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BACKGROUND AND PURPOSE

- ❖ We immunophenotyped various myeloid and lymphoid cell populations using standard multiparametric flow cytometry from healthy female individuals along with subjects with biopsy-confirmed invasive breast cancer (BCa) and non-invasive ductal carcinoma in situ (DCIS).
- ❖ Cell surface markers were included to identify myeloid-derived suppressor cell (MDSC) subsets – a heterogeneous group of immature myeloid cells that suppress T-cell responses and support tumor progression.
- ❖ We created a technique for processing flow cytometry data to be used as inputs for feedforward artificial neural networks (ANNs), or multilayer perceptrons (MLPs), in order to categorize each sample.
- ❖ The purpose of this study was to analyze immunophenotyping flow cytometry data using artificial intelligence (AI) to identify individuals presenting with early stage breast cancer (stage I/II).

METHODS

An overview of the methods are described in **Figure 1**. Peripheral blood was collected with IRB approval and informed consent. Subjects were excluded from the study if they had a previous history of cancer or were currently receiving/had received medical intervention for breast cancer. A total of 112 subjects with biopsy confirmed invasive breast cancer (BCa), 25 subjects with biopsy confirmed DCIS, and 88 control donors were included (**Table 1**). Peripheral blood mononuclear cells (PBMCs) were isolated 20 to 30 hours post-collection using standard methods. The MDSC and lymphocyte cell populations were analyzed on a BD LSRII flow cytometer (**Table 2**) and manually gated for comparison. Compensated channel values for each event were exported from FCS format to CSV format using FlowJo software.

To prepare the data for neural network input, we used a procedure wherein each channel of flow cytometry data was used as an axis in a multidimensional space (**Figure 1c**). Each axis was then divided into four segments and each event was defined by its segment location on each axis. For example, if the flow cytometry data has 3 channels, each channel is divided into four segments thus resulting in 4³ or 64 volumetric regions in the multidimensional space; these regions are referred to as voxels. Voxels of dimension greater than 3 are referred to as hypervoxels. For our study, two separate panels were used with one containing seven channels (lymphoid) and one containing ten channels (myeloid) resulting in each having 4⁷, or 16,384, hypervoxels and 4¹⁰, or 1,048,576, hypervoxels, respectively. A count was then made of the number of events falling within each hypervoxel resulting in a common feature for all samples. Each hyperspace was then converted to a one-dimensional vector and used as input for a separate NN. The outputs of the two networks were then used as an input to a third NN which integrates the two inputs generating the final classification. After data preparation, a series of feedforward ANNs were created with network inputs consisting of the numerical event counts in each hypervoxel; three datasets were then constructed: the training dataset ‘teaches’ the two output categories through backpropagation and fitting; the validation dataset evaluates the fit to minimize overfitting; and the test dataset ranks the trained networks against each other and estimates performance. Finally, a data set that was withheld from network training (Holdout) was used to evaluate overall performance of the top-ranking networks after voting.

RESULTS

Table 1. Clinical Information of study participants

	HD	DCIS	BCa
Number	88	25	112
Median Age	42	55	58.5
Age Range	18 – 71	38 - 73	30 - 82
Stage 1			63
Stage 2			28
Stage 3			10
Stage 4			2
Stage Unknown			9

Table 2. Cell surface markers used for immunophenotyping

Population	Surface Markers
eMDSC	Lineage-HLA-DR-CD33 ⁺
PMN-MDSC	CD14-CD11b-CD33-CD15-SSC ^{Hi}
M-MDSC	CD14-HLA-DR- ^{Lo}
CD4 ⁺ T Cell	CD3-CD4 ⁺
CD8 ⁺ T Cell	CD3-CD8 ⁺
B Cell	CD3-SSC ^{Lo} -CD19 ⁺
NK Cell	CD3-SSC ^{Lo} -CD56 ⁺
PMN	CD14-CD15 ⁺
Monocyte	CD14-CD15 ⁻
LOX-1 ⁺ PMN ²	CD14-CD15 ⁺ -LOX-1 ⁺

Figure 2. Manual gating analysis of the three primary subsets of MDSCs: eMDSCs (left graph), PMN-MDSCs (middle graph), and M-MDSCs (right graph) (mean ± SD)

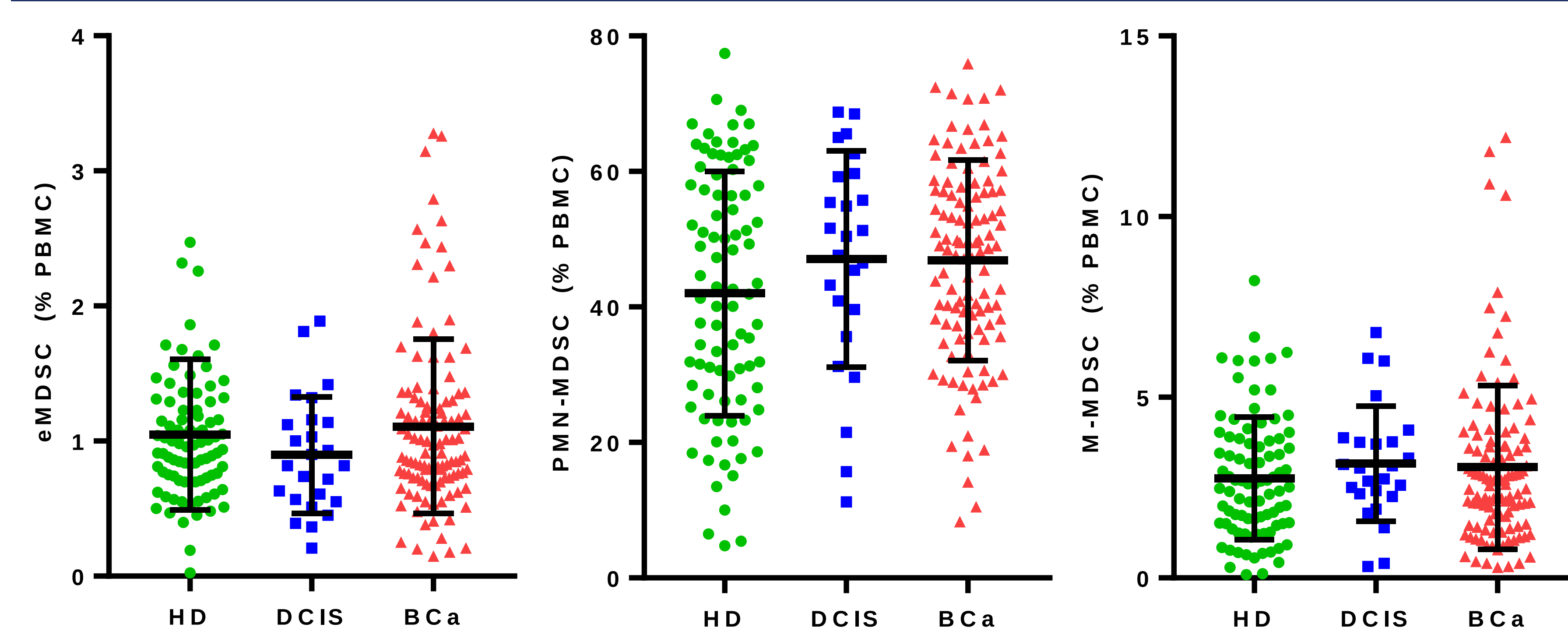


Table 3. Sensitivity/specificity analysis for early stage breast cancer detection

		Holdout		All	
		BCa	HD	BCa	HD
Samples Tested		28	19	92	88
Predicted	BCa	25	1	83	8
	HD	3	18	8	80
Sensitivity		89.3%		91.2%	
Specificity		94.7%		90.9%	
Accuracy		91.5%		91.1%	

✓ Manually gated values demonstrate the significant overlap between the three tested categories (**Figure 2**).

✓ Sensitivity and specificity of 89.3% and 94.7%, respectively, were achieved by the neural network test distinguishing between holdout early stage breast cancer and control samples (**Table 3**). For all samples, a sensitivity and specificity of 91.2% and 90.9% (including those used for training), respectively, were achieved.

METHODS Continued Figure 1. Illustrated overview of methods from sample collection to data processing and analysis.

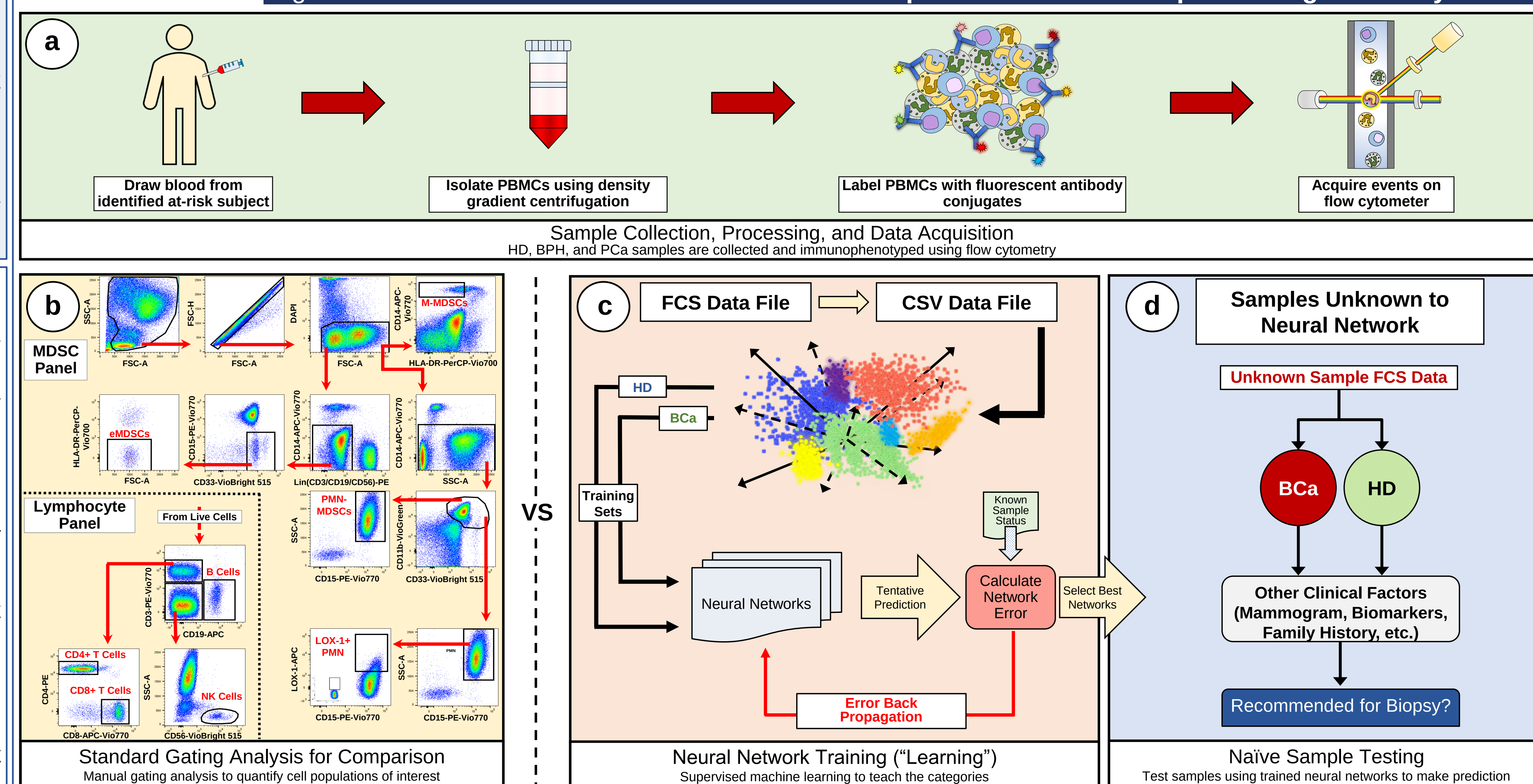
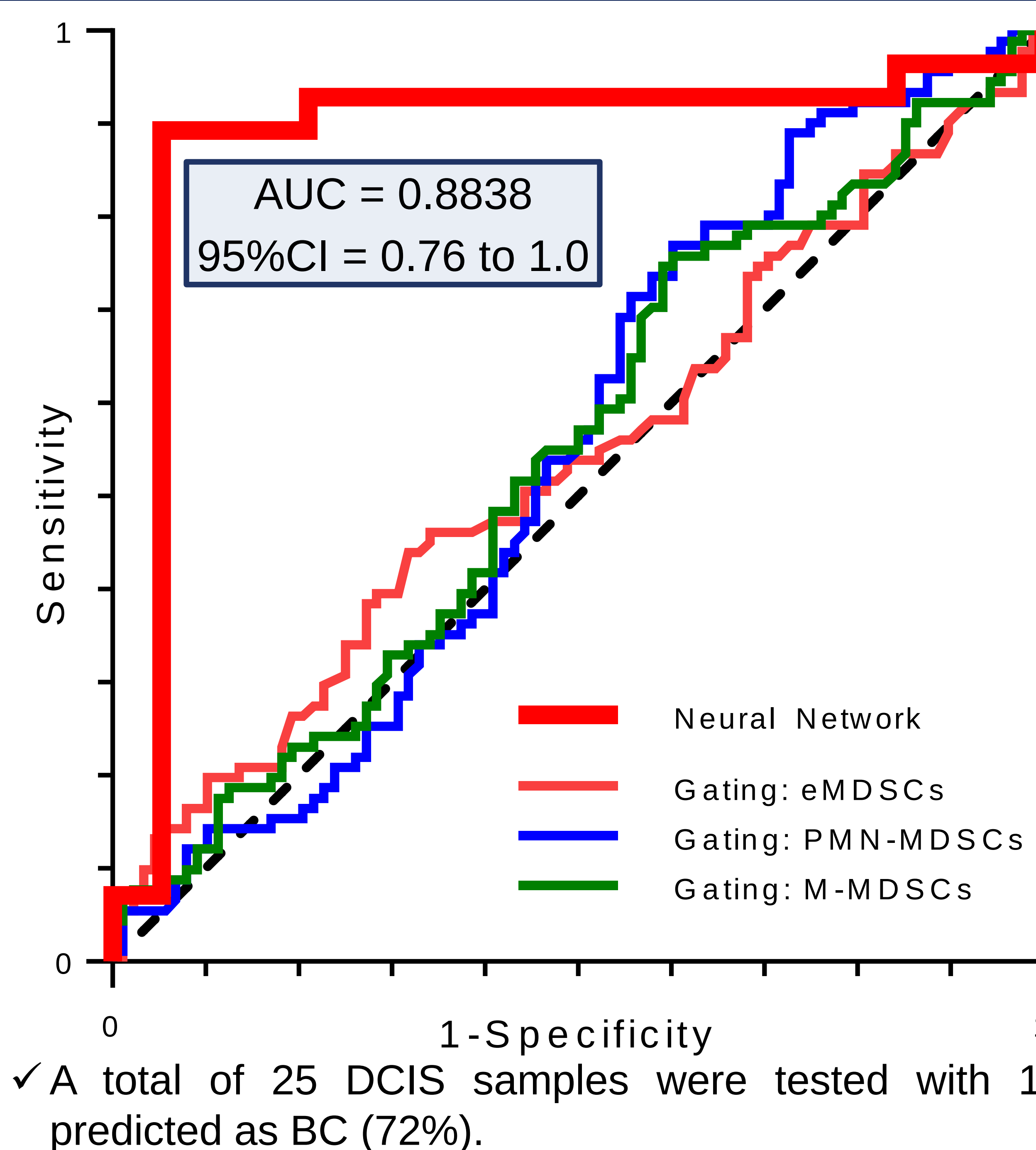


Figure 3. ROC curve analysis comparing manual gating of MDSCs to neural network performance for distinguishing between withheld early stage breast cancer subjects and healthy donor controls



✓ A total of 25 DCIS samples were tested with 18 predicted as BC (72%).

CONCLUSIONS

- Using this technique, we are able to predict the existence of early stage invasive breast cancer with 89.3% sensitivity and 94.47% specificity.
- This technique may prove beneficial in a clinical setting by pairing with a mammography exam, for which early stages of breast cancer are harder to detect than late stages, or other clinical factors to confirm the need of a biopsy.
- Additionally, 18 out of the 25 samples collected from biopsy confirmed DCIS subjects were predicted as BC. Even though they are clinically deemed as pre-cancerous (stage 0), this technique indicates its possible utility for detecting the existence of even a non-invasive breast cancer lesion.
- We expect network performance to improve as the sample number increases.
- To our knowledge, this is the first application of flow cytometry event data used to directly train artificial neural networks to detect the presence of a breast malignancy.
- We believe that this technology could be used for other types of binary classifications, such as predicting patient responses to immunotherapies and/or monitoring for the recurrence of tumors.