

MR-determined metabolic phenotype of breast cancer predicts lymphatic spread, grade and hormone status

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Introduction

MR spectroscopy describes the biochemical properties of breast cancer^{1,2}. Histopathological (tumour size, grade and number of axillary lymph node involved) and immunohistochemical (steroid hormone receptors, c-erbB2) evaluation of breast cancer specimens form the basic patient treatment plan. Additional methods may be important for better treatment strategies. High resolution magic angle spinning (HR MAS) MR spectroscopy is a high-throughput technology with the potential of becoming fully automated. It has a high degree of reproducibility, and its non-destructive nature allows additional consecutive analyses, i.e. histopathology or micro array.

Experimental

Breast cancer tissue was excised from patients with palpable breast cancer (invasive ductal carcinoma (IDC) grade I, II and III). HR MAS MR spectra were recorded on a Bruker Avance DRX600 spectrometer at 4 °C. The samples were spun at 5000 Hz. Subsequently, a pathologist scored the relative areas of normal and neoplastic epithelial elements visually. Tumour-content less than 5% in the analysed sample and neo-adjuvant treatment were exclusion criteria. Multivariate models relating spectral data to tumour grade, lymphatic spread and hormonal status were designed. Analyses included a total of 91 spectra of cancerous tissue, of which 12 were kept as blind after Kennard Stone subset selection. Multivariate methods applied were variable reduction by principal component analysis (PCA) or partial least squares regression-uninformative variable elimination (PLS-UVE), and modelling by PLS, probabilistic neural network (PNN) or cascade correlation neural network (CC NN). The predictive power of these methods was validated with leave one out cross validation and further verified by testing on blind samples.

Results and discussion

HR MAS spectra with assignments of the major metabolites are given in Figure 1. All spectra contain the same resonances, and no large differences between the groups can be observed. Validation of PNN training gave optimistic results (Table 1), with sensitivity and specificity ranging from 83.3% to 96.8%. Results obtained from blind testing with the various methods are also given in Table 1. Lymph node involvement is the most important prognostic factor in breast cancer. The PNN prediction of lymphatic spread in the blind samples was not as optimistic as the training results. In that case, prediction by PLS modelling was slightly better. However, sectional slicing of all lymph nodes had not been performed, and some patients may thus have lymphatic spread in spite of determined negative sentinel node. Three of the samples defined lymph negative by the sentinel node method are classified as lymph positive by all prediction models. Thus, detailed pathological re-examination will be performed to establish whether lymphatic spread may actually be present in these patients. Lymph node involvement has earlier been predicted from MR spectra of fine-needle aspirate biopsies (FNAB)³. However, these results could not be reproduced on core biopsies, which contain more fat than fine needle aspirate. Positive hormone receptors in invasive breast cancer are both prognostic and predictive factors. The MR based prediction of hormone status of blind samples is in close conformity with the histopathological measurements, PNN and PLS being superior to the other methods. Hormone status has also been predicted from MR spectra of FNAB⁴. When a patient is found to be lymph node negative, the breast cancer grade is one of the important factors influencing further treatment decisions. Grade II and III tumours have the same clinical implication concerning adjuvant therapy, but a difference in MR determined metabolic phenotype as proven in this work, may point out a possibility of addressing different treatments in future.

Conclusion

The tumour biology of the individual patients' disease is becoming an important factor to consider when choosing treatment for breast cancer, and MR determined metabolic phenotype may have a future role as a supplement for clinical decision making concerning adjuvant treatment, and the adaptation of more individualised treatment protocols.

References

1 Sitter et al, NMR Biomed, in press 2 Sitter et al, NMR Biomed 15, 2002 3 Mountford et al, Br J Surg 88, 2001 4 Lean et al, Technol Cancer Res Treat 3, 2004

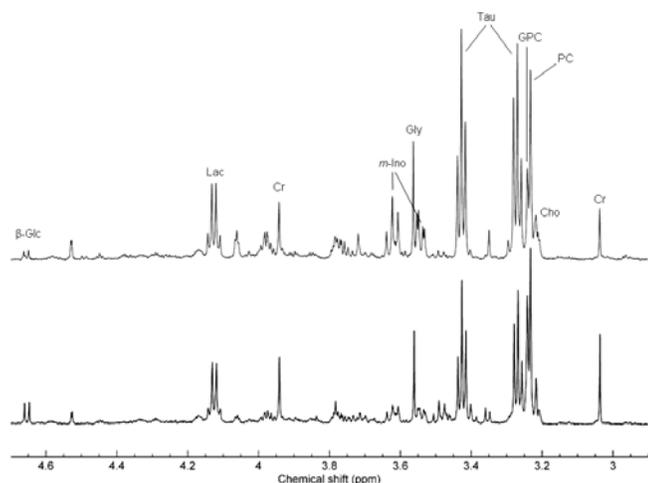


Figure 1: Region of interest for representative HR MAS MRS spectra. Both spectra are obtained from primary tumour tissue from patients with IDC3 breast cancer. The top spectrum is derived from a patient diagnosed as hormone positive, with lymphatic spread. The bottom spectrum is also from a hormone positive patient, but without proven lymphatic spread.

Table 1: Sensitivity and specificity for PNN calibration, and results from blind testing. Best predictions given in bold

Approach	Sensitivity/ Specificity (training PNN)	Blind testing: number of correct predictions of 12 samples in total			
		PNN	PLS	PLS- UVE- PNN	CC NN
Classification of tumour grade	94.4%/ 86.1%	7	NA	9	6
Classification of lymphatic spread	90.7%/ 83.3%	7	8	7	6
Classification of hormone status	84.6%/ 96.8%	11	11	7	9