HR MAS MR spectroscopy of breast cancer as a predictive tool for lymph node status

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Introduction:

Lymph node status is one of the most important prognostic factors in breast cancer patients. In addition to patient age, tumor size, grade, content of steroid hormone receptors and overexpression of c-erbb2, the lymph node status is of importance for the individual patient treatment plan. The biochemical composition of breast cancer specimens measured with high resolution magic angle spinning (HR MAS) magnetic resonance spectroscopy (MRS) [1] might give additional information important for optimising treatment strategies. The main objective of this study was to investigate the relationship between the metabolic profile of breast cancer tissue measured with HR MAS MRS and lymphatic spread in patients with breast cancer.

Experimental:

Breast cancer tissue was excised from patients with palpable breast lesions at St. Olavs University Hospital, Trondheim, Norway. Tumors diagnosed as invasive ductal carcinoma (IDC) grade I, II or III were further studied. Tissue specimens were analyzed in phosphate buffered deuterated water using a 50 µl MAS rotor (4 mm o.d.). HR MAS experiments were performed using a Bruker Avance DRX600 spectrometer at 4°C, and the samples were spun at 5 kHz. Proton spectra were acquired using a standard spin-echo sequence (cpmgpr, BRUKER) with a total echo time of 285 ms. After HR MAS analysis the relative areas of normal and neoplastic epithelial elements of the tissue specimens were scored visually by a pathologist. Specimens from patients with tumor content less than 5% were excluded from the analysis. The final database consisted of 69 breast cancer samples. Histopathological examination of the lymph nodes indicated that 37 samples were from patients with no spread and 32 samples from patients with lymphatic spread of tumor cells.

Principal component analysis (PCA) with full cross validation was performed using the spectral region from 2.9 to 4.7 ppm to reduce number of variables. The 25 first PC score values were used as input for probabilistic neural network (PNN) with full cross-validation [2] to obtain a classification of spectra based on lymph node status.

Results:

The selected spectral region for the analysis is shown in figure 1, and assignments are given for the most dominating peaks. A PCA score plot of PC2 and PC3 is shown in figure 2 and the samples from lymph node positive and negative patients are marked. The result from PNN based on 25 PCs is presented in table 1. This method gives a sensitivity and specificity of 97% and 92% respectively.

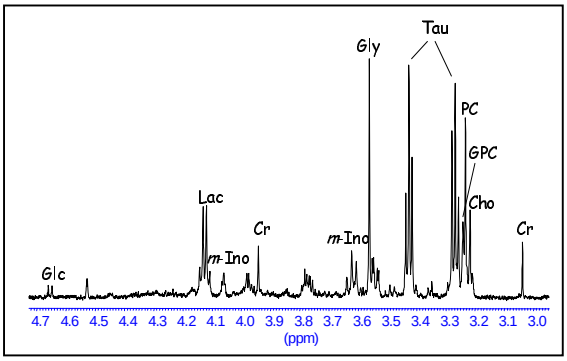

Figure 1: The selected chemical shift region.

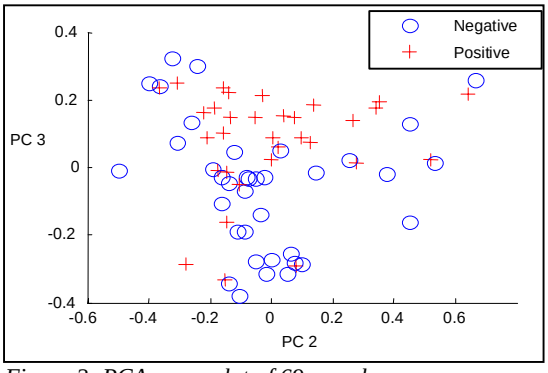

Figure 2: PCA score plot of 69 samples.

Discussion and conclusion:

Samples from lymph node positive and lymph node negative patients could not be separated based on the score plots from PCA, though a trend of clustering is seen in the score plot. However, the PNN analysis resulted in a classification of the two groups, with only 4 misclassified samples. The complexity of the metabolic pattern in cancer tissue makes it necessary to use non-linear multivariate methods like PNN. A previous study has shown similar results of classification [3]. The model will be tested further in a blinded study with unknown breast tissue samples to verify the results from this study.

References

[1] Sitter et al. *NMR Biomed* 1994;7:181
[2] Specht. *Neural Networks* 1990;3:109
[3] Mountford et al. *Brit J Surg*, 2001;88:1234

Table 1: Results from PNN

	Actual negative	Actual positive	Total
Classified as negative	34	1	35
Classified as positive	3	31	34
Total	37	32	69
Sensitivity	91.9%	96.9%	
Specificity	96.6%	91.9%	