

Supplementary material for:

# **Metabolite biomarkers of prolonged and intensified pain and distress in head and neck cancer patients undergoing radio- or chemoradiotherapy by means of NMR based metabolomics – a preliminary study**

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### The characteristics of the acquired spectra as well as the pulse sequence parameters

NOESY (Nuclear Overhauser Effect Spectroscopy) – an overview of all types of molecules.

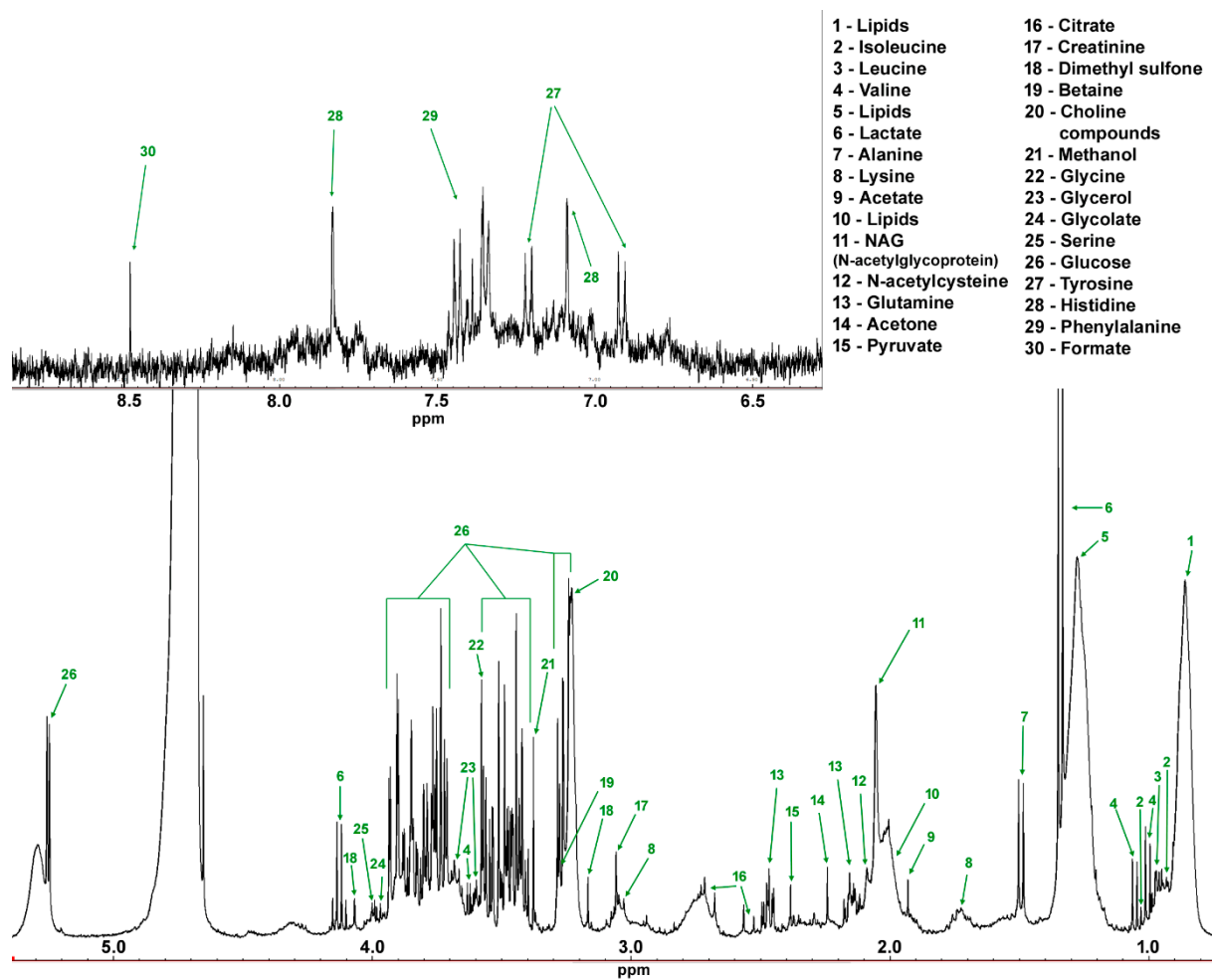
CPMG (Carr-Purcell-Meiboom-Gill) – an information on only low molecular weight metabolites.

DIFF (diffusion edited) - mainly macromolecular signals.

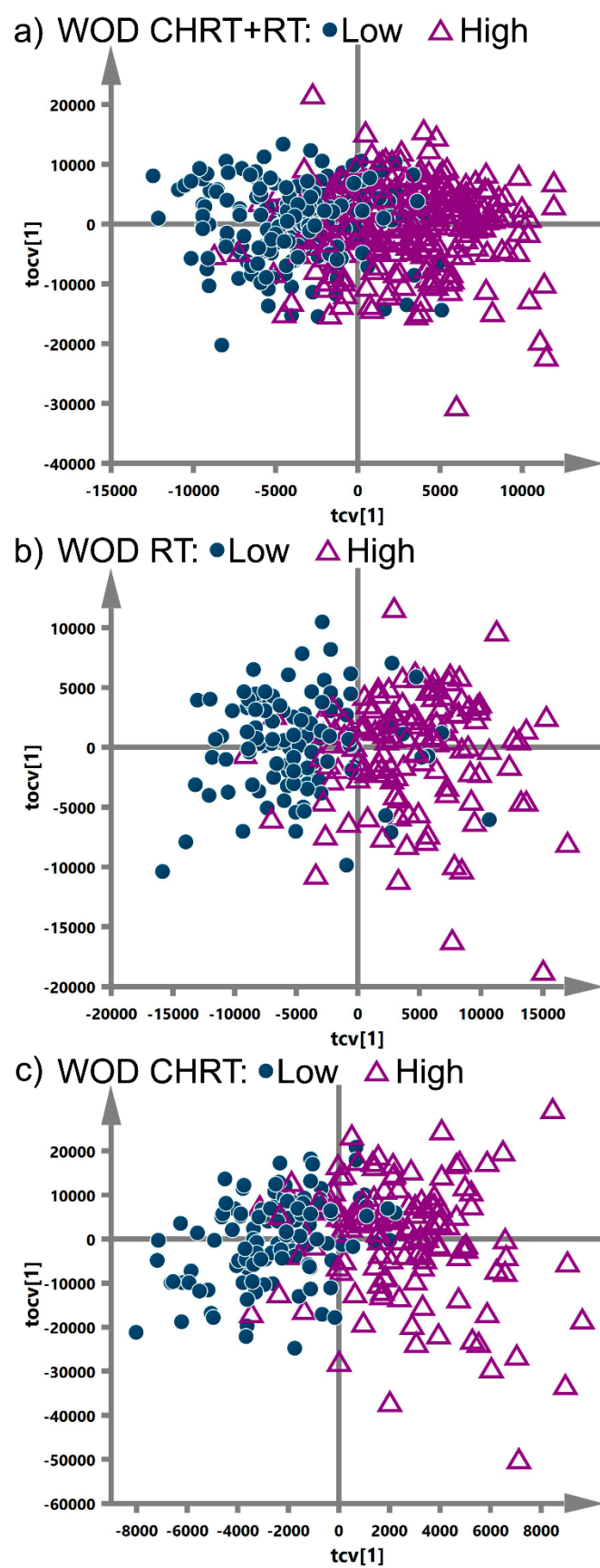
Two dimensional (2D) JRES (J-resolved) – a visualization of scalar couplings and improved metabolite identification.

**Table S1.** NMR pulse sequence parameters.

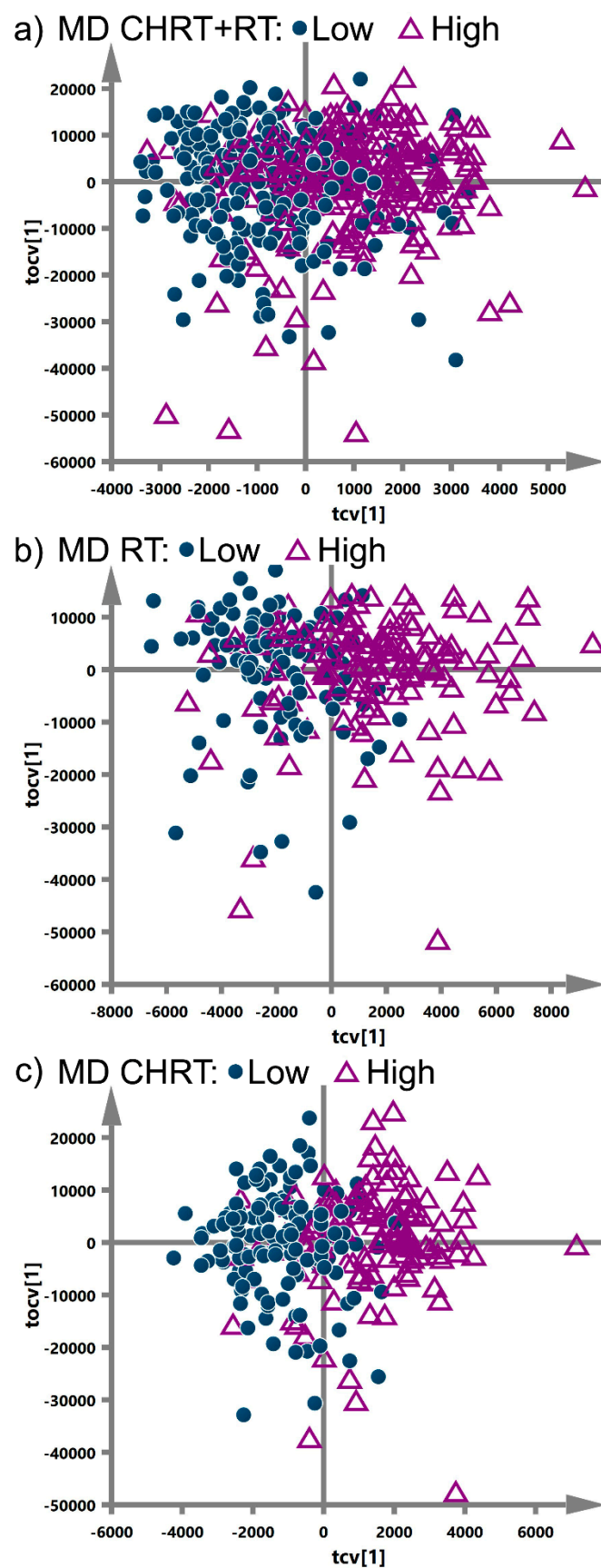
Pulse program	NOESYGPPR1D	CPMGPR1D	LEDBPGPPR2S1D	JRESGPPRQF
<b>TD</b>	65536	65536	65536	8192
<b>SW</b> [ppm]	30	20	30	16.62
<b>AQ</b> [sec]	2.73	4.09	2.73	0.62
<b>D1</b> [sec]	4	4	4	2
<b>D8</b> [sec]	0.01	-	-	-
<b>D16</b> [sec]	-	-	0.0002	0.0002
<b>D20</b> [sec]	-	0.0003	0.12	-
<b>D21</b> [sec]	-	-	0.005	-
<b>DS</b>	4	4	4	16
<b>L4</b>	-	126	-	-
<b>NS</b>	32	64	64	1
<b>DELTA1</b> [sec]	-	-	0.11572488	-
<b>DELTA2</b> [sec]	-	-	0.004172	-



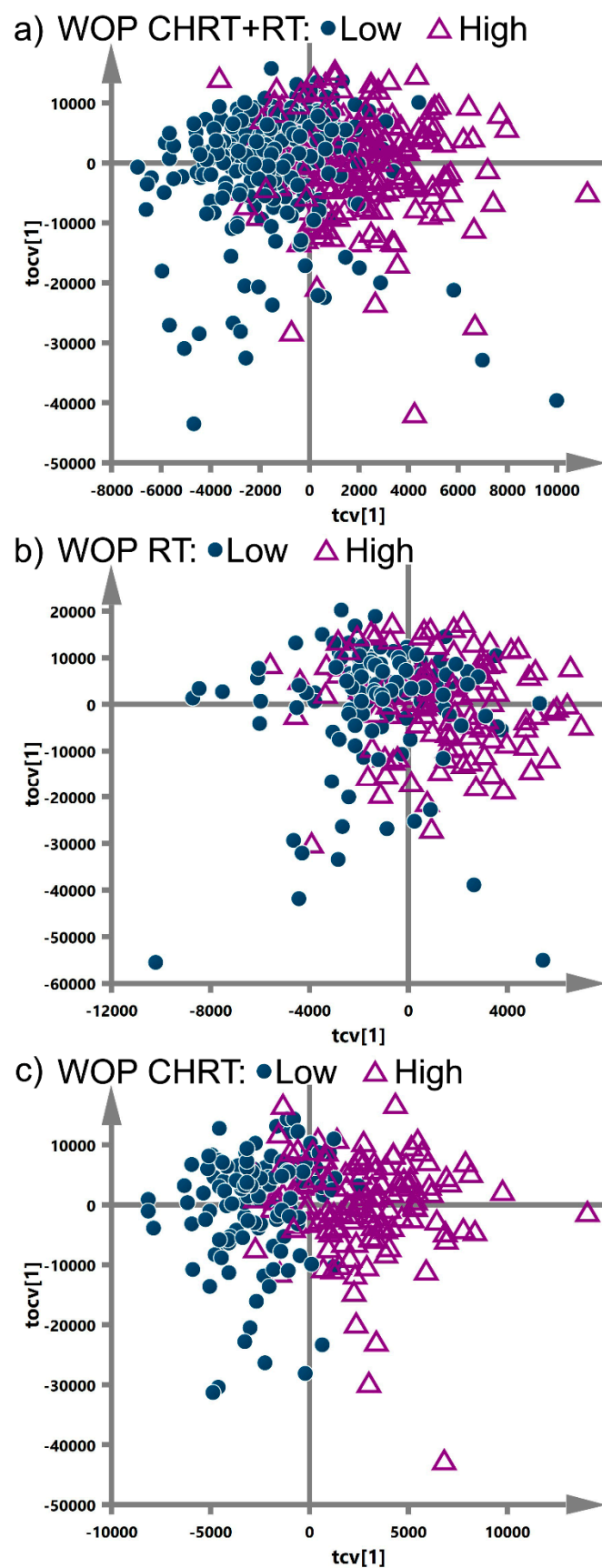
**Figure S1.** The 400 MHz  $^1\text{H}$ -NMR CPMG spectrum of blood serum from HNSCC patient treated with chemoradiotherapy. Main metabolites are indicated.



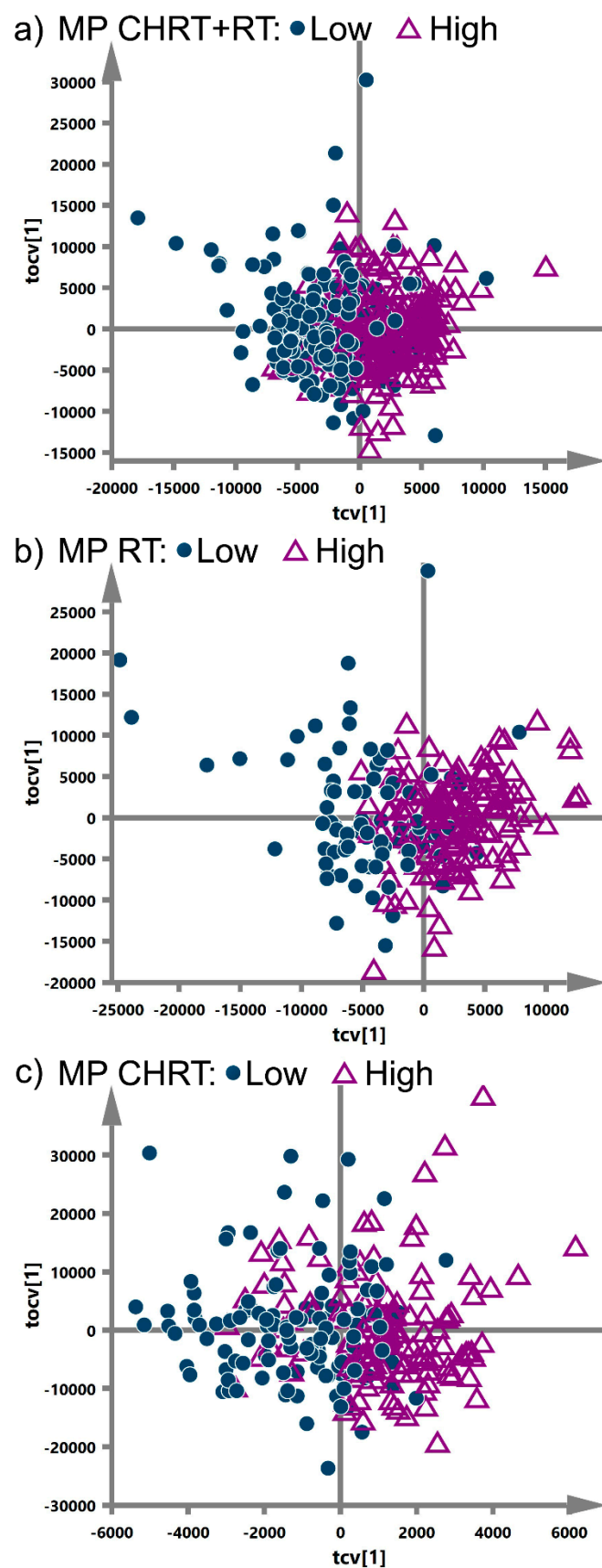
**Figure S2.** Multivariate discrimination between the L (Low) and H (High) WOD classes for CHRT+RT (a), RT (b) and CHRT (c). The OPLS-DA cross-validated scores plots show distinct separation between the classes.



**Figure S3.** Multivariate discrimination between the L (Low) and H (High) MD classes for CHRT+RT (a), RT (b) and CHRT (c). The OPLS-DA cross-validated scores plots show moderate to distinct separation between the classes.



**Figure S4.** Multivariate discrimination between the L (Low) and H (High) WOP classes for CHRT+RT (a), RT (b) and CHRT (c). The OPLS-DA cross-validated scores plots show moderate to distinct separation between the classes.



**Figure S5.** Multivariate discrimination between the L (Low) and H (High) MP classes for CHRT+RT (a), RT (b) and CHRT (c). The OPLS-DA cross-validated scores plots show only moderate separation between the classes.