ACCSCIENCE PUBLISHING

Eurasian Journal of Medicine and Oncology

ORIGINAL RESEARCH ARTICLE

Comparative serum proteomic profiling of cardiovascular death versus coronary atherosclerosis

Supplementary File

Table S1. A summary of differential protein expression analyses for the Table 3 protein panel (except ADRM1)

Number	Protein	Raw *p*	Log_2 fold change	Cohen's *d*	FDR BH
1	Vinculin	0.037	0.54	−0.70	0.04
2	S100	0.010	0.81	−0.90	0.04
3	Neutrophil elastase	0.034	0.85	−0.82	0.04
4	Dysferlin	0.029	0.88	−0.76	0.04
5	Calmodulin	0.014	0.92	−0.91	0.04
6	CD23	0.037	0.98	−0.71	0.04
7	E2F3	0.037	1.18	−0.88	0.04
8	Laminin B1/b1	0.036	1.18	−0.97	0.04
9	TRADD	0.023	1.19	−0.93	0.04
10	FLIP	0.017	1.23	−1.03	0.04
11	DP-2	0.037	1.23	−0.89	0.04
12	caspase 6 (Mch2)	0.037	1.24	−0.75	0.04
13	G-CSF	0.031	1.25	−0.81	0.04
14	Gab1	0.012	1.56	−1.46	0.04
15	mGluR1	0.028	1.67	−0.79	0.04
Sensitivity analysis					
Threshold			Count		
$p < 0.05$			15		
FDR < 0.05			15		
FDR < 0.01			0		
FDR < 0.001			0		

Notes: The same protein was averaged in normalized units before calculation of fold changes and effect sizes. Original protein-level *p*-values were adjusted for multiple testing using the Benjamini–Hochberg FDR procedure. Raw $p < 0.05$ indicates evidence against the null hypothesis of no difference. Log_2 FC was the log_2-transformed FC, calculated as log_2 (control mean/CVD mean) using normalized pixels. Positive values indicate lower abundance in CVD cases. $log_2FC \geq 0.5$ (moderate) or ≥ 1.0 (strong) indicates biologically meaningful change between groups. Cohen's *d*–standardized effect size (Cohen's *d*) was used to classify the magnitude of the effect. Absolute values of approximately 0.2, 0.5, and 0.8 are generally considered small, medium, and large effects, respectively. Benjamini–Hochberg FDR-adjusted *p*-value controlling for multiple testing across the Table 3 protein panel excluding ADRM1. FDR < 0.05; it is generally considered statistically significant after correction for multiple testing across the Table 3 protein panel excluding ADRM1.

Abbreviations: ADRM1: Adhesion regulating molecule 1 (also known as hRpn13, a proteasomal ubiquitin receptor); BH: Benjamini–Hochberg; CVD: Cardiovascular death; CD23: Low-affinity immunoglobulin epsilon Fc receptor; DP-2; Differentiation plasmid 2 (often refers to transcription factor DP-2, a dimerization partner for E2F; also known as TFDP2); E2F3: E2F transcription factor 3; FC: Fold change; FDR: False discovery rate; FLIP: FLICE (FADD-like IL-1β-converting enzyme)-like inhibitory protein (also known as CFLAR); Gab-1: GRB2-associated-binding protein 1; G-CSF: Granulocyte colony-stimulating factor; mGluR1: Metabotropic glutamate receptor 1; TRADD: Tumor necrosis factor receptor type 1-associated death domain protein.

Table S2. Ranked biomarkers according to bootstrap for Table 3

Rank	Protein	Mean difference	95 CI (lower)	95 CI (upper)	Directional stability	Stable signature	Robustness score
1	Gab1	2.58	1.13	4.07	1.00	1	0.98
2	FLIP	3.15	0.59	5.74	1.00	1	0.89
3	Laminin B1/b1	0.85	8.71	0.99	0.85	1	0.88
4	TRADD	2.64	0.36	5.08	0.99	1	0.87
5	Calmodulin	6.34	0.80	12.19	0.99	1	0.86
6	S100	6.15	1.00	12.33	0.99	1	0.86
7	DP-2	2.53	0.45	5.05	0.99	1	0.86
8	E2F3	5.07	0.44	10.03	0.99	1	0.86
9	Neutrophil elastase	6.46	0.09	13.58	0.98	1	0.84
10	G-CSF	2.39	0.05	4.85	0.98	1	0.84
11	mGluR1	2.30	0.02	4.86	0.98	1	0.84
12	Dysferlin	2.90	−0.14	6.21	0.97	0	0.73
13	caspase 6 (Mch2)	2.49	−0.06	5.20	0.97	0	0.73
14	CD23	3.01	−0.44	6.50	0.96	0	0.71
15	Vinculin	2.76	−0.58	6.15	0.95	0	0.71

Notes: Bootstrap resampling (2,000 iterations) was performed to assess the stability of the identified protein signature. Proteins exhibiting high directional stability and confidence intervals that did not cross zero were considered robust components of the cardiovascular death-associated proteomic profile (except ADRM1). A protein would generally be considered robust if: it has directional stability > 0.90; the effect remains in the same direction in > 90% of bootstrap resamples; stable signature 1; bootstrap stability > 0.90; and the bootstrap CI does not cross zero.
Abbreviations: ADRM1: Adhesion regulating molecule 1 (also known as hRpn13, a proteasomal ubiquitin receptor); CD23: Low-affinity immunoglobulin epsilon Fc receptor; DP-2; Differentiation plasmid 2 (often refers to transcription factor DP-2, a dimerization partner for E2F; also known as TFDP2); E2F3: E2F transcription factor 3; FLIP: FLICE (FADD-like IL-1β-converting enzyme)-like inhibitory protein (also known as CFLAR); Gab1: GRB2-associated-binding protein 1; G-CSF: Granulocyte colony-stimulating factor; mGluR1: Metabotropic glutamate receptor 1; TRADD: Tumor necrosis factor receptor type 1-associated death domain protein.

Table S3. Protein rating (Cohort 2) according to false discovery rate (FDR) and fold change (FC) for Table 4

Criterion	Number of proteins retained
Total proteins with FDR ≤ 0.20	575
Proteins with FDR ≤ 0.20 and FC ≥ 1.5	473
Proteins with FDR ≤ 0.20 and FC ≥ 2.0	238
Proteins with FDR ≤ 0.20 and FC ≥ 2.5	93
Proteins with FDR ≤ 0.20 and FC ≥ 3.0	34

Table S4. A summary of differential protein expression analyses for the Table 4 protein panel (Cohort 2)

Protein	Fold change	log_2FC	Cohen's *d*	*p*-value	FDR BH
Cadherin-P	21.07	4.39	1.19	0.0008	0.0053
Ang-2	18.72	4.22	0.81	0.017	0.019
TRAP	18.4	4.20	1.15	0.0012	0.0053
IL-5	12.76	3.67	1.02	0.003	0.0061
IL-1 alpha	12.32	3.62	0.73	0.029	0.030
FGF-2	9.93	3.31	0.76	0.024	0.025
FGF-1	6.44	2.68	0.71	0.035	0.035
G-CSF	4.88	2.28	0.793	0.019	0.021
GSTmu	4.80	2.26	1.12	0.0015	0.0053
Nitric Oxide Synthase, brain (bNOS)	4.61	2.20	1.14	0.0014	0.0053
DFF45/ICAD	4.39	2.13	1.04	0.0035	0.0061
Collagen II	3.93	1.97	0.99	0.0046	0.0074
IL-6	3.91	1.96	0.89	0.0097	0.012
FHIT	3.88	1.95	1.03	0.0033	0.0061
Lck (p56lck)	3.88	1.95	1.12	0.0016	0.0053
I-Kappa-B Kinase b (IKKb)	3.74	1.90	0.99	0.0044	0.0074
TNFa	3.66	1.87	1.13	0.0015	0.0053
MMP-16/MT3-MMP	3.65	1.87	1.06	0.0025	0.0058
Caspase 1	3.60	1.84	1.05	0.0028	0.0058
Inhibin alpha	3.31	1.72	1.25	0.0012	0.0053
GM-CSF	3.25	1.70	0.84	0.014	0.017
Myoglobin	3.20	1.67	0.83	0.027	0.028
MyD88	3.16	1.66	0.93	0.0091	0.011
Granzyme B	3.14	1.65	1.14	0.0019	0.0053
Oct-3	3.14	1.65	0.94	0.0073	0.010
Cadherin-E	3.12	1.64	0.92	0.0082	0.011
GluR6/7	3.12	1.64	1.04	0.0033	0.0061
p73	3.03	1.60	0.95	0.006	0.0087
CD3zeta	3.03	1.60	1.14	0.0013	0.0053
P504S	3.02	1.59	1.40	0.0001	0.0053
SODD	3.00	1.58	0.97	0.0054	0.0081
Dysferlin	3.00	1.58	1.00	0.0049	0.0077

(*cont'd...*)

Table S4. (*Continued*)

Protein	Fold change	log_2FC	Cohen's *d*	*p*-value	FDR BH
Ferritin	2.99	1.58	0.92	0.0071	0.010
CA19-9	2.99	1.58	1.21	0.00079	0.0053
Bcl-6	2.98	1.57	0.82	0.016	0.018
GluR1	2.98	1.57	1.06	0.0027	0.0058
Cullin-3 (CUL-3)	2.93	1.55	1.13	0.0020	0.0053
Caspase 8 (FLICE)	2.92	1.55	1.11	0.0019	0.0053
HDAC1	2.91	1.54	1.17	0.0019	0.0053
Ornithine Decarboxylase	2.91	1.54	1.15	0.0015	0.0053
NF kappa B/p50	2.90	1.53	1.15	0.0016	0.0053
Bonzo/STRL33/ TYMSTR	2.89	1.53	1.29	0.0022	0.0054

Notes: See the legend for Table S1. The same protein was averaged in normalized units before calculation of fold changes and effect sizes. Original protein-level *p*-values were adjusted for multiple testing using the Benjamini–Hochberg FDR procedure (for Table 4).
Abbreviations: Ang-2: Angiopoietin-2; Bcl-6: B-cell lymphoma 6 protein; Bonzo/STRL33/TYMSTR: C-X-C motif chemokine receptor 6 (CXCR6); CA19-9: Carbohydrate antigen 19-9 (also sialyl Lewis^a; a tumor marker); CD3zeta: Cluster of differentiation 3 zeta chain (CD247, T-cell receptor zeta chain); DFF45/ICAD: DNA fragmentation factor 45/inhibitor of caspase-activated DNase; FC: Fold change; FDR BH: Benjamini–Hochberg false discovery rate; FGF-1: Fibroblast growth factor 1 (also known as aFGF); FGF-2: Fibroblast growth factor 2 (also known as bFGF); FHIT: Fragile histidine triad protein; G-CSF: Granulocyte colony-stimulating factor; GluR1: Glutamate receptor 1 (AMPA-type ionotropic receptor; gene *GRIA1*); GluR6/7: Glutamate receptor 6/7 (ionotropic kainate receptor subunits; gene names *GRIK2* and *GRIK3*); GM-CSF: Granulocyte-macrophage colony-stimulating factor; GSTmu: Glutathione S-transferase mu (class Mu.); HDAC1: Histone deacetylase 1; IL: Interleukin; Lck (p56lck): Lymphocyte-specific protein tyrosine kinase (56 kDa isoform); MMP-16/MT3-MMP: Matrix metalloproteinase 16/membrane-type 3 MMP; MyD88: Myeloid differentiation primary response 88; Oct-3: Octamer-binding transcription factor 3 (also known as POU5F1, often confused with Oct-4 in humans; in mouse, Oct-3 is Pou5f1); P504S: Protein 504S (also known as AMACR: alpha-methylacyl-CoA racemase); p73: Protein 73 (tumor suppressor, p53 family member; gene *TP73*); SODD: Silencer of death domain; TNFa: Tumor necrosis factor alpha (standard symbol: TNF-α); TRAP: TNF receptor-associated periodic syndrome protein (or in some contexts tartrate-resistant acid phosphatase; also known as ACP5).

Table S5. Supplement for Table 4 (Cohort 2)

Criterion	Number of proteins retained
Total proteins with FDR ≤ 0.20	575
Proteins with FDR ≤ 0.20 and FC ≥ 1.5	473
Proteins with FDR ≤ 0.20 and FC ≥ 2.0	238
Proteins with FDR ≤ 0.20 and FC ≥ 2.5	93
Proteins with FDR ≤ 0.20 and FC ≥ 3.0	34

Abbreviations: FC: Fold change; FDR: False discovery rate.

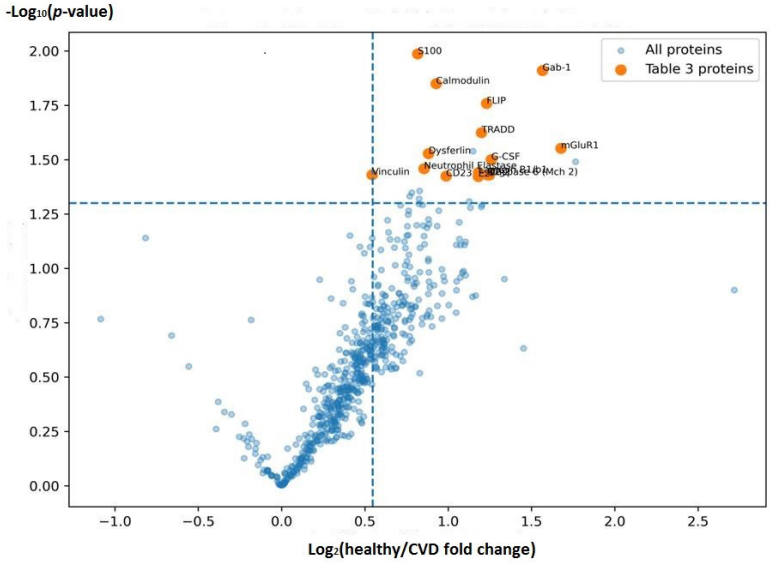

Figure S1. Volcano plot for Cohort 1 (CVD versus healthy control) for Table 3. Volcano plots combine effect size and statistical significance to identify proteins showing the strongest evidence of differential expression. All proteins from the CVD dataset are presented as background points; proteins in Table 3 are highlighted, with $p = 0.05$ as the significance threshold and fold change = 1.46 as the threshold. The volcano plot demonstrates that the proteins selected for Table 3 exhibit both biologically meaningful effect sizes and nominal statistical significance, supporting their prioritization for downstream pathway interpretation. Each point represents one protein identified in the comparative proteomic analysis of healthy controls ($n = 10$) and CVD patients ($n = 10$). The x-axis shows the $\log_2$ fold change (healthy/CVD), where positive values indicate higher abundance in healthy controls and lower abundance in CVD patients. The y-axis shows $-\log_{10}(p\text{-value})$ from the two-sample t-test; proteins located higher on the plot exhibit stronger statistical evidence for differential expression. Proteins appearing in the upper right region of the plot therefore combine large effect sizes with stronger statistical significance and represent the most prominent candidates associated with CVD. The proteins displayed correspond to those reported in Table 3.
Abbreviations: CD23: Low-affinity immunoglobulin epsilon Fc receptor; CVD: Cardiovascular death; DP-2; Differentiation plasmid 2 (often refers to transcription factor DP-2, a dimerization partner for E2F; also known as TFDP2); E2F3: E2F transcription factor 3; FLIP: FLICE (FADD-like IL-1β-converting enzyme)-like inhibitory protein (also known as CFLAR); Gab1: GRB2-associated-binding protein 1; G-CSF: Granulocyte colony-stimulating factor; mGluR1: Metabotropic glutamate receptor 1; TRADD: Tumor necrosis factor receptor type 1-associated death domain protein.

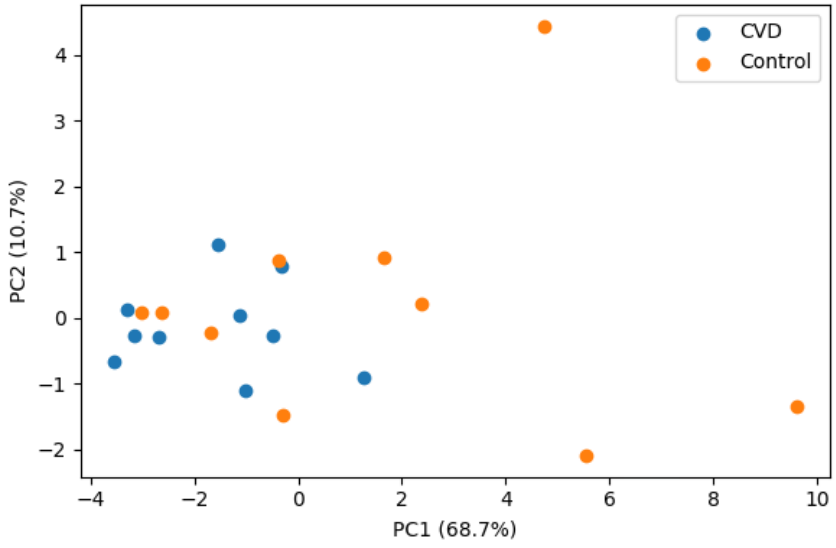

Figure S2. Principal component analysis of proteins in Table 3 assessing overall multivariate separation between the CVD and matched healthy control groups (Cohort 1)
Abbreviations: CVD: Cardiovascular death; PC: Principal component.

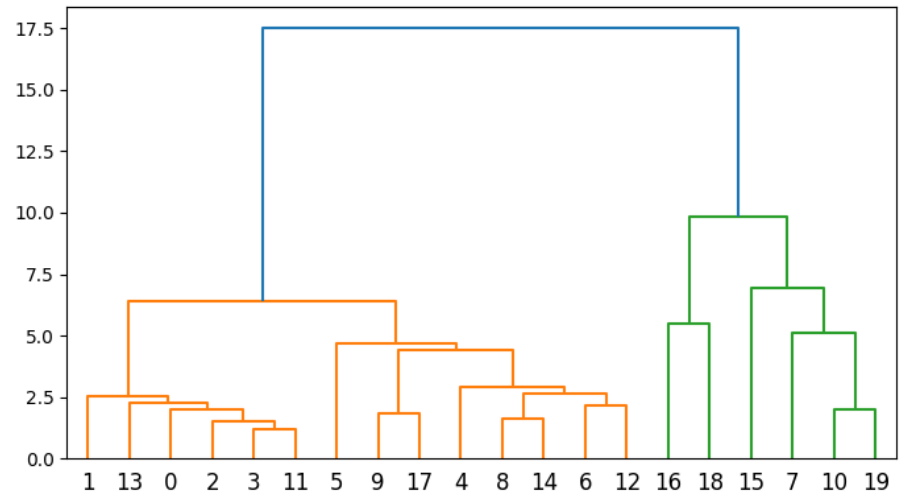

Figure S3. Hierarchical clustering of the proteins listed in Table 3 showing similarity patterns among samples in Cohort 1 (CVD versus healthy control). The higher up the horizontal line is on the Y-axis, the less similar the two merged clusters. The same evaluation is added for Table 4.
Abbreviation: CVD: Cardiovascular death.

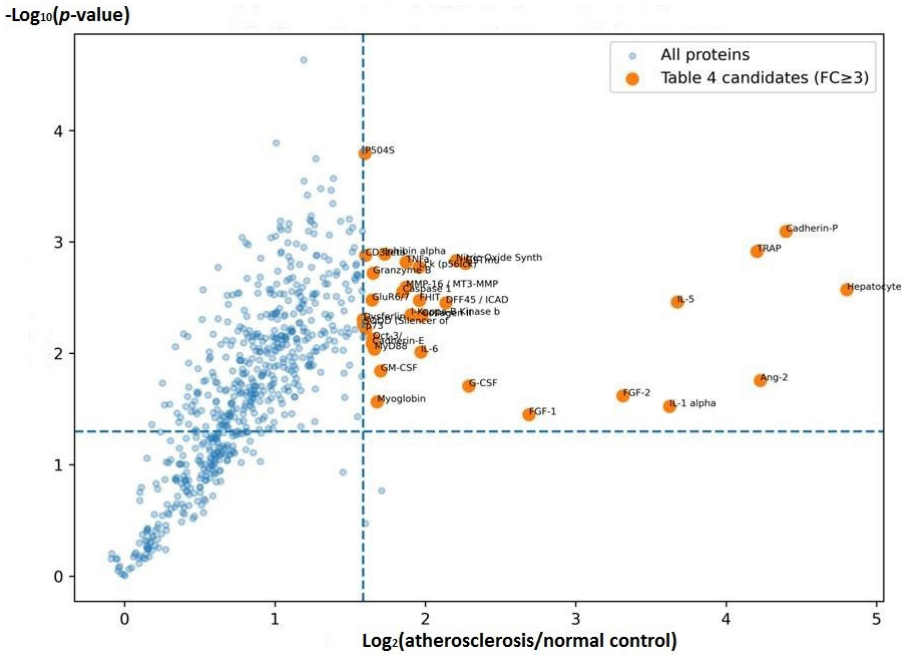

Figure S4. Volcano plot of the proteins listed in Table 4 for Cohort 2 (total dataset; severe coronary atherosclerosis versus no coronary lesions). See the legend for Figure S1.
Abbreviations: Ang-2: Angiopoietin-2; Bcl-6: B-cell lymphoma 6 protein; Bonzo/STRL33/TYMSTR: C-X-C motif chemokine receptor 6 (CXCR6); CA19-9: Carbohydrate antigen 19-9 (also sialyl Lewis[a]; a tumor marker); CD3zeta: Cluster of differentiation 3 zeta chain (CD247, T-cell receptor zeta chain); DFF45/ICAD: DNA fragmentation factor 45/inhibitor of caspase-activated DNase; FC: Fold change; FGF-1: Fibroblast growth factor 1 (also known as aFGF); FGF-2: Fibroblast growth factor 2 (also known as bFGF); FHIT: Fragile histidine triad protein; G-CSF: Granulocyte colony-stimulating factor; GluR1: Glutamate receptor 1 (AMPA-type ionotropic receptor; gene *GRIA1*); GluR6/7: Glutamate receptor 6/7 (ionotropic kainate receptor subunits; gene names *GRIK2* and *GRIK3*); GM-CSF: Granulocyte-macrophage colony-stimulating factor; GSTmu: Glutathione S-transferase mu (class Mu.); HDAC1: Histone deacetylase 1; IL: Interleukin; Lck (p56lck): Lymphocyte-specific protein tyrosine kinase (56 kDa isoform); MMP-16/MT3-MMP: Matrix metalloproteinase 16/membrane-type 3 MMP; MyD88: Myeloid differentiation primary response 88; Oct-3: Octamer-binding transcription factor 3 (also known as POU5F1, often confused with Oct-4 in humans; in mouse, Oct-3 is Pou5f1); P504S: Protein 504S (also known as AMACR: alpha-methylacyl-CoA racemase); p73: Protein 73 (tumor suppressor, p53 family member; gene *TP73*); TNFa: Tumor necrosis factor alpha (standard symbol: TNF-α); TRAP: TNF receptor-associated periodic syndrome protein (or in some contexts tartrate-resistant acid phosphatase; also known as ACP5).

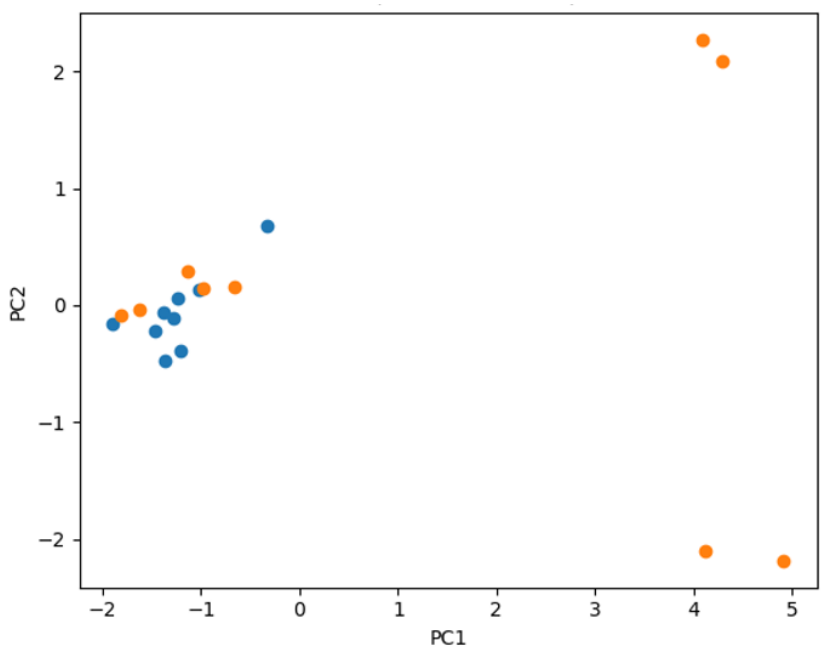

Figure S5. Principal component analysis of the proteins listed in Table 4 assessing overall multivariate separation between the severe coronary atherosclerosis and no coronary lesions groups (Cohort 2)
Abbreviation: PC: Principal component.

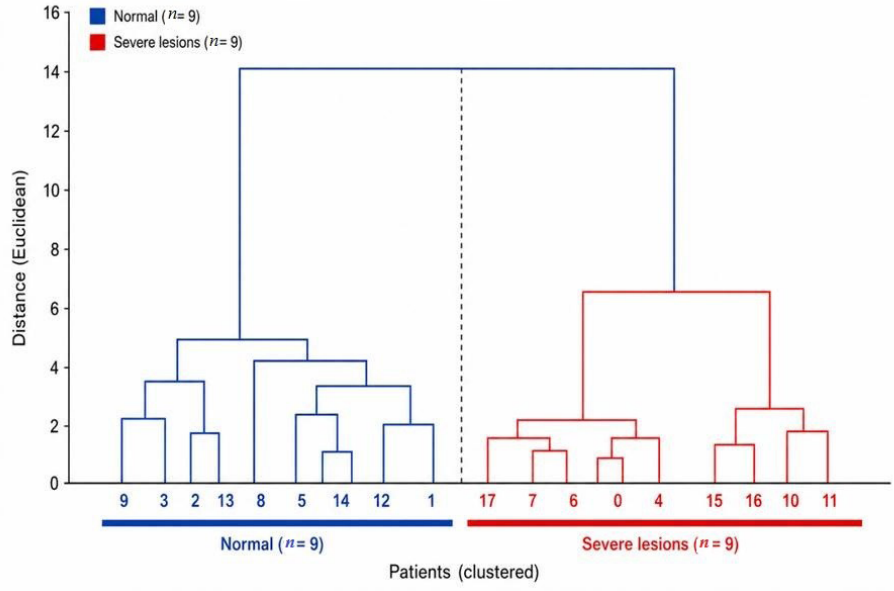

Figure S6. Hierarchical clustering of 18 patients for proteins listed in Table 4 (Cohort 2, severe coronary atherosclerosis versus no coronary lesions) for Table 4. See the legends to Figure S3.