Classification and prediction of prognostic factors of breast cancer patients by MR metabolomics

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Introduction

MR spectroscopy provides the opportunity to study the metabolite distribution of intact tissue (1). Metabolite distribution has been shown to correspond with cancer characteristics of several cancers (2,3). The purpose of the present study was to examine the feasibility of using MR metabolomics to determine the prognostic factors lymphatic spread and hormone ER receptor status of breast cancer patients.

Experimental

Tumor samples from breast cancer patients diagnosed with invasive ductal carcinoma were analyzed by high resolution magic angle spinning (HR MAS) MRS. None of the patients had received neoadjuvant treatment before surgery. The relative areas of normal and neoplastic epithelial tissue were scored by an experienced pathologist. Spectra from samples containing < 5% neoplastic tissue were excluded from further examination, resulting in a dataset of 167 (119 patients: 67 lymph node status negative and 52 positive, mean age: 61 years) and 163 (116 patients: 25 ER negative and 91 positive, mean age: 61 years) spectra for prediction of lymphatic spread and ER status, respectively. Partial least squares regression (PLS) and Bayesian Belief Network (BBN) were performed on the variable-reduced, normalized datasets. While PLS assume linear relationships between all variables, BBNs are also valid for nonlinear functions (4). For the PLS analysis, the spectral region 1.4-4.6 ppm was selected, while F-test variable selection was used on the same region before BBN analysis, resulting in 131 variables to include in the model. Patients who are deceased (survival < 5 years from surgery) were identified in the PLS score plots.

Results and discussion

The results from PLS for classification of lymphatic spread and ER status are shown in Fig. 1. Spectra from deceased patients are marked in the score plots (Fig. 1, A and C). The score for positive and negative patients are significantly different for both PC1 and PC2 for both prognostic factors (p < 0.01). Additionally, deceased patients may appear to be correlated to PC2 and PC1 for lymphatic spread and ER status, respectively. The MR predicted versus clinically measured status was significantly correlated both for lymphatic spread ($r_{calibration}$ =0.42, $r_{validation}$= 0.25) and ER status ($r_{calibration}$ =0.71, $r_{validation}$= 0.53) (p < 0,001). Results from prediction of blind samples (chosen by Kennard-Stone sample subset selection (5) for the PLS analysis, and randomly chosen for BBN) are shown in Table 1, and these results support previous findings (2). BBN is a more complex method, and gave better results for the prediction of both lymphatic spread and ER status, possibly because BBN can detect nonlinear relationships between variables. The advantage of PLS, however, is that the results can be correlated to the exact metabolite distribution (Fig. 1, B and D).
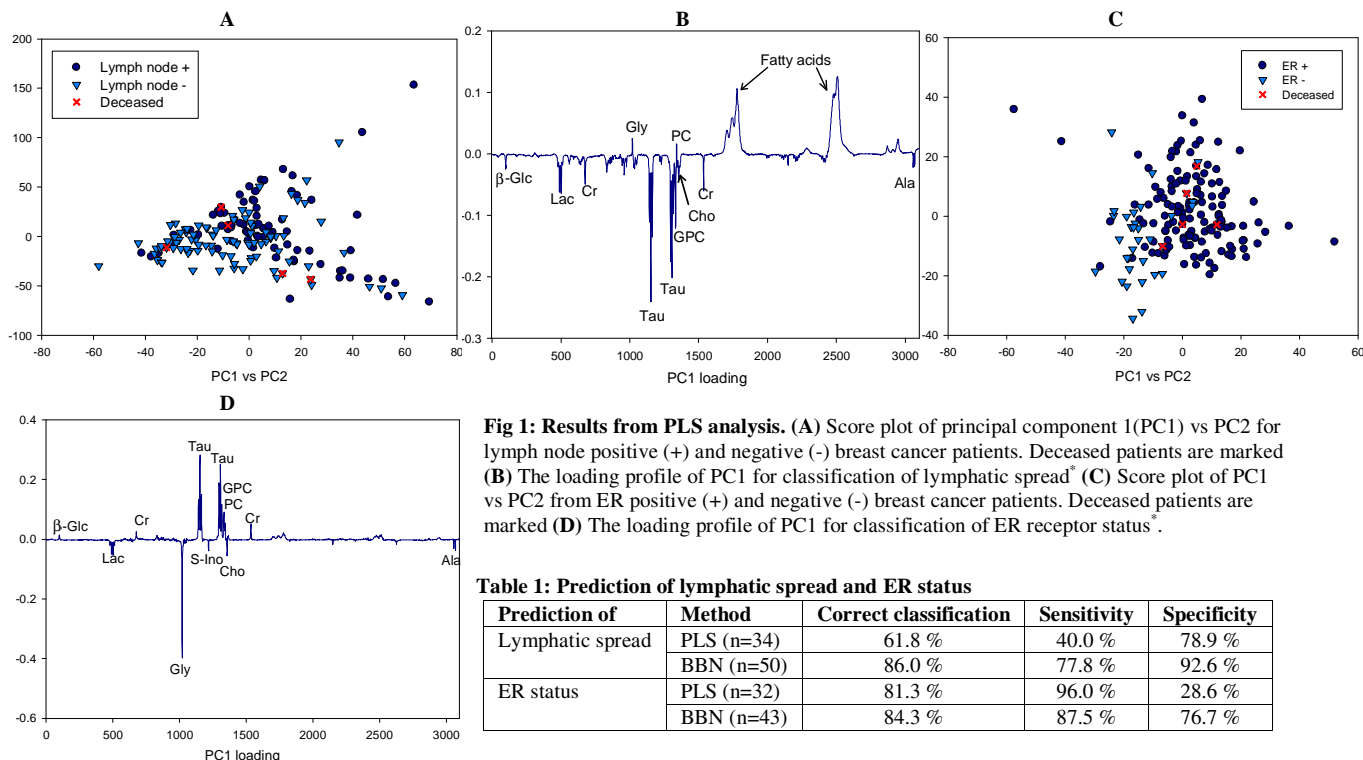

Fig 1: Results from PLS analysis. (A) Score plot of principal component 1(PC1) vs PC2 for lymph node positive (+) and negative (-) breast cancer patients. Deceased patients are marked **(B)** The loading profile of PC1 for classification of lymphatic spread* **(C)** Score plot of PC1 vs PC2 from ER positive (+) and negative (-) breast cancer patients. Deceased patients are marked **(D)** The loading profile of PC1 for classification of ER receptor status*.

Table 1: Prediction of lymphatic spread and ER status

Prediction of	Method	Correct classification	Sensitivity	Specificity
Lymphatic spread	PLS (n=34)	61.8 %	40.0 %	78.9 %
	BBN (n=50)	86.0 %	77.8 %	92.6 %
ER status	PLS (n=32)	81.3 %	96.0 %	28.6 %
	BBN (n=43)	84.3 %	87.5 %	76.7 %

* β-Glc; β-glucose, Lac; lactate, Cr; creatine, Gly; glycine, Tau; taurine, S-Ino; *scyllo*-inositol, GPC; glycerophosphocholine, PC; phosphocholine, Cho; choline, Ala; alanine

Conclusion

This study suggests that both lymphatic spread and ER receptor status can be predicted by MR metabolomics. These are important prognostic and predictive factors in clinical decision-making concerning adjuvant therapy in breast cancer. Other factors such as progesterone hormone status, cERB2, hypoxia and acidosis will also influence the total metabolite status. MR metabolomics may thus be a tool to identify subclasses of breast cancer patients related to different prognosis and outcome.

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