

**DISCOVERY OF NOVEL PLASMA PROTEIN BIOMARKERS TO PREDICT  
IMMINENT CYSTIC FIBROSIS PULMONARY EXACERBATIONS USING  
MULTIPLE REACTION MONITORING MASS SPECTROMETRY**

**ONLINE DATA SUPPLEMENT**

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**Supplemental Table S1. List of 117 isotope-labeled reference peptides and the corresponding 79 proteins evaluated with MRM-MS**

No.	Peptide	Protein	No.	Peptide	Protein
1	SYELPDGQVITIGNER	Actin, alpha cardiac muscle 1	61	VQEAHLTEDQIFYFPK	Complement component C8 gamma chain
2	IAPQLSTEELVSLGEK	Afamin	62	LSPIYNLVPVK	Complement component C9
3	AVLDVFEEGTEASAATAVK	Alpha-1-antichymotrypsin	63	VVEESELAR	Complement component C9
4	NLAVSQVVK	Alpha-1-antichymotrypsin	64	EELLPAQDIK	Complement factor B
5	ITPNLAEFAFLYR	Alpha-1-antitrypsin	65	THHDGAITER	Complement factor D
6	LSITGTVDLK	Alpha-1-antitrypsin	66	LVVLPFPK	Fetuin-B
7	ATWSGAVLAGR	Alpha-1B-glycoprotein	67	AHYGGFTVQNEANK	Fibrinogen beta chain
8	LETPDFQLFK	Alpha-1B-glycoprotein	68	QGFNVATNTDGGK	Fibrinogen beta chain
9	APHGPLYR	Alpha-2-HS-glycoprotein	69	DTVQIHDTGK	Fibrinogen gamma chain
10	HTLNQIDEVK	Alpha-2-HS-glycoprotein	70	YEASILTHDSSIR	Fibrinogen gamma chain
11	LLIYAVLPTGDVIGDSAK	Alpha-2-macroglobulin	71	HTSVQTTSSSGPFTDVR	Fibronectin
12	TEHPFVEEFVLPK	Alpha-2-macroglobulin	72	SSPVIDASTAIDAPSNLR	Fibronectin
13	ALQDQLVLVAAK	Angiotensinogen	73	TGYVFDGISR	Fibulin-1
14	DDLTVSDAFHK	Antithrombin-III	74	TGAQELLR	Gelsolin
15	FATTFYQLADSK	Antithrombin-III	75	TYPFHFDLSHGSAQVK	Hemoglobin subunit alpha
16	SLAPYAQDTQEK	Apolipoprotein A-IV	76	VGAAHEGYGAEALER	Hemoglobin subunit alpha
17	FPEVDVLTGK	Apolipoprotein B-100	77	NFSPVDAAFR	Hemopexin
18	ILGELGFASLHDLQLLGGK	Apolipoprotein B-100	78	NYNLVESLK	Heparin cofactor 2
19	TAAQNLYEK	Apolipoprotein C-II	79	SVNDLYIQK	Heparin cofactor 2
20	TYLPAVDEK	Apolipoprotein C-II	80	DGYLFQLLR	Histidine-rich glycoprotein
21	NILTSNNIDVK	Apolipoprotein D	81	FLNVLSPR	Insulin-like growth factor-binding protein 3
22	VLNQELR	Apolipoprotein D	82	VAGLLEDTFPGLLGLR	Insulin-like growth factor-binding protein complex acid labile subunit
23	LGPLVEQGR	Apolipoprotein E	83	ETAVDGELVVLVDVK	Inter-alpha-trypsin inhibitor heavy chain H2
24	VTEPISAESEQVER	Apolipoprotein L1	84	FLHVPDITFEGHFDGVPVISK	Inter-alpha-trypsin inhibitor heavy chain H2
25	ATVVYQGER	Beta-2-glycoprotein 1	85	DIFNPSPELEETLTHITK	Kininogen-1
26	IQVYSR	Beta-2-microglobulin	86	ITLPDFTGDLR	Lipopolysaccharide-binding protein
27	VNHVTLSPK	Beta-2-microglobulin	87	APGELEHGLTFSTR	Mannan-binding lectin serine protease 1
28	ALEQDLVPIK	Beta-Ala-His dipeptidase	88	STLSVGVSGTLVLLQGAR	Monocyte differentiation antigen CD14
29	SVVLPLGAVDDGEHSQNEK	Beta-Ala-His dipeptidase	89	TDCPGDALFDLLR	N-acetylmuramoyl-L-alanine amidase
30	AFVFPK	C-reactive protein	90	GLFHIDGK	Peroxisome oxidin-2
31	EDVYVVGTVLR	C4b-binding protein alpha chain	91	FLEQELETITIPDLR	Phospholipid transfer protein
32	YEIVVEAR	Cadherin-5	92	TSLEDFYLDEER	Pigment epithelium-derived factor
33	VLDALQAIK	Carbonic anhydrase 1	93	TVQAVLTVPK	Pigment epithelium-derived factor
34	IVQLIQDTR	Carboxypeptidase N catalytic chain	94	TGAVSGHSLK	Plasma kallikrein
35	NNANGVDLNR	Carboxypeptidase N catalytic chain	95	VSEGNHDIALIK	Plasma kallikrein
36	LVGGLHR	CD5 antigen-like	96	LLDSLPSDTR	Plasma protease C1 inhibitor
37	GAYPLSIEPIGVR	Ceruloplasmin	97	AVVEVDESCTR	Plasma serine protease inhibitor
38	IYHSHIDAPK	Ceruloplasmin	98	FSIEGSYQLEK	Plasma serine protease inhibitor
39	ELDESLQVAER	Clusterin	99	VILGAHQEVNLEPHVQIEVSR	Plasminogen
40	EPQDTHYLPFSLPHR	Clusterin	100	AFIQLWAFDAVK	Protein AMBP
41	AEVDDVIQVR	Coagulation factor V	101	ETLLQDFR	Protein AMBP
42	VAQVHPSTYVPGTTHDIALLR	Coagulation factor VII	102	ETSNFSGSLLR	Protein Z-dependent protease inhibitor
43	TGIVSGFGR	Coagulation factor X	103	YWGASFLQK	Retinol-binding protein 4
44	LHEAFSPVSYQHDLALLR	Coagulation factor XII	104	AEFAEVSK	Serum albumin
45	VVGGVALR	Coagulation factor XII	105	LVNEVTEFAK	Serum albumin
46	IQTHSITTYR	Coagulation factor XIII B chain	106	FRPDGLPK	Serum amyloid A-4 protein
47	LIENGYFHPVK	Coagulation factor XIII B chain	107	GPGGVWAAK	Serum amyloid A-4 protein
48	PAFSAIR	Complement C1q subcomponent subunit A	108	VVLSQGSK	Sex hormone-binding globulin
49	SLGFCDTTNK	Complement C1q subcomponent subunit A	109	GGTLGTPQTGSENDALYEYLR	Tetranectin
50	GYGFYK	Complement C1r subcomponent	110	LDTLAQEVALLK	Tetranectin
51	VSVHPDYR	Complement C1r subcomponent	111	AADDTWEPFASGK	Transferrin
52	SDFSNEER	Complement C1s subcomponent	112	GSPAINVAVHVFR	Transferrin
53	TNFDNDIALVR	Complement C1s subcomponent	113	LGEYDLR	Vitamin K-dependent protein C
54	DHENELLNK	Complement C2	114	DFAEHLIPR	Vitamin K-dependent protein Z
55	HAFILQDTK	Complement C2	115	ILAGPAGDSNVVK	von Willebrand factor
56	TGLQEVVEVK	Complement C3	116	AGEVQPELR	Zinc-alpha-2-glycoprotein
57	DHAVDLIQK	Complement C4-A	117	YSLTYIYTGLSK	Zinc-alpha-2-glycoprotein
58	VGDTLNLLNR	Complement C4-A			
59	LIDQYGYHYLQSGSLGGEYR	Complement component C7			
60	SLPVS DSVLSGFEQR	Complement component C8 gamma chain			

**Supplemental Table S2: Pearson's correlation coefficients between individual candidate and MRM-MS panel protein biomarkers and their corresponding p-values**

P-value Correlation	CD5	CRP	Peroxi redoxin- 2	Apo C-II	Hb subunit alpha	CA-1	IL-6	MPO	sCD14	NEAPC
CD5		0.74	0.17	<b>0.03</b>	0.83	0.48	0.20	0.30	0.63	<b>0.02</b>
CRP	-0.03		0.72	<b>&lt;0.01</b>	0.21	0.94	<b>&lt;0.01</b>	<b>&lt;0.01</b>	<b>&lt;0.01</b>	0.07
Peroxi redoxin -2	-0.14	0.04		0.64	<b>&lt;0.01</b>	<b>&lt;0.01</b>	0.97	0.10	0.98	0.14
Apo C-II	<b>0.21</b>	<b>0.34</b>	-0.05		0.79	0.18	<b>&lt;0.01</b>	<b>&lt;0.01</b>	0.45	0.26
Hb subunit alpha	0.02	0.12	<b>0.77</b>	0.03		<b>&lt;0.01</b>	0.61	0.15	0.86	0.9
CA-1	0.07	0.01	<b>0.74</b>	0.13	<b>0.81</b>		0.82	0.08	0.98	0.81
IL-6	0.13	<b>0.68</b>	0	<b>-0.32</b>	0.05	0.02		<b>0.01</b>	<b>&lt;0.01</b>	<b>0.04</b>
MPO	-0.10	<b>0.33</b>	-0.16	<b>-0.26</b>	-0.14	-0.17	<b>0.25</b>		0.14	0.28
sCD14	-0.05	<b>0.48</b>	0	-0.07	-0.02	0	<b>0.45</b>	0.15		<b>0.01</b>
NEAPC	<b>0.22</b>	0.18	-0.15	0.11	0.01	0.02	<b>0.20</b>	-0.11	<b>0.24</b>	

**Abbreviations:** CD5, cluster of differentiation 5; CRP, C-reactive protein; Apo-CII, apolipoprotein C-II; Hb, hemoglobin; CA-1, carbonic anhydrase-1; IL-6, interleukin-6; MPO, myeloperoxidase; sCD14, soluble cluster of differentiation 14; NEAPC, neutrophil elastase anti-proteinase complex

**Supplemental Table S3:** Pearson’s correlation coefficients between biomarker score with FEV1 % predicted and number of exacerbations requiring IV antibiotics in the previous year

Variable	Correlation	P-value
FEV1 % predicted	-0.24	0.02
Number of exacerbations requiring IV antibiotics in the previous year	0.22	0.03

**Abbreviations:** FEV1, forced expiratory volume in 1 second; IV, intravenous

**Supplemental Table S4:** P-values for comparison between biomarker scores in subjects with vs. without chronic airway infection with known CF pathogens (Refer to Supplemental Figure S1 for box plots)

Bacteria	P-value
<i>Pseudomonas aeruginosa</i>	<0.01
<i>Burkholderia cepacia complex</i>	0.34
<i>Methicillin-sensitive Staphylococcus aureus</i>	0.70
<i>Methicillin-resistant Staphylococcus aureus</i>	0.60
<i>Stenotrophomonas maltophilia</i>	0.86

**Supplemental Table S5:** Sensitivity analysis altering the threshold fold-difference for the selection of proteins for the final MRM-MS panel

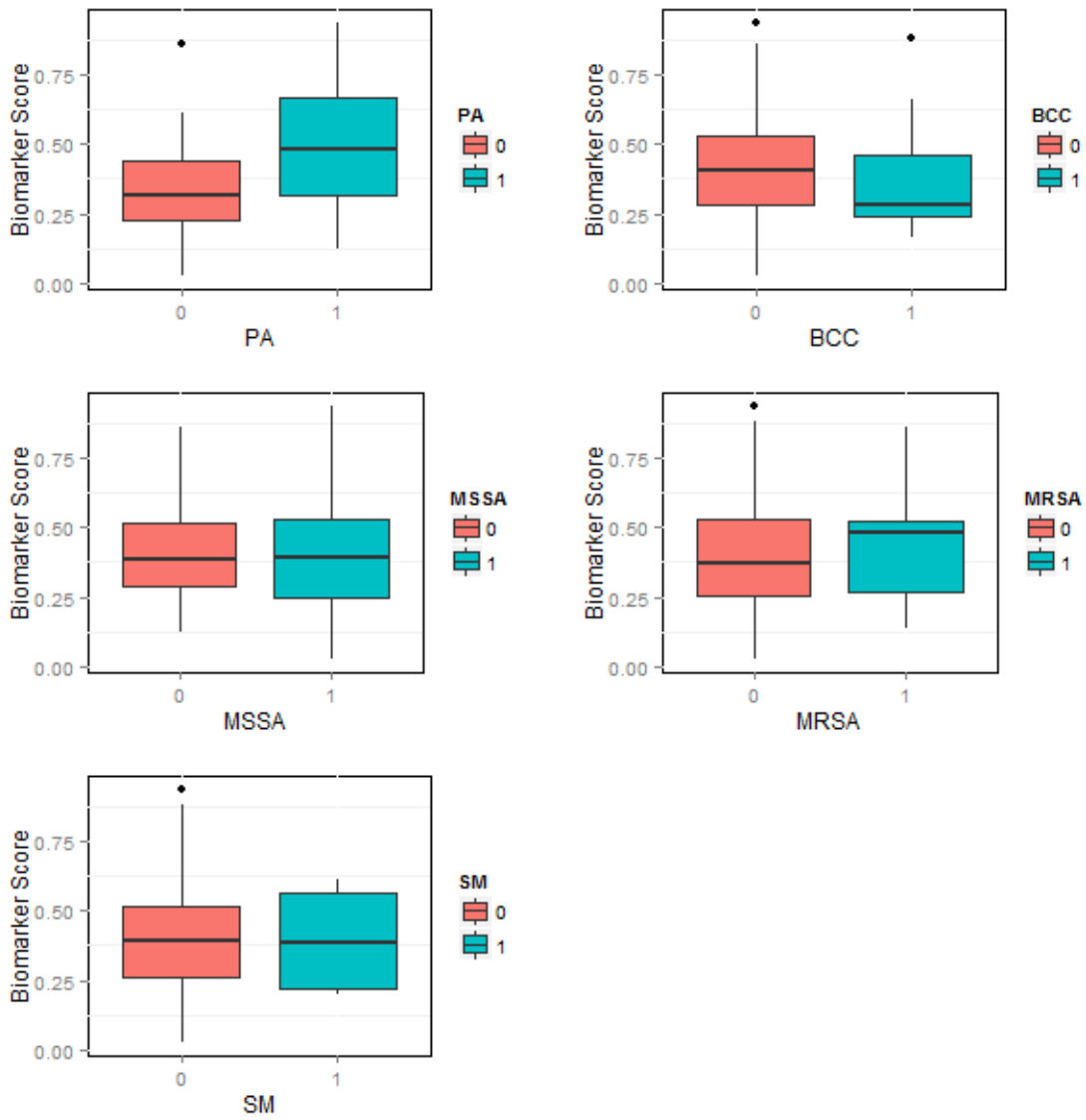
Fold Difference	Number of proteins	Discovery Cohort (n=70)			Replication Cohort (n=34)		
		AUC	Sensitivity	Specificity	AUC	Sensitivity	Specificity
1.25	11	0.52	93%	13%	0.64	100%	14%
1.50	6	0.74	90%	45%	0.73	92%	48%
1.75	3	0.49	90%	13%	0.68	92%	29%

**Supplementary Table S6:** Unadjusted and Adjusted Cox proportional hazard models of the association between biomarker score and risk of a pulmonary exacerbation

Variable	Unadjusted			Adjusted		
	HR	95% CI	P-value	HR	95% CI	P-value
<b>Biomarker score</b>	<b>6.63</b>	<b>1.26-34.85</b>	<b>0.03</b>	<b>5.08</b>	<b>0.89-29.02</b>	<b>0.07</b>
FEV1 % predicted				1.00	0.98-1.02	0.72
Male				0.76	0.33-1.73	0.51
Age				0.99	0.95-1.03	0.52
PA				1.29	0.49-3.40	0.60
BMI				1.04	0.93-1.16	0.47
CF-related diabetes				0.41	0.13-1.28	0.13
Number of IV antibiotic courses in previous year				1.47	1.11-1.95	0.01

**Abbreviations:** BMI, body mass index; FEV1, forced expiratory volume in one second; PA, *Pseudomonas aeruginosa* positive; HR, hazard ratio; CI, confidence interval

**Supplemental Figure S1:** Boxplots comparing biomarker scores in subjects with vs. without chronic airway infection with know CF pathogens.



**Abbreviations:** BCC, *Burkholderia cepacia* complex; MSSA, methicillin-sensitive *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*; PA, *Pseudomonas aeruginosa*; SM, *Stenotrophomonas maltophilia*

**Supplemental Figure S2:** Time-to-event analysis comparing the groups with high vs. low biomarker scores using a biomarker cut-off score utilized in our primary analysis (log-rank p-value = 0.15)

