**Supplementary Material**

Supplement to: Meling Stokland AE, Austdal M, Nedrebø BG, et al, Outcomes of Patients with Graves’ Disease 25 Years after Initiating Anti-Thyroid Drug Therapy

This appendix has been provided by the authors to give readers additional information about the work.

**Table of Contents: Page Number**

Supplementary Methods

Biochemical Data, Autoantibodies and Biomarkers of Inflammation 3

ThyPRO Questionnaire 3-4

Statistics 4

Supplementary Results

Validation of CXCL11 5

Supplementary Figures

Figure S1. Heat Map showing Significant Correlations 6

Figure S2. Longitudinal Changes in Inflammation, all patients 7

Figure S3.Loading Pattern for PC1 and PC2 8

Figure S4. Longitudinal Changes in Inflammation, treatment groups 9

Supplementary Tables

Table S1. Clinical and Biochemical Characteristics 10

Table S2. Status at Time of Sampling 11

Table S3. Overview of the 92 Analysed Biomarkers 12-16

Table S4. Biomarkers with Significant Differences 17-19

Table S5. Frequency of Positive Autoantibodies 20

Table S6. Results from linear mixed model analysis, all GD patients 21-22

Table S7. Results from linear mixed model analysis, ATD-groups 23-26

References 27

**Supplementary Methods**

**Biochemical Data, Autoantibodies and Biomarkers of Inflammation**

At the time of the original study TSH, T3 and FT4 in serum were measured using the auto-DELFIA hTSH Ultrakit and Auto-DELFIA FT4 and T3 kit, respectively. TRAb was measured using radioimmunoassay (DLD Diagnostika GmbH, Hamburg, Germany). Different reference intervals were used at the four study hospitals during the time period 1997-2003. Therefore, the local reference range was used to classify the thyroid- and TRAb status given in table S2 at each time point. Overt hyperthyroidism was defined as FT4 or T3 above the reference range with suppressed TSH. Subclinical hyperthyroidism and hypothyroidism were defined as TSH below and above the reference range, respectively, with FT4 and T3 in the reference range.

Autoantibodies to intrinsic factor and parietal cells were measured by QUANTA Lite, Inova Diagnostics, USA (Catalog# 708780, RRID:AB\_2943333) and NOVA Lite enzyme-linked immunosorbent assay, Inova Diagnostics, USA (Catalog#Haukeland\_001, RRID:AB\_2943409) . A value above 20 U/ml and 40 U/ml respectively was considered as positive. Tissue transglutaminase (tTG)-IgA antibodies were measured by immunofluorescence, Bioplex 2200 Celiac IgA Reagent Pack, BIO RAD, USA (Catalog# 665-2350, RRID:AB\_2943332). A value above 15 U/ml was considered as positive.

The results on CXCL11 were validated in 40 patient samples taken at baseline and 40 controls by immunoassay, human I-TAC/CXCL11 ELISA kit, Thermo Fischer Scientific, USA (Catalog# EHCXCL11X5, RRID:AB\_2943055).

**ThyPRO Questionnaires and reference data**

The thyroid-specific quality of life questionnaire (ThyPRO) consists of 85 items summarized in 13 multi-item scales (Goitre Symptoms, Hyperthyroid Symptoms, Hypothyroid Symptoms, Eye Symptoms, Tiredness, Cognitive Complaints, Anxiety, Depressivity, Emotional Susceptibility, Impaired Social Life, Impaired Daily Life, Impaired Sexlife, Cosmetic Complaints), as well as the single-item scale Overall Quality of Life. In addition, a composite score, summarizing the above mentioned scales can be made. Each item is rated on a five-point scale and transformed to 0-100 scales, with higher score indicating more health impact (1). ThyPRO reference data were generated based on a random sample of 1200 adult Danish citizens who received an invitation to participate, using the Danish Civil Registration System. The sex-distribution of the samples (80% women) was chosen to resemble the sex distribution of patients with benign thyroid disease. The questionnaires that were sent to participants included nine of the symptom scale, excluding those that are asked with attribution to thyroid disease. The participants were also asked about comorbidity and medication use. A total of 754 participants completed the survey. Responders with cancer and thyroid disease were excluded (2), leaving ThyPRO data for 707 respondents as reference data in the present study.

**Statistics**

For biomarker analyses NPX values below limit of detection (LOD) were set as LOD. Biomarkers with > 50 % of the values below LOD (n=18) were not included in further analysis.

Principal component analyses (PCA) were performed on mean-centered and unit variance scaled data with the ropls package (v1.28.2)(3). PC1 and PC2 explained a total of 42.9% of the variation, with remaining PCs explaining < 6.4% each.

The extended ANOVA simultaneous component analysis (RM-ASCA+) framework combines general linear models and principal component analysis (PCA) to decompose and visualize the separate effects of experimental factors. RM-ASCA+ can visualize multivariate differences between groups, and the possible effect of study outcome. NPX values were normalized before analysis by subtracting the mean and dividing by the standard deviation (SD) for each NPX variable. RM-ASCA+ was then performed on the linear mixed models (LMM) of the separate biomarkers. The underlying LMM was performed with the NPX data as response, time, -group and the time-group interactions as fixed effects, and individual specific random intercepts. NPX values over time were modelled by the formula (*value ~ time + (1|ID*) and the effect of time and group-time interaction was included using the formula (value~time+group+time:group+(1|ID)). P-values in the LMMs were calculated using Satterthwaite’s degrees of freedom method. Estimations of robustness of the RM-ASCA+ results were performed by bootstrapping with 1000 runs: each participant is replaced by a randomly selected participant from the same group, with replacement, and all measurements from the resampled participant are added to the resampled data set. The 2.5th and 97.5th percentiles from the bootstrapping estimations make out the error bars on both the loading and scores plot.

Correlation analyses were performed using Pearson’s correlation.

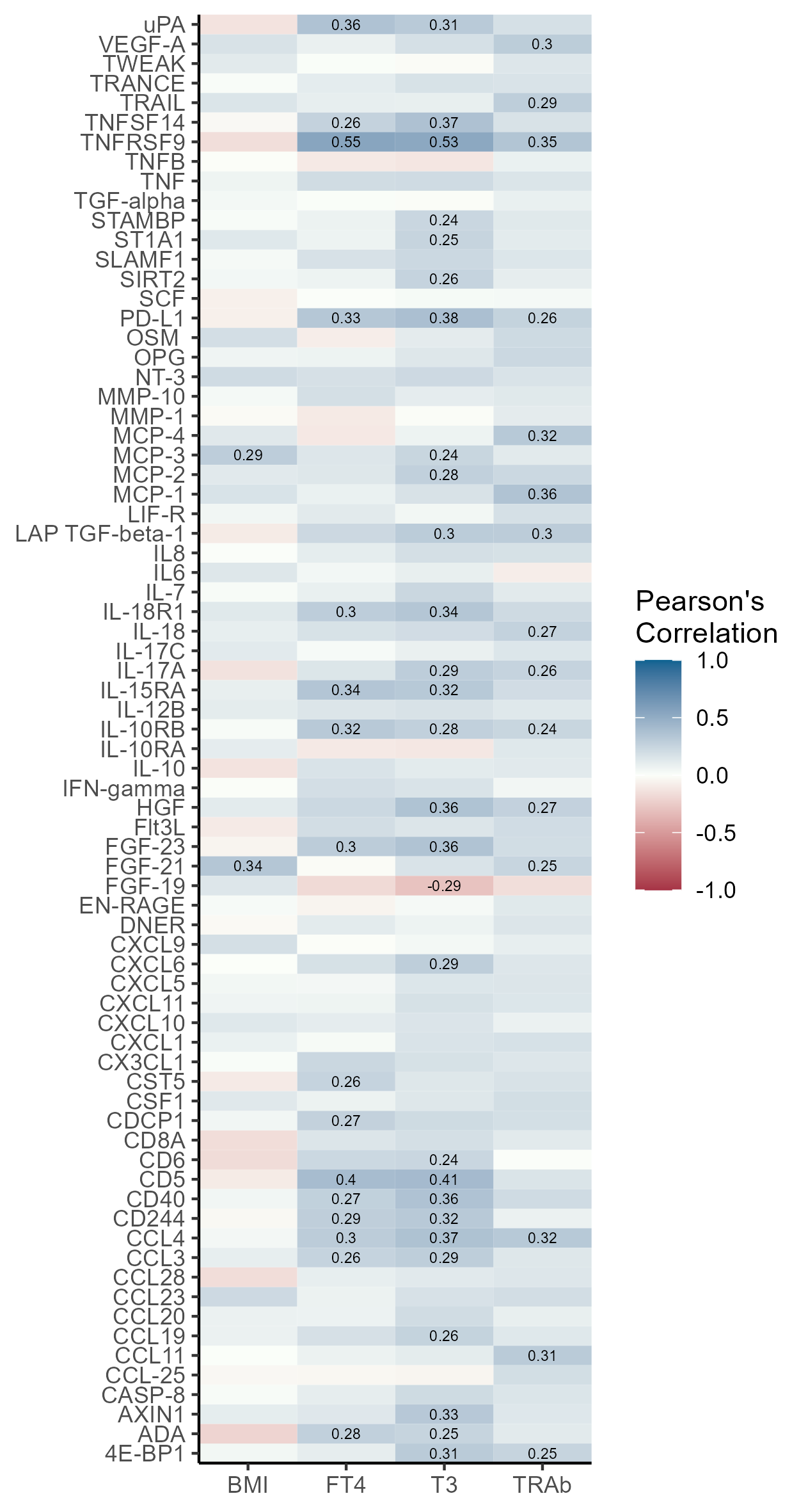
**Supplementary Results**

**Validation of CXCL11**

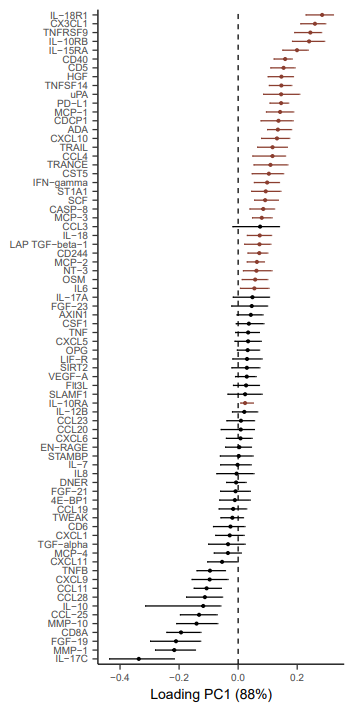
Low levels of CXCL11 was confirmed in the immunoassay with a median level of 24.96 pg/mL in GD patients vs 189.10 pg/mL in healthy controls (p=<0.001).

**Supplementary Figures**

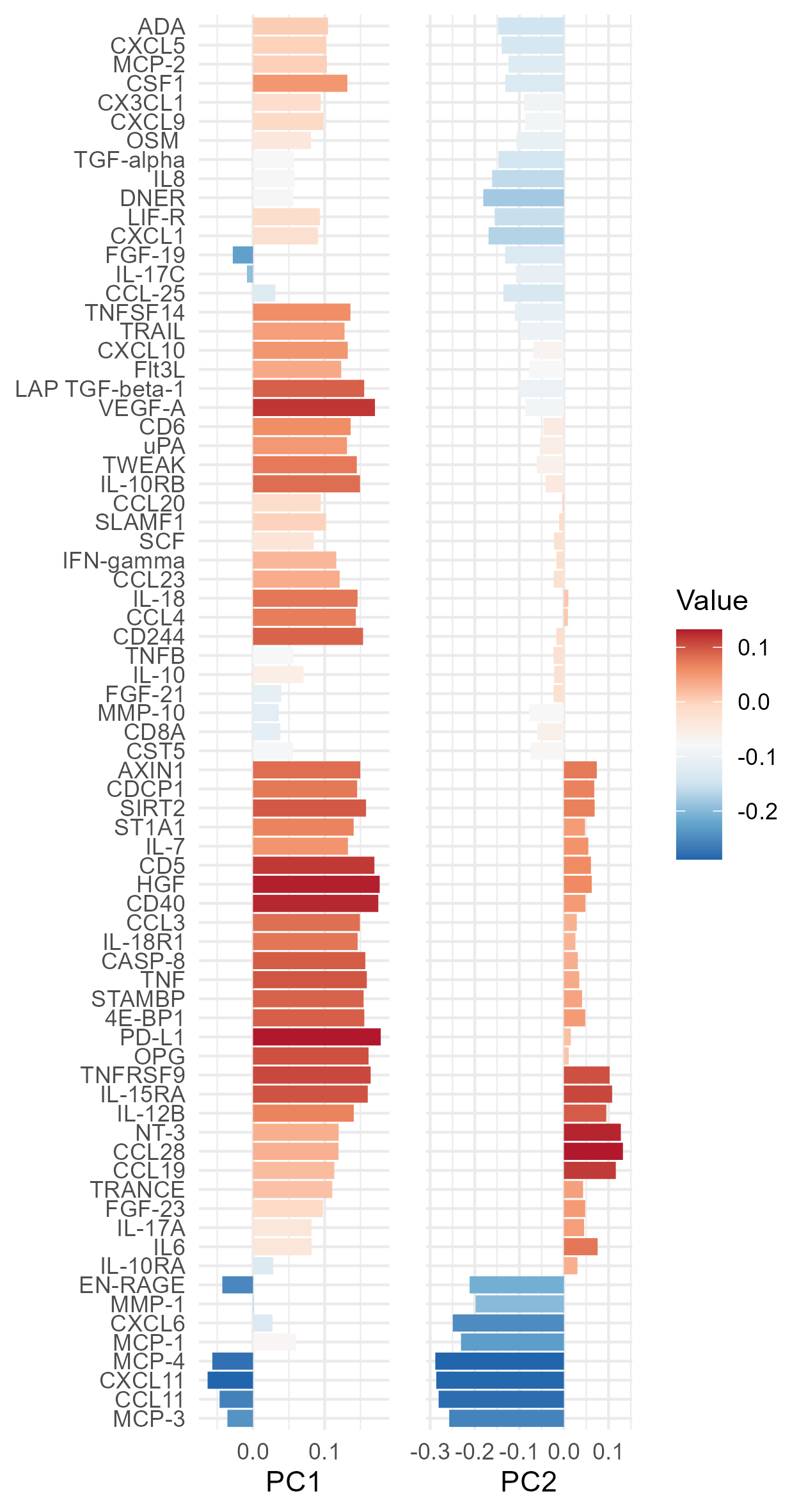
**Figure S1.** Heat map showing significant correlations between the inflammatory biomarkers, TRAb, FT4, T3 and BMI in samples taken at baseline. For significant correlations, r2 is shown in the corresponding heatmap tile.



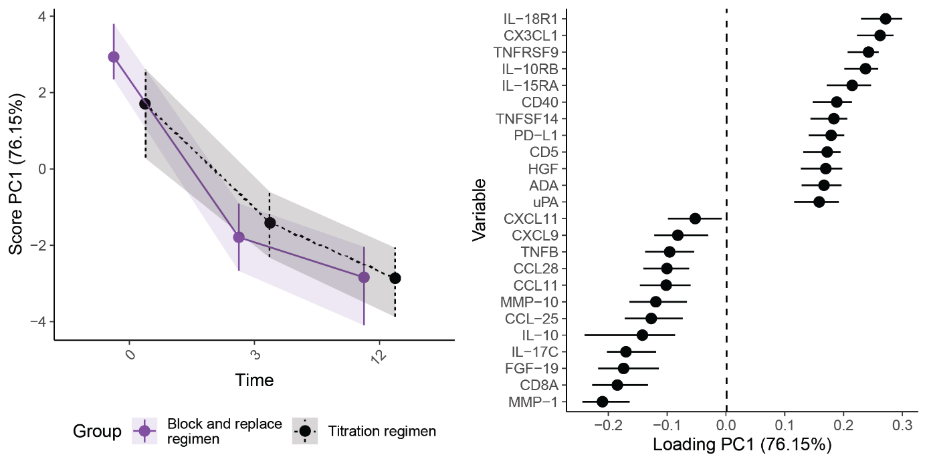
**Figure S2.** Loading plot from the RM-ASCA+ analysis of GD patients over time. Variable loadings on thelongitudinal changes in inflammation during treatment in all patients as shown in Figure 2C. High loading scores (positive or negative) indicate higher importance in describing the development over time. Vertical bars represent error bars from bootstrapping, and error bars crossing the zero line indicate that the contribution to the score plot is not robust. Biomarkers with significant positive contribution to PC1 are shown in red, these biomarkers are reduced over time in GD patients.



**Figure S3.** Loading pattern for PC1 and PC2 in GD patients compared to healthy controls. Biomarkers with red bars represent those with positive loadings (higher in GD compared to controls) and blue bars negative loadings (lower in GD compared to controls). High loading scores (positive or negative) indicate higher importance in describing the differences between the GD and healthy control groups.



**Figure S4.** Longitudinal changes in inflammation during treatment between ATD treatments groups. Results show scores (left) and loadings (right, showing only top and bottom 12 variables in the analyses) from analyses of all patients over time including group interactions at 3- and 12-months, from the multivariate RM-ASCA+ analyses. A high PC1 score (on the y-axis) indicates higher concentrations of biomarkers with positive loadings and lower concentrations of biomarkers with negative loadings. Vertical bars represent error bars from bootstrapping. All groups show clear changes over time in biomarker profiles, while no robust differences between treatment regimens is observed at any time point.



**Supplementary Tables**

**Table S1.** Clinical and Epidemiological characteristics of patients and healthy controls selected for biomarker analysis.

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Graves’ Disease** | **Healthy controls** |
| **Basic demographics** |  |  |
| Patients, n | 82 | 82 |
| Age, y (range) | 44 (18-71) | 45 (20-73) |
| Female, n (%) | 69 (84%) | 70 (85%) |
| Daily smoker, n (%) | 33 (40%) | - |
| Ex-smoker, n (%) | 8 (10%) | - |
| Body-mass-index, kg/m2 (range) | 22.5 (16.7-37.8) | - |
| **Analysed samples** |  |  |
| Number for samples per patient, n (range) | 2 (1-3) |  |
| Sample taken at baseline, n (%) | 69 (84%) |  |
| Sample taken at 3 months, n (%) | 50 (61%) |  |
| Sample taken at 12 months, n (%) | 30 (37%) |  |
| **Clinical characteristics** |  |  |
| Block-replace/titration regimen, n (%) | 47 (57%) / 35 (43%) | - |
| Relapse rate in original study, n (%) | 40 (49%) | - |
| Remission rate in original study, n (%) | 35 (43%) | - |
| Dropped out from original study1, n (%) | 7 (9%) | - |
| Normal Thyroid Function at the end of follow-up2, n (%) | 31 (38%) | - |
| Autoimmune comorbidity3 | 4 (5%) | - |
| Thyroid Eye Disease3 | 39 (48%) | - |

1 Did not complete 12 months of ATD-treatment

2Data missing on four patients

3At the time of participation in the original study

**Table S2.** Status at time of sampling for patients where biomarker analyses were performed

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Baseline** | **3 months** | **12 months** |
| **Number of samples** | 69 (46%) | 50 (34%) | 30 (20%) |
| **Treatment group** | | | |
| Block and replace | 39 (56.5%) | 33 (66%) | 16 (53%) |
| Titration | 30 (43.5%) | 17 (34%) | 14 (47%) |
| **Treatment at time of sampling** | | | |
| Untreated | 57 (82.6%) | 0 | 0 |
| Carbimazole | 6 (8.7%) | 17 (34%) | 13 (45%) |
| Carbimazole and thyroxine | 0 | 28 (56%) | 16 (52%) |
| Propylthiouracil | 0 | 2 (4%) | 1 (3%) |
| Propylthiouracil and thyroxine | 0 | 3 (6%) | - |
| Unknown/missing data | 6 (8.7%) | 0 | 0 |
| **ATD-dose at time of sampling** | | | |
| Carbimazole, mg (range) | 30 (0-45) | 20 (10-30) | 20 (5-30) |
| Propylthiouracil, mg (range) | 400 (400-400) | 300 (100-400) | 100 (100-100) |
| **Laboratory levels at time of sampling** | | | |
| TSH, mIU/L (range) | 0.05 (0.03-0..10) | 0.05 (0.03-3.50) | 1.93 (0.03-11) |
| FT4, pmol/L (range) | 67.1 (18.0-80) | 15 (4-29) | 13 (9-19.6) |
| T3, nmol/L (range)1 | 6.0 (2.4-10) | 2.1 (1.5-5.0) | 1.8 (1.3-3.3) |
| TRAb, mIU/L (range)2 | 50 (10-423) | 35 (10-421) | 10 (10-105) |
| TRAb, positive / negative2 | 61 (90%) / 7 (10%) | 37 (77%) / 11 (23%) | 10 (34%) / 19 (66%) |
| **Thyroid function at time of sampling** | | | |
| Overt hyperthyroidism | 69 (100%) | 17 (34%) | 1 (3%) |
| Subclinical hyperthyroidism | - | 25 (50%) | 8 (27%) |
| Subclinical hypothyroidism | - | - | 7 (23%) |
| Overt hypothyroidism | - | 1 (2%) | - |
| Euthyroid | - | 7 (14%) | 14 (47%) |

1Data missing in two samples

2Data missing in four samples

**Table S3.** Overview of the 92 analysed biomarkers

|  |  |  |
| --- | --- | --- |
| **Biomarker** | **LOD (pg/mL)** | **CV** |
| Adenosine deaminase (ADA) | 0.48 | 5 % |
| Artemin (ARTN) | 0.24 | 7 % |
| Axin-1 (AXIN1) | 61.00 | 6 % |
| Beta-nerve growth factor (Beta-NGF) | 0.48 | 6 % |
| Caspase-8 (CASP-8) | 0.48 | 7 % |
| C-C motif chemokine 3 (CCL3) | 0.06 | 6 % |
| C-C motif chemokine 4 (CCL4) | 1.90 | 6 % |
| C-C motif chemokine 19 (CCL19) | 15.30 | 8 % |
| C-C motif chemokine 20 (CCL20) | 7.60 | 7 % |
| C-C motif chemokine 23 (CCL23) | 30.50 | 6 % |
| C-C motif chemokine 25 (CCL25) | 3.80 | 6 % |
| C-C motif chemokine 28 (CCL28) | 61.00 | 7 % |
| T-cell surface glycoprotein CD5 (CD5) | 0.06 | 5 % |
| T cell surface glycoprotein CD6 isoform (CD6) | 0.24 | 6 % |
| CD40L receptor (CD40) | 0.01 | 5 % |
| C-X-C motif chemokine 5 (CXCL5) | 0.95 | 7 % |
| C-X-C motif chemokine 6 (CXCL6) | 7.60 | 8 % |
| C-X-C motif chemokine 9 (CXCL9) | 0.95 | 6 % |
| C-X-C motif chemokine 10 (CXCL10) | 7.60 | 7 % |
| Cystatin-D (CST5) | 1.90 | 5 % |
| Delta and Notch-like epidermal growth factor-related receptor (DNER) | 0.95 | 5 % |
| Eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) | NA | 6 % |
| Protein S100-A12 (EN-RAGE) | 122.10 | 8 % |
| Eotaxin (CCL11) | 3.80 | 5 % |
| Fibroblast growth factor 5 (FGF5) | 1.90 | 7 % |
| Fibroblast Growth factor 19 (FGF-19) | 7.60 | 6 % |
| Fibroblast growth factor 21 (FGF21) | 30.50 | 8 % |
| Fibroblast Growth factor 23 (FGF-23) | 122.10 | 9 % |
| Fms-related tyrosine kinase 3 ligand (Flt3L) | 0.01 | 6 % |
| Fractalkine (CX3CL1) | 15.30 | 7 % |
| Growth-regulated alpha protein (CXCL1) | 3.80 | 6 % |
| Hepatocyte growth factor (HGF) | 7.60 | 6 % |
| Interferon gamma (IFN-gamma) | 0.24 | 7 % |
| Interleukin-8 (IL8) | 0.03 | 6 % |
| Leukemia inhibitory factor (LIF) | 3.80 | 7 % |
| Leukemia inhibitory factor receptor (LIFR) | 30.50 | 7 % |
| Lymphotoxin-alpha (TNF-beta) | 0.24 | 6 % |
| Vascular endothelial growth factor A (VEGFA) | 0.06 | 6 % |
| T-cell surface glycoprotein CD8 alpha chain (CD8A) | NA | 9 % |
| Monocyte chemotactic protein 2 (MCP-2) | 0.06 | 6 % |
| Monocyte chemotactic protein 3 (MCP-3) | 0.48 | 7 % |
| Monocyte chemotactic protein 4 (MCP-4) | 0.24 | 6 % |
| Glial cell line-derived neurotrophic factor (GDNF) | 0.01 | 7 % |
| CUB domain-containing protein 1 (CDCP1) | 0.12 | 6 % |
| Macrophage colony-stimulating factor 1 (CSF1) | 0.00 | 5 % |
| Matrix metalloproteinase-1 (MMP-1) | 1.90 | 5 % |
| Matrix metalloproteinase-10 (MMP10) | 0.95 | 5 % |
| Monocyte chemotactic protein 1 (MCP-1) | 0.03 | 6 % |
| Natural killer cell receptor 2B4 (CD244) | 0.06 | 5 % |
| Neurotrophin-3 (NT-3) | 0.12 | 6 % |
| Neurturin (NRTN) | 3.80 | 9 % |
| Interleukin-7 (IL7) | 0.24 | 6 % |
| Oncostatin-M (OSM) | 0.03 | 5 % |
| Osteoprotegerin (OPG) | 0.24 | 6 % |
| Latency-associated peptide transforming growth factor beta-1 (LAP TGF-beta-1) | 61.00 | 7 % |
| Urokinase-type plasminogen activator (uPA) | 0.12 | 5 % |
| Interleukin-1 alpha (IL-1A) | 0.48 | 7 % |
| Interleukin-2 (IL-2) | 30.50 | 9 % |
| Interleukin-2 receptor subunit beta (IL-2RB) | 15.30 | 7 % |
| Interleukin-4 (IL4) | 0.24 | 7 % |
| Interleukin-5 (IL5) | 3.80 | 7 % |
| Interleukin-6 (IL6) | 0.12 | 6 % |
| Interleukin-10 (IL10) | 0.48 | 7 % |
| Interleukin-10 receptor subunit alpha (IL10RA) | 3.80 | 6 % |
| Interleukin-10 receptor subunit beta (IL-10RB) | 0.12 | 5 % |
| Interleukin-12 subunit beta (IL-12B) | 0.12 | 6 % |
| Interleukin-13 (IL13) | 7.60 | 14 % |
| Interleukin-15 receptor subunit alpha (IL-15RA) | 0.95 | 6 % |
| Interleukin-17A (IL-17A) | 3.80 | 8 % |
| Interleukin-17C (IL-17C) | 30.50 | 8 % |
| Interleukin-18 (IL18) | 0.06 | 6 % |
| Interleukin-18 receptor 1 (IL-18R1) | 0.06 | 5 % |
| Interleukin-20 (IL20) | 7.60 | 7 % |
| Interleukin-20 receptor subunit alpha (IL-20RA) | 1.90 | 6 % |
| Interleukin-22 receptor subunit alpha-1 (IL22RA1) | 0.24 | 7 % |
| Interleukin-24 (IL24) | 1.90 | 6 % |
| Interleukin-33 (IL33) | 3.80 | 9 % |
| C-X-C motif chemokine 11 (CXCL11) | 7.60 | 7 % |
| Programmed cell death 1 ligand 1 (PD-L1) | 3.80 | 9 % |
| Sulfotransferase 1A1 (ST1A1) | 244.10 | 6 % |
| Signaling lymphocytic activation molecule (SLAMF1) | 30.50 | 9 % |
| SIR2-like protein 2 (SIRT2) | 7.60 | 8 % |
| STAM-binding protein (STAMBP) | 7.60 | 5 % |
| Stem cell factor (SCF) | 1.90 | 5 % |
| TNF-related activation-induced cytokine (TRANCE) | 3.80 | 7 % |
| TNF-related apoptosis-inducing ligand (TRAIL) | 0.95 | 5 % |
| Thymic stromal lymphopoietin (TSLP) | 3.80 | 6 % |
| Transforming growth factor alpha (TGF-alfa) | 0.48 | 6 % |
| Tumor necrosis factor (TNF) | 0.95 | 6 % |
| Tumor necrosis factor (Ligand) superfamily, member 12 (TWEAK) | 1.90 | 6 % |
| Tumor necrosis factor receptor superfamily member 9 (TNFRSF9) | 0.03 | 5 % |
| Tumor necrosis factor ligand superfamily member 14 (TNFSF14) | 0.95 | 6 % |

**Table S4.** Distribution of 61 biomarkers with significantly different concentrations GD patients versus healthy controls. Benjamin Hochberg procedure was applied to adjust p-values for statistical significance to control for false discovery rate due to multiple comparisons. The results are reported as relative quantification between samples, by a unit named Normalized Protein expression (NPX). As NPX-values are in Log2-scale, a difference in one NPX equals a doubling in protein concentration.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Biomarker** | **Graves Disease Median (range)** | **Healthy control Median (range)** | **LOD (% < LOD)** | **CV** | **p-value\*** |
| **Increased** |  |  |  |  |  |
| C-C motif chemokine 28 (CCL28) | 2.35 (1.82-3.72) | 1.57 (1.22-2.76) | 1.22 (5%) | 7% | <0.001 |
| Hepatocyte growth factor (HGF) | 8.72 (7.08-10.79) | 7.61 (6.51-8.36) | 1.49 (0%) | 6% | <0.001 |
| Tumor necrosis factor (TNF) | 2.43 (1.26-4.15) | 2.00 (0.99-2.88) | -0.15 (0%) | 6% | <0.001 |
| Tumor necrosis factor receptor superfamily member 9 (TNFRSF9) | 8.13 (6.43-9.50) | 6.82 (5.95-7.66) | 2.06 (0%) | 5% | <0.001 |
| TNF-related activation-induced cytokine (TRANCE) | 5.42 (2.94-6.80) | 4.78 (3.59-6.27) | 1.45 (0%) | 7% | <0.001 |
| Interleukin-15 receptor subunit alpha (IL-15RA) | 1.81 (0.92-2.84) | 1.13 (0.92-1.73) | 0.92 (8%) | 6% | <0.001 |
| SIR2-like protein 2 (SIRT2) | 4.90 (2.81-10.56) | 2.81 (2.81-4.97) | 2.81 (22%) | 8% | <0.001 |
| Axin-1 (AXIN1) | 4.23 (1.51-9.39) | 2.52 (1.51-3.95) | 1.51 (8%) | 6% | <0.001 |
| Eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) | 7.54 (3.83-11.01) | 5.59 (2.59-8.92) | 1.71 (0%) | 6% | <0.001 |
| Interferon gamma (IFN-gamma) | 7.65 (5.43-11.50) | 7.02 (5.39-10.21) | 4.35 (0%) | 7% | <0.001 |
| CD40L receptor (CD40) | 12.24 (11.09-14.09) | 11.39 (10.30-12.15) | 2.21 (0%) | 5% | <0.001 |
| T-cell surface glycoprotein CD5 (CD5) | 6.32 (5.28-8.43) | 5.63 (4.67-6.09) | 0.88 (0%) | 5% | <0.001 |
| Fibroblast Growth factor 23 (FGF-23) | 1.78 (1.36-3.34) | 1.38 (1.36-2.28) | 1.36 (28%) | 9% | <0.001 |
| Interleukin-10 receptor subunit beta (IL-10RB) | 7.00 (5.21-7.62) | 6.54 (5.60-7.22) | 1.31 (0%) | 5% | <0.001 |
| Interleukin-12 subunit beta (IL-12B) | 7.16 (4.63-8.81) | 6.04 (4.73-7.31) | 0.07 (0%) | 6% | <0.001 |
| Interleukin-17A (IL-17A) | 2.01 (1.55-3.39) | 1.55 (1.55-3.69) | 1.55 (29%) | 8% | <0.001 |
| Caspase-8 (CASP-8) | 5.00 (3.46-11.04) | 3.74 (2.34-6.27) | 1.98 (0%) | 7% | <0.001 |
| STAM-binding protein (STAMBP) | 5.38 (3.51-10.25) | 4.21 (3.08-5.68) | 1.61 (0%) | 5% | <0.001 |
| C-C motif chemokine 19 (CCL19) | 10.14 (8.39-12.15) | 9.14 (8.04-12.33) | 1.53 (0%) | 8% | <0.001 |
| Latency-associated peptide transforming growth factor beta-1 (LAP TGF-beta-1) | 6.57 (4.30-9.02) | 6.17 (5.11-7.00) | 1.41 (0%) | 7% | <0.001 |
| Vascular endothelial growth factor A (VEGF-A) | 10.94 (8.88-13.20) | 10.59 (9.48-11.51) | 2.20 (0%) | 6% | <0.001 |
| Natural killer cell receptor 2B4 (CD244) | 6.40 (4.77-9.06) | 6.11 (5.42-6.63) | 1.58 (0%) | 5% | <0.001 |
| Osteoprotegerin (OPG) | 10.29 (8.38-11.02) | 9.80 (8.90-10.65) | 1.35 (0%) | 6% | <0.001 |
| Interleukin-6 (IL6) | 2.65 (1.73-6.41) | 1.66 (1.62-3.95) | 1.62 (18%) | 6% | <0.001 |
| CUB domain-containing protein 1 (CDCP1) | 3.31 (2.16-4.64) | 2.44 (1.20-3.97) | 0.55 (0%) | 6% | <0.001 |
| C-C motif chemokine 3 (CCL3) | 6.32 (4.72-7.80) | 5.59 (4.44-7.48) | 1.52 (0%) | 6% | <0.001 |
| C-C motif chemokine 4 (CCL4) | 6.79 (5.28-9.24) | 6.10 (4.72-10.30) | 1.72 (0%) | 6% | <0.001 |
| Interleukin-18 (IL18) | 9.13 (7.08-11.13) | 8.48 (7.38-9.58) | 1.05 (0%) | 6% | <0.001 |
| Interleukin-18 receptor 1 (IL-18R1) | 9.27 (7.34-10.35) | 8.43 (7.04-9.10) | 1.79 (0%) | 5% | <0.001 |
| Neurotrophin-3 (NT-3) | 2.97 (1.89-5.74) | 1.85 (1.85-5.43) | 1.85 (23%) | 6% | <0.001 |
| Interleukin-7 (IL7) | 2.09 (1.22-4.42) | 1.32 (1.22-3.83) | 1.22 (17%) | 6% | <0.001 |
| Programmed cell death 1 ligand 1 (PD-L1) | 6.02 (4.13-7.55) | 5.36 (4.03-6.77) | 2.08 (0%) | 9% | <0.001 |
| Sulfotransferase 1A1 (ST1A1) | 4.98 (2.28-8.48) | 3.35 (2.11-5.90) | 2.11 (9%) | 6% | <0.001 |
| Urokinase-type plasminogen activator (uPA) | 10.21 (8.67-10.93) | 9.93 (8.97-10.83) | 1.59 (0%) | 5% | <0.001 |
| C-C motif chemokine 23 (CCL23) | 10.81 (8.62-11.54) | 10.51 (8.71-11.45) | 1.28 (0%) | 6% | <0.001 |
| Signaling lymphocytic activation molecule (SLAMF1) | 2.41 (1.80-3.40) | 2.22 (1.80-3.25) | 1.80 (8%) | 9% | 0.001 |
| Tumor necrosis factor (Ligand) superfamily, member 12 (TWEAK) | 8.88 (6.48-10.09) | 8.62 (7.67-9.58) | 0.79 (0%) | 6% | 0.002 |
| C-X-C motif chemokine 10 (CXCL10) | 9.96 (7.10-12.10) | 9.34 (7.70-11.79) | 2.47 (0%) | 7% | <0.001 |
| C-C motif chemokine 20 (CCL20) | 7.82 (6.43-13.87) | 7.39 (6.12-10.79) | 2.19 (0%) | 7% | 0.001 |
| Stem cell factor (SCF) | 9.68 (7.74-10.59) | 9.46 (8.36-10.00) | 1.26 (0%) | 5% | 0.001 |
| T cell surface glycoprotein CD6 isoform (CD6) | 6.63 (5.31-8.37) | 6.45 (4.80-7.46) | 2.09 (0%) | 6% | 0.002 |
| Tumor necrosis factor ligand superfamily member 14 (TNFSF14) | 5.52 (3.68-9.53) | 5.19 (3.65-6.54) | 1.90 (0%) | 6% | 0.002 |
| Fractalkine (CX3CL1) | 4.67 (2.99-6.22) | 4.37 (3.26-5.40) | 1.54 (0%) | 7% | <0.001 |
| TNF-related apoptosis-inducing ligand (TRAIL) | 7.95 (6.66-8.95) | 7.78 (7.06-8.41) | 1.14 (0%) | 5% | 0.005 |
| Fms-related tyrosine kinase 3 ligand (Flt3L) | 9.80 (8.02-11.04) | 9.61 (8.37-10.49) | 2.28 (0%) | 6% | 0.01 |
| **Decreased** |  |  |  |  |  |
| C-X-C motif chemokine 11 (CXCL11) | 7.23 (4.91-9.88) | 10.88 (9.79-1.,14) | 1.53 (0%) | 7% | <0.001 |
| Eotaxin (CCL11) | 7.65 (6.14-9.40) | 9.16 (8.54-10.21) | 1.76 (0%) | 5% | <0.001 |
| Monocyte chemotactic protein 3 (MCP-3) | 1.89 (1.53-4.58) | 3.20 (2.09-4.44) | 1.53 (16%) | 7% | <0.001 |
| Monocyte chemotactic protein 4 (MCP-4) | 13.75 (11.79-16.65) | 16.76 (15.22-18.31) | 3.49 (0%) | 6% | <0.001 |
| Protein S100-A12 (EN-RAGE) | 2.83 (1.82-5.98) | 4.10 (2.46-6.42) | 1.44 (0%) | 8% | <0.001 |
| C-X-C motif chemokine 6 (CXCL6) | 8.68 (6.29-11.74) | 9.96 (8.73-11.56) | 2.04 (0%) | 8% | <0.001 |
| Matrix metalloproteinase-1 (MMP-1) | 12.16 (10.03-15.22) | 13.97 (11.25-15.86) | 1.38 (0%) | 5% | <0.001 |
| Interleukin-17C (IL-17C) | 1.69 (1.50-3.13) | 2.31 (1.50-5.22) | 1.50 (17%) | 8% | <0.001 |
| Fibroblast Growth factor 19 (FGF-19) | 8.27 (6.89-10.61) | 9.41 (6.83-11.45) | 1.86 (0%) | 6% | <0.001 |
| Monocyte chemotactic protein 1 (MCP-1) | 11.60 (9.88-13.66) | 11.91 (10.80-12.73) | 1.41 (0%) | 6% | <0.001 |
| C-C motif chemokine 25 (CCL-25) | 5.62 (3.19-7.26) | 5.91 (4.98-7.31) | 1.28 (0%) | 6% | <0.001 |
| Delta and Notch-like epidermal growth factor-related receptor (DNER) | 9.38 (7.34-10.11) | 9.53 (8.47-10.02) | 0.70 (0%) | 5% | 0.002 |
| Interleukin-8 (IL8) | 5.22 (3.51-9.02) | 5.46 (4.20-8.38) | 1.68 (0%) | 6% | 0.02 |
| T-cell surface glycoprotein CD8 alpha chain (CD8A) | 9.26 (6.88-11.05) | 9.58 (7.43-10.82) | 1.65 (0%) | 9% | 0.001 |
| Matrix metalloproteinase-10 (MMP10) | 8.76 (6.91-10.52) | 8.97 (7.57-12.28) | 1.46 (0%) | 5% | 0.02 |
| Transforming growth factor alpha (TGF-alfa) | 2.88 (1.74-4.61) | 3.07 (2.13-4.03) | 0.94 (0%) | 6% | 0.05 |

**Table S5.** Frequency of positive autoantibodies to parietal cells, intrinsic factor and tissue transglutaminase (tTG)-IgA at time of the original study in 161/182 included patients. Numbers are also given for patients with and without B12-deficiency and celiac disease as of 2022. For three patients disease status was missing. Data are presented as n (%).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Autoantibodies** | **All patients (n = 161)** | **B12-deficienct  (n= 40)** | **B12-sufficient (n=118)** | **Celiac Disease (n=6)** | **No Celiac Disease (n=152)** |
| **Parietal cells** | 18 (11%) | 5 (13%) | 13 (11%) | - | - |
| **Intrinsic Factor** | 3 (2%) | 3 (7.5%) | 0 | - | - |
| **Tissue Transglutaminase (tTG)-IgA** | 7 (4%) | 2 (5%) | 5 (4%) | 1 (17%) | 6 (4%) |

**Table S6**: **All GD patients.** Results from linear mixed model analysis of longitudinal changes in NPX variables for all GD patients. The models include main effect for time points as fixed effects, and a random intercept was included for each patient (*value ~ time + (1|ID*). The time variable was reference coded to the baseline measurement. P-values are not corrected for multiple testing.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **(Intercept)** | | **Time3** | | **Time12** | |
|  | **Effect** | **p-value** | **Effect** | **p-value** | **Effect** | **p-value** |
| **4E-BP1** | 5.11 | <0.001 | -0.12 | 0.45 | 0.21 | 0.26 |
| **ADA** | 8.04 | <0.001 | -0.60 | <0.001 | -0.75 | <0.001 |
| **AXIN1** | 2.50 | <0.001 | -0.42 | 0.01 | -0.04 | 0.83 |
| **CASP8** | 4.15 | <0.001 | -0.48 | <0.001 | -0.39 | 0.04 |
| **CCL11** | 12.56 | <0.001 | 0.34 | 0.01 | 0.72 | 0.00 |
| **CCL19** | 12.93 | <0.001 | 0.49 | <0.001 | -0.27 | 0.04 |
| **CCL20** | 7.58 | <0.001 | -0.09 | 0.55 | -0.01 | 0.98 |
| **CCL23** | 21.06 | <0.001 | -0.19 | 0.20 | 0.08 | 0.66 |
| **CCL25** | 9.66 | <0.001 | 0.55 | <0.001 | 0.78 | <0.001 |
| **CCL28** | 5.82 | <0.001 | 0.46 | <0.001 | 0.67 | <0.001 |
| **CCL3** | 10.52 | <0.001 | -0.48 | 0.01 | -0.29 | 0.18 |
| **CCL4** | 9.94 | <0.001 | -0.67 | <0.001 | -0.52 | 0.01 |
| **CD244** | 9.61 | <0.001 | -0.34 | 0.01 | -0.38 | 0.02 |
| **CD40** | 18.02 | <0.001 | -0.77 | <0.001 | -0.83 | <0.001 |
| **CD5** | 12.86 | <0.001 | -0.42 | <0.001 | -1.10 | <0.001 |
| **CD6** | 12.30 | <0.001 | 0.28 | 0.05 | 0.00 | 0.99 |
| **CD8A** | 10.72 | <0.001 | 1.01 | <0.001 | 0.96 | <0.001 |
| **CDCP1** | 7.15 | <0.001 | -0.30 | 0.06 | -1.04 | 0.00 |
| **CSF1** | 35.53 | <0.001 | -0.20 | 0.19 | -0.17 | 0.37 |
| **CST5** | 12.39 | <0.001 | -0.53 | <0.001 | -0.52 | <0.001 |
| **CX3CL1** | 8.89 | <0.001 | -1.24 | <0.001 | -1.39 | <0.001 |
| **CXCL1** | 7.73 | <0.001 | 0.06 | 0.64 | 0.22 | 0.21 |
| **CXCL10** | 12.12 | <0.001 | -0.49 | <0.001 | -0.82 | <0.001 |
| **CXCL11** | 8.20 | <0.001 | 0.11 | 0.48 | 0.42 | 0.03 |
| **CXCL5** | 7.57 | <0.001 | -0.26 | 0.07 | -0.09 | 0.59 |
| **CXCL6** | 8.08 | <0.001 | -0.10 | 0.46 | 0.01 | 0.93 |
| **CXCL9** | 10.83 | <0.001 | 0.54 | <0.001 | 0.44 | 0.04 |
| **DNER** | 23.00 | <0.001 | -0.06 | 0.57 | 0.12 | 0.36 |
| **EN-RAGE** | 3.89 | <0.001 | 0.02 | 0.89 | -0.05 | 0.74 |
| **FGF-19** | 10.63 | <0.001 | 0.69 | <0.001 | 1.40 | <0.001 |
| **FGF-21** | 4.44 | <0.001 | -0.06 | 0.70 | 0.13 | 0.45 |
| **FGF-23** | 4.13 | <0.001 | -0.35 | 0.04 | -0.13 | 0.54 |
| **Flt3L** | 19.91 | <0.001 | -0.19 | 0.13 | -0.09 | 0.53 |
| **HGF** | 14.95 | <0.001 | -0.84 | <0.001 | -0.65 | <0.001 |
| **IFN-gamma** | 7.84 | <0.001 | -0.39 | <0.001 | -0.59 | <0.001 |
| **IL10** | 2.75 | <0.001 | 0.61 | <0.001 | 0.59 | <0.001 |
| **IL10RA** | 1.82 | <0.001 | -0.07 | 0.10 | -0.16 | <0.001 |
| **IL10RB** | 18.16 | <0.001 | -1.03 | <0.001 | -1.38 | <0.001 |
| **IL12B** | 9.95 | <0.001 | 0.35 | <0.001 | -0.50 | <0.001 |
| **IL15RA** | 5.37 | <0.001 | -0.87 | <0.001 | -1.13 | <0.001 |
| **IL17A** | 5.08 | <0.001 | -0.13 | <0.46 | -0.35 | 0.11 |
| **IL17C** | 5.13 | <0.001 | 0.97 | <0.001 | 2.34 | <0.001 |
| **IL18** | 13.32 | <0.001 | -0.31 | 0.01 | -0.42 | <0.001 |
| **IL18R1** | 17.38 | <0.001 | -1.26 | <0.001 | -1.60 | <0.001 |
| **IL6** | 3.25 | <0.001 | -0.42 | <0.001 | -0.16 | 0.35 |
| **IL7** | 3.32 | <0.001 | -0.09 | 0.55 | 0.10 | 0.60 |
| **IL8** | 6.22 | <0.001 | 0.08 | 0.65 | -0.02 | 0.93 |
| **LAP TGF-beta-1** | 7.77 | <0.001 | -0.41 | 0.01 | -0.33 | 0.07 |
| **LIF-R** | 13.12 | <0.001 | -0.11 | 0.46 | -0.19 | 0.29 |
| **MCP-1** | 21.72 | <0.001 | -0.69 | <0.001 | -0.75 | <0.001 |
| **MCP-2** | 10.46 | <0.001 | -0.37 | <0.001 | -0.28 | 0.05 |
| **MCP-3** | 4.07 | <0.001 | -0.43 | <0.001 | -0.38 | <0.001 |
| **MCP-4** | 14.78 | <0.001 | 0.06 | 0.65 | 0.28 | 0.07 |
| **MMP-1** | 10.36 | <0.001 | 0.87 | <0.001 | 1.29 | <0.001 |
| **MMP-10** | 12.40 | <0.001 | 0.59 | <0.001 | 0.83 | <0.001 |
| **NT-3** | 5.49 | <0.001 | -0.49 | <0.001 | -0.16 | 0.31 |
| **OPG** | 25.04 | <0.001 | -0.22 | 0.09 | -0.11 | 0.50 |
| **PD-L1** | 10.56 | <0.001 | -0.69 | <0.001 | -0.78 | <0.001 |
| **SCF** | 20.95 | <0.001 | -0.44 | <0.001 | -0.48 | <0.001 |
| **SIRT2** | 3.01 | <0.001 | -0.29 | 0.06 | -0.02 | 0.91 |
| **SLAMF1** | 7.82 | <0.001 | -0.02 | 0.89 | -0.20 | 0.27 |
| **OSM** | 4.17 | <0.001 | -0.44 | <0.001 | -0.17 | 0.27 |
| **ST1A1** | 3.33 | <0.001 | -0.55 | <0.001 | -0.41 | 0.03 |
| **STAMBP** | 4.06 | <0.001 | -0.17 | 0.31 | 0.12 | 0.53 |
| **TGF-alpha** | 5.60 | <0.001 | 0.15 | 0.32 | 0.20 | 0.27 |
| **TNF** | 5.43 | <0.001 | 0.10 | 0.42 | -0.40 | 0.01 |
| **TNFB** | 9.34 | <0.001 | 0.73 | <0.001 | 0.26 | 0.04 |
| **TNFRSF9** | 12.07 | <0.001 | -0.75 | <0.001 | -1.66 | <0.001 |
| **TNFSF14** | 6.04 | <0.001 | -0.68 | <0.001 | -0.80 | <0.001 |
| **TRAIL** | 20.15 | <0.001 | -0.51 | <0.001 | -0.66 | <0.001 |
| **TRANCE** | 7.91 | <0.001 | -0.32 | 0.02 | -0.76 | <0.001 |
| **TWEAK** | 17.49 | <0.001 | -0.01 | 0.96 | 0.19 | 0.19 |
| **uPA** | 26.88 | <0.001 | -0.55 | <0.001 | -0.90 | <0.001 |
| **VEGF-A** | 16.65 | <0.001 | -0.29 | 0.01 | -0.02 | 0.87 |

**Table S7:** **ATD-regimen.** Results from linear mixed model analysis of longitudinal changes in NPX variables for different treatment groups (ATD-regimen). The models include main effect for time point, group, and the time-group interactions as fixed effects, and a random intercept was included for each patient (formula: *value ~ time + group + time:group + (1|ID)*). The time variable was reference coded to the baseline measurement. Treatment groups were reference coded to the blocking group. P-values are not adjusted for multiple testing.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **Intercept** | | **Time3** | | **Time12** | | **GroupTitration** | | **Time3:groupTitration** | | **Time12:groupTitration** | |
|  | **Effect** | **p-value** | **Effect** | **p-value** | **Effect** | **p-value** | **Effect** | **p-value** | **Effect** | **p-value** | **Effect** | **p-value** |
| **4E-BP1** | 5.50 | <0.001 | -0.16 | 0.45 | 0.30 | 0.27 | -0.13 | 0.59 | 0.08 | 0.82 | -0.16 | 0.68 |
| **ADA** | 9.27 | <0.001 | -0.83 | <0.001 | -0.97 | <0.001 | -0.30 | 0.21 | 0.40 | 0.10 | 0.26 | 0.37 |
| **AXIN1** | 2.78 | <0.001 | -0.46 | 0.03 | 0.08 | 0.78 | -0.12 | 0.63 | -0.01 | 0.99 | -0.24 | 0.55 |
| **CASP8** | 4.77 | <0.001 | -0.61 | 0.01 | -0.34 | 0.25 | -0.12 | 0.60 | 0.15 | 0.69 | -0.19 | 0.66 |
| **CCL11** | 12.05 | <0.001 | 0.27 | 0.06 | 0.59 | <0.001 | -0.13 | 0.57 | 0.12 | 0.63 | 0.22 | 0.44 |
| **CCL19** | 13.26 | <0.001 | 0.49 | <0.001 | -0.37 | 0.04 | -0.13 | 0.57 | -0.01 | 0.98 | 0.22 | 0.41 |
| **CCL20** | 8.25 | <0.001 | -0.17 | 0.43 | -0.10 | 0.71 | -0.26 | 0.30 | 0.15 | 0.67 | 0.22 | 0.58 |
| **CCL23** | 21.90 | <0.001 | -0.38 | 0.05 | -0.01 | 0.97 | -0.43 | 0.08 | 0.44 | 0.17 | 0.22 | 0.55 |
| **CCL25** | 9.38 | <0.001 | 0.45 | 0.01 | 0.75 | <0.001 | -0.22 | 0.34 | 0.21 | 0.47 | 0.01 | 0.99 |
| **CCL28** | 5.53 | <0.001 | 0.33 | 0.05 | 0.62 | 0.01 | -0.35 | 0.13 | 0.22 | 0.43 | 0.01 | 0.98 |
| **CCL3** | 9.89 | <0.001 | -0.63 | <0.001 | -0.17 | 0.53 | -0.35 | 0.14 | 0.48 | 0.16 | -0.17 | 0.66 |
| **CCL4** | 9.63 | <0.001 | -0.75 | <0.001 | -0.49 | 0.04 | -0.33 | 0.16 | 0.28 | 0.35 | 0.01 | 0.98 |
| **CD244** | 11.16 | <0.001 | -0.52 | 0.01 | -0.52 | 0.05 | -0.35 | 0.16 | 0.32 | 0.32 | 0.20 | 0.60 |
| **CD40** | 19.69 | <0.001 | -0.95 | <0.001 | -0.91 | <0.001 | -0.26 | 0.25 | 0.29 | 0.33 | 0.03 | 0.94 |
| **CD5** | 13.66 | <0.001 | -0.60 | <0.0001 | -1.42 | <0.001 | -0.25 | 0.28 | 0.41 | 0.12 | 0.58 | 0.06 |
| **CD6** | 12.67 | <0.001 | 0.21 | 0.28 | -0.10 | 0.68 | -0.21 | 0.39 | 0.21 | 0.51 | 0.24 | 0.52 |
| **CD8A** | 9.93 | <0.001 | 0.87 | <0.001 | 0.73 | <0.001 | -0.12 | 0.56 | 0.16 | 0.52 | 0.32 | 0.26 |
| **CDCP1** | 6.33 | <0.001 | -0.41 | 0.02 | -0.95 | <0.001 | -0.39 | 0.10 | 0.39 | 0.16 | 0.14 | 0.67 |
| **CSF1** | 37.33 | <0.001 | -0.30 | 0.14 | -0.38 | 0.16 | -0.19 | 0.45 | 0.21 | 0.53 | 0.43 | 0.27 |
| **CST5** | 12.44 | <0.001 | -0.56 | <0.001 | -0.58 | 0.02 | 0.19 | 0.43 | 0.09 | 0.78 | 0.11 | 0.76 |
| **CX3CL1** | 8.37 | <0.001 | -1.38 | <0.001 | -1.46 | <0.001 | -0.29 | 0.15 | 0.61 | 0.01 | 0.38 | 0.18 |
| **CXCL1** | 8.75 | <0.001 | 0.10 | 0.62 | 0.28 | 0.30 | -0.21 | 0.40 | -0.10 | 0.76 | -0.06 | 0.88 |
| **CXCL10** | 12.29 | <0.001 | -0.58 | <0.001 | -0.99 | <0.001 | -0.32 | 0.18 | 0.18 | 0.54 | 0.39 | 0.28 |
| **CXCL11** | 8.41 | <0.001 | 0.15 | 0.46 | 0.40 | 0.13 | -0.21 | 0.38 | -0.15 | 0.65 | 0.06 | 0.89 |
| **CXCL5** | 8.31 | <0.001 | -0.22 | 0.27 | -0.09 | 0.74 | 0.08 | 0.74 | -0.18 | 0.58 | -0.04 | 0.92 |
| **CXCL6** | 9.23 | <0.001 | -0.29 | 0.13 | -0.03 | 0.89 | -0.43 | 0.08 | 0.44 | 0.16 | 0.13 | 0.72 |
| **CXCL9** | 9.28 | <0.001 | 0.39 | 0.05 | 0.37 | 0.14 | -0.20 | 0.40 | 0.17 | 0.58 | 0.03 | 0.93 |
| **DNER** | 27.06 | <0.001 | -0.26 | 0.11 | 0.05 | 0.81 | -0.24 | 0.35 | 0.50 | 0.06 | 0.21 | 0.50 |
| **EN-RAGE** | 4.26 | <0.001 | 0.12 | 0.51 | <0.001 | 0.99 | -0.15 | 0.55 | -0.30 | 0.31 | -0.12 | 0.74 |
| **FGF-19** | 8.68 | <0.001 | 0.47 | 0.01 | 1.20 | <0.001 | -0.04 | 0.85 | 0.27 | 0.37 | -0.12 | 0.72 |
| **FGF-21** | 4.28 | <0.001 | -0.13 | 0.45 | 0.16 | 0.50 | -0.29 | 0.21 | 0.20 | 0.49 | -0.05 | 0.89 |
| **FGF-23** | 3.99 | <0.001 | -0.44 | 0.03 | -0.57 | 0.03 | -0.40 | 0.09 | 0.19 | 0.55 | 0.97 | 0.01 |
| **Flt3L** | 21.46 | <0.001 | -0.30 | 0.07 | -0.07 | 0.76 | -0.10 | 0.70 | 0.29 | 0.29 | -0.06 | 0.86 |
| **HGF** | 15.46 | <0.001 | -1.01 | <0.001 | -0.87 | <0.001 | -0.54 | 0.02 | 0.35 | 0.17 | 0.47 | 0.12 |
| **IFN-gamma** | 8.86 | <0.001 | -0.51 | 0.01 | -0.71 | 0.01 | -0.27 | 0.26 | 0.15 | 0.63 | 0.14 | 0.69 |
| **IL10** | 3.31 | <0.001 | 0.66 | <0.001 | 0.58 | <0.001 | -0.19 | 0.45 | 0.14 | 0.58 | 0.25 | 0.40 |
| **IL10RA** | 1.80 | <0.001 | -0.09 | 0.09 | -0.18 | 0.01 | 0.13 | 0.53 | 0.06 | 0.49 | 0.03 | 0.74 |
| **IL10RB** | 17.01 | <0.001 | -1.10 | <0.001 | -1.46 | <0.001 | -0.10 | 0.66 | 0.37 | 0.13 | 0.38 | 0.19 |
| **IL12B** | 9.96 | <0.001 | 0.27 | 0.06 | -0.67 | <0.001 | -0.24 | 0.31 | 0.18 | 0.46 | 0.37 | 0.19 |
| **IL15RA** | 5.44 | <0.001 | -1.07 | <0.001 | -1.41 | <0.001 | -0.41 | 0.06 | 0.55 | 0.03 | 0.66 | 0.03 |
| **IL17A** | 4.99 | <0.001 | -0.39 | 0.06 | -0.55 | 0.05 | -0.46 | 0.06 | 0.68 | 0.05 | 0.52 | 0.20 |
| **IL17C** | 2.70 | <0.001 | 0.46 | 0.02 | 1.15 | <0.001 | -0.22 | 0.32 | 0.05 | 0.88 | 0.10 | 0.79 |
| **IL18** | 14.72 | <0.001 | -0.53 | <0.001 | -0.55 | 0.01 | -0.50 | 0.04 | 0.49 | 0.07 | 0.22 | 0.48 |
| **IL18R1** | 15.32 | <0.001 | -1.32 | <0.001 | -1.53 | <0.001 | -0.65 | <0.001 | 0.59 | 0.01 | 0.36 | 0.19 |
| **IL6** | 3.27 | <0.001 | -0.43 | 0.01 | -0.42 | 0.06 | -0.36 | 0.12 | 0.03 | 0.91 | 0.58 | 0.07 |
| **IL7** | 3.84 | <0.001 | -0.11 | 0.63 | 0.16 | 0.58 | -0.10 | 0.69 | 0.00 | 0.99 | -0.10 | 0.82 |
| **IL8** | 6.07 | <0.001 | 0.01 | 0.98 | -0.03 | 0.90 | -0.34 | 0.16 | 0.14 | 0.69 | 0.06 | 0.88 |
| **LAP TGF-beta-1** | 9.03 | <0.001 | -0.49 | 0.03 | -0.40 | 0.17 | -0.35 | 0.15 | 0.02 | 0.95 | 0.10 | 0,81 |
| **LIFR** | 15.13 | <0.001 | -0.34 | 0.10 | -0.53 | 0.05 | -0.33 | 0.18 | 0.53 | 0.12 | 0.66 | 0.10 |
| **MCP1** | 19.37 | <0.001 | -0.72 | <0.001 | -0.77 | <0.001 | -0.27 | 0.26 | 0.28 | 0.35 | 0.24 | 0.49 |
| **MCP-2** | 11.40 | <0.001 | -0.46 | <0.001 | -0.37 | 0.07 | -0.38 | 0.11 | 0.14 | 0.58 | 0.17 | 0.57 |
| **MCP-3** | 3.91 | <0.001 | -0.46 | <0.001 | -0.33 | 0.04 | -0.28 | 0.23 | 0.15 | 0.44 | -0.04 | 0.86 |
| **MCP-4** | 16.21 | <0.001 | -0.01 | 0.94 | 0.19 | 0.41 | -0.18 | 0.47 | 0.20 | 0.50 | 0.27 | 0.43 |
| **MMP1** | 9.57 | <0.001 | 0.96 | <0.001 | 1.32 | <0.001 | 0.04 | 0.85 | -0.42 | 0.12 | -0.28 | 0.37 |
| **MMP10** | 10.69 | <0.001 | 0.35 | 0.07 | 0.47 | 0.07 | -0.20 | 0.38 | 0.39 | 0.21 | 0.52 | 0.16 |
| **NT-3** | 5.70 | <0.001 | -0.63 | <0.001 | -0.16 | 0.47 | -0.21 | 0.37 | 0.35 | 0.20 | 0.01 | 0.98 |
| **OPG** | 28.21 | <0.001 | -0.52 | <0.001 | -0.41 | 0.08 | -0.21 | 0.41 | 0.68 | 0.02 | 0.61 | 0.08 |
| **PD-L1** | 4.18 | <0.001 | -0.38 | 0.02 | -0.17 | 0.42 | -0.12 | 0.61 | -0.16 | 0.53 | 0.00 | 1.00 |
| **SCF** | 11.84 | <0.001 | -0.95 | <0.001 | -0.98 | <0.001 | -0.38 | 0.11 | 0.50 | 0.07 | 0.27 | 0.40 |
| **SIRT2** | 23.11 | <0.001 | -0.65 | <0.001 | -0.72 | <0.001 | 0.05 | 0.86 | 0.43 | 0.10 | 0.41 | 0.18 |
| **SLAMF1** | 3.35 | <0.001 | -0.30 | 0.18 | 0.08 | 0.78 | -0.08 | 0.74 | -0.09 | 0.81 | -0.22 | 0.61 |
| **OSM** | 7.82 | <0.001 | -0.24 | 0.20 | -0.52 | 0.04 | -0.37 | 0.13 | 0.56 | 0.07 | 0.70 | 0.06 |
| **ST1A1** | 3.53 | <0.001 | -0.49 | 0.02 | -0.28 | 0.31 | 0.03 | 0.89 | -0.26 | 0.45 | -0.32 | 0.43 |
| **STAMBP** | 4.41 | <0.001 | -0.18 | 0.44 | 0.29 | 0.33 | -0.03 | 0.89 | -0.02 | 0.95 | -0.31 | 0.47 |
| **TGF-alpha** | 6.91 | <0.001 | 0.02 | 0.91 | 0.16 | 0.59 | -0.47 | 0.06 | 0.34 | 0.36 | 0.21 | 0.62 |
| **TNF** | 5.85 | <0.001 | 0.03 | 0.85 | -0.60 | 0.01 | -0.17 | 0.49 | 0.17 | 0.52 | 0.38 | 0.23 |
| **TNFB** | 8.75 | <0.001 | 0.62 | <0.001 | 0.18 | 0.27 | -0.02 | 0.92 | 0.18 | 0.39 | 0.15 | 0.54 |
| **TNFRSF9** | 11.46 | <0.001 | -0.86 | <0.001 | -1.72 | <0.001 | -0.23 | 0.29 | 0.39 | 0.08 | 0.34 | 0.20 |
| **TNFSF14** | 7.03 | <0.001 | -0.92 | <0.001 | -0.89 | <0.001 | -0.40 | 0.08 | 0.36 | 0.25 | 0.00 | 1.00 |
| **TRAIL** | 21.38 | <0.001 | -0.69 | <0.001 | -0.86 | <0.001 | -0.26 | 0.28 | 0.42 | 0.15 | 0.35 | 0.30 |
| **TRANCE** | 8.55 | <0.001 | -0.35 | 0.06 | -0.94 | <0.001 | -0.08 | 0.74 | 0.02 | 0.96 | 0.25 | 0.48 |
| **TWEAK** | 19.61 | <0.001 | -0.18 | 0.29 | 0.04 | 0.86 | -0.29 | 0.24 | 0.44 | 0.11 | 0.39 | 0.23 |
| **uPA** | 25.65 | <0.001 | -0.87 | <0.001 | -1.11 | <0.001 | -0.39 | 0.10 | 0.89 | 0.01 | 0.53 | 0.16 |
| **VEGFA** | 19.24 | <0.001 | -0.37 | 0.03 | -0.13 | 0.56 | -0.24 | 0.34 | 0.07 | 0.79 | 0.23 | 0.48 |

**References**

1. Törring O, Watt T, Sjölin G, Byström K, Abraham-Nordling M, Calissendorff J, et al. Impaired Quality of Life After Radioiodine Therapy Compared to Antithyroid Drugs or Surgical Treatment for Graves' Hyperthyroidism: A Long-Term Follow-Up with the Thyroid-Related Patient-Reported Outcome Questionnaire and 36-Item Short Form Health Status Survey. Thyroid. 2019;29(3):322-31.

2. Cramon P, Bonnema SJ, Bjorner JB, Ekholm O, Feldt-Rasmussen U, Frendl DM, et al. Quality of life in patients with benign nontoxic goiter: impact of disease and treatment response, and comparison with the general population. Thyroid. 2015;25(3):284-91.

3. Thévenot EA, Roux A, Xu Y, Ezan E, Junot C. Analysis of the Human Adult Urinary Metabolome Variations with Age, Body Mass Index, and Gender by Implementing a Comprehensive Workflow for Univariate and OPLS Statistical Analyses. Journal of Proteome Research. 2015;14(8):3322-35.