Targeted Human Plasma Metabolomics for Lobular Breast Cancer Biomarker Discovery

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BACKGROUND

Women with lobular breast cancer (LBC) face several unique challenges compared to those with invasive ductal carcinoma (IDC). LBC often presents with several histological and clinical features, such as a diffuse growth pattern and exhibit poorer long-term outcome and a unique pattern of metastasis. Overall, LBC presents diagnostic and therapeutic challenges due to its unique growth pattern. Despite these unique features, the exact metabolic pathways involved in LBC development remains unclear. Metabolomic profiling of plasma from women with LBC may help to identify new biomarkers to understand the molecular pathways involved in the clinical characteristics of LBC. The purpose of this study is to identify a panel of metabolomic biomarkers that would improve clinical assessment of LBC using plasma samples, and to understand the intersection between LBC and IDC.

METHODS

This study employed a hybrid methodology combining random subspace sampling, ensemble modeling, and frequency-based feature selection to identify parsimonious sets of discriminative features for distinguishing between breast cancer subtypes and healthy controls. The sample included a total of 185 plasma samples from women with biopsy-confirmed BC and 56 plasma samples from healthy controls. All biospecimens were obtained from the Cooperative Human Tissue Network (CHTN) biobank. A targeted, quantitative mass spectrometry (MS)-based metabolomics approach was used to analyze 138 metabolites in plasma samples using a combination of direct injection (DI) MS and reverse-phase high performance liquid chromatography (HPLC) tandem mass spectrometry (MS/MS). Features with >30% missing values were removed, and the remaining features were normalized. A large-scale stochastic approach was implemented, generating 500,000 logistic regression models with balanced class weights. Each model utilized a randomly selected subset of features.

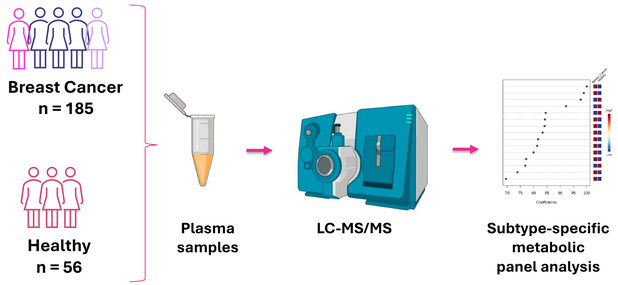

Figure 1: Methodological procedure leading to the measurement of plasma concentrations of 138 cancer specific metabolites.

STUDY POPULATION

Table 1: Characteristics of lobular breast cancer (LBC) and invasive ductal carcinoma (IDC) patients.

Total cases (N=241)		Lobular	Ductal	p-value
Population	Breast Cancer (N=185)	146	41	
	Healthy (N=56)	0	0	
Race	Black	4.88%	6.94%	
	White	92.68%	89.58%	0.558
	Other	2.44%	3,47%	
Smoking	Current	9.59%	9.76%	
	Former	25.34%	31.71%	0.481
	Never	65.07%	58.53%	
Age	Mean	59.90	56.00	0.092
BMI	Mean	30.07	29,68	0.618

RESULTS

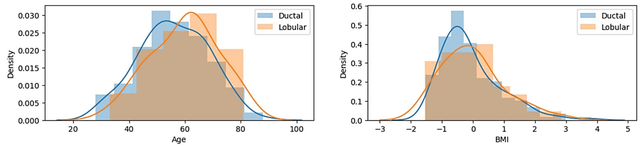

Figure 2: Statistical distribution of demographic variables for LBC and IDC patients. We observed a trend towards older age in the lobular cancer group compared to the invasive ductal carcinoma group. No differences in BMI was observed between ductal and lobular breast cancer patients

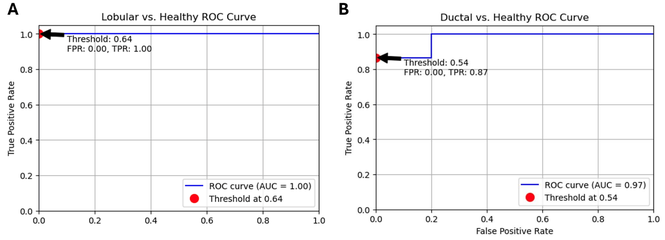

Figure 3: Multi-stage feature selection approach differentiating between breast cancer subtypes and healthy controls. The area under the receiver-operator characteristic curves (AUC) curves for cancer subtypes. (A) Lobular carcinoma vs. healthy comparison achieved 100% across all metrics, with a threshold of 0.64. (B) Ductal carcinoma vs. healthy reached an AUC of 97%, with 87% TPR and 0% FPR, and a threshold of 0.54.

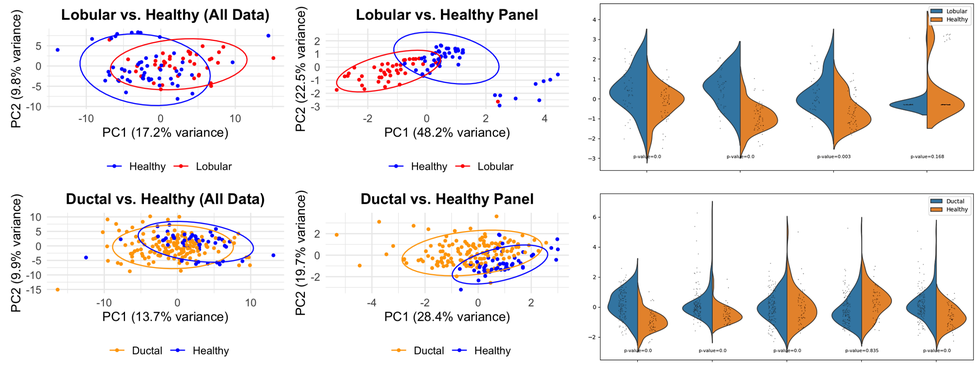

Figure 4: Contribution of plasma metabolomic differentiating between breast cancer subtypes and healthy controls. The variables from all high-performing models as measured by subtype-specific performance thresholds (>98% accuracy for lobular carcinoma vs. healthy controls and >95% accuracy for ductal carcinoma vs. healthy controls), were aggregated to create a comprehensive set of potentially significant features. The frequency of each feature across the high-performing models was calculated for each subtype comparison. Additional logistic regression models were evaluated using feature subsets derived from various frequency thresholds. Principal component analysis scatter plot show some clustering of cancer sample vs healthy control using the entire set of features (All Data). The panel-based plots show a more pronounced separation, indicating that the selected metabolites are particularly informative for distinguishing between cancer subtypes.

CONCLUSION

- **A deeper view and approach of cancer as a metabolic disease can positively impact new diagnostic dimensions by identifying specific metabolic signatures.**
- **The distinct clustering of cancer subtypes suggests that there are significant metabolic differences between ductal and lobular breast cancer impacting different pathways.**
- **The best signature for the lobular breast cancer (LBC) vs. healthy controls comparison comprised only 3 metabolites and age achieved accuracy and AUC values of 100%.**
- **The frequency-based feature selection for invasive ductal carcinoma (IDC) identified a set of five discriminative features containing four metabolites and age with an overall accuracy of 95% and a bootstrapped AUC of 97%.**
- **The targeted plasma metabolomic profiles offer promising avenues for non-invasive differentiation between ductal and lobular carcinomas, potentially transforming breast cancer diagnostics leading to more precise treatment, improved patient outcomes, and enhanced healthcare equity.**

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