A differential protein solubility approach for the depletion of highly abundant proteins in plasma using ammonium sulfate

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Supplementary information

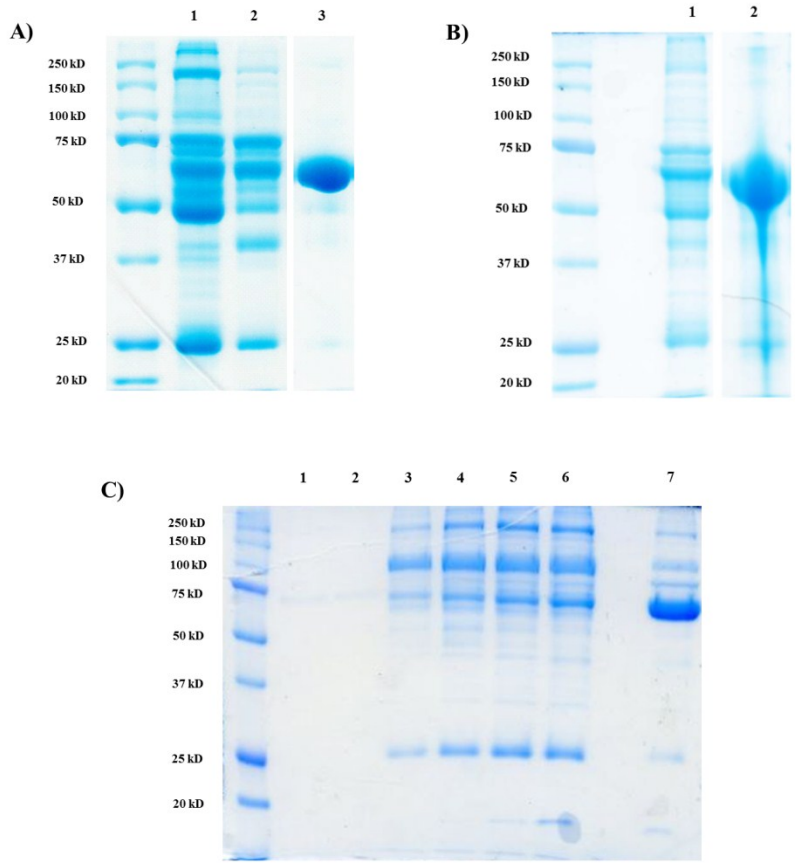

Figure S1: SDS-PAGE analysis of serum proteins precipitated with the ethanol/sodium acetate (A), TCA/acetone (B) and ammonium sulfate precipitation (C).

A) Ethanol/sodium acetate precipitation: (1) pellet obtained after 42% ethanol precipitation and (2) pellet obtained after precipitation of proteins in the supernatant with 0.8M sodium acetate (pH5.7) and (3) proteins left over in the supernatant.

B) Serum proteins are precipitated with 10% TCA/acetone (1) and (2) proteins left over in the supernatant.

C) Serum proteins are precipitated with increasing ammonium sulfate concentrations 15% (1), 25% (2), 35% (3), 40% (4), 45% (5), 50% (6) and total serum (7).

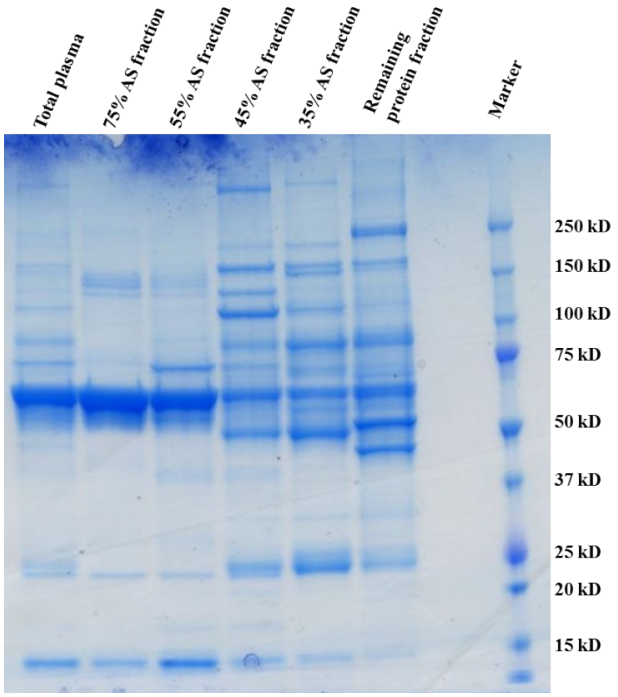

Figure S2: SDS-PAGE analysis of the protein content (10 µg) present in total plasma, 75%, 55%, 45%, 35% ammonium sulfate and remaining protein fraction. Briefly, total proteins were precipitated with 90% saturated ammonium sulfate and the precipitated proteins were then differentially extracted with different saturated concentrations of ammonium sulfate.

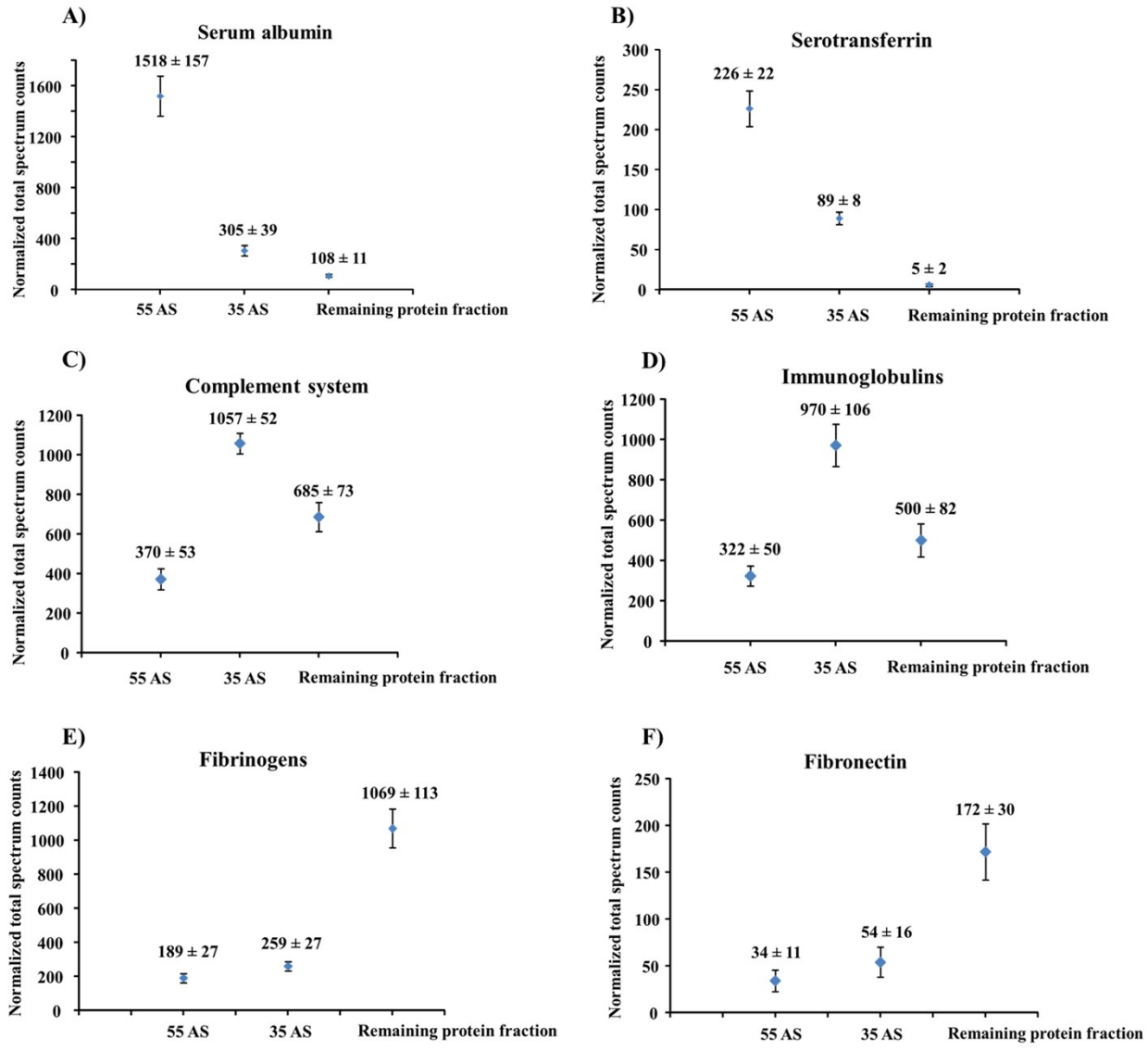

Figure S3: Total spectrum counts (Mean ± SD) across 20 plasma samples for the top two abundant proteins enriched in the 55% AS, 35% AS and remaining protein fractions. Serum albumin (A), serotransferrin (B) were consistently enriched in the 55% AS fractions, whereas complement system proteins (C) and immunoglobulins (D) were found to be consistently enriched in the 35% AS fractions. Fibrinogens (E) and fibronectin (F) were reproducibly enriched in the remaining protein fractions.

Serum albumin

A)

	SS	df	MS	F	P-value	F crit
Between Groups	71598.6	3	23866.200	0.956	0.437	3.239
Within Groups	399555.2	16	24972.2			
Total	471153.8	19				

Serotransferrin

B)

	SS	df	MS	F	P-value	F crit
Between Groups	1073.2	3	357.733	0.679	0.578	3.239
Within Groups	8430	16	526.875			
Total	9503.2	19				

Complement system

C)

	SS	df	MS	F	P-value	F crit
Between Groups	3590.95	3	1196.983	0.404	0.752	3.239
Within Groups	47410	16	2963.125			
Total	51000.95	19				

Immunoglobulins

D)

	SS	df	MS	F	P-value	F crit
Between Groups	79588.15	3	26529.383	3.212	0.051	3.239
Within Groups	132154.4	16	8259.650			
Total	211742.55	19				

Fibrinogens

E)

	SS	df	MS	F	P-value	F crit
Between Groups	172173	3	57391.133	12.949	0.0002	3.239
Within Groups	70912	16	4432.025			
Total	243086	19				

Fibronectin

F)

	SS	df	MS	F	P-value	F crit
Between Groups	3748.8	3	1249.600	1.488	0.256	3.239
Within Groups	13434	16	839.650			
Total	17183	19				

Figure S4: One-way ANOVA analysis of the spectrum counts between four categories (control (n=5), pre-diagnosis (n=5), indolent cancer (n=5) and aggressive cancer (n=5)) for the top two abundant proteins enriched in 55%, 35% AS and remaining protein fractions. ANOVA analysis was carried out for the spectrum counts observed in the 55% AS fractions for serum albumin (A), serotransferrin (B). Same kind of analysis was carried out for the spectrum counts observed in the 35% AS fractions for complement system proteins (C), immunoglobulins (D) and for spectrum counts observed in the remaining protein fractions for fibrinogens (E) and fibronectin (F).

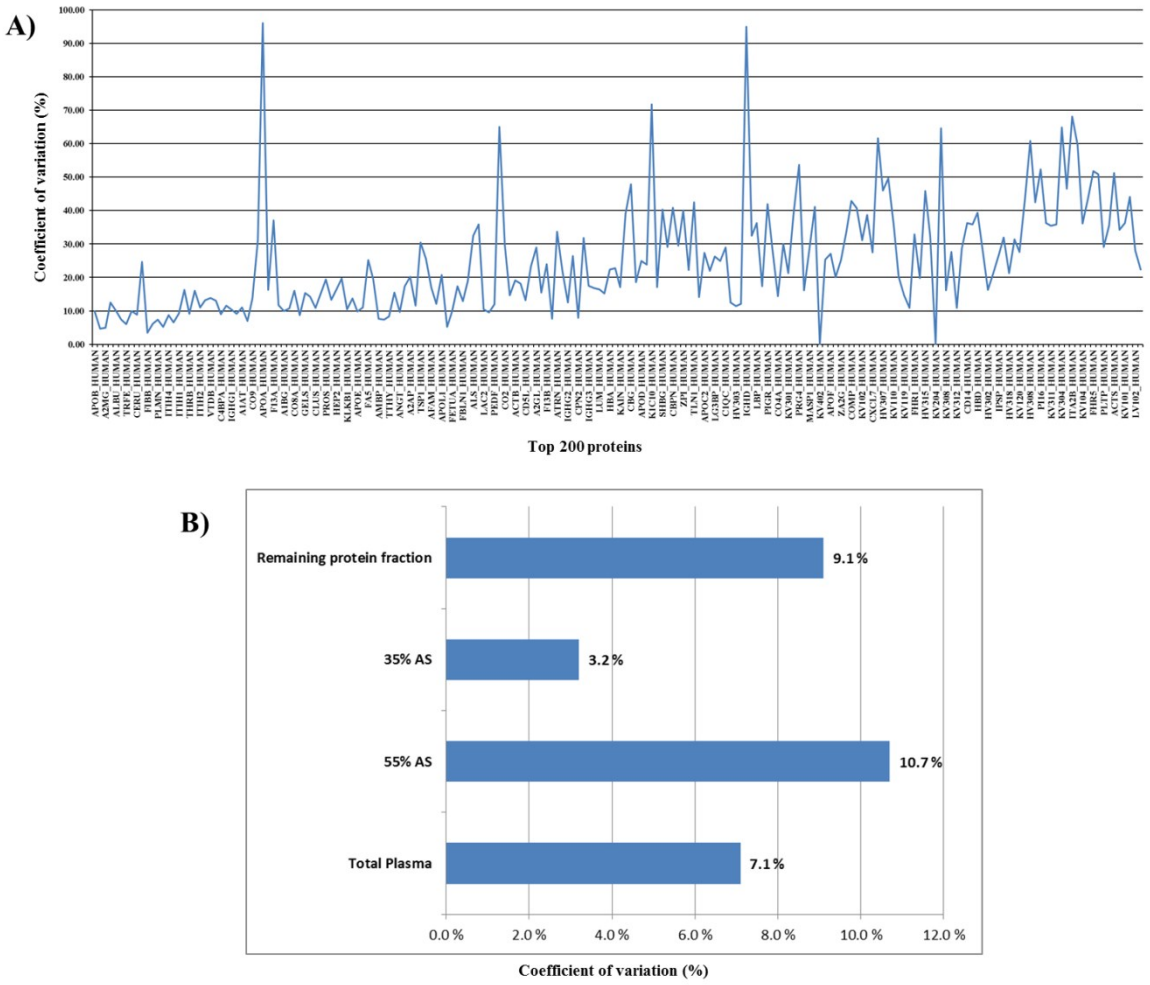

Figure S5: Line chart and bar graph displaying the coefficient of variation (CV) in the total number of unique peptides identified across 20 plasma samples for the top 200 proteins (A) and CV values for the total number of proteins identified in total plasma, 55%, 35% ammonium sulfate (AS) and remaining protein fractions from 20 different plasma samples (B).

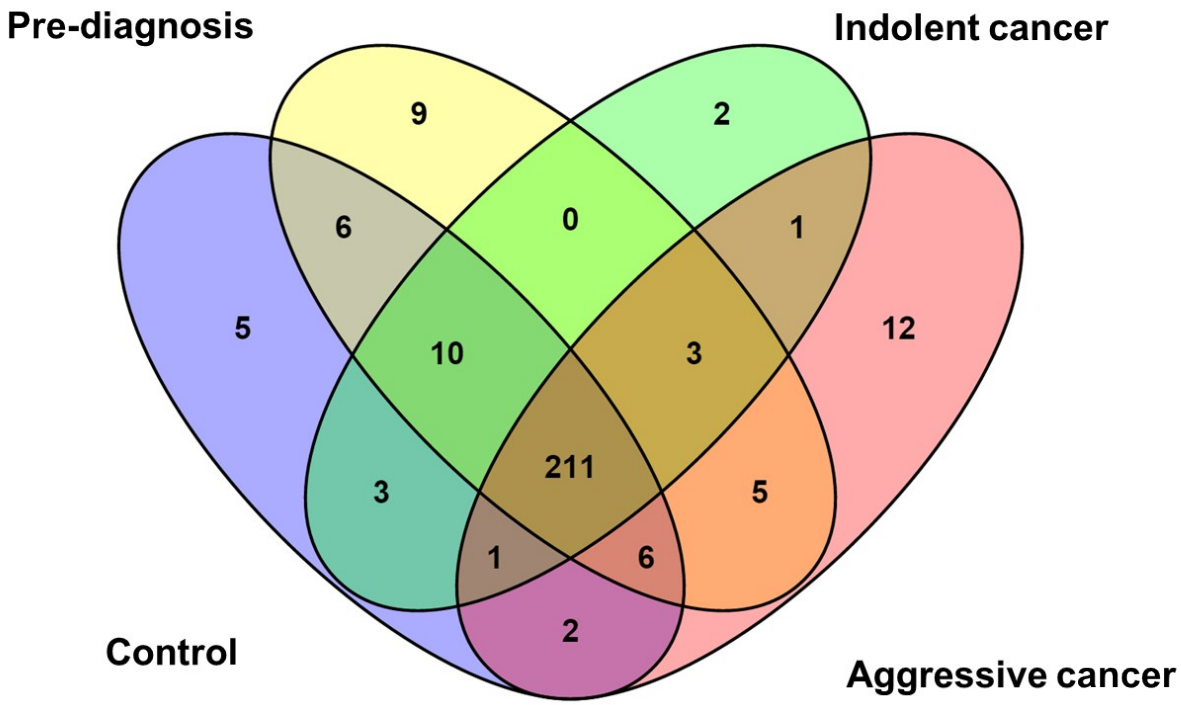

Figure S6: Venn diagram showing the distribution of identified plasma proteins between control, pre-diagnosis, indolent cancer and aggressive cancer patient samples.

Supplementary table 1: List of proteins identified in the current study across all three fractions (55%, 35% ammonium sulfate (AS) and remaining protein fractions), together with

their exclusive total spectrum counts. Exclusive spectrum counts reported here correspond to those observed in 20 different plasma samples (60 fractions).

Uniprot ID	Protein	Exclusive spectrum count		
		55% AS fractions	35% AS fractions	Remaining protein fractions
Q76LX8	A disintegrin and metalloproteinase with thrombospondin motifs 13			19
P68133	Actin, alpha skeletal muscle		5	20
P60709	Actin, cytoplasmic 1	5	176	418
Q01518	Adenylyl cyclase-associated protein 1			7
Q15848	Adiponectin 1			49
P43652	Afamin	232	334	45
P02763	Alpha-1-acid glycoprotein 1	200	27	14
P19652	Alpha-1-acid glycoprotein 2	18	2	2
P01011	Alpha-1-antichymotrypsin	504	239	89
P01009	Alpha-1-antitrypsin	1042	551	211
P04217	Alpha-1B-glycoprotein	177	536	256
P02765	Alpha-2-HS-glycoprotein	408	930	645
P08697	Alpha-2-antiplasmin	25	344	116
P01023	Alpha-2-macroglobulin	2189	5724	4750
P12814	Alpha-actinin-1			71
P01019	Angiotensinogen	155	399	116
P01008	Antithrombin-III	622	154	26
P02647	Apolipoprotein A-I	1153	1014	672
P02652	Apolipoprotein A-II	321	319	184
P06727	Apolipoprotein A-IV	323	739	508
P04114	Apolipoprotein B-100	1795	5866	3513
P02654	Apolipoprotein C-I	46	36	14
P02655	Apolipoprotein C-II	113	144	126
P02656	Apolipoprotein C-III	149	160	58
P05090	Apolipoprotein D	118	149	27
P02649	Apolipoprotein E	160	430	290
Q13790	Apolipoprotein F	57	65	42
O14791	Apolipoprotein L1	76	199	106
O95445	Apolipoprotein M		26	6
P08519	Apolipoprotein(a)	10	85	160
O75882	Attractin	131	110	
P02730	Band 3 anion transport protein			16
P98160	Basement membrane-specific heparan sulfate proteoglycan core protein			10
P02749	Beta-2-glycoprotein 1	251	583	110
Q96KN2	Beta-Ala-His dipeptidase		67	55
Q9HBI1	Beta-parvin			18
P43251	Biotinidase		92	
P02741	C-reactive protein		18	
P04003	C4b-binding protein alpha chain	240	411	1278
P20851	C4b-binding protein beta chain		4	154
O43866	CD5 antigen-like	87	200	212
Q96IY4	Carboxypeptidase B2	71	45	

P15169	Carboxypeptidase N catalytic chain	4	88	3
P22792	Carboxypeptidase N subunit 2	41	216	122
Q9NQ79	Cartilage acidic protein 1		27	4
P49747	Cartilage oligomeric matrix protein		32	119
P00450	Ceruloplasmin	1080	2541	780
P06276	Cholinesterase	29	4	
P10909	Clusterin	256	403	244
P00740	Coagulation factor IX		169	37
P12259	Coagulation factor V		142	301
P00742	Coagulation factor X		44	4
P03951	Coagulation factor XI		68	
P00748	Coagulation factor XII	29	273	285
P00488	Coagulation factor XIII A chain		4	421
P05160	Coagulation factor XIII B chain	6	13	307
P23528	Cofilin-1			59
P02745	Complement C1q subcomponent subunit A	38	53	353
P02746	Complement C1q subcomponent subunit B	78	115	385
P02747	Complement C1q subcomponent subunit C	30	58	136
P00736	Complement C1r subcomponent	47	403	659
Q9NZP8	Complement C1r subcomponent-like protein		55	
P09871	Complement C1s subcomponent	100	490	327
P06681	Complement C2	205	7	
P01024	Complement C3	2423	6965	2910
P0C0L4	Complement C4-A	59	138	118
P0C0L5	Complement C4-B	893	2940	2073
P01031	Complement C5	184	1173	1730
P13671	Complement component C6	40	368	150
P10643	Complement component C7	80	340	149
P07357	Complement component C8 alpha chain	62	517	69
P07358	Complement component C8 beta chain	6	417	207
P07360	Complement component C8 gamma chain	12	239	73
P02748	Complement component C9	269	575	291
P00751	Complement factor B	874	255	54
P08603	Complement factor H	620	1538	1022
Q03591	Complement factor H-related protein 1	11	31	37
Q02985	Complement factor H-related protein 3			19
Q9BXR6	Complement factor H-related protein 5		10	62
P05156	Complement factor I	124	399	190
Q9ULV4	Coronin-1C			2
P08185	Corticosteroid-binding globulin	97	32	
Q12805	EGF-containing fibulin-like extracellular matrix protein 1		38	146
P27105	Erythrocyte band 7 integral membrane protein			10
Q16610	Extracellular matrix protein 1	8	172	442
Q86UX7	Fermitin family homolog 3		2	39
P02792	Ferritin light chain		3	
Q9UGM5	Fetuin-B	7	112	165

P02671	Fibrinogen alpha chain	1102	1744	5769
P02675	Fibrinogen beta chain	1687	2114	10088
P02679	Fibrinogen gamma chain	654	927	3795
P02751	Fibronectin	636	1011	3240
P23142	Fibulin-1	21	209	375
Q15485	Ficolin-2		4	100
O75636	Ficolin-3		67	168
P21333	Filamin-A			226
P04075	Fructose-bisphosphate aldolase A		4	8
Q08380	Galectin-3-binding protein		16	275
P06396	Gelsolin	169	671	516
P22352	Glutathione peroxidase 3		28	80
P00390	Glutathione reductase, mitochondrial		2	
P04406	Glyceraldehyde-3-phosphate dehydrogenase	8		52
P16189	HLA class I histocompatibility antigen, A-31 alpha chain			10
P00738	Haptoglobin	948	2094	220
P00739	Haptoglobin-related protein	86	212	168
P11142	Heat shock cognate 71 kDa protein		2	
P07900	Heat shock protein HSP 90-alpha		2	
P69905	Hemoglobin subunit alpha	98	200	24
P68871	Hemoglobin subunit beta	203	404	104
P02042	Hemoglobin subunit delta	5	60	
P69892	Hemoglobin subunit gamma-2		2	
P02790	Hemopexin	973	537	263
P05546	Heparin cofactor 2	116	435	177
Q04756	Hepatocyte growth factor activator		119	4
P26927	Hepatocyte growth factor-like protein		17	80
P04196	Histidine-rich glycoprotein	130	403	168
Q14520	Hyaluronan-binding protein 2	3	72	74
P01876	Ig alpha-1 chain C region	568	1451	885
P01877	Ig alpha-2 chain C region	225	366	253
P01880	Ig delta chain C region	12	91	65
P01857	Ig gamma-1 chain C region	812	1790	839
P01859	Ig gamma-2 chain C region	415	1079	336
P01860	Ig gamma-3 chain C region	128	308	256
P01861	Ig gamma-4 chain C region	249	812	258
P01743	Ig heavy chain V-I region HG3		55	27
P23083	Ig heavy chain V-I region V35		35	11
P06331	Ig heavy chain V-II region ARH-77	21	78	65
P01766	Ig heavy chain V-III region BRO	43	231	101
P01767	Ig heavy chain V-III region BUT	5	139	71
P01768	Ig heavy chain V-III region CAM		91	3
P01769	Ig heavy chain V-III region GA		16	7
P01781	Ig heavy chain V-III region GAL	20	140	29
P01762	Ig heavy chain V-III region TRO		14	
P01779	Ig heavy chain V-III region TUR	8	25	12
P01764	Ig heavy chain V-III region VH26	46	149	51
P01776	Ig heavy chain V-III region WAS	48	194	134
P01763	Ig heavy chain V-III region WEA	6	129	35
P01834	Ig kappa chain C region	969	2238	797
P01593	Ig kappa chain V-I region AG	8	57	33
P01594	Ig kappa chain V-I region AU	48	160	68

P04430	Ig kappa chain V-I region BAN	29	76	36
P01596	Ig kappa chain V-I region CAR	50	67	47
P01597	Ig kappa chain V-I region DEE		26	8
P01602	Ig kappa chain V-I region HK102 (Fragment)	4	24	12
P01612	Ig kappa chain V-I region Mev	18	65	20
P01609	Ig kappa chain V-I region Scw	5	24	3
P01610	Ig kappa chain V-I region WEA	32	115	35
P01611	Ig kappa chain V-I region Wes	52	84	50
P01616	Ig kappa chain V-II region MIL		42	31
P06310	Ig kappa chain V-II region RPMI 6410	43	56	48
P01617	Ig kappa chain V-II region TEW	56	146	78
P01619	Ig kappa chain V-III region B6	13	112	61
P04207	Ig kappa chain V-III region CLL	27	65	27
P04206	Ig kappa chain V-III region GOL	93	245	128
P18135	Ig kappa chain V-III region HAH OS=Homo sapiens PE=2 SV=1	35	56	23
P06311	Ig kappa chain V-III region IARC/BL41		48	19
P01621	Ig kappa chain V-III region NG9 (Fragment)	6	62	12
P01622	Ig kappa chain V-III region Ti	3	26	3
P04433	Ig kappa chain V-III region VG (Fragment)	47	100	45
P01625	Ig kappa chain V-IV region Len	67	209	109
P01700	Ig lambda chain V-I region HA	11	35	8
P01703	Ig lambda chain V-I region NEWM		14	
P80748	Ig lambda chain V-III region LOI	30	102	7
P01719	Ig lambda chain V-V region DEL	3	58	4
P0CG05	Ig lambda-2 chain C regions	430	852	576
P01871	Ig mu chain C region	345	1152	1156
P04220	Ig mu heavy chain disease protein	45	135	158
Q9Y6R7	IgGFc-binding protein			405
P01591	Immunoglobulin J chain	81	187	201
P15814	Immunoglobulin lambda-like polypeptide 1		22	14
B9A064	Immunoglobulin lambda-like polypeptide 5	36	238	157
P18065	Insulin-like growth factor-binding protein 2		19	3
P17936	Insulin-like growth factor-binding protein 3		21	
P35858	Insulin-like growth factor-binding protein complex acid labile subunit	3	214	10
P08514	Integrin alpha-IIb			141
P05106	Integrin beta-3			58
Q13418	Integrin-linked protein kinase			7
P19827	Inter-alpha-trypsin inhibitor heavy chain H1	344	1129	541
P19823	Inter-alpha-trypsin inhibitor heavy chain H2	357	970	425
Q06033	Inter-alpha-trypsin inhibitor heavy chain H3	31	335	28
Q14624	Inter-alpha-trypsin inhibitor heavy	394	1410	454

	chain H4			
P29622	Kallistatin	8	189	14
P13645	Keratin, type I cytoskeletal 10	44	94	57
P02533	Keratin, type I cytoskeletal 14	8	7	4
P35527	Keratin, type I cytoskeletal 9	47	73	71
P04264	Keratin, type II cytoskeletal 1	78	116	131
P35908	Keratin, type II cytoskeletal 2 epidermal	16	47	30
P04259	Keratin, type II cytoskeletal 6B		4	3
P01042	Kininogen-1	210	686	522
P48059	LIM and senescent cell antigen-like-containing domain protein 1			11
Q14766	Latent-transforming growth factor beta-binding protein 1			10
P02750	Leucine-rich alpha-2-glycoprotein	225	97	
P18428	Lipopolysaccharide-binding protein	7	48	78
P51884	Lumican	25	230	37
P61626	Lysozyme C		35	
P48740	Mannan-binding lectin serine protease 1		96	69
O00187	Mannan-binding lectin serine protease 2		8	25
P11226	Mannose-binding protein C		54	
P08571	Monocyte differentiation antigen CD14	2	54	16
Q13201	Multimerin-1			49
P60660	Myosin light polypeptide 6			47
O14950	Myosin regulatory light chain 12B			19
P24844	Myosin regulatory light polypeptide 9			6
P35579	Myosin-9			340
Q96PD5	N-acetylmuramoyl-L-alanine amidase	63	632	305
P14543	Nidogen-1			4
O00151	PDZ and LIM domain protein 1			11
Q6UXB8	Peptidase inhibitor 16		29	
P04180	Phosphatidylcholine-sterol acyltransferase	7	96	9
P80108	Phosphatidylinositol-glycan-specific phospholipase D	4	223	198
P55058	Phospholipid transfer protein		25	25
P36955	Pigment epithelium-derived factor	199	215	
P03952	Plasma kallikrein	57	328	83
P05155	Plasma protease C1 inhibitor	220	394	97
P05154	Plasma serine protease inhibitor	31	18	
P00747	Plasminogen	496	926	1302
P02775	Platelet basic protein	72	32	17
P02776	Platelet factor 4		9	9
P08567	Pleckstrin			25
P01833	Polymeric immunoglobulin receptor	2	24	65
P20742	Pregnancy zone protein		75	149
P07737	Profilin-1		5	5
P27918	Properdin	14	55	3
P02760	Protein AMBP	164	438	249
P05109	Protein S100-A8	19		2
P06702	Protein S100-A9	19	5	
Q9UK55	Protein Z-dependent protease inhibitor		84	

Q92954	Proteoglycan 4		91	41
P00734	Prothrombin	371	724	425
P14618	Pyruvate kinase PKM			17
Q15404	Ras suppressor protein 1			11
P61224	Ras-related protein Rap-1b			9
P02753	Retinol-binding protein 4	284	347	108
P49908	Selenoprotein P	12	86	9
P02787	Serotransferrin	4202	1677	100
P02768	Serum albumin	28216	5750	2011
P0DJI8	Serum amyloid A-1 protein	49	68	40
P0DJI9	Serum amyloid A-2 protein	9	8	5
P35542	Serum amyloid A-4 protein	161	95	24
P02743	Serum amyloid P-component	94	230	32
P27169	Serum paraoxonase/arylesterase 1	225	482	405
P04278	Sex hormone-binding globulin		46	57
O00391	Sulfhydryl oxidase 1		18	
Q9Y490	Talin-1			804
P05452	Tetranectin	61	95	
P07996	Thrombospondin-1	10	22	463
P35443	Thrombospondin-4			38
P05543	Thyroxine-binding globulin	147	48	
P37802	Transgelin-2			6
P02766	Transthyretin	536	303	182
P06753	Tropomyosin alpha-3 chain			4
P67936	Tropomyosin alpha-4 chain		9	65
P68363	Tubulin alpha-1B chain			103
P68366	Tubulin alpha-4A chain			33
P07437	Tubulin beta chain			86
Q9H4B7	Tubulin beta-1 chain			140
Q14667	UPF0378 protein KIAA0100		2	
P50552	Vasodilator-stimulated phosphoprotein			10
Q6EMK4	Vasorin		47	
P18206	Vinculin			123
P02774	Vitamin D-binding protein	1084	1027	376
P04070	Vitamin K-dependent protein C		21	9
P07225	Vitamin K-dependent protein S	83	224	501
P04004	Vitronectin	273	419	224
P25311	Zinc-alpha-2-glycoprotein	43	82	
Q15942	Zyxin			17
P04275	von Willebrand factor	30	57	1167

Supplementary table 2: Unique proteins identified in the plasma of control, pre-diagnosis, indolent cancer and aggressive cancer patients.

Control	**Pre-diagnosis**
Actin, cytoplasmic 2	Adenylyl cyclase-associated protein 1 (CAP 1)
Ig heavy chain V-III region TUR	LIM and senescent cell antigen-like-containing

	domain protein 1
Ig kappa chain V-I region Scw	Beta-parvin
Immunoglobulin lambda-like polypeptide 1	HLA class I histocompatibility antigen
Ankyrin repeat and sterile alpha motif domain-containing protein 1B	Ras suppressor protein 1
	14-3-3 protein zeta/delta
	Nidogen-1
	Coronin-1C
	Zinc finger homeobox protein 3
Indolent cnacer	**Aggressive cancer**
Ig lambda chain V-I region HA	Serum amyloid A-2 protein (SAA2)
Insulin-like growth factor-binding protein 6	Heat shock cognate 71 kDa protein
	Heat shock protein HSP 90-alpha
	Fructose-bisphosphate aldolase A
	Ig kappa chain V-III region VH
	Keratin, type II cytoskeletal 6B
	Tropomyosin alpha-3 chain
	Ferritin light chain
	Formin-like protein 1
	Hemoglobin subunit gamma-2
	Glutathione reductase, mitochondrial
	Protein KIAA0100

Supplementary table 3: Total protein concentrations obtained for control (n=5), pre-diagnosis (n=5), indolent cancer (n=5) and aggressive cancer (n=5) samples in 55%, 35% ammonium sulfate and remaining protein fractions. Protein concentrations were measured using the Bradford protein assay.

	Total protein concentrations (µg)														
	55% AS					**35% AS**					**Remaining protein fraction**				
Control	708.54	889.90	593.43	737.24	772.23	249.99	447.76	315.30	287.28	365.03	70.44	177.42	97.23	111.31	171.08
Pre-diagnosis	662.04	632.62	868.10	685.13	616.80	330.11	338.24	312.89	320.28	339.08	91.24	89.49	131.21	112.18	92.70
Indolent cancer	721.68	714.17	790.18	663.17	592.29	274.13	335.26	337.60	235.67	243.07	123.99	101.44	93.92	107.20	84.05
Aggressive cancer	589.00	712.41	727.82	714.80	593.01	251.60	267.19	378.94	377.47	376.82	114.31	86.31	102.04	147.05	142.85