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Biomarker-based clustering of patients with chronic obstructive pulmonary disease

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Research in Context:

Evidence before the study:

Multiple markers of inflammation, such as acute phase proteins and the complement system, cytokines and chemokines and their receptors, inhibitors of cytokine signaling, adhesion molecules, immunoglobulins, growth factors, proteins involved in tissue remodeling, hormones and adipokines, coagulation, and plasma carrier proteins have shown to be associated with COPD. These data suggest that to better understand the complexity of COPD on a systemic level, a variety of serum markers encompassing the wider scope of inflammatory processes are needed. Previous studies showed that combinations of biomarkers can improve predictive outcome of relevant cross-sectional and longitudinal outcome. Only a limited number of studies used large-scale proteomics to find different subgroups of well characterized COPD patients.

Added value of this study:

Biomarker-based clustering in clinically well-characterized patients with COPD resulted in four distinct clusters. Although a similar degree of airflow limitation was seen among the clusters, they were associated with distinct clinical phenotypes. Pathway analysis revealed components of the RAGE pathway to be enriched in three clusters, suggesting a systemic role for this pathway in COPD.

Implications of all the available evidence:

This study provides new insight into identifying and understanding relevant inflammatory interactions in the heterogenous disease COPD. Furthermore, the evidence provided by this study may aide in generating new hypotheses into the similarities and differences observed subgroups of COPD patients.

ABSTRACT

Rationale: COPD has been repeatedly associated with single biomarkers of systemic inflammation, ignoring the complexity of inflammatory pathways.

Objectives: This study aimed to cluster patients with COPD based on systemic markers of inflammatory processes and to evaluate differences in their clinical characterization and examine how these differences may relate to altered biological pathways.

Methods: Two hundred thirteen patients with moderate to severe COPD in a clinically stable state were recruited and clinically characterized, which included a venous blood sample for analysis of serum biomarkers. Patients were clustered based on the overall similarity in systemic levels of 57 different biomarkers. To determine interactions among the regulated biomarkers, protein networks and biological pathways were examined for each patient cluster.

Results: Four clusters were identified: two clusters with lower biomarker levels (I and II) and two clusters with higher biomarker levels (III and IV) with only a small number of biomarkers with similar trends in expression. Pathway analysis indicated that 3 of the 4 clusters were enriched in RAGE and Oncostatin M pathway components. Although the degree of airflow limitation was similar, the clinical characterization of clusters ranged from (I) better functional capacity, health status and fewer comorbidities, (II) more underweight, osteoporosis and more static hyperinflation, (III) more metabolically deranged and (IV) older subjects with worse functional capacity and higher comorbidity load.

Conclusions: These new insights may help to understand the functionally relevant inflammatory interactions in the pathophysiology of COPD as a heterogeneous disease.

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INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) has been shown to be consistently associated with systemic inflammation (1). Most COPD studies only focused on one or a limited number of markers of systemic inflammation revealing the heterogeneity within and between the different systemic inflammatory biomarkers (1-3). Multiple other inflammatory biomarkers exist, and for example, acute phase proteins and the complement system(1, 4, 5), cytokines and chemokines and their receptors (6-10), inhibitors of cytokine signaling (11), adhesion molecules (12, 13), immunoglobulins (14), growth factors (10, 15, 16), proteins involved in tissue remodeling (17, 18), hormones and adipokines (19, 20), coagulation (21, 22), and plasma carrier proteins (23, 24) have all been shown to be associated with COPD. These data suggest that to better understand the complexity of COPD on a systemic level, a variety of serum markers encompassing a wider scope of inflammatory processes are needed. Pinto-Plata and colleagues previously showed a pattern of systemic biomarkers in patients with COPD that can be associated with different clinical variables known to predict disease outcome, including the degree of airflow limitation, lung transfer factor, functional capacity, the Body-mass index, airflow Obstruction, Dyspnea and Exercise (BODE) index and exacerbation frequency (25, 26). Additionally, in studies such as COPDGene and Eclipse, combinations of biomarkers improved predictive value for relevant cross-sectional and longitudinal COPD outcomes (27) .

Only a limited number of studies used large-scale proteomics to find different subgroups of COPD. In a post-hoc analysis of the Treatment of Emphysema with a Selective Retinoid Agonist (TESRA) trial, 87 peripheral blood biomarkers in 396 former smokers with emphysema were analyzed with multiplex platforms and included in a cluster analysis (28). A small subgroup of participants with increased inflammatory biomarkers was identified, presented with less emphysema and similar lung function, but worse quality of life compared to the other clusters.

In the present manuscript, we hypothesized that a cluster analysis of a composite panel of biomarkers could express the heterogeneity of different mechanistic pathways in COPD and could

to some extent be associated with the phenotypic expressions of COPD, including co-morbidities/systemic phenotypes.

Therefore we aimed to perform a cluster analysis on a broad set of biomarkers, related to inflammatory processes previously associated with COPD. Furthermore, we aimed to compare clinical characteristics among the identified clusters. Finally, we aimed to analyze the pathways enriched in each cluster which may suggest alterations in biologically relevant pathways, thus contributing insight into COPD pathophysiology.

METHODS

Study design

This is a secondary analysis of the baseline data from the prospective CIRO Co-morbidity study (29). In brief, patients with COPD (forced expiratory volume <80% predicted (30)), aged 40 to 80 years, and in a clinically stable state were recruited during the baseline assessment of a comprehensive pulmonary rehabilitation program at CIRO (31). During a three-day assessment patients were clinically characterized, including multiple comorbidities, as described previously (29).

Venous blood sampling and laboratory analysis

Venous blood was sampled in the fasted state. A total of 57 biomarkers were examined (Table S1). Measurement of leptin, fetuin A (Quantikine;R&D Systems, Minneapolis, Minnesota) and Human Fibrinogen (Abcam; Cambridge, UK) was performed using ELISA. Measurements of other biomarkers were carried out on multiplex platforms (Meso Scale Discovery (Gaithersburg, Maryland) and Myriad RBM (Austin, Texas)). If the level of a specific biomarker was below the detection threshold, the value was set to the threshold level. Eight biomarkers with >30% missing values were excluded from the analysis (B-Lymphocyte Chemoattractant, Eotaxin-1, Eotaxin-3,

Interleukin-1 alpha, Interleukin-12 subunit p70, Interleukin-15, Interleukin-17 and MHC class I chain-related protein-A.) Measurement of leucocytes, hemoglobin, hematocrit, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides, bilirubin, creatinine, and glucose were determined in the CIRO+ laboratory (validated, custom made arrays). Glucose, hemoglobin, total cholesterol, and creatinine were not evaluated as biomarkers, but as markers of comorbidities: hyperglycemia, anemia, dyslipidemia and renal impairment, respectively.

All markers classified in one of the categories that have shown to be previously linked with the pathophysiology of COPD (see introduction) were included in the analysis (see table 2 for the classification). Those classified as others were included in the analysis because of availability and previous shown association with COPD, namely Ferritin(32), Beta-2-Microglobulin (B2M)(33), Myoglobin,(34) Osteonectin(35), Osteopontin(36), Bilirubin(37), Leucocytes,(1) Low-density lipoprotein (LDL) and High-density lipoprotein (HDL)(38).

Statistics

All statistical analyses were performed using Viscovery SOMine 7.1 by Viscovery Software GmbH, (www.viscovery.net). Self-organizing maps (SOMs, also known as Kohonen maps) were used to create an ordered representation of the data.(39) The SOM method can be viewed as a non-parametric regression technique that converts multi-dimensional data spaces into lower dimensional abstractions. A SOM generates a non-linear representation of the data distribution and allows the user to visually identify homogenous data groups.

Patients were ordered by their overall similarity regarding their systemic levels of biomarkers. Based on the created SOM model, clusters were generated using the SOM-Ward Cluster algorithm of Viscovery, a hybrid algorithm that applies the classical hierarchical method of Ward on top of the SOM topology. The method begins by defining each individual node as a separate cluster. In each step of the algorithm, two clusters with minimal distance according to the SOM-Ward distance measure are merged. This measure heeds the Ward distances as well as the positioning

of two clusters in the map picture by defining that the distance of non-adjacent clusters is always infinite, limiting merging to topologically neighboring clusters. A detailed description of SOMs can be found in the supplemental methods section.

Summary variables on the systemic levels of biomarkers and clinical characteristics for the study sample and for each cluster are presented as mean ± standard deviation for quantitative variables and percentage for discrete variables. Viscovery automatically identified for each cluster the attributes that significantly differ from the average of the whole study sample of 213 patients using the integrated two-sided T-test with a confidence of 95 and 99%.

Pathway analysis

To determine interactions between similarly regulated biomarkers, protein networks were made for each cluster using String(40), including the serum markers for which an official gene symbol could be identified (Supplemental table S1). The minimum required interaction score was set to high confidence (0.7) and all possible active interactions were allowed. The edges of the network display predicted molecular modes of action and unconnected nodes were not shown. Gene ontology (GO) of biological processes as well as pathway enrichment were analysed in each cluster (Supplemental tables S2-9) (40, 41).

RESULTS

General patient characteristics

A total of 213 out of 255 patients admitted to CIRO were eligible for the study. These patients had moderate to very severe COPD, with a substantial smoking history, moderately impaired diffusion capacity, increased static lung volumes, and multimorbidity (Table 1).

Clustering based on the systemic levels of the biomarkers

Fifty-seven serum biomarkers were used to cluster the 213 patients with COPD, which resulted in four clusters with distinct biomarker profiles. Table 2 describes how these clusters are constituted and which biomarkers have significantly higher or lower levels compared to the mean values of the whole sample. Fifteen biomarkers were *not* significantly different between the four clusters at the 99% confidence level

In cluster 1 ('Lower-level cluster I'), the levels of 17 biomarkers were significantly lower compared to the mean values of the whole sample, while two biomarkers were significantly higher. In cluster 2 ('Lower-level cluster II'), the systemic levels of 19 biomarkers were significantly lower compared to the mean values of the whole sample, while three biomarkers were significantly higher. Only three biomarkers were significantly lower in both clusters. In cluster 3 ('Higher-level cluster I'), the systemic levels of 3 biomarkers were significantly lower compared to the mean values of the whole sample, while 12 biomarkers were significantly higher. In cluster 4 ('Higher-level cluster II'), the systemic level of only one biomarker was significantly lower compared to the mean values of the whole sample, while the levels of 27 biomarkers were significantly higher. Only four biomarkers were found to be significantly higher in both clusters 3 and 4.

Clinical characteristics and objectively identified comorbidities related to the different clusters

Tables 3 and 4 summarize the clinical characteristics and the comorbidities of the four clusters, respectively. Spirometry results were similar among different clusters as were the mean pack years, mean score on mMRC dyspnea scale and the Charlson comorbidity index. Additionally, no difference in exacerbations in the last 12 months was observed among clusters.

While patients in cluster 1 had a similar level of airway obstruction and diffusion capacity, these patients exhibited less static hyperinflation, better mouth pressures, a higher peak work rate, a longer 6-minute walking distance and were less often current smokers. Subjects in cluster 1 had lower prevalence of low muscle mass and a better bone mineral density compared to the whole

study population. In addition, subjects had better renal function, a higher BMI, lower blood cholesterol and lower SGRQ levels.

Cluster 2 had a lower mean age, BMI and FFMI and were more likely to be underweight. In contrast to cluster 1, cluster 2 had more hyperinflation. Bone mineral density was worse, with a corresponding higher prevalence of osteoporosis. Conversely, atherosclerosis and dyslipidaemia were less prevalent with also a lower Framingham cardiovascular risk score.

Cluster 3 had a higher SGRQ score and higher scores on the depression score of the Hospital Anxiety and Depression Score (HADS). Cluster 3 had a higher BMI, triglycerides and cholesterol, but worse renal function and more anaemia.

Cluster 4 had the highest mean age. The mean score on the updated BODE index and for the Framingham risk score within 10 years were increased in cluster 4, suggesting an overall more severe disease status. Although the degree of airflow obstruction was similar compared to the other clusters, diffusion capacity was lower. Exercise capacity was significantly less compared to the whole study population and there was a higher mMRC, a lower haemoglobin oxygen saturation and a higher proportion of patients on long term oxygen therapy. Cluster 4 had a higher number of comorbidities including a significantly higher degree of renal impairment, lower bone mineral density than in other clusters and a significantly increased Framingham cardiovascular risk and a higher cardiac infarction injury score.

A pathway analysis based on the identified clusters of biomarkers

Protein levels that were altered in the same direction (significantly up- or down-regulated) per cluster were used to generate networks and predict enriched pathways (Figure 1). In all clusters there were significant degrees of protein-protein interactions ($p<3.5xe^{-11}$; Figure 1). Interestingly, when we enriched for pathways associated with these protein networks, the most significant were common between cluster 1, 3 and 4, namely the Oncostatin M and receptor for advanced glycation end products (RAGE) pathways (Supplemental tables 2,4 and 5). In cluster 2, cytokine-

cytokine receptor interactions and Interleukin-1 regulation of extracellular matrix were the two most significantly enriched pathways (Supplemental table 3).

DISCUSSION

This is the first study to cluster a sample of well-characterized patients with COPD based on a set of biomarkers. This resulted in four clusters with distinct biomarker patterns. Although the clusters had a similar degree of airflow limitation, distinct patient profiles could, to a certain extent, be attributed to the different biomarker clusters. Additionally, those clusters appeared connected and enriched for numerous signaling pathways.

A large heterogeneity in the levels of the different biomarkers was observed in this study, which corroborates previous findings (3, 25-27). The current clustering resulted in two lower-level clusters (1 and 2) and two higher-level clusters (3 and 4). Interestingly, only three biomarkers in clusters 1 and 2 and only four biomarkers in clusters 3 and 4 overlapped in the same direction. In contrast, four biomarkers in the low-level and three in the high-level clusters exhibited opposing expression patterns. These findings illustrate that different biological processes can be involved in patients with a high inflammatory state, as well as in patients with a low inflammatory state. Fifteen biomarkers were not significantly altered in any of the four clusters, however multiple of these biomarkers have been found to be altered in COPD and/or shown to play a role in the pathogenesis and could thus point to common pathogenic mechanisms. (e.g. Alpha-2-Macroglobulin (A2Macro) (42), factor VII (21), ferritin (32, 43, 44), fetuin A (23), Interleukin-1 beta (IL-1 beta) (6), Interleukin-8 (IL-8) (1), Monocyte chemotactic protein-4 (MCP-4) (7), Macrophage Migration Inhibitory Factor (MIF) (8), Matrix Metalloproteinase-9 (MMP-9) (17), Thymus and activation-regulated chemokine (TARC) (9) and Vitamin D-Binding Protein (VDBP) (24)).

The degree of airflow limitation was comparable between clusters, indicating the limited value of the degree of airflow limitation in predicting systemic levels of the biomarkers and vice versa.

Interestingly, there were some significant differences in clinical characteristics between the clusters. Overall, the lower-level biomarker clusters have less disease manifestations compared to the high-level clusters. This is in line with Pinto-Plata and colleagues who reported that patients in the highest quartile of a broader set of inflammatory biomarkers were more clinically compromised and had higher mortality (26). Cluster 1 could be considered as metabolically healthier with fewer comorbidities compared to the whole study population. Moreover, this cluster showed one of the smallest protein interaction networks, suggesting limited systemic alterations. Clinically, cluster 2 relates in some way to the cachectic or implosive cluster or phenotype (29). This finding might be in contrast with previous data suggesting that cachectic patients have increased levels of systemic inflammation (45) as the majority of the systemic biomarkers were decreased.

The clear differences in body composition between cluster 1 and 2 may help to explain some biomarker differences between the groups, such as the low leptin, high adiponectin and BDNF levels in cluster 2. The higher levels of the selective competitive inhibitor of IL-1, IL-1Ra, in cluster 1 confirms a robust low inflammatory status. Indeed, the lack of upregulation of IL-1Ra in cluster 2 may be underlying the implosive phenotype rather than upregulation of other pro-inflammatory mediators as seen in the high clusters. Conversely, cluster 2 exhibited decreased levels of IL-23, which is involved in the proliferation and maintenance of Th17 cells and, thus, proper mucosal defenses via, for example, the induction and production of sIgA. Importantly, sIgA levels were also lower in cluster 2 which could indicate a specific defect in mucosal immunity (46).

Cluster 4 is clearly the most inflamed cluster in terms of the number of significantly altered biomarkers (27/57 increased). Perhaps this high level of inflammation is unsurprising due to the clinical characteristics of this cluster (Table 1) as age, smoking and cardiovascular risk have indeed been repeatedly associated with increased inflammatory status (1).

Cluster 3, the other "high-level" biomarker cluster is clearly different from cluster 4 in terms of number (12 vs 27) and type of increased markers. On the clinical level, this cluster was mainly characterized by metabolic dysregulation. It is likely that the biomarker pattern in this cluster is partly driven by increased fat mass. The adipokine leptin was slightly increased in this cluster. The adipokines BDNF and IGFBP2, which have been implicated in the pathogenesis of metabolic syndrome(47), were bidirectionally different between clusters 3 and 4. Increased levels of IL12p40 have been found in the serum of obese adolescents compared to normal weight controls (48). Also PAI-1, a major inhibitor of fibrinolysis, which was elevated in cluster 3, but not in cluster 4, has been shown to significantly correlate with components of metabolic syndrome (22).

Through examination of protein-protein interactions and pathway analysis we can begin to shed light on the underlying molecular mechanisms that may be partially responsible for aspects of disease in the different clusters. Interestingly, we found that 3 of the 4 clusters were enriched in the Oncostatin M and RAGE pathways. Ample evidence has already implicated the RAGE pathway in the pathogenesis of COPD. This evidence includes protection from experimental emphysema in RAGE deficient mice and the identification of the *AGER*, the gene encoding RAGE protein, as a susceptibility gene in genome wide association studies (49). Due to the direction of alterations of key molecules associated with the RAGE pathway (Supplementary tables 2, 4 and 5) we would predict that the RAGE pathway is downregulated in cluster 1 and upregulated in clusters 3 and 4, which fits the role of RAGE as a major contributor to inflammation and the data obtained in animal studies. Additionally, RAGE deficient mice were not prone to weight gain even when fed a high fat diet (50). We see that our COPD patients in cluster 1 show clinical characteristics of lower cholesterol and normal body weight, suggesting that these clinical manifestations may be due to systemic downregulation of the RAGE pathway. Conversely, we find that where the RAGE pathway is predicted to be increased, significantly higher body weight and higher cholesterol are observed (cluster 3). Additionally, an increase in RAGE is reported to be associated with platelet activation and, in agreement, we find a significant increase in thrombocytes and PAI-1 in cluster 3

and a significant decrease in cluster 1 (51). The enrichment of the RAGE pathway in cluster 4 only partially overlaps with cluster 3, with additional effects on inflammatory mediators such as IL-6 and sVCAM1. Important to note is that our data set included mostly inflammatory mediators downstream of RAGE, whereas ligands activating the pathway are underrepresented. Levels of the various ligands identified for RAGE likely also differ between clusters, which could be a reason for the observed discriminatory downstream effects between clusters 3 and 4 in particular. As the RAGE signaling pathway is furthermore inhibited by soluble RAGE (of which levels are in general lower in COPD patients), this is yet another unexplored mechanism through which the pathway could be differentially regulated between clusters and contributing to the pathogenesis and clinical characteristics between patients and clusters. Due to the prevalence of RAGE components and enrichment of the pathway, it would be interesting to determine these additional aspects, as well as genetic variations of *AGER* in our identified COPD clusters in future studies.

Oncostatin M (OSM) pathways were highly significant in the same direction and in the same clusters as RAGE. This is not entirely surprising since a number of biomarkers are indeed shared between both pathways (supplemental Tables 1, 2 and 4). OSM is a pleiotropic cytokine of the IL-6 family of cytokines, which like RAGE, contains multiple positive feedback loops to enhance its pro-inflammatory actions. Moreover, it has previously been shown to be increased in COPD and is involved in airway remodeling (52, 53). Interestingly, it was also demonstrated using RAGE deficient mice that RAGE mediates hypomethylation in the promoter of *OSM* and that the resulting elevated expression of OSM is likely to contribute to the observed effects of RAGE in cigarette-induced airway inflammation and emphysema development (54).

Previously, a subanalysis of the TESRA study was the first to use proteomics to subtype stable ex-smoking COPD patients.(28) The authors included 87 biomarkers measured in 396 COPD patients and identified 3 different clusters. They report that 18 biomarkers were different between the three clusters, but do not report the distribution of markers among the three clusters. Similarly to

our results BDNF, Fibrinogen, Osteoprotegerin, Stem Cell Factor and Vascular Endothelial Growth Factor (VEGF) had discriminating power in differentiating the clusters. The authors specifically pointed out a smaller cluster of participants with increased inflammatory biomarkers, impaired quality of life yet less emphysema, which could be reminiscent of cluster 4 in the current study, as this cluster was also characterized by increased markers of Alpha-1 Antitrypsin, CRP, Haptoglobin and Tumor Necrosis Factor Receptor I (TNF-R1). Although diffusion capacity was lower in cluster 4, which commonly relates to the degree of emphysema. In TESRA, subjects were characterized with less detail as no information on e.g., comorbidities and functional performance is provided. Most COPD patients have multiple chronic conditions which are strongly interconnected. Previously, five different comorbidity clusters have been identified in COPD patients of the CIROCO cohort (55). The present study highlights the complexity of the pathophysiology, as the clinical characteristics of patients in the four observed clusters of circulating biomarkers did not seem to correspond well with the previously described comorbidity clusters (55). This likely reflects the heterogeneity of patients with COPD, where different clinical traits and diseases coexist to varying degrees within the same individual, and there is an inability to delineate mutually exclusive clusters of (mechanisms of) disease(s) (56).

The current results are rather hypothesis generating than definitive and there are several limitations to the current study. Indeed, our findings need to be corroborated, using additional cohorts of individuals to determine the robustness of our clustering approach. We have thus far only examined patients with moderate to severe COPD, but not those in early stages of the disease. Including early-stage individuals and following them over time, might gain additional insight into systemic changes in inflammation and how they may mirror the development of clinical characteristics. Although a large set of biomarkers was used, this still does not reflect the complexity of biological interactions in the human body. Moreover, there is considerable debate over how reflective systemic inflammation is of local inflammation in the airways. However, using a relatively non-invasive sampling method, such as whole blood sampling, one can relatively easily

examine numerous biomarkers without causing unnecessary stress to the patient. Recognising the fact that the discussion is valid only for the biomarkers we have explored, our findings may help to shed light on the underlying pathogenic processes involved in COPD.

Biomarker-based clustering in patients with COPD resulted in four clusters, illustrating the heterogeneity of biomarkers in patients with COPD. A dichotomy in clusters was seen based on mainly lower/higher levels of biomarkers, which in turn resulted in a unique biomarker composition for each cluster. Although, the degree of airflow limitation was similar among the clusters, differences in clinical characterization were seen. Pathway analysis revealed components of the RAGE pathway to be enriched in three of the four clusters, suggesting a systemic role for this pathway in COPD. These new insights may help to understand the functionally relevant inflammatory interactions in the pathophysiology of COPD as a heterogeneous disease.

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Table 1. General characteristics

Values are shown as mean with the standard deviation in parenthesis unless otherwise indicated. Abbreviations: BMI: body mass index, FFMI: fat-free mass index, mMRC: modified Medical Research Council, FEV1: Forced expiratory volume in the first second, FVC: forced vital capacity, ITGV: intra-thoracic gas volume, DLCO: diffusion capacity for carbon monoxide, 6MWD: six-minute walking distance, SGRQ: saint-George Respiratory Questionnaire, BODE: Body mass index – Obstruction – Dyspnea – Exercise capacity index.

	All subjects n=213
Age, years	63.6 (7.0)
Male, %	59
BMI, kg/m^2	26.2 (5.1)
FFMI, kg/m^2	17.0 (2.4)
mMRC dyspnea grade	2.1 (1.1)
Current smoker, %	28
Pack years	46 (26)
Long-term oxygen therapy, %	17
FEV1, liters	1.40 (0.54)
FEV1, % predicted	51.2 (16.9)
FEV1/FVC	0.40 (0.11)
ITGV, % predicted	148 (33)
DLCO, % predicted	56 (17)
6MWD, meters	470 (106)
SGRQ, total score	51.3 (17.5)
Updated BODE score	2.9 (2.5)
Framingham 10-year risk %	9.4 (6.7)

Table 2. Systemic levels of biomarkers of inflammation, chemoattraction, cell activation, tissue destruction and tissue repair per cluster

Legend: Values are shown as mean with the standard deviation in parenthesis. Eight biomarkers were measured but excluded from the statistical analysis because of >30% missing values (B-Lymphocyte Chemoattractant, Eotaxin-1, Eotaxin-3, Interleukin-1 alpha, Interleukin-12 subunit p70, Interleukin-15, Interleukin-17 and MHC class I chain-related protein-A.)

Biomarker	Cluster 1 (n=64) Lower-level Cluster I	Cluster 2 (n=64) Lower-level Cluster II	Cluster 3 (n=46) Higher-level Cluster I	Cluster 4 (n=39) Higher-level Cluster II
Multiplex platforms				
Acute phase proteins & complement system				
Complement C3 (C3), mg/ml	1.2 (0.2)	1.1 (0.2)	1.3 (0.2)	1.5 (1.0)
C-reactive protein (CRP), ng/ml	3011 (2795)	1773 (1859)	8214 (8153)	9725 (8480)
Fibrinogen, ug/ml	6417 (2826)	6870 (4533)	6465 (2374)	8879 (7911)
Serum Amyloid A (SAA), ng/mL	3059 (2705)	1889 (1403)	6239 (7311)	10745 (7810)
Cytokines & Chemokines & their receptors				
Eotaxin-2, pg/ml	1022 (632)	1398 (940)	1371 (954)	1337 (965)
Interleukin-1 beta (IL-1 beta), pg/mL	4.8 (1.2)	4.3 (1.3)	4.2 (1.1)	5.0 (1.6)
Interleukin-12 Subunit p40 (IL-12p40), ng/mL	0.6 (0.2)	0.5 (0.1)	0.5 (0.1)	0.6 (0.1)
Interleukin-23 (IL-23), ng/mL	1.4 (0.4)	1.2 (0.3)	1.2 (0.3)	1.4 (0.3)
Interleukin-6 (IL-6), pg/mL	2.9 (1.6)	1.9 (1.7)	3.4 (1.7)	6.3 (8.2)
Interleukin-8 (IL-8), pg/mL	12 (5)	13 (5)	14 (6)	14 (5)
Interferon γ -Inducible T cell α Chemoattractant (ITAC), pg/mL	43 (22)	44 (24)	55 (25)	64 (47)
Interferon γ–Inducible Protein-10 (IP-10), pg/mL	93 (41)	73 (39)	90 (30)	142 (98)
Interferon-γ (IFN-γ), pg/mL	0.5 (0.3)	0.4 (0.4)	0.5 (0.2)	1.3 (2.6)
Macrophage Migration Inhibitory Factor (MIF), ng/mL	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)
Monocyte chemotactic protein-1 (MCP-1), pg/mL	511 (190)	469 (130)	606 (159)	513 (200)
Monocyte chemotactic protein-4 (MCP-4), pg/mL	688 (235)	678 (207)	762 (210)	778 (455)
T-Cell-Specific Protein RANTES (RANTES), ng/mL	19 (8)	23 (10)	31 (10)	25 (14)
Thymus and activation-regulated chemokine (TARC), pg/mL	524 (414)	636 (384)	784 (620)	770 (828)
Interleukin-2 receptor alpha (IL-2 receptor alpha), pg/mL	2427 (694)	2020 (535)	2695 (927)	3429 (1240)
Tumor necrosis factor receptor 2 (TNF-R2), ng/mL	6.0 (1.6)	4.9 (1.2)	6.0 (1.8)	9.8 (5.0)
Tumor Necrosis Factor Receptor I (TNF-R1), pg/mL	1850 (476)	1531 (436)	1905 (588)	2603 (919)
Osteoprotegerin (OPG), pM	7.0 (1.9)	6.7 (1.1)	7.2 (1.3)	8.9 (2.0)
Inhibitors of cytokine signaling				
Interleukin-1 receptor antagonist (IL-1ra), pg/mL	423 (132)	336 (74)	372 (99)	388 (118)
Adhesion molecules				
Soluble intercellular adhesion molecule 1 (sICAM1), ng/mL	282 (76)	292 (76)	321 (102)	389 (113)
Soluble vascular Cell Adhesion Molecule-1 (sVCAM-1), ng/mL	520 (116)	493 (106)	517 (142)	719 (396)
Immunoglobulins				
Immunoglobulin A (IgA), mg/ml	2.9 (1.6)	2.4 (1.2)	3.1 (1.4)	3.6 (2.9)
Immunoglobulin M (IgM), mg/ml	2.1 (1.4)	1.8 (1.9)	1.7 (1.1)	2.5 (4.2)
Growth factors				
Angiopoietin-2 (ANG-2), ng/ml	3.7 (1.8)	3.8 (1.3)	4.4 (1.4)	5.1 (1.3)
Brain-Derived Neurotrophic Factor (BDNF), ng/ml	18.1 (4.9	22 (5)	23 (7)	18 (7)

Stem Cell Factor (SCF), pg/mL	397 (93)	330 (83)	354 (88)	473 (163)
Vascular Endothelial Growth Factor (VEGF), pg/ml	242 (111)	258 (122)	354 (159)	290 (170)
Tissue remodeling				
Alpha-1-Antitrypsin (AAT), mg/ml	1.9 (0.3)	2.0 (0.4)	1.9 (0.4)	2.3 (0.6)
Latency-Associated Peptide of Transforming Growth Factor beta 1 (LAP TGF-b1), ng/ml	9.4 (2.8)	12 (3)	13 (3)	10 (3)
Matrix Metalloproteinase-3 (MMP-3), ng/ml	19 (14))	13 (7)	14 (7)	23 (16)
Matrix Metalloproteinase-9 (MMP-9), ng/ml	147 (72)	130 (62)	153 (69)	159 (83)
Tissue Inhibitor of Metalloproteinases 1 (TIMP-1), ng/ml	150 (23)	163 (27)	195 (30)	199 (65)
Hormones & Adipokines				
Adiponectin, ug/ml	4.4 (2.1)	7.0 (3.3)	5.1 (2.7)	7.4 (4.7)
Leptin, ng/ml	14.8 (12.3)	8.2 (8.8)	16.8 (14.6)	13.7 (14.8)
Erythropoietin (EPO), IU/ml	11.3 (5.2)	8.4 (2.6)	11 (7)	13 (4)
Osteocalcin (OCN/BGLAP, ng/ml	117 (20)	127 (18)	116 (17)	135 (25)
Coagulation				
Alpha-2-Macroglobulin (A2Macro), mg/ml	1.7 (0.4)	1.7 (0.4)	1.7 (0.6)	1.9 (0.7)
Factor VII, ng/ml	447 (140)	406 (99)	403 (127)	422 (119)
Plasminogen Activator Inhibitor 1 (PAI-1), ng/ml	195 (45)	228 (53)	290 (56)	220 (112)
Plasma carrier proteins				
Fetuin A, ug/ml	574 (292)	706 (364)	603 (303)	652 (420)
Haptoglobin, mg/ml	1.7 (0.9)	1.6 (1.1)	2.9 (1.4)	3.5 (3.1)
Insulin-like Growth Factor-Binding Protein 1 (IGFBP-1), ng/ml	2978 (2432)	4368 (2771)	3204 (2178)	4504 (2870)
Insulin-like Growth Factor-Binding Protein 2 (IGFBP-2), ng/ml	79 (34)	110 (53)	80 (35)	132 (45)
Vitamin D-Binding Protein (VDBP), ug/ml	288 (108)	300 (91)	291 (122)	396 (727)
Other				
Ferritin, ng/ml	180 (142)	151 (128)	190 (160)	183 (203)
Beta-2-Microglobulin (B2M), ug/ml	2.1 (0.5)	1.8 (0.3)	2.1 (0.4)	2.9 (1.0)
Myoglobin, ng/ml	69 (37)	74 (111)	54 (28)	96 (62)
Osteonectin, ng/ml	1145 (344)	1394 (230)	1720 (322)	1318 (394)
Osteopontin, ng/ml	22 (7)	22 (8)	22 (7)	36 (14)
Bilirubin, mmol/l	11.6 (4.9)	14 (6)	10 (5)	12 (3)
Leucocytes, $*10^9$/l	6.7 (1.4)	6.9 (1.9)	8.1 (1.4)	8.5 (2.4)
Low-density lipoprotein (LDL), mmol/l	2.7 (0.9)	3.1 (0.9)	3.2 (1.2)	2.9 (1.0)
High-density lipoprotein (HDL), mmol/l	1.7 0.4)	1.7 (0.5)	1.7 (0.5)	1.5 (0.5)

Legend:

	= A significantly higher level compared to the remaining three clusters (P-value <0.01)
	= A tendency for a significantly higher level compared to the remaining three clusters (P-value between 0.05 and 0.01)
	= A significantly lower level compared to the remaining three clusters (P-value <0.01)
	= A tendency for a significantly lower level compared to the remaining three clusters (P-value between 0.05 and 0.01)

Table 3. The clinical characteristics per cluster

Values are shown as mean with the standard deviation in parenthesis unless otherwise indicated. Abbreviations: FEV1: Forced experiatory volume in the first second, FVC: forced vital capacity, DLCO: diffusion capacity for carbon monoxide, ITGV: intra-thoracic gas volume, RV: residual volume, TLC: total lung capacity, PaCO2: partial arterial pressure for carbiondioxide, PaO2: partial arterial oxygen pressure, SaO2: arterial hemoglobin oxygen saturation, PImax: maximal inspiratory mouth pressure, PEmax: maximal expiratory mouth pressure, mMRC: modified Medical Research Council, LTOT: long term oxygen treatment, 6MWD: six-minute walking distance, SGRQ: saint-George Respiratory Questionnaire, BODE: Body mass index – Obstruction – Dyspnea – Exercise capacity index.

Clinical characteristics	**Cluster 1 Lower-level Cluster I**	**Cluster 2 Lower-level Cluster II**	**Cluster 3 Higher-level Cluster I**	**Cluster 4 Higher-level Cluster II**
Women, %	34	47	48	33
Age, years	65 (7)	61 (6)	62 (6)	69 (6)
Lung function				
FEV1, l	1.5 (0.5)	1.4 (0.5)	1.4 (0.5)	1.3 (0.6)
FEV1, % predicted	54 (16)	51 (19)	50 (15)	48 (16)
FEV1/FVC, %	41 (12)	40 (11)	40 (11)	38 (9)
DLCO, % predicted	60 (17)	54 (16)	58 (17)	48 (14)
ITGV, % predicted	139 (32)	156 (35)	147 (34)	152 (29)
RV, % predicted	153 (45)	175 (54)	162 (44)	165 (39)
TLC, % predicted	116 (17)	123 (16)	119 (17)	116 (16)
PaCO2, kPa	5.3 (0.6)	5.2 (0.5)	5.5 (0.7)	5.4 (0.6)
PaO2, kPa	9.6 (1.1)	9.6 (1.2)	9.3 (0.9)	9.2 (0.9)
SaO2, %	95.1 (1.9)	95.2 (1.7)	94.7 (2.0)	94.4 (1.8)
PImax, % predicted	85 (24)	77 (25)	78 (22)	75 (18)
PEmax, % predicted	64 (20)	62 (22)	60 (17)	56 (17)
COPD-specific characteristics				
mMRC dyspnea grade	2.0 (1.1)	2.0 (1.1)	2.1 (0.9)	2.4 (1.3)
LTOT use, %	11	14	17	31
Pack years	44 (21)	47 (28)	48 (30)	47 (23)
Current smoker, %	14	31	37	36
Hospital admissions for COPD last 12 months, n	0.51 (1.17)	0.42 (1.17)	0.49 (0.84)	0.69 (1.28)
Steroid/antibiotic courses for COPD last 12 months, n	1.53 (1.95)	1.42 (1.61)	1.01 (1.32)	1.92 (1.92)
Total amount of exacerbations last 12 month	2.05 (2.60)	1.85 (2.41)	1.54 (1.81)	2.55 (2.18)
GOLD group A – B – C – D, %	19 – 30 – 20 – 30	18 – 26 – 15 - 40	11 – 40 – 7 – 40	15 – 18 – 15 – 51
Physical fitness				
6MWD, m	493 (91)	497 (101)	461 (91)	402 (126)
Peak work rate, watts	86 (30)	76 (23)	75 (27)	58 (26)
Peak work rate, % predicted	63 (25)	62 (25)	61 (29)	47 (22)
CWRT, s	409 (264)	408 (329)	296 (191)	234 (187)
Health status				
SGRQ symptoms domain, points	54 (19)	53 (21)	57 (18)	57 (25)
SGRQ activity domain, points	65 (22)	68 (22)	73 (17)	69 (27)
SGRQ impact domain, points	35 (19)	38 (18)	47 (16)	43 (26)
SGRQ total score, points	48 (19)	50 (17)	57 (12)	53 (23)

Prognostic indices				
Updated BODE index, points	2.2 (1.8)	2.7 (2.3)	2.6 (2.0)	4.6 (3.7)
Framingham Risk Score, points	9.7 (6.1)	7.5 (5.9)	9.3 (7.2)	12.3 (7.2)

Legend:

	= A significantly higher level compared to the remaining three clusters (P-value <0.01)
	= A tendency for a significantly higher level compared to the remaining three clusters (P-value between 0.05 and 0.01)
	= A significantly lower level compared to the remaining three clusters (P-value <0.01)
	= A tendency for a significantly lower level compared to the remaining three clusters (P-value between 0.05 and 0.01)

Table 4. Comorbidities per biomarker-based cluster

Values are shown as mean with the standard deviation in parenthesis unless otherwise indicated. Abbreviations: eGFR: estimated glomerular filtration rate, BMI: body mass index, FFMI: fat-free mass index, HDL: high-density lipoprotein, HADS: hospital anxiety and depression score, c-IMT: carotid intima-media thickness

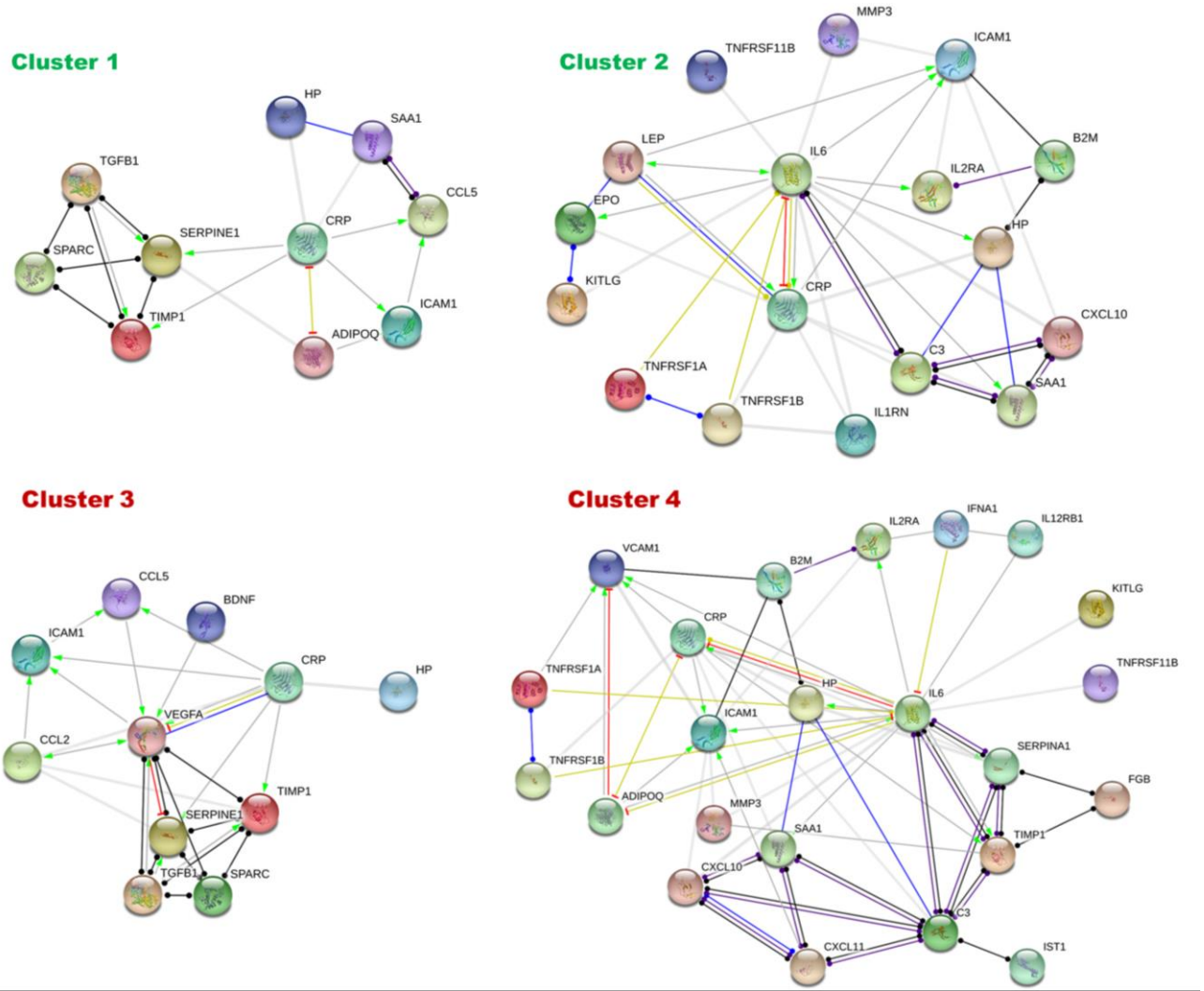
	Cluster 1 Lower-level Cluster I	Cluster 2 Lower-level Cluster II	Cluster 3 Higher-level Cluster I	Cluster 4 Higher-level Cluster II
Number of Comorbidities, n	3.5 (1.5)	3.4 (1.5)	3.7 (1.7)	4.3 (1.8)
Charlson comorbidity index, points	1.6 (0.7)	1.5 (0.9)	1.6 (0.8)	1.8 (1.1)
eGFR, ml/min	78 (21)	83 (23)	87 (25)	66 (20)
Creatinin, µmol/L	93 (20)	78 (15)	84 (14)	99 (32)
Renal Impairment, % patients	23	14	15	41
Hemoglobin, mmol/L	9.0 (0.6)	9.1 (0.7)	9.1 (0.9)	8.7 (0.7)
Hematocrit, %	44 (4)	44 (4)	44 (5)	42 (4)
Anemia, % patients	2	2	11	10
Systolic Blood Pressure, mm Hg	140 (17)	136 (25)	138 (15)	145 (28)
Diastolic Blood Pressure, mm Hg	83 (8)	82 (11)	83 (9)	83 (12)
Hypertension, % patients	45	44	48	62
BMI, kg/m^2	27.4 (4.8)	24.2 (4.9)	28.1 (5.4)	25.2 (4.2)
Obesity, % patients	28	16	33	18
Underweight, % patients	9	25	4	15
FFMI, kg/m^2	17.6 (2.0)	16.1 (2.5)	17.5 (2.8)	16.9 (2.0)
Muscle Wasting, % patients	14	50	20	26
Glucose, mmol/L	5.8 (1.0)	5.7 (0.8)	5.9 (1.0)	5.6 (0.9)
Hyperglycemia, % patients	56	55	61	44
Triglycerides, mmol/L	1.6 (0.7)	1.3 (0.5)	2.0 (1.3)	1.4 (0.7)
Cholesterol, mmol/L	5.0 (1.0)	5.4 (1.0)	5.8 (1.5)	5.1 (1.1)
Cholesterol/HDL ratio	3.2 (1.0)	3.3 (0.9)	3.8 (1.3)	3.6 (1.1)
Dyslipidemia, % patients	41	22	52	33
Thrombocytes, $x10^9/L$	235 (57)	253 (65)	334 (138)	260 (72)
HADS Anxiety, points	5.5 (3.)	6.7 (4.3)	7.0 (3.8)	6.0 (3.7)
Anxiety, % patients	16	23	25	22
HADS Depression, points	5.1 (3.5)	5.5 (3.9)	6.7 (3.3)	5.7 (3.2)
Depression, % patients	16	15	20	14
c-IMT, mm	1.0 (0.2)	0.9 (0.2)	1.0 (0.2)	1.0 (0.2)
Atherosclerosis, % patients	61	35	57	62
Cardiac infarction injury score (CIIS)	10.4 (7.2)	11.2 (5.4)	11.3 (5.7)	13.3 (7.0)
T-score Total Body	-0.6 (1.1)	-1.4 (1.3)	-0.8 (1.2)	-1.4 (1.5)
Osteoporosis, % of patients	22	41	22	41

Legend:

	= A significantly higher level compared to the remaining three clusters (P-value <0.01)
	= A tendency for a significantly higher level compared to the remaining three clusters (P-value between 0.05 and 0.01)
	= A significantly lower level compared to the remaining three clusters (P-value <0.01)
	= A tendency for a significantly lower level compared to the remaining three clusters (P-value between 0.05 and 0.01)

Figure 1: Network interactions of predicted up- or down- regulated proteins by cluster.

Legend: Proteins that were similarly and significantly up- (clusters 3 & 4 indicated in red) or down-regulated (clusters 1 & 2 indicated in green) were entered into String [35], all possible interaction sources were allowed and subjected to a minimum required high confidence interaction score (0.7). Disconnected nodes are not shown. All clusters had a p-value of at least $3.53e^{-11}$. The mode of interaction is depicted as an arrow (positive interaction), inhibition (negative interaction) or ball (unspecified interaction). Gene names, with corresponding biomarker names in parenthesis, used in this figure: ADIPOQ(Adiponectin), BDNF(BDNF), B2M (B2M), C3(C3), CCL2(MCP-1), CCL5 (RANTES), CRP(CRP), CXCL10(IP-10), CXCL11(ITAC), EPO(EPO), HP(Haptoglobin), ICAM1(ICAM-1), IFNG(IFN-γ)IL1RN(IL-1ra), IL2RA(IL-2 receptor alpha), IL6 (IL-6), IL12RB1 (IL12p40), BGLAP(OCL), KITLG (SCF), LEP(Leptin), MMP3 (MMP-3), SAA1(SAA), SERPINA1(AAT),SERPINE1 (PAI-1), SPARC(ONN), TGFB1(LAP TGF-b1), TIMP1 (TIMP-1), TNFRSF11B(OPG), TNFRSF1A(TNFRI), TNFRSF1B (TNFR2), VEGFA(VEGF), VCAM1(VCAM-1)



Supplement on data analysis and statistical methods

Self-organizing maps (SOMs)

SOMs were introduced by Teuvo Kohonen in 1982 (1) and constitute self-organized formation of topologically correct feature maps (2) as a simple model for biological neural networks. They are a form of unsupervised artificial neural networks and learn statistical patterns in a recursive manner. SOMs consist of a (usually two-dimensional) grid of learning units (which are also called nodes or neurons). Each node i has an associated reference vector, w_i, which stands for the pattern represented in the specific node.

Throughout the SOM learning algorithm, data records are presented to the SOM. The best-matching node for a record x is determined by evaluating the Euclidean distance $|w_i - x|$ to all the reference vectors. Now, the best-matching node j and it's neighboring nodes (on the two dimensional grid) learn the information of the new data record by adapting their weight vectors towards the direction of x, namely

$$w_i(t+1) = w_i(t) + \alpha(t) \cdot h_{ji}(t) \cdot (x - w_i(t))$$

where h_{ji} is the neighboring function of node i to the best-matching node j of the data vector x and α is the learning rate of the SOM. In our case we have

$$h_{ji}(t) = exp\left(-\frac{|z_j - z_i|}{2\sigma(t)}\right)$$

where z_i and z_j are the coordinates of the corresponding nodes on the two-dimensional grid. The radius σ is called tension.

By presenting all the available data vectors to the SOM, the winning nodes and their neighbours will be attracted to the corresponding parts of the multi-dimensional data distribution. By doing this repeatedly, the nodes represent the data distribution increasingly well, and neighboring nodes on the grid will represent similar parts of the data. We obtain a topology-preserving, two-dimensional representation of the multi-dimensional data distribution.

The nodes of the trained SOM can be interpreted as micro-clusters, which group very similar data records (here: patients) into the representing nodes. The SOM then interpolates through the data distribution like a non-linear regression surface. Figuratively speaking, a SOM can be seen as an elastic membrane having an inner tension, defined by the neighboring function, that moves freely within the data space, and becomes "attracted" to individual data vectors, respectively dense regions in the data space, until it reaches an equilibrium in which the inner tension and the forces pulling the nodes to the individual data vectors balance out.

Since adjacent nodes of the trained SOM correspond to neighboring regions in the data space, properties of the data distribution can be viewed by visualizing single variables over the SOM and comparing them with each other. This way, correlations and dependencies can easily be observed. In addition, the representation of the SOM allows clustering algorithms to be applied to the data. By clustering the frequency weighted SOM nodes. This has the advantage that clustering techniques can be used, which were not feasible using the original data due to runtime issues and susceptibility to statistical noise.

Learning parameters of the SOM model

The most precise training schedule of Viscovery SOMine 7.1, “Accurate” was used to calculate the SOM model on a hexagonal grid of 21 rows and 23 columns (where every second row has one node less, such that the SOM consists of 472 nodes).

Both, the learning rate α and the time dependence of the tension σ, are managed internally by Viscovery SOMine to guarantee optimal convergence properties. Only the final tension can be set by the user. In our model, a tension of 0.5 was used, which corresponds to a weak remaining interaction between directly adjacent nodes and a small smoothing effect by the SOM.

The following list of 58 biomarkers was chosen to constitute the data space for the training of the SOM: A2macro, AAT, Adiponectin, ANG2, B2M, BDNF, Bilirubin, C3, Eotaxin2, EPO, FactorVII, Ferritin, FetuinA, Fibrinogen, Haptoglobin, HDL, hsCRP, ICAM1, IFN, IgA, IGFBP1, IGFBP2, IgM, IL12p40, IL1beta, IL1ra, IL23, IL2Ralpha, IL6, IL8, IP10, ITAC, LAPTGFB1, LDL, Leptin, Leucocytes, MCP1, MCP4, MIF, MMP3, MMP9, Myoglobin, OCL1, ONN1, OPG, OPN1, PAI1, RANTES, SAA, SCF, SICAM1, SVCAM1, TARC, TIMP1, TNF-R1, TNF-R2, VDBP, and VEGF.

Hence, only these variables have been given a weight > 0 to influence the order of the resulting SOM and the cluster model. All other variables mentioned are mapped onto the nodes after the ordering process has been finished. All weighted variables have been scaled to equal variance in order to make the scales comparable and assigned the same weight, namely 1, to contribute equally to the order of the SOM. Eventual correlations between weighted variables have been compensated internally by Viscovery SOMine, adapting the weights accordingly to get a more balanced map.

Clustering method

Based on the created SOM model, clusters were generated using the SOM-Ward Cluster algorithm of Viscovery, a hybrid algorithm that combines the local ordering information of the map, i.e. the SOM topology, with Ward's classical hierarchical cluster algorithm producing very cohesive clusters.

The method begins by defining each individual node as a separate cluster. In each step of the algorithm, two clusters with minimal distance according to the SOM-Ward distance measure are merged. This measure heeds the Ward distances as well as the positioning of two clusters in the map picture by defining that the distance of non-adjacent clusters is always infinite, limiting merging to topologically neighboring clusters. (3)

References

(1) Kohonen, T. (2001). Self-Organizing Maps. Springer Series in Information Sciences, vol 30. Springer, Berlin, Heidelberg.

(2) Kohonen, T. (1982), Self-organized formation of topologically correct feature maps. *Biol. Cybern.* **43**, 59–69 (1982)

(3) Viscovery Software GmbH, Manual of Viscovery SOMine 7.1

Supplementary Table 1: Description of the biomarkers and their gene symbol included in the pathway analysis.

Biomarker name	Official gene symbol	Brief description (from NCBI gene)
Adiponectin	ADIPOQ	Expressed exclusively in adipose tissue, but found circulating in the plasma. Involved in metabolic and hormonal processes.
Alpha-1-Antitrypsin (AAT)	SERPINA1	Secreted. It is a serine protease inhibitor that targets numerous proteolytic enzymes including elastase, thrombin and plasmin.
Alpha-2-Macroglobulin (A2Macro)	A2M	A protease inhibitor for a broad spectrum of proteases such as trypsin and collagenase.
Angiopoietin-2 (ANG-2)	ANGPT2	Ligand for the endothelial tyrosine kinase receptor and is an antagonist of angiopoietin-1.
B Lymphocyte Chemoattractant (BLC)	CXCL13	Preferentially promotes the migration of B lymphocytes expressing the receptor BLR-1.
Beta-2-Microglobulin (B2M)	B2M	Serum protein associated with major histocompatibility complex (MHC) class 1 heavy chain.
Brain-Derived Neurotrophic Factor (BDNF)	BDNF	Member of the nerve growth factor family that binds to BDNFR and promotes neuronal survival in the adult brain.
Complement C3 (C3)	C3	Plays a central role in the activation of the complement system and is required to activate classical and alternative complement pathways.
Eotaxin-1	CCL11	Part of a superfamily of secreted proteins involved in the immunoregulatory and inflammatory process. This chemokine is specific for eosinophils
Eotaxin-2	CCL24	Part of a superfamily of secreted proteins involved in the immunoregulatory and inflammatory process. Displays chemotactic activity on resting T lymphocytes, but not monocytes or activated T lymphocytes.
Eotaxin-3	CCL26	Part of a superfamily of secreted proteins involved in the immunoregulatory and inflammatory process. Displays chemotatic activity for blood eosinophils and basophils.
Factor VII	F7	A coagulation factor essential for hemostasis.
Ferritin	FTH1/FTL (heavy and light chains, respectively)	The major intracellular iron storage protein. It is composed of light and heavy chains.
Fibrinogen	FG -A/B/G	A blood borne glycoprotein involved in clotting, cell adhesion and spreading.
Haptoglobin	HP	Bind free plasma hemoglobin.
Immunoglobulin A (IgA)	IGHA1	Immunoglobulin A heavy constant alpha
Immunoglobulin M (IgM)	IGHM	Immunoglobulin M heavy constant

Insulin-like Growth Factor-Binding Protein 2 (IGFBP-2)	IGFBP2	Can be found intracellularly or in the bloodstream. High expression levels promote growth of several different tumors or may be used to predict patient recovery.
Intercellular Adhesion Molecule 1 (ICAM-1)	ICAM1	A surface glycoprotein typically expressed on endothelial cells and immune cells. It binds to CD11/CD18 type integrins.
Interleukin-1 alpha (IL-1 alpha)	IL1A	A pleiotropic cytokine produced by monocytes and macrophages. It is involved in various immune responses and inflammation processes.
Interleukin-1 beta (IL-1 beta)	IL1B	A cytokine produced by macrophages and processed to its active form by caspase-1. An important mediator of inflammatory processes.
Interleukin-1 receptor antagonist (IL-1ra)	IL1RN	Inhibits the activities of IL1A and IL1B and modulates IL1 mediated responses.
Interleukin-2 receptor alpha (IL-2 receptor alpha)	IL2RA	One of the subunits of the IL2 receptor (together with IL2RB and IL2RG).
Interleukin-12 Subunit p40 (IL-12p40)	IL12B	Expressed by activated macrophages and induce the development of Th1 cells.
Interleukin-12 Subunit p70 (IL-12p70)	IL12p70	The heterodimer of IL12A and IL12B. Produced by dendritic cells, macrophages and B-cells in response to antigenic stimulation. Stimulates production of interferon gamma and tumor necrosis factor alpha from T and natural killer (NK)cells
Interleukin-15 (IL-15)	IL15	Regulates activation and proliferation of T and NK cells. Activates JAK kinases and a variety of transcription activators such as STATs -3,-5 and -6.
Interleukin-17 (IL-17)	IL17A	A proinflammatory cytokine produces by activated T cells that regulates kinases of the NFkB and MAPK pathways. Can stimulate the induction/production of IL6, COX2 and nitric oxide.
Interleukin-23 (IL-23)	IL23A	Can activate the transcription of STAT4 and produce interferon gamma.
Latency-Associated Peptide of Transforming Growth Factor beta 1 (LAP TGF-b1)	TGFB1	Secreted ligand of TGF-beta which can activate SMAD family transcription factors.
Macrophage Migration Inhibitory Factor (MIF)	MIF	A lymphokine involved in cell-mediated immunity, immunoregulation and inflammation. Suppresses anti-inflammatory effects of glucocorticoids through the regulation of macrophage function.
Matrix Metalloproteinase-3 (MMP-3)	MMP3	Involved in the breakdown of the extracellular matrix. MMP3 is involved in the degradation of fibronectin, laminin, collagens and proteoglycans.
Matrix Metalloproteinase-9 (MMP-9)	MMP9	Involved in the breakdown of the extracellular matrix. MM9 degrades type IV and V collagens.
MHC class I chain-	MICA	Expressed on the cell surface but seems to act non-canonically as it does not associate beta-2-

related protein A (MICA)		microglobin. Functions as a stress-induced antigen broadly recognized by intestinal epithelial gamma delta T cells.
Myoglobin	MB	Part of the globin superfamily. Predominantly express in skeletal and cardiac muscles. Plays a role in regulating nitric oxide levels.
Osteoprotegerin (OPG)	TNFRSF11B	Member of the TNF receptor superfamily that functions as a negative regulator of bone resorption. A key regulator of osteoclast development.
Plasminogen Activator Inhibitor 1 (PAI-1)	SERPINE1	Member of the serine proteinase inhibitor superfamily which acts as an inhibitor of fibrinolysis.
Stem Cell Factor (SCF)	KITLG	A pleotropic factor that appears to act in cell migration and is a requirement in hematopoesis.
T-Cell-Specific Protein RANTES (RANTES)	CCL5	A chemokine involved in the immunoregulatory and inflammatory process. Functions as a chemoattractant for blood monocytes, memory T helper cells and eosinophils.
Tissue Inhibitor of Metalloproteinases 1 (TIMP-1)	TIMP1	Natural inhibitors of MMPs, but can also promote cell proliferation and may have anti-apoptotic functions.
Tumor Necrosis Factor Receptor I (TNFRI)	TNFRSF1A	A receptor found in both membrane and soluble forms that interacts with TNF-alpha. Plays a role in cell survival, apoptosis and inflammation.
Tumor necrosis factor receptor 2 (TNFR2)	TNFRSF1B	Forms a heterocomplex with TNF receptor 1 that mediates the recruitment of two anti-apoptotic proteins that possess E3 ligase activity.
Vascular Cell Adhesion Molecule-1 (VCAM-1)	VCAM1	A type I membrane protein that mediates leukocyte-endothelial cell adhesion and signal transduction.
Vascular Endothelial Growth Factor (VEGF)	VEGFA	Induces proliferation and migration of vascular endothelial cells and is essential for angiogenesis.
Vitamin D-Binding Protein (VDBP)	GC	A multifunctional protein found in the plasma, ascitic fluid, cerebrospinal fluid and on the surfaces of many cells. It binds to vitamin D.
Erythropoietin (EPO)	EPO	Binds to the erythropoietin receptor to promote red blood cell production in the bone marrow. Expression is upregulated under hypoxic conditions.
High-density lipoprotein (HDL)	APOA1	Encodes the major component of HDL in the plasma which promotes cholesterol efflux from tissues to the liver for excretion.
Low-density lipoprotein (LDL)	APOE	Essential for the normal catabolism of triglyceride-rich lipoprotein constituents.
C-reactive protein (CRP)	CRP	Able to recognize foreign pathogens and damaged cells of the host and initiate initiation of host defense.
Interferon-γ (IFN-γ)	IFNG	A protein secreted by innate and adaptive immune cells that triggers a cellular response to viral and microbial infections.
Interleukin-6 (IL-6)	IL6	Functions in the inflammation and maturation of B cells. Primarily produced at sites of acute and chronic inflammation.
Interleukin-8 (IL-8)	CXCL8	Secreted by mononuclear macrophages, neutrophils, eosinophils, T lymphocytes, epithelial cells and

		fibroblasts. Functions as a chemotactic factor for neutrophils. Bacterial and viral products induce expression of IL8.
Interferon γ–Inducible Protein-10 (IP-10)	CXCL10	Encodes a chemokine. Binds to CXCR3 receptor that results in stimulation of monocytes, NK and T cell migration and modulation of adhesion molecule expression.
Interferon γ -Inducible T cell α Chemoattractant (ITAC)	CXCL11	A chemokine. Induces a chemotactic response in activated T cells and is the dominant ligand for CXCR3 receptor.
Leptin	LEP	Secreted by white adipocytes into circulation and plays a role in regulating energy homeostasis. Also involved in regulation of immune and inflammatory responses.
Osteocalcin (OCL)	BGLAP	Encodes a highly abundant bone protein secreted by osteoblasts which regulates bone remodeling. Serum osteocalcin levels may negatively correlate to metabolic syndrome.
Osteonectin (ONN)	SPARC	Encodes a matrix-associated protein that is required for the collagen in bone to become calcified.
Osteopontin (OPN)	SPP1	Involves in the attachment of osteoclasts to the mineralized bone matrix. Also acts as a cytokine that upregulates the expression of IFNG and IL12.
Serum Amyloid A (SAA)	SAA1	A major acute phase protein that is highly expressed in response to inflammation and tissue injury. Plays an important role in HDL and cholesterol metabolism and homeostasis, respectively.

Supplementary Table 2: Top 20 pathways in Cluster 1

Term	P-value	Adjusted P-value	Odds Ratio	Combined Score	Genes
Oncostatin M	2.18E-13	3.30E-10	38.585209	1124.859446	CRP;ANGPT2;CCL5;ADIPOQ;IGFBP2;SERPINE1;HP;TIMP1;ICAM1
RAGE pathway	2.76E-12	2.09E-09	133.3333333	3548.682453	ANGPT2;TGFB1;ADIPOQ;SERPINE1;TIMP1;ICAM1
Vitamin B12 metabolism	5.43E-08	2.73E-05	102.5641026	1715.805366	CRP;CCL5;SERPINE1;ICAM1
Response to elevated platelet cytosolic calcium	3.63E-07	1.37E-04	64.25702811	952.7785806	TGFB1;SPARC;SERPINE1;TIMP1
Interleukin-11 pathway	5.99E-07	1.81E-04	173.9130435	2491.836273	TGFB1;TIMP1;ICAM1
TWEAK regulation of gene expression	9.87E-07	2.49E-04	148.1481481	2048.607828	CCL5;SERPINE1;ICAM1
Selenium pathway	1.14E-05	0.002455403	66.66666667	758.894624	CRP;SERPINE1;ICAM1
Folate metabolism	1.32E-05	0.002489863	63.49206349	713.393721	CRP;SERPINE1;ICAM1
Interleukin-6 regulation of target genes	4.75E-05	0.005980516	190.4761905	1896.039371	TIMP1;ICAM1
Urokinase-type plasminogen activator (uPA) and uPAR-mediated signaling	4.44E-04	0.037269056	63.49206349	490.1006395	TGFB1;SERPINE1
Influenza factor interactions with host	0.004492068	0.242250831	222.2222222	1201.209337	TGFB1
Eosinophils in the chemokine network of allergy	0.005985243	0.282428646	166.6666667	853.0763944	CCL5
Visceral fat deposits and the metabolic syndrome	0.005985243	0.291539248	166.6666667	853.0763944	ADIPOQ
Transcription factor regulation of microRNAs related to cardiac hypertrophy	0.005985243	0.301257223	166.6666667	853.0763944	TGFB1
Inhibition of matrix	0.00673104	0.30799637	148.1481481	740.892540	TIMP1

metalloproteinases	6			8	
Dissolution of fibrin clot	0.005985243	0.311645403	166.6666667	853.0763944	SERPINE1
B lymphocyte cell surface molecules	0.008221088	0.335509272	121.2121212	581.9457822	ICAM1
Monocyte and its surface molecules	0.008221088	0.344828974	121.2121212	581.9457822	ICAM1
CTL mediated immune response against target cells	0.009709044	0.349063256	102.5641026	475.353583	ICAM1
TGF-beta signaling in gastrointestinal stem cells	0.008221088	0.35468123	121.2121212	581.9457822	TGFB1

Supplementary Table 3: Top 20 pathways in Cluster 2

Term	P-value	Adjusted P-value	Odds Ratio	Combined Score	Genes
Cytokine-cytokine receptor interaction	5.61E-15	8.47E-12	41.92872117	1375.873735	CXCL10;IL6;KITLG;EPO;IL23A;IL2RA;LEP;TNFRSF11B;TNFRSF1B;TNFRSF1A
Interleukin-1 regulation of extracellular matrix	7.20E-10	5.43E-07	55.55555556	1169.565723	C3;IL1RN;IL6;MMP3;TNFRSF11B;ICAM1
Thymic stromal lymphopoietin (TSLP) pathway	1.35E-08	6.79E-06	61.72839506	1118.604074	CXCL10;IL6;IL2RA;TNFRSF11B;ICAM1
Leptin influence on immune response	3.72E-08	1.40E-05	50.50505051	864.0427745	CRP;IL1RN;IL6;IL2RA;TNFRSF11B
Erythrocyte differentiation pathway	2.77E-07	5.97E-05	222.2222222	3355.71187	IL6;KITLG;EPO
Cells and molecules involved in local acute inflammatory response	4.13E-07	7.79E-05	196.0784314	2882.35841	C3;IL6;ICAM1
Hematopoietic cell lineage	1.02E-06	1.40E-04	50.50505051	696.6882003	IL6;KITLG;EPO;IL2RA
TWEAK regulation of gene expression	1.77E-06	2.22E-04	123.4567901	1635.392515	CXCL10;IL6;ICAM1
Inflammatory response pathway	2.45E-06	2.84E-04	111.1111111	1435.608352	IL2RA;TNFRSF1B;TNFRSF1A
Vitamin B12 metabolism	1.32E-05	0.001241833	64.1025641	720.4129978	CRP;IL6;ICAM1
SODD/TNFR1 signaling pathway	5.02E-05	0.003297152	185.1851852	1833.160517	TNFRSF1B;TNFRSF1A
CTL mediated immune response against target	5.93E-05	0.003732289	170.9401709	1663.682827	B2M;ICAM1

cells					
Interleukin-17 signaling pathway	7.98E-05	0.004632824	148.1481481	1397.978819	IL6;KITLG
Hematopoiesis regulation by cytokines	7.98E-05	0.004818137	148.1481481	1397.978819	IL6;EPO
Acetylation and deacetylation of RelA in the nucleus	9.11E-05	0.005095845	138.8888889	1292.133026	TNFRSF1B;TNFRSF1A
Transcriptional activity regulation by PML	1.16E-04	0.005841261	123.4567901	1118.700756	TNFRSF1B;TNFRSF1A
Modulation of interferon signaling by chaperones	1.91E-04	0.008757636	96.61835749	827.1677915	TNFRSF1B;TNFRSF1A
NF-kappaB signaling pathway	2.09E-04	0.009267855	92.59259259	784.6951641	TNFRSF1B;TNFRSF1A
Low-density lipoprotein (LDL) pathway during atherogenesis	0.005388466	0.12915212	185.1851852	967.313805	IL6
Activation of C3 and C5	0.005388466	0.131235219	185.1851852	967.313805	C3

Supplementary Table 4: Top 20 pathways in Cluster 3

Term	P-value	Adjusted P-value	Odds Ratio	Combined Score	Genes
Oncostatin M	4.95E-13	3.74E-10	46.76995031	1325.181907	CRP;CCL5;SERPINE1;HP;CCL2;TIMP1;ICAM1;VEGFA
RAGE pathway	2.57E-13	3.89E-10	181.8181818	5270.643369	TGFB1;SERPINE1;CCL2;TIMP1;ICAM1;VEGFA
Vitamin B12 metabolism	4.45E-11	2.24E-08	174.8251748	4167.009539	CRP;CCL5;SERPINE1;CCL2;ICAM1
Response to elevated platelet cytosolic calcium	4.93E-10	1.86E-07	109.5290252	2347.167916	TGFB1;SPARC;SERPINE1;TIMP1;VEGFA
TWEAK regulation of gene expression	8.63E-10	2.61E-07	269.3602694	5621.642225	CCL5;SERPINE1;CCL2;ICAM1
Leptin influence on immune response	2.07E-09	5.20E-07	82.6446281	1652.697978	CRP;CCL5;CCL2;TIMP1;VEGFA
Selenium pathway	2.38E-08	5.13E-06	121.2121212	2127.882057	CRP;SERPINE1;CCL2;ICAM1
Folate metabolism	2.90E-08	5.47E-06	115.4401154	2003.550262	CRP;SERPINE1;CCL2;ICAM1
Interleukin-11 pathway	2.18E-07	3.29E-05	237.1541502	3637.807935	TGFB1;TIMP1;ICAM1
Malaria	2.54E-06	3.20E-04	106.9518717	1377.881166	TGFB1;CCL2;ICAM1
Interleukin-6 regulation of target genes	2.49E-05	0.001882589	259.7402597	2753.049316	TIMP1;ICAM1
Fibroblast growth factor 1	8.87E-05	0.005825605	139.8601399	1304.875962	SERPINE1;VEGFA
Neurophilin interactions with VEGF and VEGF receptor	0.002747205	0.094279093	363.6363636	2144.425861	VEGFA
Kit receptor transcriptional targets	0.002747205	0.09647163	363.6363636	2144.425861	VEGFA
MSP/RON receptor signaling pathway	0.003295826	0.103681183	303.0303033	1731.848048	CCL2
Low-density lipoprotein (LDL) pathway during atherogenesis	0.003295826	0.105887165	303.0303033	1731.848048	CCL2
Influenza factor interactions with host	0.003295826	0.10818906	303.0303033	1731.84804	TGFB1

				8	
Eosinophils in the chemokine network of allergy	0.004392244	0.130044883	227.2727273	1233.61703	CCL5
Transcription factor regulation of microRNAs related to cardiac hypertrophy	0.004392244	0.132645781	227.2727273	1233.61703	TGFB1
Dissolution of fibrin clot	0.004392244	0.135352838	227.2727273	1233.61703	SERPINE1

Supplementary Table 5: Top 20 pathways in Cluster 4

Term	P-value	Adjusted P-value	Odds Ratio	Combined Score	Genes
Oncostatin M	6.76E-14	1.02E-10	27.20751917	825.0626424	CRP;FGB;IL6;ANGPT2;ADIPOQ;IGFBP2;MMP3;HP;TIMP1;B2M;ICAM1
RAGE pathway	9.58E-13	7.23E-10	89.74358974	2483.577873	IL6;ANGPT2;VCAM1;ADIPOQ;TNFRSF11B;TIMP1;ICAM1
TNF-alpha effects on cytokine activity, cell motility, and apoptosis	3.23E-10	1.62E-07	39.88603989	871.7021773	CXCL10;IL6;CXCL11;VCAM1;IL2RA;IGFBP2;ICAM1
Cells and molecules involved in local acute inflammatory response	5.28E-09	1.59E-06	180.9954751	3449.754807	C3;IL6;VCAM1;ICAM1
TWEAK regulation of gene expression	3.86E-08	9.71E-06	113.960114	1945.38053	CXCL10;IL6;VCAM1;ICAM1
Integrin beta-2 pathway	5.21E-08	1.12E-05	106.1007958	1779.299731	C3;FGB;VCAM1;ICAM1
Thymic stromal lymphopoietin (TSLP) pathway	1.01E-07	1.90E-05	42.73504274	688.5228567	CXCL10;IL6;IL2RA;TNFRSF11B;ICAM1
Leptin influence on immune response	2.75E-07	4.62E-05	34.96503497	528.1388796	CRP;IL6;IL2RA;TNFRSF11B;TIMP1
Interleukin-6 regulation of target genes	7.03E-07	9.65E-05	164.8351648	2335.338597	SERPINA1;TIMP1;ICAM1
Inflammatory response pathway	7.74E-06	9.73E-04	76.92307692	905.362349	IL2RA;TNFRSF1B;TNFRSF1A
SODD/TNFR1 signaling pathway	1.06E-04	0.006426335	128.2051282	1172.864094	TNFRSF1B;TNFRSF1A
CTL mediated immune response against target cells	1.26E-04	0.007026572	118.3431953	1062.96856	B2M;ICAM1
Adhesion and diapedesis of lymphocytes	1.46E-04	0.007898584	109.8901099	970.1903532	VCAM1;ICAM1
Erythrocyte differentiation pathway	1.69E-04	0.007968166	102.5641026	890.9158971	IL6;KITLG
Interleukin-17 signaling pathway	1.69E-04	0.008225203	102.5641026	890.9158971	IL6;KITLG
Acetylation and deacetylation of RelA in the nucleus	1.93E-04	0.008563958	96.15384615	822.4708919	TNFRSF1B;TNFRSF1A
Transcriptional activity regulation by PML	2.45E-04	0.010017702	85.47008547	710.4571392	TNFRSF1B;TNFRSF1A
Modulation of interferon signaling by chaperones	4.04E-04	0.014196982	66.88963211	522.6348271	TNFRSF1B;TNFRSF1A
Low-density lipoprotein (LDL) pathway during atherogenesis	0.007775574	0.152482038	128.2051282	622.6625614	IL6
Activation of C3 and C5	0.007775574	0.154488381	128.2051282	622.6625614	C3

Supplementary Table 6: Top 20 GO terms-Biological Processes in *Cluster 1*

Term ID	term description	false discovery rate	matching proteins in your network
GO:0009605	response to external stimulus	8.19E-09	ADIPOQ,ANGPT2,BDNF,CCL24,CCL5,CRP,HP,ICAM1,IGFBP2,SAA1,SERPINE1,SPARC,TGFB1
GO:0070887	cellular response to chemical stimulus	4.19E-07	ADIPOQ,ANGPT2,BDNF,CCL24,CCL5,HP,ICAM1,IGFBP2,SAA1,SERPINE1,SPARC,TGFB1,TIMP1
GO:0006954	inflammatory response	4.68E-07	CCL24,CCL5,CRP,HP,ICAM1,SAA1,TGFB1,TIMP1
GO:0030334	regulation of cell migration	4.68E-07	ADIPOQ,ANGPT2,CCL24,CCL5,ICAM1,SERPINE1,SPARC,TGFB1,TIMP1
GO:0071310	cellular response to organic substance	5.14E-07	ADIPOQ,ANGPT2,BDNF,CCL24,CCL5,ICAM1,IGFBP2,SAA1,SERPINE1,SPARC,TGFB1,TIMP1
GO:0009617	response to bacterium	6.06E-07	ADIPOQ,CCL5,CRP,HP,ICAM1,SERPINE1,SPARC,TGFB1
GO:0010469	regulation of signaling receptor activity	6.89E-07	ADIPOQ,BDNF,CCL24,CCL5,SAA1,SERPINE1,TGFB1,TIMP1
GO:0048518	positive regulation of biological process	7.91E-07	ADIPOQ,ANGPT2,BDNF,CCL24,CCL5,CRP,HP,ICAM1,IGFBP2,IST1,SAA1,SERPINE1,SPARC,TGFB1,TIMP1
GO:0030155	regulation of cell adhesion	8.66E-07	ADIPOQ,ANGPT2,CCL5,ICAM1,IGFBP2,SAA1,SERPINE1,TGFB1
GO:0022603	regulation of anatomical structure morphogenesis	9.02E-07	ADIPOQ,ANGPT2,BDNF,CCL24,ICAM1,IST1,SERPINE1,SPARC,TGFB1
GO:0032879	regulation of localization	9.02E-07	ADIPOQ,ANGPT2,BDNF,CCL24,CCL5,CRP,ICAM1,SAA1,SERPINE1,SPARC,TGFB1,TIMP1
GO:1901700	response to oxygen-containing compound	1.11E-06	ADIPOQ,ANGPT2,CCL5,HP,ICAM1,IGFBP2,SERPINE1,SPARC,TGFB1,TIMP1
GO:0051239	regulation of multicellular organismal process	2.31E-06	ADIPOQ,ANGPT2,BDNF,CCL24,CRP,ICAM1,IST1,SAA1,SERPINE1,SPARC,TGFB1,TIMP1
GO:0051240	positive regulation of multicellular organismal process	2.31E-06	ADIPOQ,ANGPT2,BDNF,CCL24,ICAM1,IST1,SAA1,SERPINE1,SPARC,TGFB1
GO:0002687	positive regulation of leukocyte migration	3.41E-06	CCL24,CCL5,ICAM1,SERPINE1,TGFB1
GO:0051704	multi-organism process	3.41E-06	ADIPOQ,ANGPT2,CCL5,CRP,HP,ICAM1,IGFBP2,IST1,SERPINE1,SPARC,TGFB1
GO:0048522	positive regulation of cellular process	3.74E-06	ADIPOQ,BDNF,CCL24,CCL5,CRP,HP,ICAM1,IGFBP2,IST1,SAA1,SERPINE1,SPARC,TGFB1,TIMP1
GO:0006952	defense response	4.42E-06	CCL24,CCL5,CRP,HP,ICAM1,SAA1,SERPINE1,TGFB1,TIMP1
GO:0034284	response to monosaccharide	4.42E-06	ADIPOQ,ANGPT2,ICAM1,SPARC,TGFB1
GO:0050900	leukocyte migration	4.42E-06	ANGPT2,CCL24,CCL5,ICAM1,SAA1,TGFB1

Supplementary Table 7: Top 20 GO terms-Biological Processes in *Cluster 2*

Term ID	term description	false discovery rate	matching proteins in your network
GO:0006954	inflammatory response	1.62E-16	C3,CRP,CXCL10,EPO,HP,ICAM1,IL1RN,IL23A,IL2RA,IL6,SAA1,TNFRSF11B,TNFRSF1A,TNFRSF1B
GO:0019221	cytokine-mediated signaling pathway	5.40E-15	B2M,CXCL10,EPO,ICAM1,IL1RN,IL23A,IL2RA,IL6,LEP,MMP3,SAA1,TNFRSF11B,TNFRSF1A,TNFRSF1B
GO:0006952	defense response	3.82E-13	B2M,C3,CRP,CXCL10,EPO,HP,ICAM1,IL1RN,IL23A,IL2RA,IL6,SAA1,TNFRSF11B,TNFRSF1A,TNFRSF1B
GO:0002376	immune system process	1.14E-12	B2M,C3,CRP,CXCL10,EPO,HP,ICAM1,IL1RN,IL23A,IL2RA,IL6,KITLG,LEP,SAA1,TNFRSF11B,TNFRSF1A,TNFRSF1B
GO:0009617	response to bacterium	1.14E-12	B2M,C3,CRP,CXCL10,EPO,HP,ICAM1,IL23A,IL6,TNFRSF11B,TNFRSF1A,TNFRSF1B
GO:0002526	acute inflammatory response	8.65E-11	CRP,EPO,HP,ICAM1,IL1RN,IL6,SAA1
GO:0006950	response to stress	1.30E-10	B2M,C3,CRP,CXCL10,EPO,HP,ICAM1,IL1RN,IL23A,IL2RA,IL6,LEP,MMP3,SAA1,TNFRSF11B,TNFRSF1A,TNFRSF1B
GO:0006953	acute-phase response	4.98E-10	CRP,EPO,HP,IL1RN,IL6,SAA1
GO:0009605	response to external stimulus	1.18E-09	B2M,C3,CRP,CXCL10,EPO,HP,ICAM1,IL23A,IL6,LEP,SAA1,TNFRSF11B,TNFRSF1A,TNFRSF1B
GO:0006955	immune response	3.34E-09	B2M,C3,CXCL10,HP,ICAM1,IL1RN,IL23A,IL2RA,IL6,SAA1,TNFRSF11B,TNFRSF1A,TNFRSF1B
GO:0070887	cellular response to chemical stimulus	5.84E-09	B2M,CXCL10,EPO,HP,ICAM1,IL1RN,IL23A,IL2RA,IL6,LEP,MMP3,SAA1,TNFRSF11B,TNFRSF1A,TNFRSF1B
GO:0051239	regulation of multicellular organismal process	9.61E-09	B2M,C3,CRP,CXCL10,EPO,ICAM1,IL1RN,IL23A,IL2RA,IL6,LEP,SAA1,TNFRSF11B,TNFRSF1A,TNFRSF1B
GO:0032496	response to lipopolysaccharide	9.68E-09	B2M,CXCL10,EPO,ICAM1,IL6,TNFRSF11B,TNFRSF1A,TNFRSF1B
GO:0050727	regulation of inflammatory response	2.32E-08	C3,IL23A,IL2RA,IL6,LEP,SAA1,TNFRSF1A,TNFRSF1B
GO:0050793	regulation of developmental process	2.51E-08	B2M,C3,CRP,CXCL10,EPO,ICAM1,IL1RN,IL23A,IL2RA,IL6,LEP,TNFRSF11B,TNFRSF1A,TNFRSF1B
GO:0033993	response to lipid	3.88E-08	B2M,CXCL10,EPO,ICAM1,IL1RN,IL6,LEP,TNFRSF11B,TNFRSF1A,TNFRSF1B
GO:0048583	regulation of response to stimulus	3.88E-08	B2M,C3,CXCL10,EPO,ICAM1,IL1RN,IL23A,IL2RA,IL6,KITLG,LEP,MMP3,SAA1,TNFRSF11B,TNFRSF1A,TNFRSF1B
GO:0010469	regulation of signaling receptor activity	4.13E-08	CXCL10,EPO,IL1RN,IL23A,IL6,KITLG,LEP,SAA1,TNFRSF11B
GO:0048519	negative regulation of biological	5.80E-08	B2M,C3,CRP,CXCL10,EPO,HP,ICAM1,IL1RN,IL23A,IL2RA,IL6,LEP,MMP3,SAA1,TNFRSF11

	process		B,TNFRSF1A,TNFRSF1B
GO:0065009	regulation of molecular function	7.77E-08	B2M,C3,CXCL10,EPO,HP,ICAM1,IL1RN,IL23A,IL2RA,IL6,KITLG,LEP,SAA1,TNFRSF11B,TNFRSF1B

Supplementary Table 8: Top 20 GO terms-Biological Processes in *Cluster 3*

Term ID	term description	false discovery rate	matching proteins in your network
GO:0009617	response to bacterium	1.20E-07	CCL2,CCL5,CRP,HP,ICAM1,SERPINE1,SPARC,TGFB1
GO:0002685	regulation of leukocyte migration	2.25E-07	CCL2,CCL5,ICAM1,SERPINE1,TGFB1,VEGFA
GO:0009605	response to external stimulus	3.58E-07	BDNF,CCL2,CCL5,CRP,HP,ICAM1,SERPINE1,SPARC,TGFB1,VEGFA
GO:0030334	regulation of cell migration	3.58E-07	CCL2,CCL5,ICAM1,SERPINE1,SPARC,TGFB1,TIMP1,VEGFA
GO:0006954	inflammatory response	4.93E-07	CCL2,CCL5,CRP,HP,ICAM1,TGFB1,TIMP1
GO:0002576	platelet degranulation	8.46E-07	SERPINE1,SPARC,TGFB1,TIMP1,VEGFA
GO:0002687	positive regulation of leukocyte migration	8.46E-07	CCL5,ICAM1,SERPINE1,TGFB1,VEGFA
GO:0010469	regulation of signaling receptor activity	8.46E-07	BDNF,CCL2,CCL5,SERPINE1,TGFB1,TIMP1,VEGFA
GO:0032496	response to lipopolysaccharide	8.46E-07	CCL2,CCL5,ICAM1,SERPINE1,SPARC,TGFB1
GO:0010941	regulation of cell death	1.01E-06	BDNF,CCL2,CCL5,HP,ICAM1,SERPINE1,TGFB1,TIMP1,VEGFA
GO:0071222	cellular response to lipopolysaccharide	1.11E-06	CCL2,CCL5,ICAM1,SERPINE1,TGFB1
GO:0032879	regulation of localization	1.17E-06	BDNF,CCL2,CCL5,CRP,ICAM1,SERPINE1,SPARC,TGFB1,TIMP1,VEGFA
GO:0070887	cellular response to chemical stimulus	1.85E-06	BDNF,CCL2,CCL5,HP,ICAM1,SERPINE1,SPARC,TGFB1,TIMP1,VEGFA
GO:0050731	positive regulation of peptidyl-tyrosine phosphorylation	2.41E-06	BDNF,CCL5,ICAM1,TGFB1,VEGFA
GO:0006952	defense response	2.61E-06	CCL2,CCL5,CRP,HP,ICAM1,SERPINE1,TGFB1,TIMP1
GO:0050920	regulation of chemotaxis	2.74E-06	CCL2,CCL5,SERPINE1,TGFB1,VEGFA
GO:0006887	exocytosis	3.04E-06	CCL5,HP,SERPINE1,SPARC,TGFB1,TIMP1,VEGFA
GO:0070374	positive regulation of ERK1 and ERK2 cascade	3.10E-06	CCL2,CCL5,ICAM1,TGFB1,VEGFA
GO:0030335	positive regulation of cell migration	4.17E-06	CCL5,ICAM1,SERPINE1,SPARC,TGFB1,VEGFA
GO:0048523	negative regulation of cellular process	5.04E-06	BDNF,CCL2,CCL5,CRP,HP,ICAM1,SERPINE1,SPARC,TGFB1,TIMP1,VEGFA

Supplementary Table 9: Top 20 GO terms-Biological Processes in *Cluster 4*

Term ID	term description	false discovery rate	matching proteins in your network
GO:0006952	defense response	9.12E-15	B2M,C3,CRP,CXCL10,CXCL11,FGB,HP,ICAM1,IFNA1,IL12RB1,IL2RA,IL6,SAA1,SERPINA1,TIMP1,TNFRSF11B,TNFRSF1A,TNFRSF1B,VCAM1
GO:0006954	inflammatory response	9.12E-15	C3,CRP,CXCL10,CXCL11,HP,ICAM1,IL2RA,IL6,SAA1,SERPINA1,TIMP1,TNFRSF11B,TNFRSF1A,TNFRSF1B,VCAM1
GO:0019221	cytokine-mediated signaling pathway	2.41E-13	B2M,CXCL10,CXCL11,ICAM1,IFNA1,IL12RB1,IL2RA,IL6,MMP3,SAA1,TIMP1,TNFRSF11B,TNFRSF1A,TNFRSF1B,VCAM1
GO:0034097	response to cytokine	2.41E-13	ADIPOQ,B2M,CXCL10,CXCL11,FGB,ICAM1,IFNA1,IL12RB1,IL2RA,IL6,MMP3,SAA1,TIMP1,TNFRSF11B,TNFRSF1A,TNFRSF1B,VCAM1
GO:0002376	immune system process	5.89E-13	ANGPT2,B2M,C3,CRP,CXCL10,CXCL11,FGB,HP,ICAM1,IFNA1,IL12RB1,IL2RA,IL6,IST1,KITLG,SAA1,SERPINA1,TNFRSF11B,TNFRSF1A,TNFRSF1B,VCAM1
GO:0009617	response to bacterium	5.89E-13	ADIPOQ,B2M,C3,CRP,CXCL10,CXCL11,FGB,HP,ICAM1,IL6,TNFRSF11B,TNFRSF1A,TNFRSF1B,VCAM1
GO:0071345	cellular response to cytokine stimulus	8.77E-13	B2M,CXCL10,CXCL11,FGB,ICAM1,IFNA1,IL12RB1,IL2RA,IL6,MMP3,SAA1,TIMP1,TNFRSF11B,TNFRSF1A,TNFRSF1B,VCAM1
GO:0009605	response to external stimulus	2.78E-12	ADIPOQ,ANGPT2,B2M,C3,CRP,CXCL10,CXCL11,FGB,HP,ICAM1,IFNA1,IGFBP2,IL6,OPHN1,SAA1,TNFRSF11B,TNFRSF1A,TNFRSF1B,VCAM1
GO:0006955	immune response	2.79E-12	B2M,C3,CXCL10,CXCL11,FGB,HP,ICAM1,IFNA1,IL12RB1,IL2RA,IL6,IST1,SAA1,SERPINA1,TNFRSF11B,TNFRSF1A,TNFRSF1B,VCAM1
GO:0051707	response to other organism	2.79E-12	ADIPOQ,B2M,C3,CRP,CXCL10,CXCL11,FGB,HP,ICAM1,IFNA1,IL6,TNFRSF11B,TNFRSF1A,TNFRSF1B,VCAM1
GO:0006950	response to stress	7.81E-12	ADIPOQ,ANGPT2,B2M,C3,CRP,CXCL10,CXCL11,FGB,HP,ICAM1,IFNA1,IL12RB1,IL2RA,IL6,MMP3,SAA1,SERPINA1,TIMP1,TNFRSF11B,TNFRSF1A,TNFRSF1B,VCAM1
GO:0071310	cellular response to organic substance	4.02E-11	ADIPOQ,ANGPT2,B2M,CXCL10,CXCL11,FGB,ICAM1,IFNA1,IGFBP2,IL12RB1,IL2RA,IL6,MMP3,SAA1,TIMP1,TNFRSF11B,TNFRSF1A,TNFRSF1B,VCAM1
GO:0070887	cellular response to chemical stimulus	5.84E-11	ADIPOQ,ANGPT2,B2M,CXCL10,CXCL11,FGB,HP,ICAM1,IFNA1,IGFBP2,IL12RB1,IL2RA,IL6,MMP3,SAA1,TIMP1,TNFRSF11B,TNFRSF1A,TNFRSF1B,VCAM1
GO:0048583	regulation of response to stimulus	2.41E-10	ADIPOQ,ANGPT2,B2M,C3,CXCL10,CXCL11,FGB,ICAM1,IFNA1,IGFBP2,IL12RB1,IL2RA,IL6,KITLG,MMP3,OPHN1,SAA1,TIMP1,TNFRSF11B,TNFRSF1A,TNFRSF1B,VCAM1
GO:0001775	cell activation	6.29E-10	B2M,C3,CXCL10,FGB,HP,ICAM1,IFNA1,IL6,IST1,SAA1,SERPINA1,TIMP1,TNFRSF1B,VCAM1

GO:0051704	multi-organism process	6.29E-10	ADIPOQ,ANGPT2,B2M,C3,CRP,CXCL10,CXCL11,FGB,HP,ICAM1,IFNA1,IGFBP2,IL6,IST1,TNFRSF11B,TNFRSF1A,TNFRSF1B,VCAM1
GO:0002526	acute inflammatory response	7.63E-10	CRP,HP,ICAM1,IL6,SAA1,SERPINA1,VCAM1
GO:1901700	response to oxygen-containing compound	2.58E-09	ADIPOQ,ANGPT2,B2M,CXCL10,CXCL11,HP,ICAM1,IGFBP2,IL6,MMP3,TIMP1,TNFRSF11B,TNFRSF1A,TNFRSF1B,VCAM1
GO:0050896	response to stimulus	3.38E-09	ADIPOQ,ANGPT2,B2M,C3,CRP,CXCL10,CXCL11,FGB,HP,ICAM1,IFNA1,IGFBP2,IL12RB1,IL2RA,IL6,IST1,KITLG,MMP3,OPHN1,SAA1,SERPINA1,TIMP1,TNFRSF11B,TNFRSF1A,TNFRSF1B,VCAM1
GO:0032101	regulation of response to external stimulus	3.44E-09	ADIPOQ,ANGPT2,C3,CXCL10,CXCL11,FGB,IL12RB1,IL2RA,IL6,SAA1,TNFRSF1A,TNFRSF1B