate the association of each (available) gene encoding selenoprotein (mRNA) and serum Se concentrations, adjusted for age, sex and BMI. Restricted cubic splines, using 4 knots at the percentiles recommended by Harrel, were utilized to visualize the variations of each expression across Se concentration. In addition, the association to 2-year all-cause mortality for the mRNA expression of each gene-encoding selenoprotein was investigated in a multivariable Cox regression, adjusted for the previously published BIOSTAT-CHF model [6]. A correction of multiple testing was performed using the Benjamini-Hochberg procedure. All mRNA expression data of genes

encoding selenoproteins were available, except for glutathione peroxidase 1 (GPX1).

In addition, the cytokines data of the conducted experiment were compared between supplemented vs. non-supplemented samples in each setting separately using the Mann Whitney *U* Test and corrected for multiple testing using the Benjamini-Hochberg procedure.

3. Results

3.1. Patients with lower Se have a worse clinical profile

Se serum levels were measured in 2328 (92.5 %) of the 2516 patients. The mean age in the cohort was 68.8 (12.0) years and the mean Se level was 89.1 (24.8) µg/mL. Patients were divided into quartiles, with the first quartile (Q1) having the lowest Se levels and the fourth quartile (Q4) having the highest levels (mean Se Q1 60.6 [9.1] µg/L vs. Q4 122.0 [18.1] µg/L, *p < 0.001*). Patients with lower levels of Se were older, more likely to be female higher, have NT-proBNP levels and a higher inflammatory profile (Supplementary Table S1). Subsequent analysis of the sub-cohort (N = 582) where transcriptomics were successfully measured showed similar results as the aforementioned published characteristics (Supplementary Table S2).

3.2. Biomarkers predict Se concentration in the MCP linear regression model

An MCP penalized linear regression model was developed to identify the determinants of Se levels. In this model, we included both the 356 biomarkers from the OLINK panels, as well as the described 20 clinically relevant parameters. Patients with missing data were deleted from the model, resulting in 1847 (79.3 %) out of 2328 patients entering the regression model. This model, in turn, resulted in 81 selected features, of which 44 had mFDR <5 % (Fig. 1A). All identified variables were biomarkers measured in the OLINK panels, except for age and BMI. In total, 20 variables were positively associated with Se levels and 24 variables were negatively associated with Se levels. Differentially expressed biomarkers that were associated with high Se levels (i.e., have positive coefficients) included: EGFR, ERbB3/HER3, IFN-gamma-R1, CD4, IL-10, GDF-15 and GH, while the variables that were associated with low Se levels (i.e. have negative coefficients) included: PCSK9, FGF21, SPON2, TNFRSF4, CEACAM1, IL4RA and PAI (Fig. 1A–Supplementary Table S3). PPI analysis of differentially expressed biomarkers showed significant interaction enrichment between nodes (P < 1.0e-16). EGFR and IL10 stand centrally and are connected to several biomarkers, regardless of their association with Se (Fig. 1B). KEGG Pathway enrichment analysis highlighted cytokine-cytokine receptor interaction (hsa04630, P = 6.31e-12; e.g., GDF15, IFNGR1 and IL10) and JAK-STAT signaling (hsa04630; P = 0.0002; e.g. EGFR, IFNGR1 and IL4RA) as most significant enriched pathways (Supplementary Table S4).

3.3. Low vs. high serum selenium exhibits distinct whole blood transcriptomes

Differential expression analysis using the LIMMA package resulted in 4613 differentially expressed genes (DEGs) between low and high serum selenium, after adjustment for age, sex and BMI. Within this set, the DEGs reaching the combined threshold ($|logFC|>0.25$; $P_{FDR}<0.05$) were selected, resulting in a final total of 148 DEGs. 55 % of DEGs (n = 81) were associated with lower Se status (upregulated in the blood of patients in the lowest tertile of serum selenium concentrations) and 45 % of DEGs (n = 67) were associated with high Se status (upregulated in the blood of patients in the tertile with the highest serum selenium concentrations) (Supplementary Table S5). Several of these genes were inflammatory-related genes such as CLEC4D, IL7R, IL2RB, IL18RAP, CD2 and CD28. Reactome pathway enrichment showed a separation based on DEGs between the patient groups based on immune system
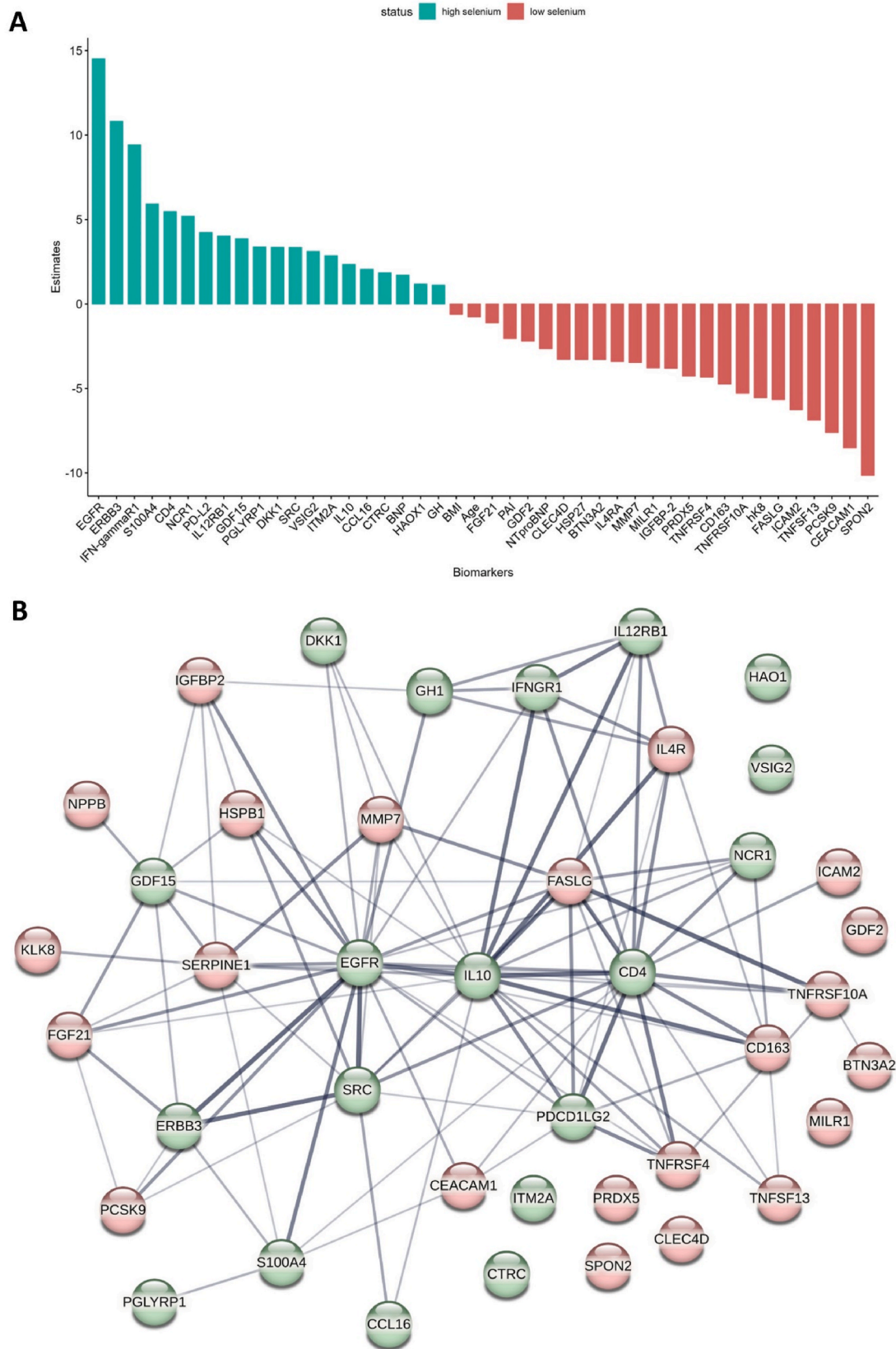
Fig. 1. Panel A: Selected biomarkers that were associated with serum selenium concentrations using MCP penalized linear regression model. All selected biomarkers with marginal FDR <0.05 were included.

In green, variables positively associated with serum selenium. In red, variables negatively associated with serum selenium. Panel B: Proten-protein interaction network of the 42 identified proteins based on STRING database, where epidermal growth factor receptor (EGFR) and IL-10 are at the central of the network as they are connected with several proteins.

activation. Where the DEGs associated with low selenium showed enrichment for the "innate immune system" (hsa168249; n = 14 DEGs; p = 0.0352; e.g., CLEC4D, TLR5 and TNFAIP6), the DEGs associated with high selenium showed enrichment for the "adaptive immune system" (hsa1280218, n = 17 DEGs; p = 4.92e-07; e.g., NFATC2; CD28 and CD96) (Supplementary Tables S6–S8).

3.4. Inflammatory pathways and T lymphocytes are prominent in pathway enrichment analyses

Assessing the enrichment in the biological process of the Gene Ontology database for both differentially expressed biomarkers and DEGs demonstrated great overlap in processes. For differentially expressed biomarkers, the most enriched pathways with the highest strength were related to adaptive immunity, including regulation of adaptive immune response, (GO:0002822, p = 0.00019), positive regulation of lymphocyte proliferation (GO:0050671, p = 0.0013), and regulation of lymphocyte-mediated immunity (GO:0002706, p = 0.0019) (Fig. 2A). While analyzing the DEGs, similar results were observed with specificity towards T cells (Fig. 2B). Notably, as for the Reactome pathway analyses, when differentially expressed biomarkers and DEGs associated with high Se status were analyzed separately, mainly GO-terms related to the adaptive immune system (i.e. T cell and lymphocyte activation) were enriched (Supplementary Table S7, Supplementary Table S9), while DEGs associated with low Se status resulted mainly in less specific immune processes such as exocytosis, response to other organisms or stimulus (Supplementary Table S8, Supplementary Table S10).

The findings of the enrichment analysis are further emphasized, using gene expression data of 18 immune cells from the Human Protein Atlas repository [28]. DEGs that were associated with high selenium levels were mainly expressed in immune cells that are involved in adaptive immunity, including T regulatory cells, memory CD4 and CD8 T cells. On the other hand, those with low Se status were mainly expressed in cells involved in innate immunity such as neutrophils, eosinophils, basophils, and monocytes (Fig. 2C).

3.5. Selenium supplementation dampens cytokine expression in human PBMCs

Several of the measured cytokines were (after correction for multiple testing) significantly lower in the samples supplemented with Se compared to those without supplementation, providing proof-of-concept on the potential effects of selenium on cytokine concentrations. These include CD40 Ligand, GM-CSF, IFN-gamma, IL10, IL-15, IL17A, IL-18, IL-2, IL-6 and MIP-3a (Fig. 3A). In the samples enriched for T cells, most of these cytokines remained significantly lower in the Se-supplemented samples, including CD40 Ligand, GM-CSF, IL-15, IL17E, and MIP-3a. Moreover, the following cytokines were additionally significantly modulated upon enrichment for T cells activation compared to the basal state, including IL12, IL-1B, IL-33, IL-4, IL-5, and IL-9. The absolute differences between the supplemented samples vs. non-supplemented were lower in the setting with T cell enrichment compared to the setting without T cells enrichment (Fig. 3B).

3.6. Blood selenoprotein mRNA expression associated with serum Se

Using age and sex-adjusted linear regression, we investigated the association between the mRNA expression levels of selenoproteins in whole blood and serum Se levels. mRNA expression levels of *SELENOH*, *MSRB1* and *GPX4* were found to be associated with serum Se concentrations. Only *MSRB1* and *GPX4* were associated with serum Se after correction for multiple testing using Benjamini-Hochberg (Fig. 4).

3.7. GPX4, SELENOK and SELENOS were associated with all-cause mortality

mRNA expression of seven selenoproteins was found to be associated with all-cause mortality in the patient subset. Three of these genes-encoding selenoproteins remained significantly associated with all-cause mortality after multiple testing and after adjustment of the BIOSTAT-CHF mortality prediction model. Higher mRNA expression levels of *SELENOS* (HR: 0.39, 95 %CI: 0.22 to 0.70) were associated with lower mortality risk, while higher mRNA expression levels of *GPX4* (HR: 1.47, 95 %CI: 1.13 to 1.91) and *SELENOK* (HR: 1.47, 95 %CI: 1.17 to 1.85) were associated with higher mortality risk (Supplementary Fig. 2).

4. Discussion

In this study, we found an association between serum selenium concentrations and several immunoregulatory processes in patients with HF. Using the extensive data from the BIOSTAT-CHF cohort, 42 biomarkers plus age and BMI have been identified as determinants of Se serum concentrations. Enrichment analysis of the identified biomarkers demonstrated several enriched processes related to the regulation of leukocytes in general, as well as T lymphocyte activation and differentiation. Moreover, using 17,900 whole blood transcripts of protein-coding genes, various inflammation-related genes were significantly differentiated, resulting in several enriched pathways related to the adaptive immune system in patients with high Se status. Furthermore, the individual enriched transcripts associated with high Se status were mainly expressed in cells directly involved in adaptive immunity, while those with low Se status were mainly enriched in cells involved in innate immunity, as observed using online repository data. The mRNA expression of two selenoproteins, GPX4 and MSRB1, were associated with prognosis and serum selenium, respectively. Furthermore, as a proof-of-concept, the immunomodulatory effects of selenium have been observed in an experimental *in vitro* setting, as PBMCs that were supplemented with selenium over a period of 5 days showed an overall dampening of excreted cytokine concentrations than those without supplementation. This result does not reveal the underlying mechanism of the effects by selenium on the immune system, but adds potential causality to the associative findings in patients with HF, and therewith opens a new door for research in immunomodulatory therapies for HF.

Cytokines are critical signaling molecules in the immune system. They facilitate the proliferation and differentiation of immune cells and enhance the effector activities of lymphocytes and phagocytes. As such, the main driving factor of the enriched processes we observed is the cytokines, which are often imbalanced in the context of HF [30].

The enriched processes related to adaptive immunity (i.e. pathways related to T cells) are in line with several experimental studies that indicate that Se stimulates T cell differentiation and improves the quality of the immune response [31–33]. On the other hand, low Se status was associated with a more unrelated immune response (Supplementary Table S8) and with higher enrichment of innate immunity, findings that are in line with preclinical studies [33]. The distinct cytokines profiles that can be modulated by Se status have direct consequences on the biological microenvironment as they have direct effects on immune cells activation as well as differentiation. For instance, in several preclinical studies, inducing Se deficiency led to changes in various cytokines, including a significant decrease of IL10, IL-12beta, IFN-gamma and a significant increase of IL-6 [34], effects that are in line with our DEBs. Using human PBMC, we showed that almost two thirds of the measured cytokines were lower in the supplemented cultures, with or without T cells enrichment of the PBMC cultures. Therefore, Se may have the potential to modulate the immune response in HF.

Se may have favorable immunomodulatory effects in the context of HF by rebalancing the immune response. Current evidence indicates that Se supplementation increases Tregs cells [35–38], but likely decreases Th17 cells [39]. Several studies report the beneficial effects of Tregs
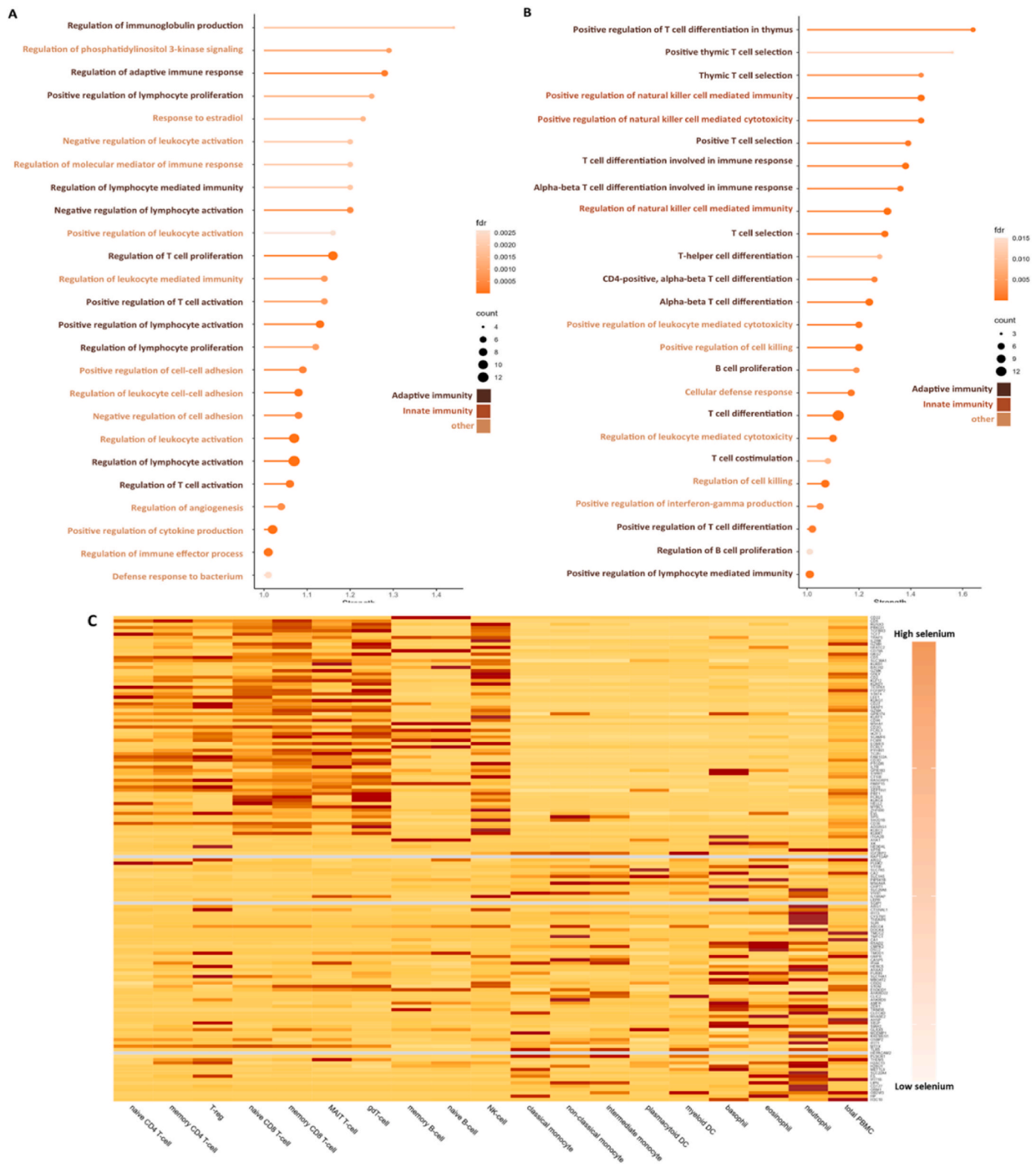
Fig. 2. The outcomes of the enrichment analysis. A. The top enriched pathways based on the biomarkers. B. The top enriched pathways based on RNA transcripts. The 25 top processes with the lowest FDR as well as with a strength value of higher than 1.0 were included. C. The expression of the enriched 148 RNA transcripts in 18 types of immune cells from an online repository data. The transcripts at the upper half are associated with high selenium concentrations, while those at the lower half are associated with low selenium concentrations. It is noticeable that RNA transcripts associated with high selenium are more expressed in NK-cell, B cells subtypes and T cells subtypes and those associated with lower selenium are more expressed in neutrophils and monocytes. The shading line at the right side indicates only selenium status and does not represent statistical data. Supplementary Fig. 1 provides higher quality figure of Fig. 2C.

A. Cytokines concentrations in the basal state in human PBMCs

B. Cytokines concentrations in human PBMCs enriched with CD3/CD28 beads
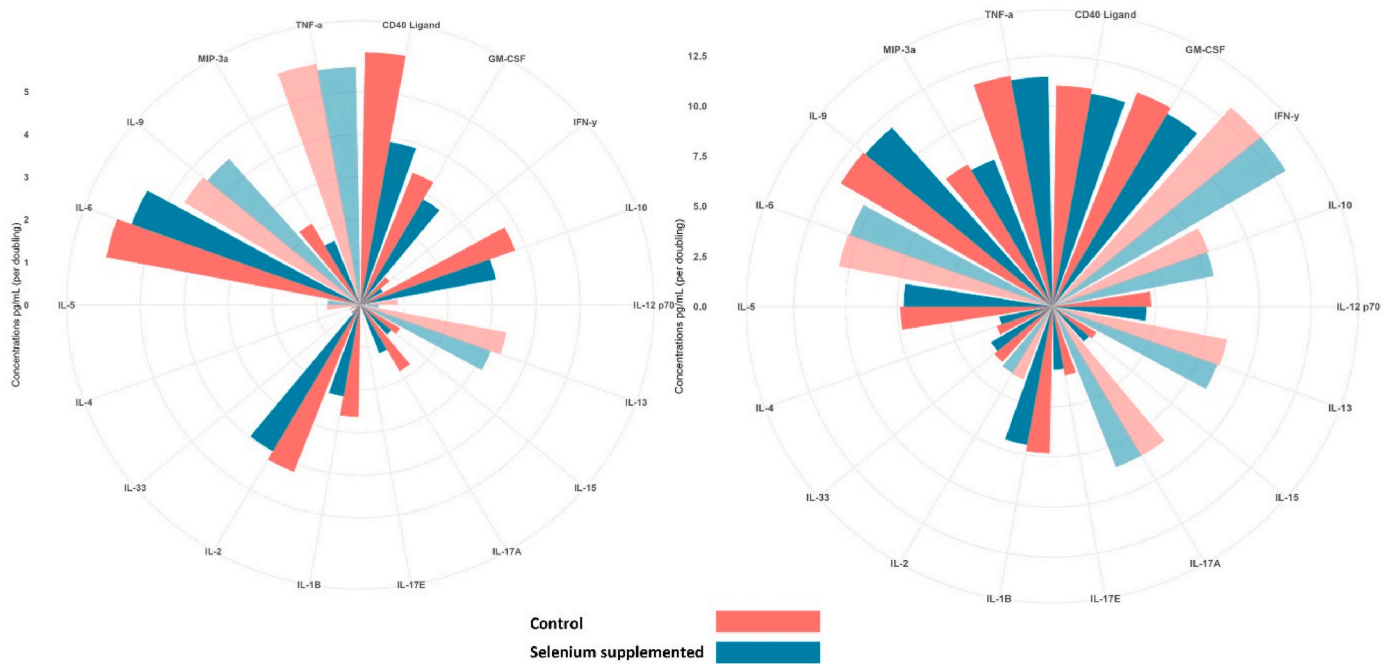

Fig. 3. Cytokines concentrations in A. the basal state and in B. T cells activation state. Darker colors indicate statistical significance after multiple corrections (P < 0.05). In general in the basal state are larger than in T cells activation state. The larges differences were observed in CD40 Ligand, IL-17E and IL1B. The duration of the intervention was 5 days, and per group included 4–5 samples.
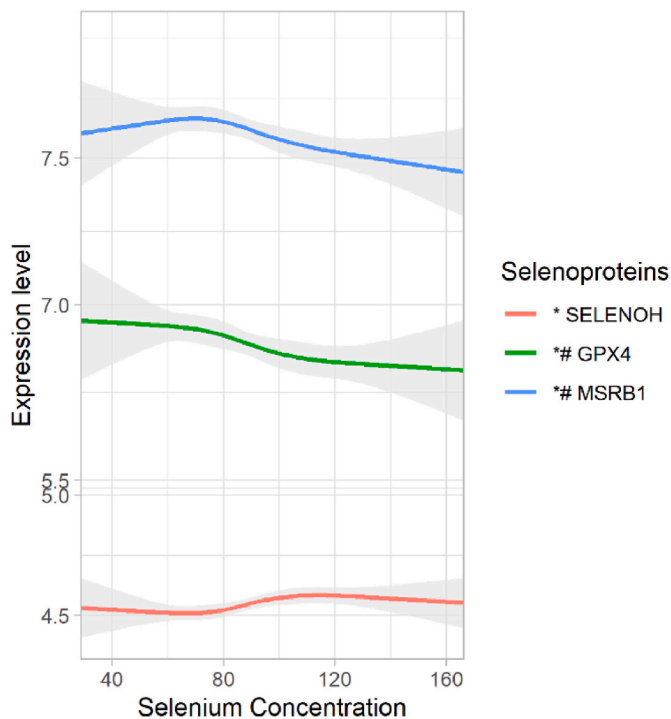

Fig. 4. The mRNA expression of the 3 selenoproteins in relation to serum selenium concentrations. All of these selenoproteins were significantly associated with serum selenium (<0.05). *# indicates significance after multiple correction. * indicates statistical significance.

themselves on the failing heart [40,41]. Our in-vitro findings support the notion that Se is likely to have global immunomodulatory effects by affecting several crucial cytokines, which provides an explanation of the enriched pathways in human data as they mainly include regulatory processes which are in essence driven by cytokines. However, these findings do not provide specific direction towards specific T cells sub-types, which might be due to the lack of in-vivo pathological processes or due to the duration of the intervention. Nevertheless, they provide proof-of-concept of the findings observed in patients with HF.

The association between GPX4 and mortality may indicate the importance of Se in regulating ferroptosis, an essential pathological mechanism involved in cardiovascular disease [42] as well as immune cell survival and activity [43]. Additionally, our data indicate that MSRB1 is closely related to selenium concentration and it had the highest expression. It has been shown that MSRB1 depletion caused inflammasome activation and exacerbated inflammation in a model for sepsis [44]. MSRB1's role in regulating inflammation is further emphasized by its role in promoting the production of IL10 [45], a known anti-inflammatory cytokine that may improve left ventricular function [46] and inhibit the conversion of fibroblasts to myofibroblasts (the type cells that exacerbates HF) [47]. Besides the systematic effects, previous studies demonstrated that, compared to other selenoproteins, MSRB1 has the largest increase in expression during cardiac hypertrophy [48] and its depletion led to an increase of oxidized glutathione and reduction of free and protein thiols [49], which are closely involved in HF progression [50]. Since it is well established that the expression and the activity of MSRB1 are dependent on the bioavailability of the dietary Se [51], the aforementioned pathophysiological consequences are likely to be relevant in patients with HF and Se deficiency.

Higher Se status could have direct effects on the functionality of the immune cells [52]. For instance, it is proven that higher dietary Se leads to higher levels of selenoproteins and several selenoproteins do have regulatory functions inside the T lymphocytes [52]. An important

function is redox hemostasis, the imbalance of which leads to immune cells malfunction [53,54]. It has been shown that Se concentrations within immune cells themselves have an influence on the half-life of reactive oxygen species (ROS) as many selenoproteins neutralize ROS via various pathways [55]. Our data indicate a strong association between SELENOK and mortality. It has been shown that SELENOK, a widely distributed selenoprotein, can protect cardiomyocytes from oxidative stress-induced toxicity [56] and that SELENOK knockout mice exhibit impaired immune response by disrupting calcium flux during activation of immune cells [57]. Similar regulatory mechanisms involved in endoplasmic-reticulum stress and inflammatory cytokines have been linked to SELENOH and SELENOS [58,59], both of which were strongly associated with serum selenium and mortality, respectively. Collectively, our findings indicate that selenoproteins associated with serum Se or have prognostic value are also involved in immuno-modulation processes, oxidative stress and ferroptosis.

Our results show that the mRNA expressions of selenoproteins varied based on Se levels. A frequently observed shift point was Se serum concentration close to 120 μg/L, which is close to the suggested optimal concentration of Se levels [60,61]. Remarkably, while it is well established that the expression of these proteins is dependent on Se levels, our results show that only the mRNA expression of three selenoproteins were significantly associated with serum Se levels. This association could be masked by difficult to measure factors such as the intracellular expression of selenoproteins, cellular intake of Se, and lack of correlation between mRNA levels and protein levels. For this exact reason, the strong association between serum Se and MSRB1 expression suggests that its physiological actions are likely to be systematic, reinforcing our findings in the enrichment analysis. In contrast to what is expected, higher concentrations of selenium were associated with lower expressions of GPX4 and MSRB1. One potential explanation for this observation is the reduced requirement for elevated expression of these proteins at higher selenium concentrations due to an already enough production at the proteomic level.

Evidence from clinical studies showed some effects of selenium supplementations on the immune cells, including cells involved in adaptive immunity (i.e. lymphocytes) as well as innate immunity [62, 63]. Our *in-vitro* experiment showed larger differences in the cytokines in the basal state, supporting the notion of the global immunomodulatory effects, especially as neutrophils and monocytes form more than 60 % of the white blood cells population and are important source of cytokines. Given that patients with HF have a chronic state of inflammation with dysregulated immune response, which makes them more vulnerable to hospitalization as a result of infection [64], investigating the effects of selenium supplementation in these patients is of particular interest.

5. Strengths and limitations

This cohort includes a large number of patients who were suboptimally treated at the moment of inclusion, reflecting the biological processes that underlie HF. A limited number of biomarkers were included in the study, several of which were related to inflammation, which creates some unavoidable bias. However, the findings of this study are in line with several experimental studies related to Se. Moreover, the enrichment analyses of the biomarkers and transcriptomics showed similar results, while they are at two different biological levels. The analyzed transcriptomics data are taken from blood and the observed differences could be driven by different amounts of circulating immune cells. While collecting data from blood might create some bias towards immune system cells, one should recognize that several studies that examined the transcriptomics of human subjects' cardiac tissues showed indeed several enriched inflammatory pathways [65,66], emphasizing the importance and the relevance of the findings of the current study. Nevertheless, additional validation of the current findings using single cell RNA-sequencing in the context of HF as well as in

experimental settings, including the wide range of immune cells, could provide further mechanistical insights, especially in defining the transition of the immune response. In the provided proof-of-concept experiment, additional focus was given on T-cells as several of the enriched pathways were mainly related to these cells. Investigating the effects of selenium on the various immune cells subtypes is nevertheless warranted.

6. Conclusion

In a cohort of HF patients, higher Se levels were associated with immune processes mainly related to adaptive immunity, whereas low Se levels were associated with less specific immune responses. This is supported by the enriched pathways of the highly differentiated RNA transcripts, which also show more advanced immune processes to be enriched in high Se levels vs. nonspecific processes in low levels. MSRB1 might have an essential regulatory role as it was strongly associated with serum Se. These data provide evidence for a potential pathophysiological link between low Se levels and dysregulated immune response in patients with HF. By inducing global effects on the abundance of cytokines, Se supplementation may have the potential to modulate the immune responses in patients with HF, optimize the functionality of immune cells and stimulate cardioprotective processes. An interventional randomized clinical trial is needed to prove causality in patients with HF.

CRediT authorship contribution statement

Ali A. Al-Mubarak: Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **George Markousis Mavrogenis:** Writing – review & editing, Methodology. **Xuanxuan Guo:** Writing – review & editing, Visualization, Methodology. **Marco De Bruyn:** Writing – review & editing, Methodology. **Mintu Nath:** Writing – review & editing, Resources, Data curation. **Simon P.R. Romaine:** Writing – review & editing, Data curation. **Niels Grote Beverborg:** Writing – review & editing, Methodology. **Karla Arevalo Gomez:** Writing – review & editing, Methodology. **Sietske N. Zijlstra:** Writing – review & editing, Methodology. **Dirk J. van Veldhuisen:** Writing – review & editing, Resources, Funding acquisition, Data curation. **Nilesh J. Samani:** Funding acquisition, Data curation. **Adriaan A. Voors:** Writing – review & editing, Funding acquisition, Data curation. **Peter van der Meer:** Writing – review & editing, Conceptualization. **Nils Bomer:** Writing – review & editing, Supervision, Formal analysis, Conceptualization.

Declaration of competing interest

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Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2024.103046.

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