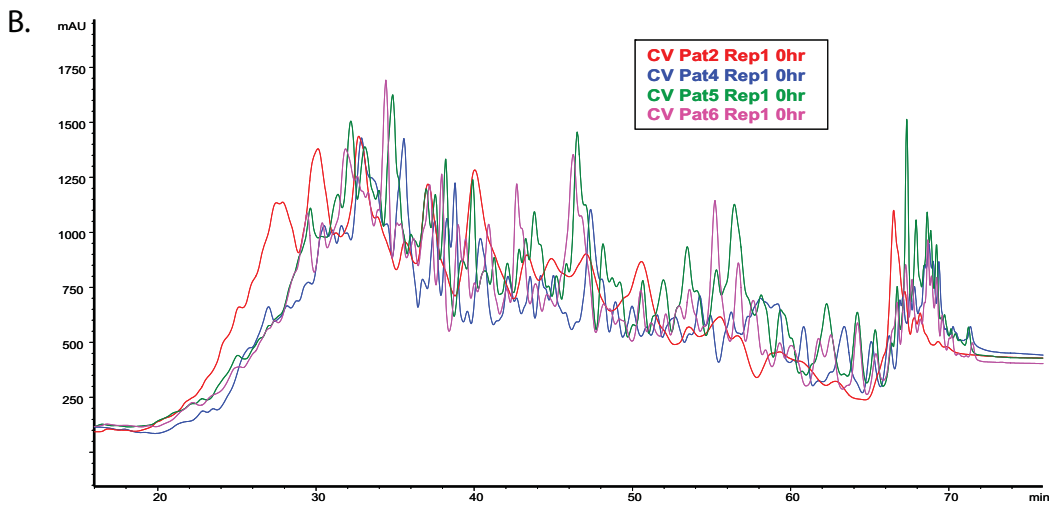
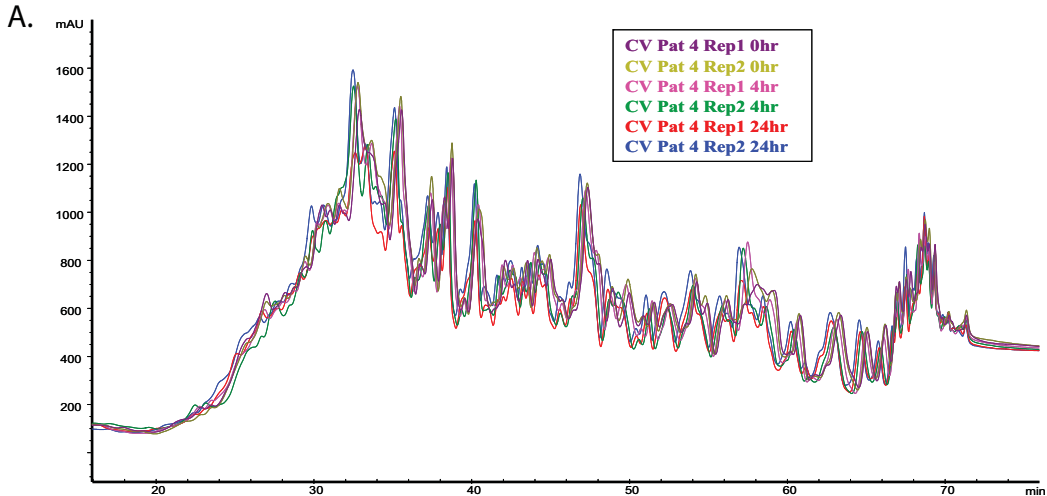
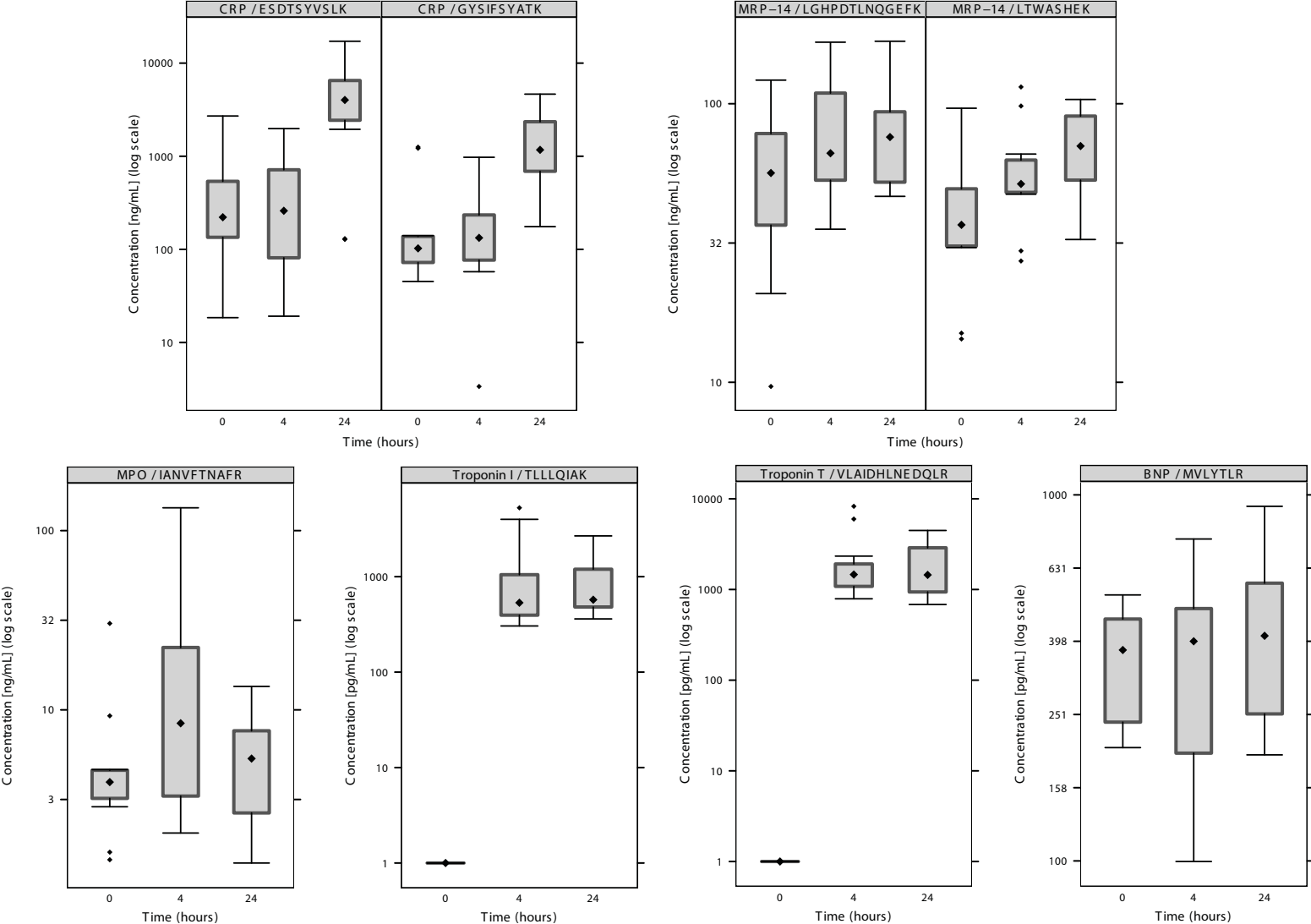


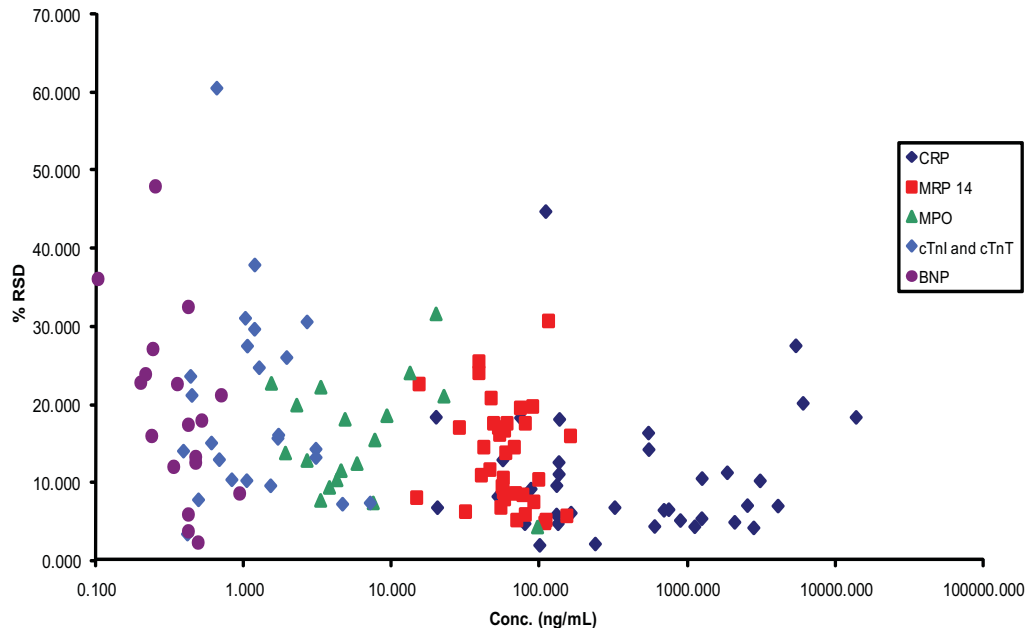
Supplemental Figure 1: Calibration curve of all the peptides in SCX background. Linear regression analysis of the standard addition data for the peptides of CRP (A), MRP-14 (B), MPO (C), cTnI (D), cTnT (E), NTproBNP and BNP32 (F), IL-33 (G), and sCD40L (H).

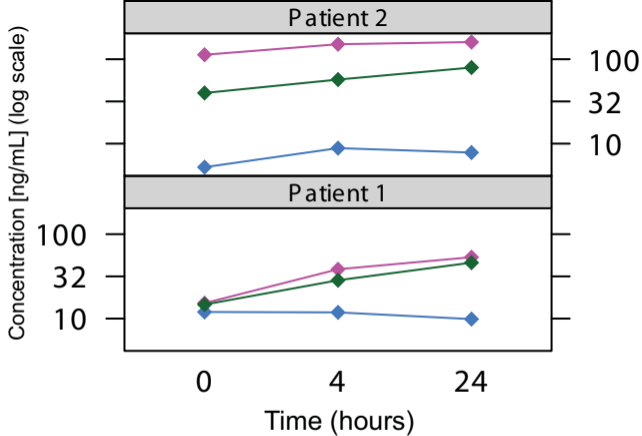


Supplemental Figure 2: SCX profile of patient plasma digest. Overlaid SCX chromatograms for two process replicates at baseline, 4 hours and 24 hours for one patient (**A**) and one replicate of the baseline sample for 4 different patients (**B**).



Supplemental Figure 3: Quantitation of all 6 proteins across all the patient plasma samples. Box and whisker plots showing median concentration and the range of concentrations measured by MRM assay at baseline, 4h and 24h for CRP (A), MRP 14 (B), MPO (C), cTnI (D), cTnT (E), and NT-proBNP (F).





Supplemental Figure 5: Comparison of MRM assay with the Immunoassay for MRP 14. Line plots of Log₁₀ [concentration] of MRP 14 at the baseline, 4h, and 24h as measured by MRM with 2 different signature peptides (LGHPDTLNQGEFK in pink and LTWASHEK in green) and by immunoassay (blue) for 2 patients.

Supplemental Table 1. Target proteins and their signature peptides. Both [¹²C] and [¹³C] versions of signature peptides were synthesized. Uniformly labeled amino acids and transitions used for quantification are indicated in boldface type.

Protein	MW (kDa)	Signature Peptide	MH+ (mono)	Z (Q1)	MRM Transitions ^b			
					Q1	Q3		
NT-proBNP	11	EVATEGIR	874.5	2	437.7	474.3	575.3	646.4
		EVATEG[¹³ C ₆]IR	880.5	2	440.8	480.3	581.3	652.4
		MVLYTLR	895.5	2	448.3	552.3	665.4	764.5
		MVLYT[¹³ C ₆]LR	901.5	2	451.3	558.3	671.4	770.5
		MoxVLYTLR	911.5	2	456.3	552.3	665.4	764.5
		MoxVLYT[¹³ C ₆]LR	917.5	2	459.3	558.3	671.4	770.5
MRP14	13	LTWASHEK	971.5	2	486.3	500.3	571.3	757.4
		[¹³ C ₆]LTWASHEK	977.5	2	489.3	500.3	571.3	757.4
		LGHPDTLNQGEFK	1455.7	3	485.9	621.3	722.4	734.4
		LGHPDT[¹³ C ₆]LNQGEFK	1461.7	3	487.9	621.3	722.4	740.4
BNP 32	15	ISSSSGLGC _{amc} K ^a	995.5	2	498.2	708.3	795.4	882.4
		ISSSSG[¹³ C ₆]LGC _{amc} K ^a	1001.5	2	501.3	714.4	801.4	888.4
		MVQGS GC _{amc} FGR ^a	1098.5	2	549.7	683.3	740.3	868.4
		M[¹³ C ₅]VQGS GC _{amc} FGR ^a	1103.5	2	552.3	683.3	740.3	868.4
		MoxVQGS GC _{amc} FGR ^a	1114.5	2	557.7	683.3	740.3	868.4
		Mox [¹³ C ₅] VQGS GC _{amc} FGR ^a	1119.5	2	560.3	683.3	740.3	868.4
Troponin I	24	TLLLQIAK	899.6	2	450.3	459.3	572.4	685.5
		TLL[¹³ C ₆]LQIAK	905.6	2	453.3	459.3	578.4	691.5
		NITEIADLTQK	1245.7	2	623.3	675.4	788.5	1018.5
		NITEIAD[¹³ C ₆]LTQK	1251.7	2	626.4	681.4	794.5	1024.6
		NIDALSGMEGR	1162.6	2	581.8	636.3	749.4	935.4
		NIDA[¹³ C ₆]LSGMEGR	1168.6	2	584.8	636.3	755.4	941.5
CRP	25	ESDTSYVSLK	1128.5	2	564.8	609.4	696.4	797.4
		ESDTSYVS[¹³ C ₆]LK	1134.6	2	567.8	615.4	702.4	803.5
		GYSIFSYATK	1136.6	2	568.8	716.4	829.5	916.5
		GYS[¹³ C ₆]IFS YATK	1142.6	2	517.8	716.4	835.5	922.5
sCD40L	29	TTSVLQWAEK	1162.6	2	581.8	661.3	774.4	960.5
		TTSV[¹³ C ₆]LQWAEK	1168.6	2	584.8	661.3	780.4	966.5

		EASSQAPFIASLC _{amc} LK ^a	1621.8	2	811.4	691.4	1048.6	1119.6
		EASSQAPFIASLC _{amc} [¹³ C ₆]LK ^a	1627.8	2	814.4	697.4	1054.6	1125.6
		SQFEGFVK	941.5	2	471.2	579.3	726.4	837.4
		SQFEGF [¹³ C ₅]VK	946.5	2	473.8	584.3	731.4	842.4
		SLSLLNCEEIK	1305.7	2	653.3	792.4	905.4	1105.6
		SLSLLNCEE [¹³ C ₆]IK	1311.7	2	656.4	798.4	911.5	1111.6
IL 33	31	DNHLALIK	923.5	2	462.3	480.2	551.3	664.3
		DNHLA [¹³ C ₆]LIK	929.6	2	465.3	480.2	551.3	670.4
		TDPGVFIGVK	1032.6	2	516.8	662.4	719.5	816.5
		TDPGVFIG [¹³ C ₅]VK	1037.6	2	519.3	667.4	724.5	821.5
		DFWLHANNK	1144.6	3	382.2	441.7	583.3	696.4
		DFW [¹³ C ₆]LHANNK	1150.6	3	384.2	444.7	583.3	702.4
		VLLSYYESQHPSNESGDGVDGK	2381.1	3	794.4	903.4	1028.4	1085.0
		VLLSYYESQHPSNESGDG [¹³ C ₅]VDGK	2386.1	3	796.0	905.9	1030.9	1087.5
Troponin T	34	VLAIDHLNEDQLR	1535.8	3	512.6	662.3	718.9	774.4
		VLAIDHLNEDQ [¹³ C ₆]LR	1541.8	3	514.6	665.4	721.9	780.4
		SFMPNLVPPK	1129.6	2	565.3	764.5	789.4	895.5
		SFMPNL [¹³ C ₅]VPPK	1134.6	2	567.8	769.5	794.4	900.5
		SFMoxPNLVPPK	1145.6	2	573.3	764.5	805.4	911.5
		SFMoxPNL [¹³ C ₅]VPPK	1150.6	2	575.8	769.5	810.4	916.5
		YEINVLR	906.5	2	453.8	501.3	614.4	743.4
		YEINV [¹³ C ₆]LR	912.5	2	456.8	507.3	620.4	749.5
MPO	84	IPC _{amc} FLAGDTR ^a	1149.6	2	575.3	632.3	779.4	939.4
		IPC _{amc} F [¹³ C ₆]LAGDTR ^a	1155.6	2	578.3	638.4	785.4	945.5
		IANVFTNAFR	1152.6	2	576.8	755.4	854.5	968.5
		IAN [¹³ C ₅]VFTNAFR	1157.6	2	579.3	755.4	859.5	973.5

^a Cysteines synthesized as carbamidomethyl cysteines

^b All three transitions per peptide were monitored. However, LOD and LOQ values reported in table 1 as well as transition used for the quantification of peptides in patient plasma is the transition in boldface type.

Supplemental Table 2. Summary of MRM measurements for all patients and timepoints.

Sample	C reactive protein										Myeloid related protein 14										
	ESDTSYVSLK					GYSIFSYATK					LTWASHEK					LGHPDTLNQGEFK					
	Technical replicates		Process replicates			Technical replicates		Process replicates			Technical replicates		Process replicates			Technical replicates		Process replicates			
	Avg. Conc. (ng/mL)	Intra-assay % CV ^a	Avg S/N ^b	Avg. Conc. (ng/mL)	Inter-assay % CV ^c	Avg. Conc. (ng/mL)	Intra-assay % CV ^a	Avg S/N ^b	Avg. Conc. (ng/mL)	Inter-assay % CV ^c	Avg. Conc. (ng/mL)	Intra-assay % CV ^a	Avg S/N ^b	Avg. Conc. (ng/mL)	Inter-assay % CV ^c	Avg. Conc. (ng/mL)	Intra-assay % CV ^a	Avg S/N ^b	Avg. Conc. (ng/mL)	Inter-assay % CV ^c	
Patient 1	rep 1 Baseline	163.48	1.3	395	161.44	4.1	58.09	3.5	475	51.66	5.6	15.01	7.8	24	14.66	32.9	20.82	16.4	52	15.24	42.6
	rep 1 4 hr	61.69	1.5	543	99.13	41.4	3.37	2.6	43	55.84	87.1	29.63	7.7	249	28.44	10.4	41.33	17.0	190	38.39	17.0
	rep 1 24 hr	1946.77	4.7	11153	2774.73	32.8	175.54	1.9	1810	1107.26	78.9	59.37	8.9	96	45.96	31.2	57.80	9.3	257	53.36	13.7
	rep 2 Baseline	159.40	5.9	332			45.23	8.1	215			14.31	0.2	25			9.66	4.7	50		
	rep 2 4 hr	136.57	1.2	334			108.31	6.6	226			27.25	15.6	53			35.45	16.9	269		
	rep 2 24 hr	3602.68	1.9	5206			2038.98	2.3	13156			32.56	2.2	303			48.92	13.6	498		
Patient 2	rep 1 Baseline	574.43	12.9	551	539.14	12.6	135.33	9.5	59	133.85	3.6	48.43	7.4	70	39.83	24.7	121.56	15.0	97	112.92	30.8
	rep 1 4 hr	429.98	12.5	421	542.21	24.2	120.18	12.4	60	133.67	13.5	65.97	12.8	118	57.40	19.5	166.33	3.7	201	150.55	12.1
	rep 1 24 hr	4483.56	13.2	2936	5346.39	24.8	499.11	1.9	585	593.32	17.7	71.09	6.5	343	79.70	26.5	167.61	13.9	83	159.64	12.3
	rep 2 Baseline	503.85	9.3	368			132.37	5.3	125			31.22	7.9	13			104.29	28.2	72		
	rep 2 4 hr	654.43	8.3	376			147.17	5.0	102			48.82	9.2	36			134.77	4.4	132		
	rep 2 24 hr	6209.22	21.6	3461			687.53	3.4	1231			88.30	14.9	465			151.67	6.7	124		
Patient 3	rep 1 Baseline	132.16	16.7	100	134.87	10.0	62.72	6.8	33	73.50	18.0	61.29	8.2	130	55.95	11.9	73.15	16.4	201	73.68	11.3
	rep 1 4 hr	106.90	4.8	343	131.93	21.2	57.56	7.8	273	108.85	45.8	50.28	14.0	88	48.81	10.1	63.12	12.3	327	58.86	3.8
	rep 1 24 hr	3085.78	0.4	7736	3065.50	6.4	962.68	9.2	1275	1240.67	27.3	45.24	15.4	35	46.44	13.5	51.96	10.9	307	52.28	10.7
	rep 2 Baseline	137.59	7.4	74			84.28	15.1	92			50.61	8.2	60			74.22	10.6	273		
	rep 2 4 hr	156.96	2.1	456			160.14	30.2	94			47.33	10.2	147			54.60	4.0	356		
	rep 2 24 hr	3045.21	10.2	6521			1518.66	6.2	1859			47.65	14.0	83			52.60	12.9	318		
Patient 4	rep 1 Baseline	18.49	7.6	15	19.76	9.8	83.42	7.6	98	86.53	7.3	41.54	6.1	50	68.95	43.7	36.04	4.8	276	59.18	44.2
	rep 1 4 hr	19.17	5.7	24	20.17	6.5	96.01	2.9	96	78.64	24.1	98.13	0.4	532	106.50	9.1	95.56	4.8	816	110.19	15.5
	rep 1 24 hr	127.96	3.9	263	129.14	5.6	691.57	3.4	211	739.44	10.1	103.60	9.0	77	97.98	9.1	46.51	4.9	93	66.40	34.8
	rep 2 Baseline	21.03	15.8	17			89.64	5.2	83			96.36	2.5	248			82.31	12.4	631		
	rep 2 4 hr	21.17	3.8	123			61.27	3.9	224			114.87	4.4	613			124.82	2.5	709		
	rep 2 24 hr	130.33	8.6	296			787.30	5.2	941			92.36	4.4	109			86.29	10.9	309		
Patient 5	rep 1 Baseline	338.33	3.1	928	319.09	7.8	140.19	1.0	317	128.75	10.3	43.95	15.7	222	38.23	19.5	46.10	9.7	57	41.69	14.7
	rep 1 4 hr	589.47	1.9	948	686.02	16.3	212.01	1.6	577	235.44	11.0	52.82	2.2	268	55.65	8.2	51.73	7.5	296	57.62	12.6
	rep 1 24 hr	6816.15	17.4	7058	5980.60	19.9	2701.60	3.2	6547	2063.22	34.0	69.89	3.7	431	67.79	6.7	86.07	1.5	323	79.42	9.9
	rep 2 Baseline	299.85	6.2	800			117.31	6.2	261			32.50	15.7	54			37.27	10.8	276		
	rep 2 4 hr	782.57	5.4	1608			258.87	1.3	468			58.48	8.8	147			63.51	3.7	298		
	rep 2 24 hr	5145.05	3.3	11938			1424.85	3.5	7116			65.69	8.0	384			72.76	6.2	760		
Patient 6	rep 1 Baseline	2705.32	5.6	881	2514.99	9.4	1253.28	2.8	1453	1235.80	3.5	30.45	3.9	115	31.01	4.4	51.85	5.3	143	56.61	8.1
	rep 1 4 hr	1980.51	9.4	980	1837.44	11.2	975.07	2.9	1623	885.36	11.4	59.73	5.2	179	54.67	11.0	82.69	6.5	121	76.09	10.9
	rep 1 24 hr	10140.82	10.7	9282	13656.18	30.5	3453.21	7.0	5670	4048.91	16.7	81.17	4.8	232	88.86	14.1	79.22	5.8	149	90.34	14.3
	rep 2 Baseline	2324.66	3.7	4166			1218.32	4.5	2532			31.56	4.9	161			61.37	6.6	340		
	rep 2 4 hr	1694.37	5.2	4050			795.64	4.4	2573			49.61	3.9	203			69.49	4.8	348		
	rep 2 24 hr	17171.53	13.1	3544			4644.61	3.0	13024			96.55	17.7	364			101.46	4.9	149		

Supplemental Table 2 cont.

Sample	Myeloperoxidase					Troponin I					Troponin T					NT-proBNP					
	IANVFTNAFR					TLLLQIAK					VLAIHNLNEDQLR					MVLTYTLR					
	Technical replicates		Process replicates			Technical replicates		Process replicates			Technical replicates		Process replicates			Technical replicates		Process replicates			
	Avg. Conc. (ng/mL)	Intra-assay % CV ^a	Avg S/N ^b	Avg. Conc. (ng/mL)	Inter-assay % CV ^c	Avg. Conc. (ng/mL)	Intra-assay % CV ^a	Avg S/N ^b	Avg. Conc. (ng/mL)	Inter-assay % CV ^c	Avg. Conc. (ng/mL)	Intra-assay % CV ^a	Avg S/N ^b	Avg. Conc. (ng/mL)	Inter-assay % CV ^c	Avg. Conc. (ng/mL)	Intra-assay % CV ^a	Avg S/N ^b	Avg. Conc. (ng/mL)	Inter-assay % CV ^c	
Patient 1	rep 1 Baseline	3.92	5.0	37	4.24	10.5	n/d ^d	-	-	-	n/d ^d	-	-	-	0.44	3.6	141	0.42	6.9		
	rep 1 4 hr	20.33	14.3	8	22.38	17.9	3.98	7.9	40	4.62	15.8	8.27	5.2	189	7.12	17.6	0.76	16.3	36	0.70	15.9
	rep 1 24 hr	9.39	5.6	4	7.46	28.8	0.54	6.5	19	0.49	11.4	0.68	11.9	10	0.82	20.6	0.93	4.5	15	0.93	5.4
	rep 2 Baseline	4.56	8.5	53			n/d	-	-	-	n/d	-	-	-	0.40	4.6	96				
	rep 2 4 hr	24.43	15.2	25			5.26	1.8	32			5.98	4.7	10	0.65	12.9	7				
	rep 2 24 hr	5.52	2.3	69			0.44	3.1	17			0.96	2.2	23	0.93	7.2	170				
Patient 2	rep 1 Baseline	30.35	17.8	9	19.81	68.2	n/d ^d	-	-	-	n/d ^d	-	-	-	0.50	2.6	19	0.42	20.6		
	rep 1 4 hr	134.05	3.0	59	95.80	40.6	0.55	31.5	2	0.65	42.1	1.57	24.0	5	1.18	43.3	0.47	2.1	24	0.49	5.1
	rep 1 24 hr	13.52	18.2	11	13.22	15.4	2.67	14.9	9	2.66	19.3	0.92	8.6	2	1.94	60.0	0.44	15.7	18	0.52	19.9
	rep 2 Baseline	9.26	33.9	6			n/d	-	-	-	n/d	-	-	-	0.34	2.5	20				
	rep 2 4 hr	57.56	1.0	33			0.76	46.9	3			0.79	30.0	3	0.51	1.0	24				
	rep 2 24 hr	12.93	15.4	10			2.65	26.7	11			2.96	16.8	10	0.60	10.4	22				
Patient 3	rep 1 Baseline	3.57	9.3	29	3.78	8.4	n/d ^d	-	-	-	n/d ^d	-	-	-	0.53	8.1	107	0.47	16.6		
	rep 1 4 hr	8.18	17.6	28	9.23	17.1	1.00	13.4	12	1.05	17.9	1.44	16.5	15	1.26	19.3	0.41	12.0	72	0.41	8.9
	rep 1 24 hr	6.22	3.9	8	5.80	11.1	0.42	5.8	4	1.17	73.7	2.81	12.8	17	3.06	8.0	0.55	7.0	105	0.47	20.1
	rep 2 Baseline	3.99	2.8	27			n/d	-	-	-	n/d	-	-	-	0.41	11.1	87				
	rep 2 4 hr	10.27	8.9	21			1.09	23.2	6			1.09	18.4	8	0.42	12.0	100				
	rep 2 24 hr	5.38	12.5	16			1.93	17.9	10			3.30	5.3	38	0.39	11.5	77				
Patient 4	rep 1 Baseline	4.64	4.8	9	4.53	7.8	n/d ^d	-	-	-	n/d ^d	-	-	-	0.25	6.3	8	0.24	12.3		
	rep 1 4 hr	8.67	12.3	21	7.65	17.5	0.52	1.1	4	0.41	28.7	2.33	9.2	18	1.71	41.6	0.23	13.5	6	0.20	22.0
	rep 1 24 hr	5.32	7.0	50	4.82	17.0	0.67	13.2	7	0.60	15.6	4.48	9.3	10	3.05	51.9	0.25	18.2	23	0.25	32.7
	rep 2 Baseline	4.41	10.6	16			n/d	-	-	-	n/d	-	-	-	0.22	15.4	9				
	rep 2 4 hr	6.63	7.5	49			0.30	3.9	8			1.08	15.5	23	0.17	19.6	8				
	rep 2 24 hr	4.32	18.2	8			0.53	2.4	6			1.63	7.3	9	0.25	44.5	3				
Patient 5	rep 1 Baseline	1.61	14.8	15	1.53	15.2	n/d ^d	-	-	-	n/d ^d	-	-	-	0.47	26.4	46	0.42	23.9		
	rep 1 4 hr	2.05	12.5	14	2.27	16.4	0.39	21.1	11	0.43	19.0	1.49	8.5	22	1.51	6.2	0.32	24.1	26	0.35	18.3
	rep 1 24 hr	2.42	8.5	12	1.91	30.5	0.74	10.8	16	0.68	13.2	2.27	11.4	45	1.69	38.9	0.43	2.9	41	0.33	34.4
	rep 2 Baseline	1.46	17.4	8			n/d	-	-	-	n/d	-	-	-	0.36	14.6	28				
	rep 2 4 hr	2.49	14.9	12			0.48	12.7	14			1.53	4.4	18	0.39	6.1	34				
	rep 2 24 hr	1.40	11.5	12			0.61	5.5	33			1.11	3.6	36	0.23	16.4	18				
Patient 6	rep 1 Baseline	3.71	5.6	13	3.30	14.6	n/d ^d	-	-	-	n/d ^d	-	-	-	0.23	20.8	12	0.22	16.3		
	rep 1 4 hr	3.43	14.5	12	3.31	14.6	0.40	9.1	8	0.39	9.5	0.94	22.5	5	1.02	21.3	0.10	22.2	6	0.10	23.1
	rep 1 24 hr	2.46	7.4	14	2.67	11.6	0.36	17.8	6	0.44	24.1	0.79	5.5	19	1.04	27.1	0.19	4.8	12	0.24	26.6
	rep 2 Baseline	2.88	5.0	18			n/d	-	-	-	n/d	-	-	-	0.20	9.7	28				
	rep 2 4 hr	3.18	16.9	17			0.37	10.6	12			1.10	21.3	7	0.11	28.1	8				
	rep 2 24 hr	2.87	10.0	29			0.52	12.8	21			1.29	7.4	20	0.28	22.7	43				

^a Intra-assay % CV is calculated by the following equation: Stnd. Dev /Avg. Area ratio x 100

^b s/n is calculated by dividing peak height at the apex by 150cps.

^c Inter-assay % CV is calculated based on the average of all 3 technical replicates of both process replicates.

^d n/d indicates no detection of analyte.

Supplemental Table 3. Percent recovery as determined by MRM and clinical immunoassay.

Protein	% Recovery after depletion	
	MRM ^{a, c}	Immunoassay ^{b, c}
NT-proBNP	NM	65
MRP-14	NM	33
BNP32	NM	NM
cTnl	4	NM
CRP	84	61
sCD40L	NM	NM
IL-33	93	NM
cTnT	0.5	16
MPO	NM	40

^a Based upon MRM measurements with std protein in normal plasma

^b Based upon immunoassay measurements in patient plasma.

^c % Recovery is calculated by the following equation:

$(\text{Measured protein conc. after depletion}) / (\text{Measured protein conc. before depletion}) \times 100$

NM indicates not measured

Supplemental Table 4. Results of clinical immuoassays for CRP, cTnT, MPO, MRP14 and NT-proBNP for patients 1-3.

Sample		CRP			cTnT			MPO			MRP-14 ^a			NT-proBNP		
		ng/mL		% Recovery	ng/mL		% Recovery	ng/mL		% Recovery	ng/mL		% Recovery	ng/mL		% Recovery
		Non Depl	Depl		Non Depl	Depl		Non Depl	Depl		Non Depl	Depl		Non Depl	Depl	
Patient 1	0 hr	470.0	320.0	68	< 0.01	< 0.01	-	33.9	17.5	52	12.0	4.2	34.9	0.9	0.6	67.2
	4 hr	480.0	250.0	52	12.58	2.09	17	89.4	48.8	55	11.8	5.7	47.8	1.1	0.7	63.3
	24 hr	16200.0	7080.0	44	4.94	0.85	17	63.9	25.7	40	9.9	6.2	63.0	3.3	1.8	54.2
Patient 2	0 hr	1100.0	810.0	74	< 0.01	< 0.01	-	30.0	15.1	50	5.3	9.3	177.2	0.9	0.6	68.0
	4 hr	1140.0	800.0	70	5.68	0.55	10	148.2	113.3	76	8.8	13.1	148.3	0.9	0.6	67.2
	24 hr	8420.0	6160.0	73	6.52	< 0.01	0	42.2	20.0	47	7.8	12.8	163.0	1.6	1.1	68.9
Patient 3	0 hr	390.0	230.0	59	< 0.01	< 0.01	-	59.7	18.1	30	4.8	26.2	543.6	1.2	0.7	60.9
	4 hr	460.0	380.0	83	1.04	0.19	18	87.7	34.7	40	QNS ^b	14.7	-	1.0	0.7	69.2
	24 hr	7470.0	5580.0	75	4.31	0.70	16	65.5	23.8	36	QNS ^b	12.9	-	1.1	0.7	66.1

^a MRP-14 variability in Immunoassay measurement is presently unknown.

^b QNS indicates quantity not sufficient.