

Title. Sequential serum metabolomic profiling after radiofrequency ablation of hepatocellular carcinoma reveals different response patterns according to aetiology

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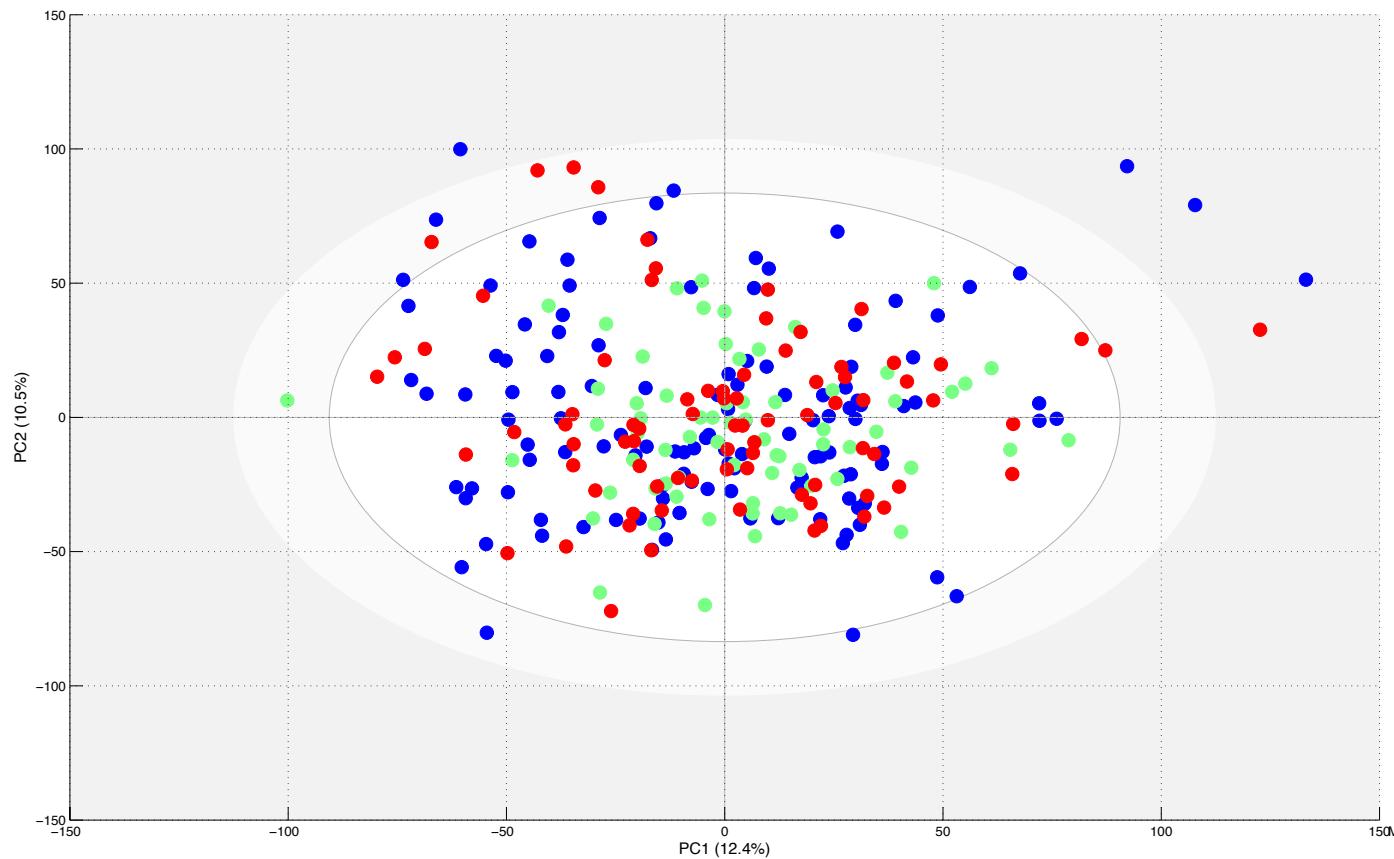


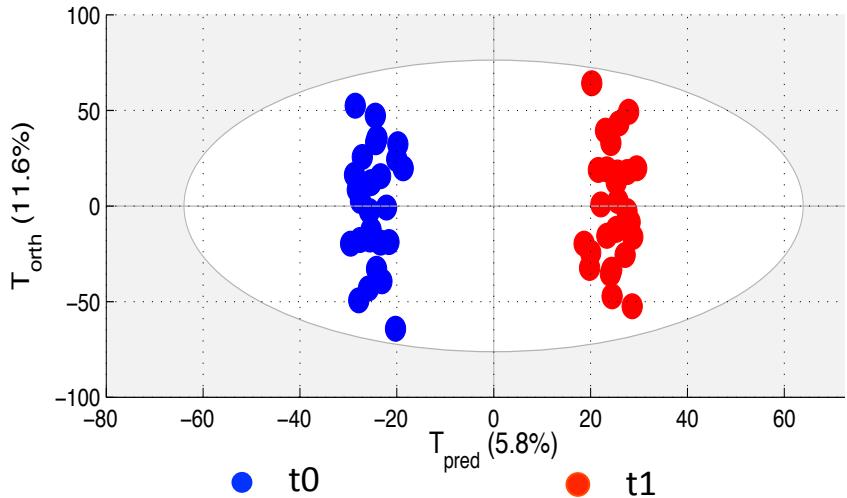
Fig. S1. PCA score plot of the 273 samples obtained from the sera of HCC patients pre-RFA (blue), 1 day post-RFA intervention (green) and after 1-4 months post-RFA (red). Four samples were considered as outliers and excluded for the following analysis.

Table S1. Risk factors for HCC recurrence after RFA procedure. HR, hazard ratio; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase.

	Cox univariate analysis	Cox multivariate analysis
Viral-HCC	HR=1.9 [1.1-3.4] <i>P</i> =0.02	HR=1.8 [1.01-3.4] <i>P</i> =0.04
AST>48 IU/mL	HR=1.8 [1.03-3.3] <i>P</i> =0.03	—
ALT>36 IU/mL	HR=2.1 [1.1-3.8] <i>P</i> =0.01	—
GGT>114 IU/mL	HR=2.6 [1.4-4.9] <i>P</i> =0.002	HR=2.6 [1.4-4.8] <i>P</i> =0.003

A

Non Viral-HCC patients

**B**

Viral-HCC patients

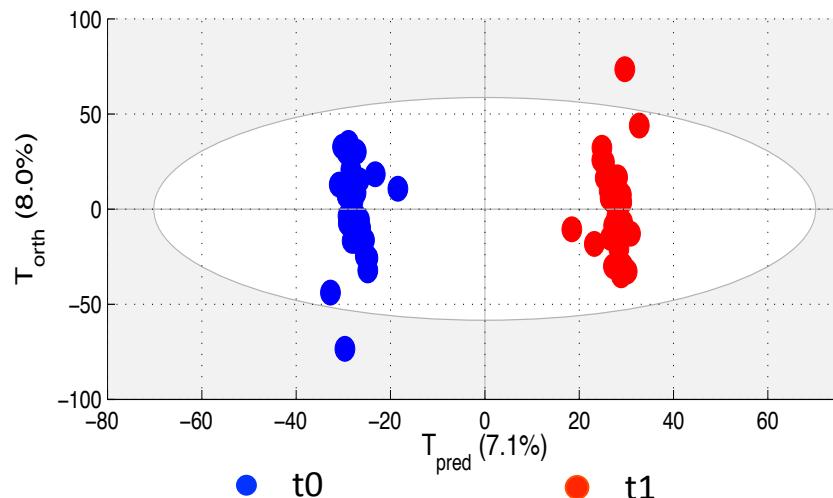
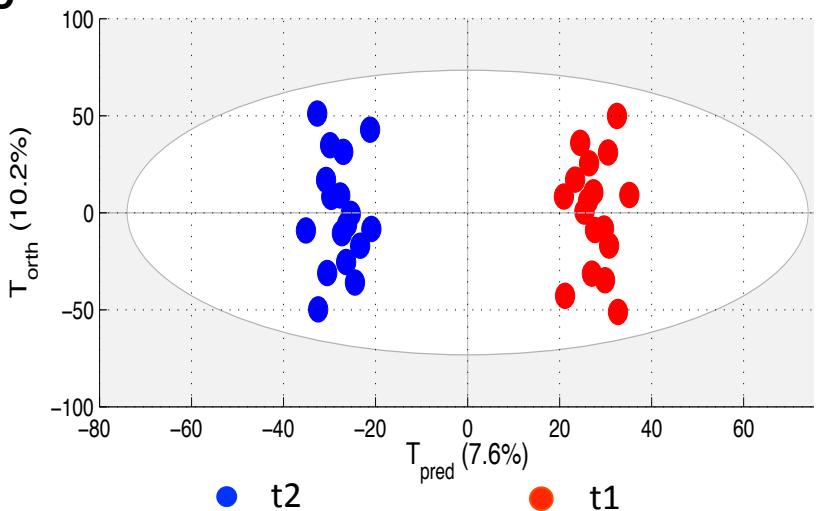
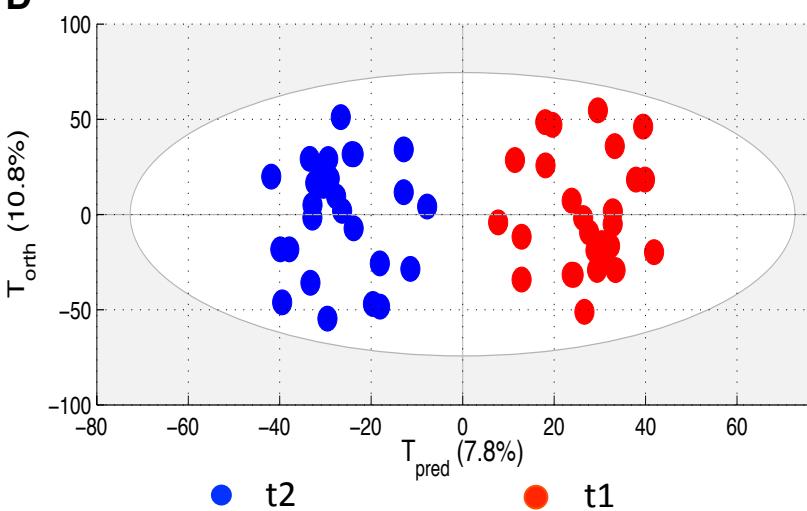
**C****D**

Fig. S2. Multilevel OPLS-DA score plots of the within-subject variation obtained from the sera of HCC patients pre-RFA (t0) and post-RFA intervention (t1) comparing non-viral HCC patients (A) (27 patients) and viral HCC patients (B) (34 patients). Panels C and D represent score plots of the within-subject variation obtained from the sera taken from one to four months after intervention (t2) and post-RFA intervention (t1) comparing non-viral HCC patients (C) (17 patients) and viral HCC patients (D) (27 patients).

Table S2 : Discriminant metabolites observed in sera of non-viral and viral HCC patients by ^1H NMR spectroscopy, according to the loading plot in the pre-ablation period (t0) group and in the one-day post-ablation (t1) group and according to the loading plot in the one from four months post-ablation period (t2) group and in the one-day post-ablation (t1) group.

Metabolite	Chemical shift (ppm) and multiplicity [#]	<u>t0 vs t1</u>		<u>t2 vs t1</u>	
		Non-Viral (A)	Viral (B)	Non-Viral (C)	Viral (D)
I. Metabolites affected by the RFA intervention					
2	Isoleucine	0.93 ^t ; 1.01 ^d	7.4x10 ⁻⁸	1.1x10 ⁻⁷	1.7x10 ⁻⁷
5	Lactate	1.32 ^d ; 4.11 ^q	8.1x10 ⁻⁵	8.8x10 ⁻⁷	4.4x10 ⁻⁵
9	Glutamine	2.15 ^m ; 2.44 ^m	1.9x10 ⁻⁶	1.1x10 ⁻⁸	4.0x10 ⁻³
17	PC/GPC	3.22 ^s	4.3x10 ⁻⁶	1.2x10 ⁻⁸	3.3x10 ⁻⁹
22	3-phenylpropionate	7.25 ^t ; 7.35 ^d	7.0x10 ⁻⁹	9.1x10 ⁻¹⁴	1.2x10 ⁻⁷
II. Metabolites affected by the RFA procedure depending on cirrhosis aetiology					
1	Lipids	0.83 ^b ; 1.23 ^b ; 1.57 ^b	2.0x10 ⁻⁶	4.1x10 ⁻⁸	7.3x10 ⁻⁶
13	Aspartate	2.69 ^{dd} ; 2.80 ^{dd}	2.2x10 ⁻⁴	2.7x10 ⁻⁷	2.0x10 ⁻⁸
16	Choline	3.19 ^s	2.2x10 ⁻⁵	5.6x10 ⁻⁴	-
19	Glucose	3.24 ^{dd} ; 3.41 ^t ; 3.42 ^t ; 3.46 ^m ; 3.49 ^t ; 3.54 ^{dd} ; 3.71 ^t ; 3.73 ^m ; 3.84 ^m ; 3.9 ^{dd} ; 5.23 ^d	5.0x10 ⁻³	2.0x10 ⁻⁸	3.3x10 ⁻⁹
III. Metabolites affected by the RFA procedure and identified for one of the cohorts					
4	3-hydroxybutyrate	1.19 ^d ; 4.16 ^m	3.9x10 ⁻⁷	-	3.6x10 ⁻⁴
6	Acetate	1.91 ^s	1.0x10 ⁻²	4.1x10 ⁻¹⁰	2.3x10 ⁻⁴
7	N-acetyl glyccoprotein	2.02 ^s	2.3x10 ⁻³	-	1.5x10 ⁻⁵
10	Acetone	2.22 ^s	7.0x10 ⁻⁴	-	7.0x10 ⁻³
14	Malonate	3.14 ^s	-	-	3.0x10 ⁻⁵
18	Methanol	3.35 ^s	4.4x10 ⁻⁵	4.4x10 ⁻⁵	1.1x10 ⁻⁵
20	Myo-Inositol	4.06 ^t	-	-	1.8x10 ⁻⁶
21	Glycerol	3.56 ^{dd} ; 3.64 ^{dd}	1.1x10 ⁻⁵	-	2.4x10 ⁻⁵

Chemical shift and multiplicity correspond to those found in the ^1H -NMR spectra of the patients' sera.

ppm : parts per million. s, singlet; b, broad; d, doublet; dd, doublet of doublet; t, triplet; m, multiplet.

PC/GPC : phosphatidylcholine/glycerophosphocholine

p^{*} : p-value