Identification of the chemical shifts of a series of metabolites appearing in the ^{1}H-NMR spectrum of blood plasma by spiking

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Background

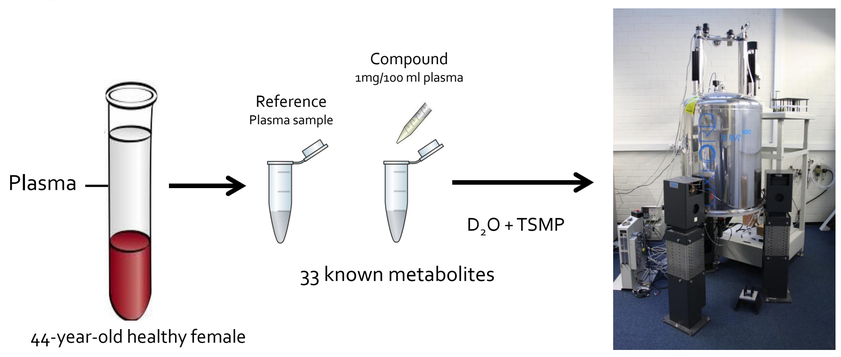
Instead of using reported chemical shift values of metabolites, which differ seriously from study to study due to differences in e.g. biofluid, temperature and pH, the chemical shifts of metabolites could be determined more accurately by spiking the biofluid itself.

Objective

To assign proton chemical shifts and J-coupling constants of known plasma metabolites measured with 400MHz ^{1}H-NMR spectroscopy and to compare the literature based on settings of integration regions (IR) in the ^{1}H-NMR spectra of plasma metabolites with those based on spiking experiments towards their power to discriminate between lung cancer patients and controls.

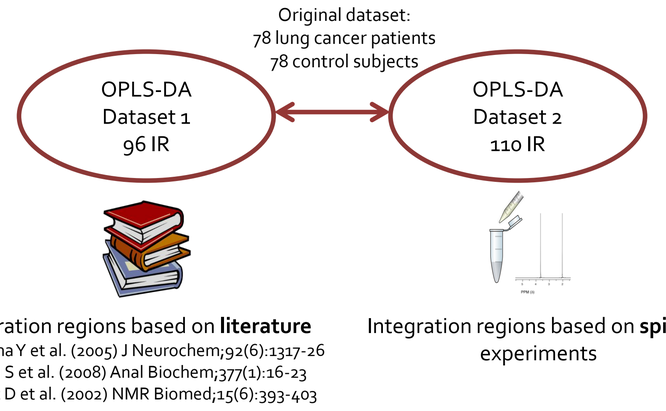
Methods

1. Spiking experiment



2. Multivariate data analysis

At first, the independent samples t-test (IBM SPSS version 20.0, Chicago, IL, USA) was implemented in order to evaluate differences in integration values of all defined IRs between lung cancer patients and control subjects. Additionally, Benjamini-Hochberg procedure was done in order to correct for multiple testing. Second, orthogonal partial least squares discriminant analyses (OPLS-DA) were performed on both datasets using SIMCA-P$^+$ (version 12.0, Umetrics, Umea, Sweden).

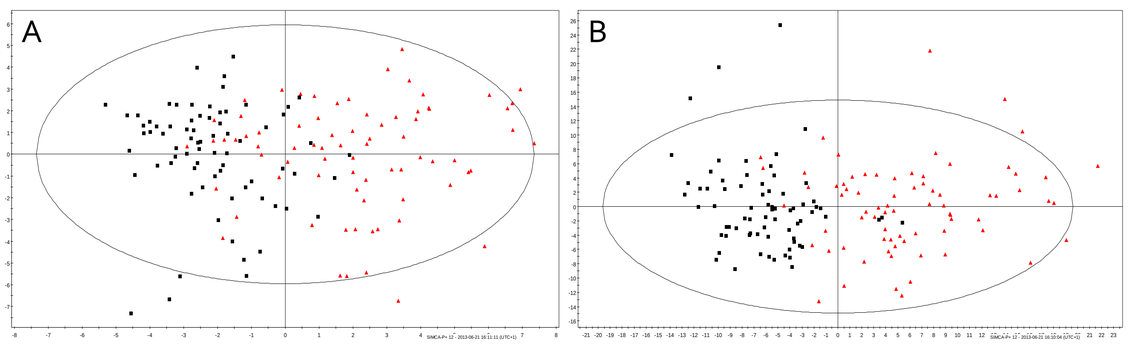


Shirayama Y et al. (2005) J Neurochem;92(6):1317-26
Tiziani S et al. (2008) Anal Biochem;377(1):16-23
Trehout D et al. (2002) NMR Biomed;15(6):393-403

Results

Chemical shifts and J-coupling constants of the 33 spiked metabolites are presented in Table 1 (see on the right). Figure 1 illustrates the difference of sensitivity and specificity between OPLS-DA of both datasets, i.e. based on literature or spiking experiments.

Figure 1 | OPLS-DA multivariate analysis carried out on 78 lung cancer patients (▲) and 78 controls (■): (A) based on 96 IR determined by literature chemical shift information and (B) based on 110 IR determined by spiking experiments



Sensitivity: 78.95%
Specificity: 89.19%

Sensitivity: 86.67%
Specificity: 95.89%

Table 1 | Proton chemical shifts (δ in ppm) and coupling constants (J in Hz) for low-molecular-weight plasma metabolites. Chemical shifts are reported with reference to TSMP singlet resonance at 0.015 ppm and multiplicity definitions are: s, singlet; d, doublet; dd: double doublet; t, triplet; dt, double triplet; q, quartet; p, pentaplet; o, octaplet; m, multiplet. Assignments of the molecular groups follow the IUPAC-IUB nomenclature. br: broad peak area, difficult J-coupling assignment. The multiplicity given here was observed in conventional 1D-spectra recorded at 400 MHz (Varian Inova spectrometer).

Compound	Group	δ (ppm) in plasma	Multiplicity	J (Hz)	Connectivity
Amino acid metabolism					
Histidine (His)	$^{\alpha}CH$	4,012	dd	8	α-β
	$^{\beta}CH_2$	3,150	dd	4,9	α-β'
		3,260	dd	15,5	β-β'
	$^{\gamma}CH$	7,802	s	none	
	$^{\delta}CH$	7,086	s	none	
Arginine (Arg)	$^{\alpha}CH$	3,690	t	6,1	α-β
	$^{\beta}CH_2$	1,700	m	br	β-β'
	$^{\gamma}CH_2$	1,875	m	br	β-γ
				br	β'-γ
	$^{\delta}CH_2$	3,266	t	6,9	δ-γ
Methionine (Met)	$^{\alpha}CH$	3,885	dd	5,4	α-β
				1,5	α-β'
	$^{\beta}CH_2$	2,230	m	br	β-β'
	$^{\gamma}CH_2$	2,673	t	7,6	β-γ, β'-γ
	$^{\delta}CH_3$	2,167	s	none	
Glutamine (Gln)	$^{\alpha}CH$	3,786	t	6,2	α-β
	$^{\beta}CH_2$	2,160	m	5,9	α-β'
				14,5	β-β'
	$^{\gamma}CH_2$	2,480	m	6,3	β-γ
				9,2	β-γ'
				9,2	β'-γ
				6,4	β'-γ'
				15,3	γ-γ'
Lysine (Lys)	$^{\alpha}CH$	3,760	t	6,0	α-β
	$^{\beta}CH_2$	1,900	m	br	β-β'
	$^{\gamma}CH_2$	1,500	m	br	β-γ, β'-γ
	$^{\delta}CH_2$	1,751	p	7,5	γ-δ
	$^{\epsilon}CH_2$	3,030	t	7,5	δ-ε
Glycine (Gly)	$^{\alpha}CH_2$	3,586	s	none	
Isoleucine (Ile)	$^{\alpha}CH$	3,968	d	4,0	α-β
	$^{\beta}CH$	2,020	m	br	β-δ
	$^{\gamma}CH_3$	1,040	d	7,0	β-γ
	$^{\delta}CH_2$	1,504	p	7,4	γ-δ
	$^{\epsilon}CH_3$	0,970	t	7,4	δ-ε
Leucine (Leu)	$^{\alpha}CH$	3,769	dd	7,0	α-β
				1,5	α-β'
	$^{\beta}CH_2$	1,742	m	br	β-γ
	$^{\gamma}CH$	1,742	m	br	β'-γ
	$^{\delta}CH$	1,003	d	4,7	γ-δ
		0,987	d	4,7	γ-δ'
Alanine (Ala)	$^{\alpha}CH$	3,790	q	7,2	α-β
	$^{\beta}CH_3$	1,509	d	14,3	β-β',β''
				14,3	β'-β''
Serine (Ser)	$^{\alpha}CH$	3,845	dd	5,3	α-β
	$^{\beta}CH_2$	3,982	dd	4,1	α-β'
		4,002	dd	12,2	β-β'
Proline (Pro)	$^{\alpha}CH$	4,162	dd	6,3	α-β
				8,9	α-β'
	$^{\beta}CH_2$	2,382	m	br	β-β'
	$^{\gamma}CH_2$	2,060	m	14,0	γ-β
				7,0	γ'-β, γ-β', γ'-β'
				7,0	γ-γ'
	$^{\delta}CH_2$	3,365	dt	14,1	δ-γ
		3,441	dt	7,0	δ'-γ, δ-γ', δ'-γ'
				7,0	δ-δ'
Phenylalanine (Phe)	$^{\alpha}CH$	3,955	dd	5,2	α-β
	$^{\beta}CH_2$	3,310	dd	7,7	α-β'
		3,140	dd	14,4	β-β'
	$^{\gamma}CH$	7,351	d	7,2	γ-δ
	$^{\delta}CH$	7,454	t	7,2	δ-ε
	$^{\epsilon}CH$	7,397	t		
Cysteine (Cys)	$^{\alpha}CH$	3,973	dd	5,7	α-β
	$^{\beta}CH_2$	3,112	dd	4,3	α-β'
		3,052	dd	14,7	β-β'
Valine (Val)	$^{\alpha}CH$	3,635	d	4,3	α-β
	$^{\beta}CH$	2,305	o	7,1	β-γ
	$^{\gamma}CH_3$	1,074	d	7,1	β-γ'
	$^{\delta}CH_3$	1,021	d		
Tryptophan (Trp)	$^{\alpha}CH$	4,085	dd	5,2	α-β
	$^{\beta}CH_2$	3,340	dd	8,1	α-β'
				15,3	β-β'
	$^{\gamma}CH$	7,351	s	none	
	$^{\delta}CH$	7,770	d	7,8	δ-ε
	$^{\epsilon}CH$	7,229	t	7,8	ε-ζ
	$^{\zeta}CH$	7,310	t	7,8	ζ-η
	$^{\eta}CH$	7,570	d		
Tyrosine (Tyr)	$^{\alpha}CH$	3,954	dd	5	α-β
	$^{\beta}CH_2$	3,230	dd	7,6	α-β'
		3,075	dd	14,2	β-β'
	$^{\gamma}CH$	6,924	d	8,5	γ-γ'
				8,4	δ'-γ'
				8,4	γ-δ
	$^{\delta}CH$	7,222	d	8,5	δ-δ'
Asparagine (Asn)	$^{\alpha}CH$	3,999	q	7,8	α-β
	$^{\beta}CH_2$	2,962	dd	4,3	α-β'
		2,845	dd	16,7	β-β'
Threonine (Thr)	$^{\alpha}CH$	3,596	d	4,9	α-β
	$^{\beta}CH$	4,276	o	6,6	β-γ
	$^{\gamma}CH_3$	1,358	d		
Aspartate (Asp)	$^{\alpha}CH$	3,930	dd	3,7	α-β
	$^{\beta}CH_2$	2,850	dd	8,9	α-β'
		2,702	dd	17,5	β-β'
Glutamate (Glu)	$^{\alpha}CH$	3,788	dd	7,1	α-β
	$^{\beta}CH_2$	2,162	m	4,9	α-β'
				14,7	β-β'
	$^{\gamma}CH_2$	2,388	m	8,41	β-γ'
				6,88	β'-γ'
				6,4	β-γ
				8,4	β'-γ
				15,9	γ-γ'
Creatine	$^{\alpha}CH_2$	3,068	s	none	
		3,962	s	none	
Creatinine	$^{\alpha}CH_3$	3,075	s	none	
	$^{\beta}CH_2$	4,087	s	none	
Glucose metabolism					
Glucose					
α-anomer	$^{\alpha}CH$	5,216	d	3,8	α-β
	$^{\beta}CH$	3,519	dd	9,6	β-γ
	$^{\gamma}CH$	3,698	t	9,4	γ-δ
	$^{\delta}CH$	3,395	t	9,9	δ-ε
	$^{\epsilon}CH$	3,822	m	1,5	ε-ζ
	$^{\zeta}CH$	3,826	dd	6	ε-ζ'
	$^{\zeta}CH$	3,749	dd	12,1	ζ-ζ'
β-anomer	$^{\alpha}CH$	4,630	d	8	α-β
	$^{\beta}CH$	3,230	dd	9,1	β-γ
	$^{\gamma}CH$	3,473	t	9,4	γ-δ
	$^{\delta}CH$	3,387	t	8,9	δ-ε
	$^{\epsilon}CH$	3,450	m	1,6	ε-ζ
	$^{\zeta}CH$	3,882	dd	5,4	ε-ζ'
	$^{\zeta}CH$	3,707	dd	12,3	ζ-ζ'
Inositol	$^{\alpha}CH$	3,563	dd	2,9	α-β
	$^{\beta}CH$	4,097	t	10	α-ζ
	$^{\gamma}CH$	3,563	dd	2,9	β-γ
	$^{\delta}CH$	3,655	t	9,8	γ-δ
	$^{\epsilon}CH$	3,306	t	9,3	δ-ε
	$^{\zeta}CH$	3,655	t	9,3	ε-ζ
Glycolyse					
Lactate	$^{\alpha}CH$	4,138	q	6,9	α-β
	$^{\beta}CH_3$	1,354	d		
Pyruvate	$^{\alpha}CH_3$	2,402	s	none	
Krebs Cycle					
Citrate	$^{\alpha\text{-}\beta}CH_2$	2,642	q	15,7	α-β
				60,2	α-α',β-β'
α-ketoglutarate	$^{\alpha}CH_2$	3,040	t	6,9	α-β
	$^{\beta}CH_2$	2,470	t		
Succinate	$^{\alpha}CH_2$	2,439	s	none	
	$^{\beta}CH_2$	2,439	s	none	
Malate	$^{\alpha}CH$	4,300	dd	10,2	α-β
	$^{\beta}CH_2$	2,700	dd	3	α-β'
		2,400	dd	15,4	β-β'
Lipid metabolism					
Acetate	CH_3	1,948	s	none	
Acetoacetate	CH_3	2,319	s	none	
β-hydroxybutyrate	$^{\alpha}CH$	2,400	dd	7,3	α-β
	$^{\beta}CH_2$	2,300	dd	14,4	β-β'
	$^{\gamma}CH_3$	1,200	d	6,3	β-γ

Conclusion

Instead of relying on chemical shifts of metabolites derived from literature, spiking experiments seem to be a better approach in order to more accurately assign the chemical shifts of plasma metabolites in a ^{1}H-NMR spectrum. Consequently, it could lead to an enhancement of the discriminative power of the cluster analysis and a better understanding and/or explanation of the clinical relevance of study findings.

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