Breast cancer diagnosis based on lipid profiling by probe electrospray ionization mass spectrometry

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Introduction

Breast cancer is the most common cancer in women, and detection at an early stage of the disease assures a good prognosis. Initial screening is often based on imaging techniques such as mammography, but many women have dense breasts, which makes the sensitivity of mammography unsatisfactory[1]. Pathological examination of a tissue biopsy is a routine approach in establishing a breast cancer diagnosis, but this technique requires multiple steps over several days. No alternative or supportive procedures are currently available in a clinical setting. More accurate and less time-consuming diagnostic methods using small specimens are needed.

Previous studies[2−4] have been directed towards characterization of tissues, including intensive research for new genetic or metabolic markers. Using metabolomics, markers in tissues and body fluids can be identified[3,4]. Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis is good at identifying breast cancer-specific molecules, but it is a time-consuming procedure, starting from preparation of the column to purifying specific components from tissue extracts. To mitigate this situation, probe electrospray ionization mass spectrometry (PESI-MS) is applied to a biological system[5], enabling the analysis of almost 'raw' samples with no specific, laborious pretreatments.

PESI-MS is an ambient ionization-based mass spectrometry (MS) method that surpasses the original electrospray ionization technique in features such as the rapidity of analysis, simplicity of the equipment and procedure, and the low cost per capita[6]. For example, PESI-MS has been used by the present authors for the rapid analysis of biological samples to discriminate cancers[7,8]. Additionally, machine learning has been combined with PESI-MS to diagnose various diseases with reference to information such as medical images and clinical data[9,10].

Methods

Full details of the study design, methods employed, and statistical analysis can be found in *Appendix S1* (supporting information).

Results and Discussion

PESI-MS and LC-MS/MS were used to study breast cancer as summarized in *Fig. 1*. To create a database, 46 sets of spectra were acquired from small samples (10 mg) of normal and cancerous tissues in both positive and negative ion modes of PESI-MS. Breast cancer-specific spectra in each ion mode were analysed by eMSTAT Solution software (Shimadzu Corporation, Kyoto, Japan).

Partial least squares discriminant analysis (PLS-DA) plots showed clear differences between the non-cancerous and cancerous groups with both ion modes (*Fig. 2a,b*). This suggests that the instrumentation can distinguish breast cancer from normal breast tissue, even with specimens much smaller than those obtained by core-needle biopsy.

All mass spectra obtained from 23 patients with breast cancer were further analysed by logistic regression to establish an algorithm for distinguishing breast cancer from normal breast tissue. To test the predictive power of the constructed algorithm, leave-one-out cross-validation was performed. Ninety-two data sets (23 non-cancerous and 23 cancerous samples analysed using two ion modes) were used to evaluate the potential of the system for breast cancer diagnosis. According to the relative cumulative frequency distribution plots, the thresholds of probability were 0·480–0·625 and 0·370–0·465 in the positive and negative ion modes respectively (*Fig. S1*, supporting information). At these points, sensitivity, specificity and accuracy in the positive ion mode were all 95·7 per cent, whereas those in the negative ion mode were 87·0, 91·3 and 89·1 per cent respectively (*Table S1*, supporting information). The area under the receiver operating characteristic

Fig. 1 Study design
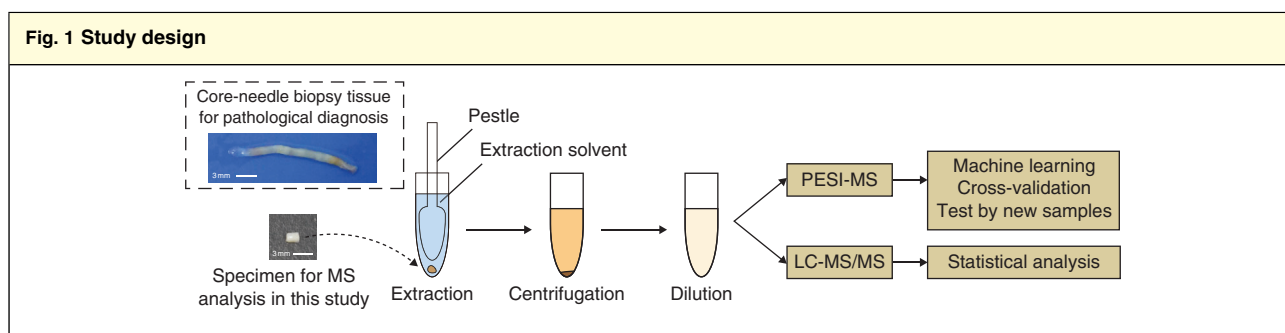
A representative 10-mg fragment of specimen is shown on the left with core-needle biopsy tissue for comparison of sizes. The procedure of sample preparation for the probe electrospray ionization mass spectrometry (PESI-MS) and liquid chromatography tandem mass spectrometry (LC-MS/MS) analyses is depicted on the right.

curve (AUC) was calculated to be 0·967 and 0·910 for the positive and negative ion modes respectively (*Fig. 2c,d*).

The system was further validated using specimens from additional patients and 24 data sets (6 non-cancerous and 6 cancerous samples in both ion modes) and the generated algorithm. This testing achieved complete (100 per cent) discrimination in the positive ion mode (*Table S2*, supporting information). Data from the negative ion mode yielded one mismatch among the non-cancerous samples (92 per cent accuracy).

Collectively, these results strongly indicate that this diagnostic system can distinguish breast cancer from normal breast tissue. Previous reports showed the usefulness of this system in discriminating squamous cell carcinoma of head and neck regions from normal mucosa[11], and the present study of breast cancer indicates that this technology can be applied to epithelial tissues regardless of their origin. Moreover, because breast cancer is heterogeneous and comprises glands and a large proportion of stroma, this study has revealed the versatility of the instrumentation.

The present study demonstrates that this system can discriminate breast cancer from normal breast tissue without identifying the molecules. However, it is vital to focus on the specific molecules that are critical to the pathogenesis and pathophysiology of breast cancer. To address this issue, LC-MS/MS was used to enumerate the candidate molecular markers. Some species of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were increased significantly in breast cancer (*Table S3*, supporting information). AUC values were calculated for those species with significantly lower *P* values: PE(36:2), PE(38:4), PE(36:1), PC(36:0), PC(40:7) and PC(42:6) (*Fig. 2e; Fig. S2*, supporting information). Interestingly, none of the specific molecules exceeded the power of the instrumentation employing machine learning with positive ion mode. Because PC(42:6) gave the highest AUC value (0·952) among the individual molecules, this can

be assumed to be a candidate molecular marker of breast cancer. Although data for PE(36:2), PE(38:4), PE(36:1), PC(40:6) and PC(30:0) have been reported in the previous studies[12,13], the finding of PC(42:6) as a potential molecular marker is novel. This result may be attributed to study population differences and/or the precision of the LC-MS/MS used here.

The expression of oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER) 2 in breast cancer provides valuable guidance for systemic treatments. The expression of the proliferation marker Ki-67 is used to establish the aggressiveness of the cancer. All of these can be combined as surrogate markers for the molecular subtypes of breast cancer. To investigate whether these profiles were predicted by this instrumentation, 28 samples judged as cancer were classified by machine learning into two subgroups according to the value of each criterion. Spectra were acquired by PESI-MS and analysed by eMSTAT Solution™ software. PLS-DA could discriminate two groups categorized by the signature of Ki-67, HER2, histological tumour grade or PR (*Fig. S3*, supporting information). In this study, a high Ki-67 value overlapped completely with a low ER value, but pT and pN groups were less separated (*Fig. S3*, supporting information). These results suggest that the instrumentation has the potential to assess several breast cancer properties, such as aggressiveness. To launch a diagnostic system for breast cancer subgrouping on to the market, more mass spectra and clinical data need to be entered into the database.

This approach using PESI-MS requires only a small amount of tissue with minimal pretreatments, and enables instantaneous judgement using machine learning. Ambient MS techniques, including PESI-MS, have been studied in diagnostics. Rapid evaporative ionization mass spectrometry (REIMS) and desorption electrospray ionization (DESI) MS were used to study breast cancer diagnosis[14,15]. REIMS has been commercialized as an intelligent knife

Fig. 2 Probe electrospray ionization mass spectrometry combined with logistic regression analysis showed better prediction of breast cancer than biomarkers based on liquid chromatography tandem mass spectrometry
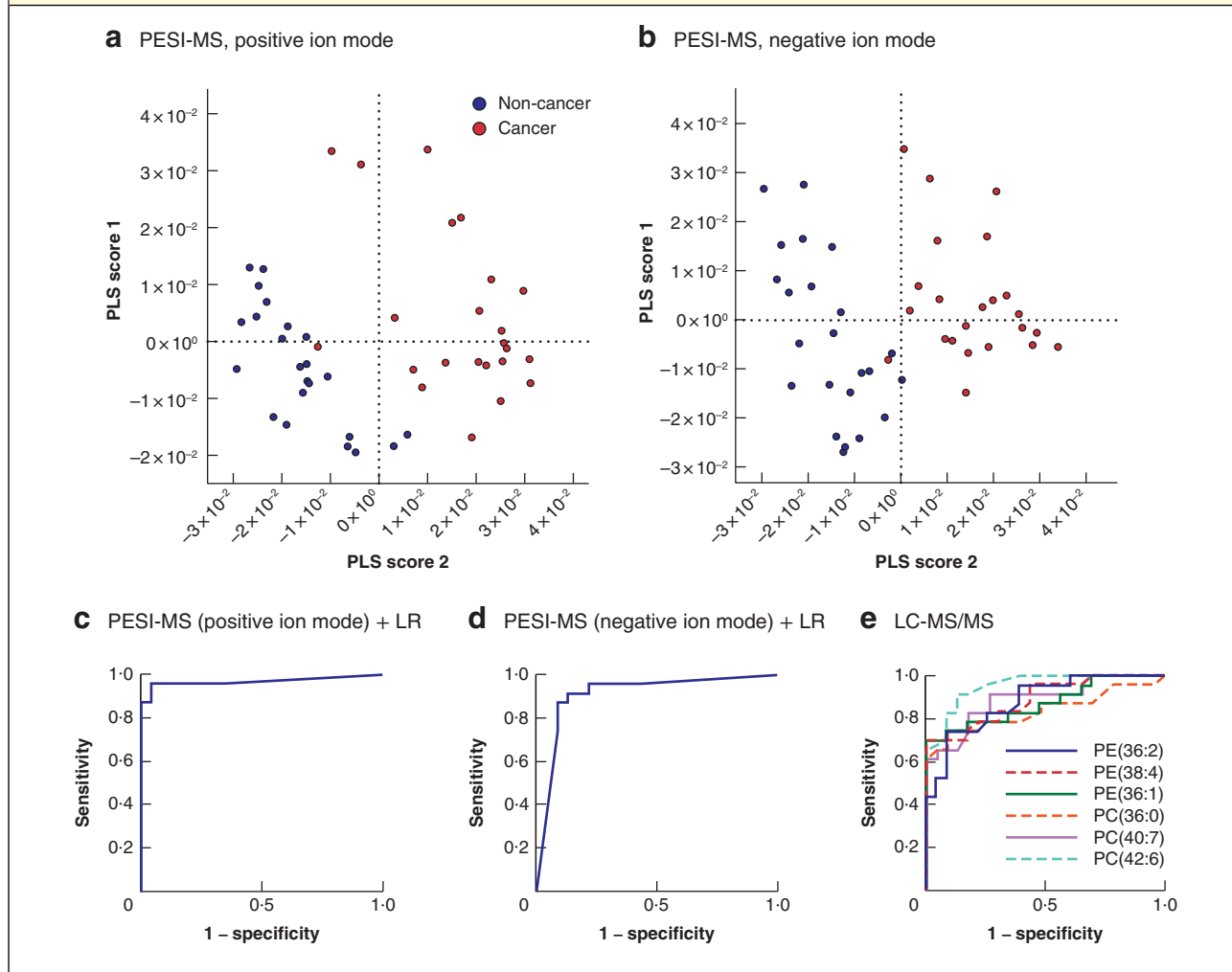
a,b Scatter plots of partial least squares discriminant analysis scores of mass spectra from 46 specimens in **a** positive and **b** negative ion modes. **c,d** Depiction of receiver operating characteristic (ROC) curves for **c** positive and **d** negative ion modes using sensitivity and specificity scores. Area under the ROC curve (AUC) values indicate the evaluation of the discrimination algorithm: **c** AUC = 0·967; **d** AUC = 0·910. **e** ROC curves giving AUC values for phosphatidylethanolamine (PE) (36:2): AUC = 0·870; PE(38:4): AUC = 0·860; PE(36:1): AUC = 0·886; phosphatidylcholine (PC) (36:0): AUC = 0·836; PC(40:7): AUC = 0·889; PC(42:6): AUC = 0·952. PESI-MS, probe electrospray ionization mass spectrometry; PLS, partial least squares; LR, logistic regression; LC-MS/MS, liquid chromatography tandem mass spectrometry.

(iKnife™; Waters Corporation, Milford, Massachusetts, USA), which analyses the mass spectra of vapours released by an electric surgical knife. The MasSpec Pen (Ms Pen Technologies Inc., Tulsa, Oklahoma, USA), combined with DESI-MS, can analyse directly the surface of the solid specimens[16]. Recently, another ambient MS system, internal extractive electrospray ionization mass spectrometry (iEESI-MS), was used for the discrimination of lung cancer by analysis of metabolites and lipids in tissue extracts[17]. This would also be applicable for extracts of breast cancer tissue. As PESI-MS is available for solid and liquid samples[18,19], the combination of MS analysis with existing procedures such as core-needle biopsy and liquid biopsy will be more useful and more accurate for breast cancer screening in the future. Before clinical implementation, rigorous validation studies with different MS methods will be mandatory.

Acknowledgements

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Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the article.