Temperature effect on the HRMAS spectra of human brain tumour biopsies, their pattern recognition analysis and their post-HRMAS histopathology

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Purpose/Introduction

Recent studies aimed to profile tumour biopsies by HRMAS are performed at 1-4ºC to minimize spectral pattern changes[1,2], while other studies[3,4] used higher temperatures (20-30ºC). Our purpose was to investigate the effect of temperature used to record HRMAS of human brain tumour biopsies in the accuracy of classification obtained using pattern recognition (PR) analysis.

Sequence	T	PCs#	LOO-CV (%)
Pulse and acquire	0ºC	7	92.1
	37ºC	6	79.6
Spin echo without J modulation 30ms	0ºC	9	95.2
	37ºC	9	88.9
Spin echo without J modulation 136ms	0ºC	8	95.2
	37ºC	8	93.8
Spin echo with J modulation 30ms	0ºC	9	90.8
	37ºC	8	88.9
Spin echo with J modulation 136ms	0ºC	9	79.4
	37ºC	9	88.9

Table 1. Summary of results of the Principal Component Analysis and the accuracy achieved with the classifier. PCs# number of principal components used for classifier development

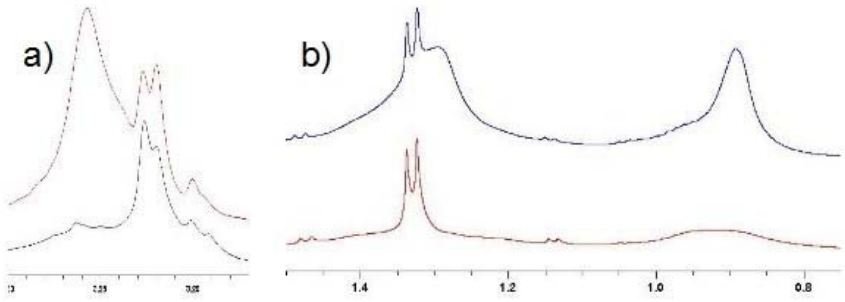

Figure 1. Main changes detected on a glioblastoma sample when acquiring spectra at 0ºC (lower) and at 37ºC (upper) at the choline-containing compounds (a) and the mobile lipids[5] (b) regions using a pulse and acquire sequence

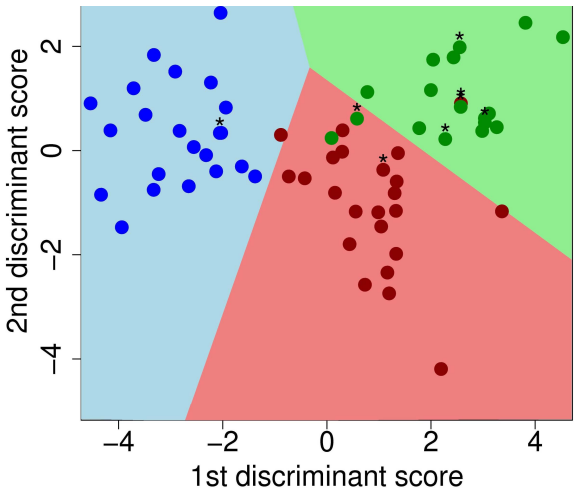

*Figure 2. Classification plot for the three classes studied using a spin echo without J modulation 136ms sequence aquired at 37ºC. Blue colour stands for meningioma, red for glioblastoma and green for low grade glioma. Boundaries are the decision boundaries of the clasifier. *labels potentially unusable cases after post-HRMAS histopathology.*

Samples and Methods

20 meningothelial meningioma (MM), 22 glioblastoma multiforme (GBM) and 17 low grade glioma (LGG) biopsies were analyzed. HRMAS spectra were obtained at 0ºC and 37ºC with presat and spin echo (30 and 136ms with and without phase modulation) sequences. Spectra were processed with 0.5Hz line broadening, phased and baseline corrected. Calibration was carried out setting the lactate doublet central position at 1.33ppm. If lactate was too low, the central position of the alanine doublet (1.45ppm) or the creatine methyl singlet (3.03ppm) were used.

Unit length normalization was applied. Mean class spectra were calculated. All statistical procedures were carried out using R.

Principal Component Analysis (PCA) was applied to the whole set of spectra. Principal components chosen explained at least 80% of the variance. Linear Discriminant Analysis (LDA) and Leave-one-out Cross-validation (LOO-CV) were applied to the selected variables to develop and test the classifier. No independent test set was used for classifier testing purposes.

46 out of 59 samples were analyzed by post-HRMAS histopathology to control sample integrity.

Results

Mean spectra demonstrated clear changes in the spectral pattern due to temperature, specifically in the lipid/macromolecule regions (figure 1). These changes were reversible for the three tumour types investigated (n=3 for MM, n=3 for GBM and n=2 for LGG). See also [6].

Classifier accuracy was above 80% for almost all instances (table 1), except when using the pulse and acquire at 37ºC and for the spin echo with J modulation 136ms at 0ºC acquisition conditions. In general, classifier success was higher when acquiring spectra at 0ºC, except for the spin echo with J modulation 136ms sequences.

Eight of the samples analyzed by post-HRMAS histopathology were necrosed or had less than 50% recognizable tumour tissue (4 samples) or were not evaluable (2 because of the small amount of sample and 2 because of artifacted sample). These samples were not discarded from the PCA analysis but are singled out in figure 2.

Discussion/conclusion

A better overall classification of the cases studied seems to be achieved at 0ºC (figure 2). This could be caused by the additional pattern variability introduced by the changes in the lipid/macromolecule region when acquiring spectra at 37ºC. Since it has been recently described that there may exist molecular subtypes of GBM[7,8], the acquisition of HRMAS spectra at 37ºC could be probing this differential tumour type heterogeneity.

The acquisition protocols used at 37ºC did not preclude histopathology analysis of post-HRMAS samples.

References

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