

turbidimetric method, using assays supplied by Randox Laboratories Limited. TC/HDL ratio and apoB/apoA-I ratios were calculated.

HDL profiling was assessed with surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS)^{6,7}.

HDL-associated proteins were measured directly from fresh frozen plasma and non-HDL components were eliminated using anti-apoA-I antibodies. SELDI-TOF-MS analysis was performed with a PBS IIc protein chip reader using an automated data collection protocol within protein-chip software. Data were collected up to 200 kDa. Laser intensity was set in a range from 190 to 220 relative units and the focus mass was set to 28 kDa for anti-apoA-I capture. Protein spectra were measured with about 100 shots at 13 positions per SELDI spot. The sensitivity of detection was set at signal/noise ratio > 5. Statistical analyses were performed using SPSS version 16.0. The differences between groups were tested using independent t test, chi-square test, or Mann-Whitney U test, as appropriate.

RESULTS

Clinical and demographic characteristics of all patients are

shown in Table 1. There were no significant differences between etanercept- and adalimumab-treated patients (data not shown).

Absolute changes in lipid levels were assessed using generalized estimating equation analysis. HDL and apoA did not change in EULAR nonresponders. In the EULAR good responders, however, HDL and apoA-II increased significantly after 1 and 4 months of therapy (Table 2).

At baseline there were no significant differences in HDL profiling between EULAR good responders and nonresponders. In total, 14 out of 133 mass charge markers changed during therapy, of which 4 were associated with SAA-1 and -2 (11092, 11540, 11696, 11750 m/z)⁷. After 1 month of therapy, marker 11750 was significantly higher in nonresponders compared to good responders ($p = 0.036$; Appendix 1 and Appendix 2). After 4 months of therapy, all markers

Table 1. Demographic and clinical characteristics at baseline. Mean values (SD), or mean \pm SD, or count (percentage) are shown unless indicated.

Characteristic	All Patients, n = 100	EULAR Responders, n = 50	EULAR Nonresponders, n = 50
Age, yrs	52 \pm 10	49 \pm 11*	56 \pm 8*
Female, %	87	88	86
Disease duration, yrs	5 (2–13)	5 (2–10)	7 (3–15)
RF-positive, %	67	72	62
Erosive disease, %	66	66	66
DAS28	4.7 \pm 1.0	4.9 \pm 0.8	4.6 \pm 1.2
ESR, mm/h	16 (8–33)	15 (8–24)	18 (8–39)
CRP, mg/l	6 (3–19)	6 (3–21)	6 (3–17)
HAQ	1.1 \pm 0.6	1.1 \pm 0.6	1.1 \pm 0.7
Prior DMARD	2.9 \pm 1.1	2.8 \pm 1.1	2.9 \pm 1.2
MTX use, %	79	86	72
MTX dose, mg/wk	21.5 \pm 6.3	21.4 \pm 5.9	21.5 \pm 6.9
Prednisolone use, %	26	26	26
Prednisolone dose, mg/day	6.7 \pm 4.2	5.2 \pm 3.0	8.2 \pm 4.7
Other DMARD than MTX, %	29	36	22
Cardiovascular risk factors			
Body mass index	25.5 \pm 4.7	24.9 \pm 4.0	26.1 \pm 5.2
Hypertension, %	23	14	22
Diabetes mellitus, %	4	2	2
Current smoking, %	26	26	26
Statin use, %	12	8	16
Lipid profile			
Total cholesterol, mmol/l	5.0 \pm 1.0	4.9 \pm 1.0	5.0 \pm 1.0
Triglycerides, mmol/l	1.3 \pm 0.5	1.2 \pm 0.5	1.3 \pm 0.5
HDL cholesterol, mmol/l	1.3 \pm 0.5	1.2 \pm 0.4	1.3 \pm 0.5
LDL cholesterol, mmol/l	3.3 \pm 0.8	3.3 \pm 0.8	3.4 \pm 0.8
Total cholesterol/HDL ratio	4.4 \pm 1.9	4.4 \pm 2.0	4.4 \pm 1.8
ApoA-I, mg/dl	178 \pm 36	178 \pm 33	179 \pm 39
ApoA-II, mg/dl	30 \pm 7	29 \pm 6	31 \pm 8
ApoB, mg/dl	122 \pm 31	120 \pm 33	125 \pm 29
ApoE, mg/dl	4.4 \pm 2.1	4.7 \pm 2.5	4.1 \pm 1.6
ApoCIII, mg/dl	9.9 \pm 3.3	9.3 \pm 2.9	10.6 \pm 3.6
ApoB/ApoA-I ratio	0.7 \pm 0.3	0.7 \pm 0.3	0.7 \pm 0.3

* Patients with European League Against Rheumatism (EULAR) good response were significantly younger than EULAR nonresponders ($p < 0.001$). IQR: interquartile range; RF: rheumatoid factor; DAS28: Disease Activity Score of 28 joints; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; DMARD: disease-modifying antirheumatic drugs; MTX: methotrexate; HAQ: Health Assessment Questionnaire; HDL: high-density lipoprotein; LDL: low-density lipoprotein; apo: apolipoproteins.

Table 2. Changes in lipid levels according to EULAR response at 1 and 4 months of therapy.

	Baseline	1 Month	4 Months
EULAR nonresponders			
Total cholesterol, mmol/l	5.03 ± 0.96	5.09 ± 1.10	4.98 ± 1.03
Triglycerides, mmol/l	1.32 ± 0.54	1.29 ± 0.53	1.23 ± 0.49
HDL cholesterol, mmol/l	1.29 ± 0.50	1.30 ± 0.51	1.30 ± 0.46
LDL cholesterol, mmol/l	3.40 ± 0.76	3.42 ± 0.82	3.34 ± 0.78
Total cholesterol/HDL ratio	4.39 ± 1.76	4.41 ± 1.69	4.23 ± 1.65
ApoA-I, mg/dl	1.79 ± 0.39	1.78 ± 0.32	1.79 ± 0.31
ApoA-II, mg/dl	0.31 ± 0.08	0.32 ± 0.09	0.31 ± 0.08
ApoB, mg/dl	1.25 ± 0.29	1.26 ± 0.33	1.21 ± 0.28
ApoE, mg/dl	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.01
Apo-CIII, mg/dl	0.11 ± 0.04	0.11 ± 0.04	0.10 ± 0.03
ApoB/ApoA-I ratio	0.75 ± 0.34	0.73 ± 0.21	0.74 ± 0.31
EULAR good responders			
Total cholesterol, mmol/l	4.88 ± 0.96	4.78 ± 0.88	4.95 ± 1.02
Triglycerides, mmol/l	1.19 ± 0.53	1.18 ± 0.60	1.26 ± 0.60
HDL cholesterol, mmol/l	1.24 ± 0.40	1.27 ± 0.44*	1.30 ± 0.39*
LDL cholesterol, mmol/l	3.30 ± 0.83	3.16 ± 0.75	3.26 ± 0.85
Total cholesterol/HDL ratio	4.38 ± 1.98	4.16 ± 1.53*	4.16 ± 1.66*
ApoA-I, g/l	1.78 ± 0.33	1.75 ± 0.25	1.78 ± 0.33
ApoA-II, g/l	0.29 ± 0.06	0.31 ± 0.06*	0.31 ± 0.06*
ApoB, g/l	1.20 ± 0.33	1.14 ± 0.30*	1.18 ± 0.31*
ApoE, g/l	0.05 ± 0.03	0.04 ± 0.03	0.05 ± 0.02
Apo-CIII, g/l	0.09 ± 0.03	0.09 ± 0.03	0.10 ± 0.03
ApoB/ApoA-I ratio	0.70 ± 0.26	0.67 ± 0.21*	0.67 ± 0.23*

* Generalized estimating equation analyses were used to test for longitudinal changes in lipids. There were significant differences at 1 and 4 months of therapy compared to baseline for HDL ($p = 0.006$), TC/HDL ratio ($p = 0.014$), ApoA-II ($p = 0.004$), ApoB ($p = 0.013$), and ApoB/ApoA-I ratio ($p = 0.001$). EULAR: European League Against Rheumatism; HDL: high-density lipoprotein; LDL: low-density lipoprotein; apo: apolipoproteins.

representing SAA-1 and -2 were significantly higher in nonresponders than in good responders (all $p < 0.05$).

In addition, dynamics in markers associated with SAA-1 and -2 were assessed in EULAR good and nonresponders. SAA did not change in nonresponders at both 1 and 4 months of therapy. In contrast, in good responders after 1 month of therapy, marker 11750 decreased significantly compared to baseline ($p < 0.05$), whereas a clear decreasing trend in signal was observed in the other 3 markers. After 4 months of therapy, markers 11092, 11540, 11696 ($p < 0.05$), and 11750 ($p < 0.001$) decreased significantly compared to baseline (Figure 1).

DISCUSSION

Our study reaffirms the beneficial effect of TNF inhibitors on structural changes in HDL protein composition^{5,8} and demonstrates, to our knowledge for the first time, that these findings apply only to RA patients with a good EULAR response, because HDL profiling did not change in EULAR nonresponders.

There is strong evidence that HDL is atheroprotective, although during inflammation the HDL protein composition changes, rendering HDL to a proinflammatory and atherogenic state⁹. For example, in response to inflammation,

SAA replaces apoA-I from the HDL particle, resulting in a decreased ability of HDL to protect LDL from oxidation¹⁰.

The CV risk reduction by TNF inhibitors in patients with RA appears not to be mediated by quantitative changes of the lipids, because the effect on the atherogenic index is very modest³. Our data suggest that atheroprotective changes in HDL particle are the consequence of inflammation reduction by potent anti-TNF therapy. This was illustrated by the mass charge markers representing HDL-associated SAA-1 and -2, because these markers decreased significantly in good EULAR responders but did not change in EULAR nonresponders.

Inherent to the design of our study, selection bias should be taken into account. Moreover, the SELDI-TOF-MS approach was used to assess HDL profiling because of its strong applicability in high-throughput sample measurement. This method has limitations such as lack of direct protein identification⁶. However, protein profiling and not protein identification was the major objective in our study, justifying this approach.

Despite the limitations, our study clearly demonstrates that successful reduction of inflammation in RA patients with anti-TNF therapy restores the HDL protein composition to an atheroprotective profile, underscoring the

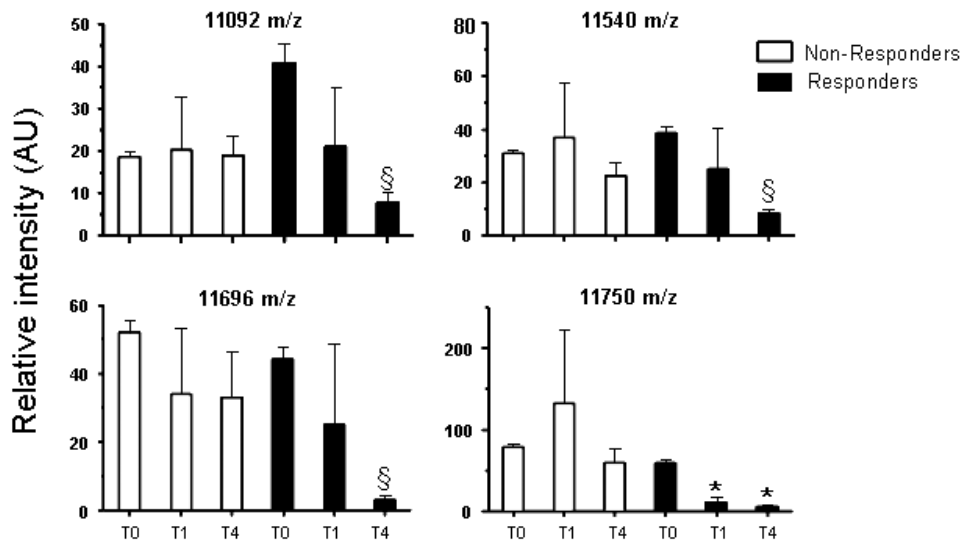


Figure 1. Dynamics of the serum amyloid A (SAA)-1 and -2 markers stratified for EULAR response. The mean values (\pm 1 SD) of the 4 markers correspond with the SAA isoforms as present in the nonresponders and responders. The corresponding timepoints are baseline (T0), 1 month (T1), and after 4 months after the start of therapy (T4). § p < 0.05, * p < 0.001 using 1-way ANOVA with Bonferroni multiple comparison. AU: arbitrary unit.

need for tight inflammation control in patients with RA, to decrease the CV risk. However, it remains to be determined in longterm prospective studies whether these changes in the HDL protein composition translate into meaningful reduction of CV risk in patients with RA.

ACKNOWLEDGMENT

We are grateful to the Division of Research and Education of the Jan van Breemen Research Institute I Reade, which receives support from the Dutch Arthritis Foundation, for help in conducting the study. We thank the research nurses Marga Kammeijer-Rippen, Anne-Marie Abrahams, Martine Kos, and Astrid Twisk for performing clinical assessments. We also thank Margret de Koning for preparation of the assays.

REFERENCES

1. Avina-Zubieta JA, Choi HK, Sadatsafavi M, Etminan M, Esdaile JM, Lacaille D. Risk of cardiovascular mortality in patients with rheumatoid arthritis: A meta-analysis of observational studies. *Arthritis Rheum* 2008;59:1690-7.
2. Westlake SL, Colebatch AN, Baird J, Curzen N, Kiely P, Quinn M, et al. Tumour necrosis factor antagonists and the risk of cardiovascular disease in patients with rheumatoid arthritis: A systematic literature review. *Rheumatology* 2011;50:518-31.
3. Van Sijl AM, Peters MJ, Knol DL, de Vet RH, Sattar N, Dijkmans BA, et al. The effect of TNF-alpha blocking therapy on lipid levels in rheumatoid arthritis: A meta-analysis. *Semin Arthritis Rheum* 2011;41:393-400.
4. Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A, et al. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA* 2009;302:1993-2000.
5. van Eijk IC, de Vries MK, Levels JH, Peters MJ, Huizer EE, Dijkmans BA, et al. Improvement of lipid profile is accompanied by atheroprotective alterations in high-density lipoprotein composition upon tumor necrosis factor blockade: A prospective cohort study in ankylosing spondylitis. *Arthritis Rheum* 2009;60:1324-30.
6. Levels JH, Bleijlevens B, Rezaee F, Aerts JM, Meijers JC. SELDI-TOF mass spectrometry of high-density lipoprotein. *Proteome Sci* 2007;5:15.
7. Levels JH, Geurts P, Karlsson H, Maree R, Ljunggren S, Fornander L, et al. High-density lipoprotein proteome dynamics in human endotoxemia. *Proteome Sci* 2011;9:34.
8. Watanabe J, Charles-Schoeman C, Milao Y, Elashoff D, Lee YY, Katselis G, et al. Proteomic profiling following immunoaffinity capture of HDL: Association of acute phase proteins and complement factors with pro-inflammatory HDL in rheumatoid arthritis. *Arthritis Rheum* 2012;64:1828-37.
9. Heinecke JW. The HDL proteome: A marker — and perhaps mediator — of coronary artery disease. *J Lipid Res* 2009;50 Suppl:S167-71.
10. Rohrer L, Hersberger M, von Eckardstein A. High density lipoproteins in the intersection of diabetes mellitus, inflammation and cardiovascular disease. *Curr Opin Lipidol* 2004;15:269-78.

Appendix 1. Details of mass charge markers in nonresponders (signal in relative units).

Marker	Non responders (Signal in RU)												
	M/Z (Da)	N	Baseline			1 month			4 months				
			Minimum	Maximum	Mean	N	Minimum	Maximum	Mean	N	Minimum	Maximum	Mean
1	3011.00	40.00	-0.55	20.12	1.46	42.00	-0.06	3.25	0.92	42.00	-0.20	4.28	0.08
2	3042.00	40.00	0.00	38.67	4.03	41.00	0.00	8.55	1.47	42.00	-0.05	14.28	2.37
3	3084.00	40.00	-0.15	42.88	11.63	41.00	0.08	49.76	10.14	42.00	0.00	35.02	9.98
4	3221.00	40.00	-0.40	153.44	38.09	41.00	0.00	314.44	53.62	42.00	0.10	209.57	47.80
5	3324.00	40.00	0.16	427.76	127.63	41.00	0.05	612.00	157.22	42.00	0.04	485.07	139.24
6	3380.00	40.00	-0.19	18.21	4.32	41.00	-0.02	126.78	10.45	42.00	0.00	627.97	19.47
7	3440.00	40.00	-0.09	25.77	5.18	41.00	0.10	130.81	16.42	42.00	-0.12	645.47	23.93
8	3495.00	40.00	-0.62	11.48	0.93	41.00	-0.15	27.84	1.48	42.00	-0.18	139.20	3.96
9	3984.00	40.00	-0.15	42.48	2.21	41.00	0.07	21.85	2.02	42.00	-0.06	3.59	0.54
10	3984.00	40.00	0.00	97.22	25.08	41.00	0.00	67.68	22.03	42.00	0.00	95.39	16.25
11	4186.00	40.00	-0.05	90.29	8.81	41.00	0.00	37.77	5.90	42.00	0.00	37.02	4.78
12	4331.00	40.00	0.00	117.31	13.08	41.00	-0.04	113.20	11.88	42.00	0.00	25.37	3.92
13	4483.00	40.00	1.20	163.04	66.42	41.00	0.02	322.99	80.30	42.00	1.90	234.50	62.65
14	4722.00	40.00	0.05	202.21	21.32	41.00	0.00	207.17	26.58	42.00	0.00	149.64	25.92
15	4837.00	40.00	0.34	173.96	28.93	41.00	0.00	622.04	48.06	42.00	0.00	118.33	18.51
16	5140.00	40.00	-0.06	42.94	2.54	41.00	0.03	15.16	3.92	42.00	0.00	13.79	2.28
17	5403.00	40.00	-0.36	39.56	4.86	41.00	-0.07	63.03	5.00	42.00	0.00	50.95	4.06
18	5798.00	40.00	0.00	16.45	3.07	41.00	0.00	53.54	7.49	42.00	-0.16	47.96	4.23
19	5845.00	40.00	0.00	139.55	14.72	41.00	0.00	74.45	7.38	42.00	0.00	111.74	9.81
20	6297.00	40.00	0.36	125.08	32.75	41.00	0.00	148.22	37.08	42.00	0.00	155.15	30.32
21	6480.00	40.00	54.23	283.98	116.56	41.00	291.04	5078.09	1337.31	42.00	394.88	3088.66	1205.89
22	6658.00	40.00	1178.93	4769.05	2348.48	41.00	902.27	7045.20	2542.65	42.00	644.55	4907.08	2284.19
23	6868.00	40.00	212.57	747.38	414.72	41.00	112.65	1031.31	458.83	42.00	105.58	897.38	405.01
24	7099.00	40.00	0.93	112.24	47.47	41.00	0.00	289.08	58.63	42.00	12.14	202.27	52.92
25	7051.00	40.00	0.05	185.08	27.56	41.00	0.00	130.15	17.52	42.00	0.00	299.61	21.41
26	7805.00	40.00	0.00	337.83	84.05	41.00	0.00	491.80	72.77	42.00	1.08	345.21	72.41
27	7985.00	40.00	-0.31	66.54	7.35	41.00	0.00	148.13	6.87	42.00	0.00	64.05	4.68
28	8100.00	40.00	-0.11	257.29	16.59	41.00	-0.01	59.86	3.29	42.00	-0.02	111.90	6.00
29	8181.00	40.00	0.00	222.98	25.77	41.00	0.00	294.60	17.11	42.00	0.11	86.53	17.34
30	8250.00	40.00	45.21	1421.48	204.81	41.00	36.62	593.24	152.41	42.00	49.65	534.30	157.81
31	8398.00	40.00	0.00	441.20	28.30	41.00	0.00	269.09	15.86	42.00	-0.02	216.21	14.88
32	8480.00	40.00	2.43	199.45	27.66	41.00	0.21	103.90	16.52	42.00	0.00	123.72	21.17
33	8181.00	40.00	3.30	1178.40	215.14	41.00	18.60	918.88	187.83	42.00	10.23	546.26	154.13
34	8731.00	40.00	80.16	1841.03	437.52	41.00	129.54	1147.77	388.92	42.00	137.89	808.65	360.05
35	8807.00	40.00	5.34	1056.97	171.73	41.00	5.14	418.45	146.03	42.00	13.27	392.00	135.84
36	8858.00	40.00	74.43	2012.80	449.27	41.00	142.89	1554.17	423.91	42.00	130.87	712.75	379.79
37	8960.00	40.00	289.85	6380.85	1251.10	41.00	340.32	2988.83	1165.47	42.00	346.76	2661.80	1056.95
38	9059.00	40.00	5.82	901.55	174.96	41.00	0.00	426.67	163.09	42.00	2.51	314.67	146.46
39	9174.00	40.00	203.17	3888.15	828.99	41.00	266.55	2226.17	724.54	42.00	240.87	1351.83	690.26
40	9336.00	40.00	0.00	373.85	347.84	41.00	14.16	933.55	223.86	42.00	3.62	2797.90	275.72
41	9100.00	40.00	3.88	4034.04	1240.02	41.00	14.45	286.26	124.63	42.00	0.00	287.88	119.67
42	9397.00	40.00	3.36	62.55	14.82	41.00	4.60	33.57	12.93	42.00	1.83	25.59	10.96
43	9399.00	40.00	1.71	47.99	8.92	41.00	0.00	22.22	6.89	42.00	1.35	51.79	7.99
44	9402.00	40.00	4.45	320.87	52.51	41.00	1.48	962.21	44.88	42.00	2.73	191.89	34.10
45	9418.00	40.00	58.84	715.63	174.58	41.00	50.47	367.22	179.87	42.00	78.52	438.16	180.70
46	9469.00	40.00	161.91	2067.89	555.81	41.00	214.51	1281.97	533.15	42.00	207.73	903.67	498.28
47	9558.00	40.00	49.71	956.24	237.17	41.00	104.47	836.27	243.66	42.00	52.57	775.84	229.17
48	9682.00	40.00	33.87	454.45	148.54	41.00	4.56	451.64	165.22	42.00	5.15	274.65	151.85
49	9782.00	40.00	17.22	336.30	152.37	41.00	12.67	634.35	183.80	42.00	27.86	395.53	168.12
50	9839.00	40.00	0.00	377.05	103.59	41.00	0.00	632.31	101.13	42.00	0.00	364.23	114.71
51	9930.00	40.00	0.00	138.53	50.51	41.00	0.00	358.97	53.90	42.00	2.65	171.27	57.37
52	9981.00	40.00	2.54	240.53	64.53	41.00	0.07	214.89	85.77	42.00	20.84	328.82	117.48
53	10117.00	40.00	2.21	1641.17	159.78	41.00	0.96	520.49	115.44	42.00	20.05	1310.68	172.07
54	10346.00	40.00	1.28	319.40	45.83	41.00	2.10	135.24	39.41	42.00	0.00	252.80	38.86
55	10350.00	40.00	0.00	82.70	23.29	41.00	-0.15	89.57	31.26	42.00	0.06	214.00	33.42
56	10730.00	40.00	2.89	172.07	72.82	41.00	0.77	486.68	74.19	42.00	0.77	322.02	79.68
57	10889.00	40.00	0.49	705.72	47.55	41.00	0.00	706.49	28.13	42.00	0.16	187.82	21.41
58	10960.00	40.00	78.90	607.23	159.85	41.00	61.59	315.86	148.35	42.00	61.88	409.59	146.90
59	11992.00	40.00	0.00	180.89	18.75	41.00	0.00	513.13	20.24	42.00	0.36	1330.09	190.02
60	11212.00	40.00	9.36	328.77	48.54	41.00	0.71	414.07	50.52	42.00	10.01	171.75	44.96
61	11299.00	40.00	-0.14	210.48	16.20	41.00	-0.01	486.34	18.88	42.00	-0.03	95.09	10.31
62	11427.00	40.00	0.81	127.46	26.51	41.00	0.12	1030.05	38.96	42.00	-0.14	181.01	19.51
63	11493.00	40.00	0.00	195.16	20.21	41.00	0.00	277.47	17.46	42.00	0.00	177.17	16.72
64	11540.00	40.00	-0.03	240.17	30.87	41.00	0.41	820.64	26.84	42.00	0.00	138.92	22.86
65	11590.00	40.00	0.00	319.18	50.76	41.00	0.02	1476.23	67.75	42.00	0.00	255.66	41.34
66	11698.00	40.00	-0.08	667.84	52.11	41.00	-0.12	768.65	34.06	42.00	-0.06	622.51	33.15
67	11790.00	40.00	-0.14	867.85	79.05	41.00	0.00	3702.51	132.53	42.00	-0.21	486.91	59.69
68	11973.00	40.00	2.45	211.60	50.56	41.00	12.72	134.53	59.28	42.00	0.60	210.80	57.40
69	12202.00	40.00	38.68	288.05	127.60	41.00	46.01	571.01	139.58	42.00	55.90	425.83	143.82
70	12431.00	40.00	1.55	163.19	63.10	41.00	32.12	120.48	70.24	42.00	0.42	444.90	81.35
71	12523.00	40.00	0.47	67.47	29.79	41.00	0.31	143.20	36.15	42.00	8.86	257.70	43.42
72	12662.00	40.00	27.84	369.84	117.05	41.00	25.62	191.45	110.02	42.00	375.21	110.36	
73	12926.00	40.00	18.01	523.25	170.64	41.00	18.58	459.40	149.84	42.00	24.84	796.66	166.78
74	13145.00	40.00	10.52	203.34	56.07	41.00	0.38	293.96	58.49	42.00	0.23	67.07	77.27
75	13524.00	40.00	0.00	127.77	13.41	41.00	0.04	87.31	13.45	42.00	0.01	74.67	17.39
76	13955.00	40.00	10.46	2095.50	169.70	41.00	13.77	2148.99	64.72	42.00	13.03	131.86	53.92
77	14115.00	40.00	781.68	2375.07	1121.35	41.00	786.62	1489.83	1083.54	42.00	799.93	1384.03	1029.54
78	14217.00	40.00	718.24	1567.46	1025.97	41.00	725.82	1523.28	1042.93	42.00	795.43	1444.46	1010.11
79	14599.00	40.00	360.33	827.19	532.83	41.00	308.54	896.44	544.30	42.00	378.66	774.57	529.09
80	14999.00	40.00	21.71	194.40	63.87	41.00	0.77	22.68	17.08	42.00	0.00	108.40	56.51
81	15702.00	40.00	-0.24	213.76	11.59	41.00	-0.04	27.08	6.01	42.00	0.00	59.51	8.02
82	15958.00	40.00	-0.21	68.70	7.55	41.00	-0.01	52.26	6.85	42.00	-0.07	20.86	6.54
83	16877.00	40.00	0.00	224.05	12.82	41.00	-0.04	85.10	5.35	42.00	-0.14	21.35	5.14

Appendix 2. Details of mass charge markers in responders (signal in relative units).

Marker	Responders (Signal in RU)													
	MZ (Da)	N	Baseline			1 month			4 months					
			Minimum	Maximum	Mean	N	Minimum	Maximum	Mean	N	Minimum	Maximum	Mean	
1	3011.00	28.00	0.00	4.43	0.94	25.00	0.21	0.82	0.56	21.00	0.00	0.25	11.72	1.15
2	3042.00	28.00	0.00	23.03	3.08	25.00	0.00	18.85	2.56	21.00	-0.32	6.45	1.34	
3	3084.00	28.00	0.00	30.49	10.29	25.00	-0.25	21.98	7.57	21.00	-0.10	29.85	8.36	
4	3221.00	28.00	1.54	264.25	53.94	25.00	0.81	159.40	47.38	21.00	3.89	157.59	53.50	
5	3234.00	28.00	51.08	338.52	144.79	25.00	36.45	340.32	136.93	21.00	52.41	359.97	138.86	
6	3380.00	28.00	-0.01	64.13	7.86	25.00	0.08	15.81	6.41	21.00	-0.20	30.12	5.68	
7	3440.00	28.00	-0.10	109.57	11.62	25.00	-0.02	23.75	4.44	21.00	-0.09	52.52	6.85	
8	3485.00	28.00	-0.15	26.29	1.53	25.00	-0.25	9.25	0.85	21.00	-0.08	6.06	1.28	
9	3864.00	28.00	0.00	22.80	2.15	25.00	0.00	123.44	9.10	21.00	-0.13	60.99	4.60	
10	3964.00	28.00	0.14	76.34	19.86	25.00	0.00	152.65	24.62	21.00	0.15	52.02	20.96	
11	4186.00	28.00	-0.06	30.62	6.53	25.00	0.00	26.50	5.77	21.00	0.00	19.16	4.91	
12	4331.00	28.00	0.00	35.86	4.75	25.00	-0.05	93.32	7.76	21.00	0.00	49.65	5.14	
13	4463.00	28.00	0.00	139.14	57.28	25.00	1.41	230.36	64.69	21.00	0.83	163.58	66.45	
14	4722.00	28.00	0.00	89.56	21.32	25.00	-0.31	116.35	27.66	21.00	0.24	149.22	27.89	
15	4837.00	28.00	-0.57	101.80	24.00	25.00	0.45	736.13	51.30	21.00	0.00	106.54	23.41	
16	5140.00	28.00	-0.02	64.18	4.33	25.00	0.00	36.50	3.88	21.00	0.00	16.68	2.03	
17	5403.00	28.00	-0.02	49.09	6.21	25.00	-0.01	48.66	5.32	21.00	0.00	183.28	12.05	
18	5786.00	28.00	0.00	22.67	4.25	25.00	0.00	27.28	4.01	21.00	0.00	21.14	5.16	
19	5845.00	28.00	-0.07	70.46	7.86	25.00	0.00	40.87	7.37	21.00	0.00	404.48	23.69	
20	6297.00	28.00	0.66	76.41	28.82	25.00	0.00	76.37	25.00	21.00	0.61	89.52	26.03	
21	6460.00	28.00	709.44	4098.74	1424.51	25.00	587.60	2778.14	1344.18	21.00	537.49	2763.11	1275.60	
22	6856.00	28.00	1337.04	4752.88	2486.71	25.00	1265.69	4863.39	2428.27	21.00	1036.00	5184.15	2338.90	
23	6860.00	28.00	120.36	694.35	404.50	25.00	165.19	1223.51	423.57	21.00	171.32	893.53	423.03	
24	7099.00	28.00	0.00	155.43	50.75	25.00	0.00	291.36	52.74	21.00	0.92	250.17	56.33	
25	7651.00	28.00	0.00	133.85	24.46	25.00	0.04	304.92	37.15	21.00	0.00	266.55	29.80	
26	7805.00	28.00	11.75	291.14	67.27	25.00	0.25	587.47	86.84	21.00	0.52	428.46	106.23	
27	7985.00	28.00	2.18	48.33	6.13	25.00	0.00	49.27	5.57	21.00	0.00	209.34	11.50	
28	8100.00	28.00	-0.08	58.99	6.69	25.00	0.00	38.51	4.24	21.00	0.04	36.15	5.28	
29	8183.00	28.00	0.00	188.82	22.48	25.00	0.00	378.88	25.46	21.00	0.00	218.80	23.98	
30	8250.00	28.00	52.21	147.77	33.33	25.00	22.52	427.33	119.12	21.00	11.63	303.67	123.83	
31	8386.00	28.00	0.35	176.16	16.00	25.00	0.05	207.16	15.11	21.00	0.12	51.48	10.29	
32	8460.00	28.00	2.59	199.49	24.33	25.00	0.00	207.52	21.87	21.00	0.21	113.10	17.94	
33	8618.00	28.00	5.08	866.63	170.89	25.00	0.77	1411.73	174.25	21.00	25.03	413.73	145.51	
34	8731.00	28.00	37.05	684.76	433.33	25.00	116.96	1770.78	393.52	21.00	92.44	661.24	352.64	
35	8907.00	28.00	4.51	330.09	133.90	25.00	14.07	920.11	160.04	21.00	22.91	398.99	144.03	
36	8856.00	28.00	105.27	944.85	414.85	25.00	111.11	2064.89	430.48	21.00	103.71	869.39	356.43	
37	8960.00	28.00	406.18	2406.98	1034.42	25.00	277.33	4446.20	1115.11	21.00	272.31	2435.66	979.53	
38	8959.00	28.00	2.18	676.77	161.61	25.00	11.42	966.11	165.69	21.00	4.14	351.97	122.27	
39	9174.00	28.00	188.70	1817.01	700.87	25.00	164.69	2875.14	714.22	21.00	164.69	1622.66	619.58	
40	9398.00	28.00	26.57	3066.70	422.62	25.00	14.77	2813.62	438.22	21.00	6.27	2691.94	337.25	
41	9394.00	28.00	190.99	1003.97	250.97	25.00	24.54	917.80	241.80	21.00	7.20	447.61	101.40	
42	9397.00	28.00	5.10	35.42	12.54	25.00	0.00	80.94	13.90	21.00	0.00	36.43	12.08	
43	9399.00	28.00	2.57	32.71	7.55	25.00	0.00	14.59	6.10	21.00	2.68	37.55	7.65	
44	9402.00	28.00	2.54	140.38	41.93	25.00	4.32	168.06	40.48	21.00	4.94	254.75	44.08	
45	9418.00	28.00	55.13	1027.96	194.96	25.00	54.50	583.37	175.65	21.00	64.98	331.58	169.54	
46	9469.00	28.00	214.24	833.26	489.78	25.00	217.90	1794.13	513.71	21.00	185.02	1249.74	498.24	
47	9558.00	28.00	88.70	1231.53	264.74	25.00	59.44	1123.53	286.80	21.00	43.10	1134.58	248.24	
48	9692.00	28.00	36.85	346.29	142.74	25.00	16.52	266.81	137.36	21.00	19.89	362.97	139.68	
49	9762.00	28.00	43.44	353.66	146.88	25.00	0.87	549.82	150.93	21.00	0.00	500.64	136.09	
50	9839.00	28.00	0.00	192.61	62.33	25.00	3.44	156.31	57.66	21.00	8.31	428.14	87.23	
51	9930.00	28.00	5.87	143.25	55.93	25.00	0.00	164.77	37.09	21.00	1.21	170.70	44.14	
52	9961.00	28.00	1.17	412.27	116.53	25.00	0.00	412.25	99.04	21.00	0.00	448.18	36.17	
53	10117.00	28.00	11.23	2019.12	245.45	25.00	26.19	1702.81	238.33	21.00	8.30	1385.92	199.99	
54	10346.00	28.00	1.52	436.09	66.48	25.00	0.09	239.92	58.92	21.00	0.22	621.25	68.09	
55	10550.00	28.00	0.30	154.77	23.59	25.00	0.77	75.32	26.38	21.00	0.00	148.18	36.17	
56	10703.00	28.00	0.50	199.28	104.29	25.00	0.91	296.06	81.35	21.00	19.10	183.66	90.05	
57	10869.00	28.00	0.50	281.65	27.35	25.00	0.28	1106.82	56.04	21.00	0.39	157.00	19.73	
58	10960.00	28.00	89.03	637.23	164.97	25.00	74.81	405.79	150.80	21.00	77.52	260.49	141.16	
59	11092.00	28.00	584.76	481.67	403.77	25.00	28.21	389.21	213.00	21.00	0.00	384.44	74.83	
60	11212.00	28.00	5.99	247.94	49.68	25.00	2.10	78.62	35.42	21.00	5.30	279.93	45.70	
61	11299.00	28.00	-0.04	161.36	21.07	25.00	-0.15	42.96	4.48	21.00	-0.12	13.04	3.52	
62	11427.00	28.00	0.00	359.11	40.55	25.00	0.00	82.28	10.92	21.00	0.00	44.14	8.94	
63	11482.00	28.00	0.00	131.74	13.74	25.00	0.04	124.51	9.84	21.00	0.00	63.01	5.83	
64	11540.00	28.00	0.00	227.82	38.37	25.00	0.29	393.44	24.90	21.00	0.00	16.75	8.71	
65	11590.00	28.00	0.34	229.71	35.05	25.00	0.00	87.05	13.90	21.00	1.12	23.79	11.79	
66	11696.00	28.00	-0.09	379.73	44.12	25.00	-0.06	588.82	25.00	21.00	-0.20	386.39	23.06	
67	11728.00	28.00	0.16	471.67	59.42	25.00	-0.06	150.08	10.60	21.00	-0.27	32.43	6.75	
68	11973.00	28.00	0.15	247.03	48.49	25.00	15.65	170.08	45.12	21.00	1.02	55.25	25.93	
69	12220.00	28.00	75.82	463.92	140.28	25.00	62.07	328.22	135.97	21.00	58.97	171.39	119.60	
70	12431.00	28.00	4.56	211.13	63.88	25.00	6.55	222.76	69.29	21.00	0.42	119.11	31.87	
71	12523.00	28.00	7.48	91.15	36.03	25.00	1.98	253.51	40.18	21.00	11.13	69.30	31.00	
72	12682.00	28.00	29.92	215.79	91.45	25.00	28.16	3035.85	200.96	21.00	5.95	256.94	76.80	
73	12926.00	28.00	37.80	705.29	185.92	25.00	41.13	426.29	165.21	21.00	29.04	501.16	123.33	
74	13145.00	28.00	8.17	84.23	34.23	25.00	0.85	300.69	75.30	21.00	0.00	199.78	58.94	
75	13524.00	28.00	-0.10	87.57	16.71	25.00	0.19	56.74	15.65	21.00	0.00	59.65	11.78	
76	13955.00	28.00	23.36	776.87	119.89	25.00	19.93	777.89	91.46	21.00	15.77	158.71	63.17	
77	14115.00	28.00	857.19	1425.43	1075.63	25.00	804.81	1404.77	1033.21	21.00	805.85	1892.53	1104.85	
78	14217.00	28.00	198.32	1472.87	1005.09	25.00	826.14	1358.63	1035.07	21.00	787.31	1716.76	1073.11	
79	14559.00	28.00	331.05	897.25	510.52	25.00	338.30	781.68	539.92	21.00	340.72	1099.83	577.10	
80	15199.00	28.00	21.05	117.75	57.56	25.00	16.38	84.01	54.01	21.00	32.45	162.24	66.44	
81	15702.00	28.00	0.07	33.94	8.87	25.00	0.25	81.51	11.37	21.00	0.00			