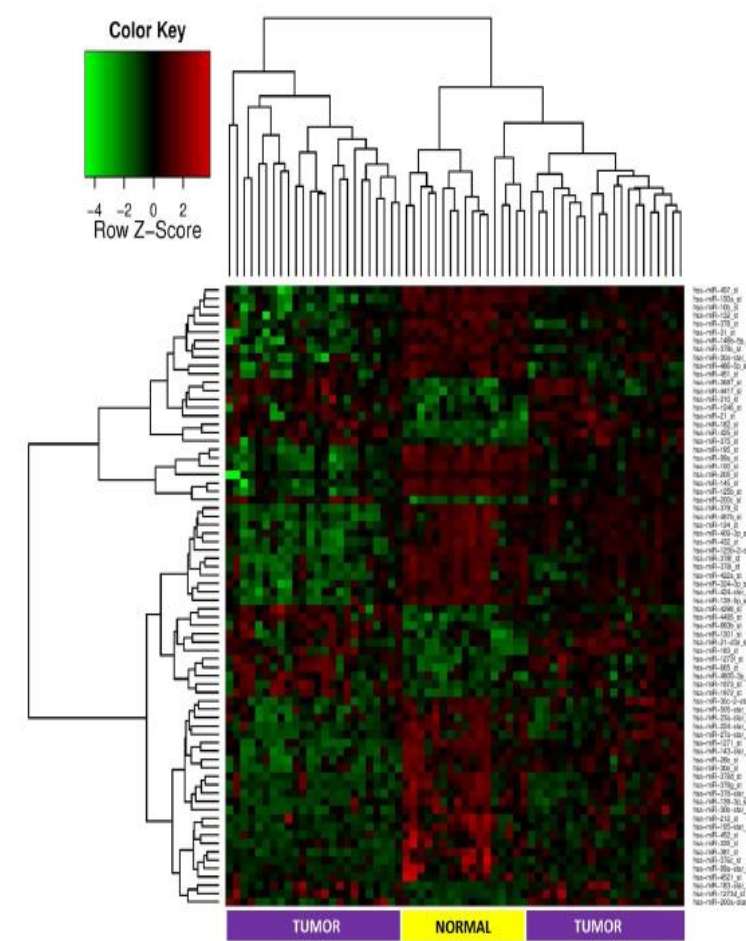# Potential Value of Circulating microRNA as Diagnostic Biomarkers for Breast Cancer in Lebanese Women

Farah Nassar[1*], Maha Itani[2*], Arafat Tfayli[1], Rosemary Boustany[3], Joelle Makoukji[3], Ghada Chamandi[2], Abdul Rahman Itani[2], Rabih Talhouk[4], Lifang Hou[5], Nathalie Zgheib[6], and Rihab Nasr[2]

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Background

Figure 1. microRNA Microarray Analysis. 74 differentially expressed miRNA in BC tissues from Lebanese BC patients compared to normal adjacent tissues.

- Breast cancer (BC) is the most prevalent type of cancer in Lebanese women.
- Mammography is recommended for women above 40 years for BC screening in Lebanon, yet 17.33% of Lebanese BC patients are younger than 40 [1]. Detecting BC in its early stages would decrease its fatality rate.
- We previously showed dysregulation of microRNA in tumors vs. normal adjacent tissues from early stage BC Lebanese patients.
- miRNA are stable in body fluids and expressed differentially in tumor vs. normal samples making them promising diagnostic biomarkers for BC.

Aim

This research study aims to investigate the expression of circulating microRNA in early stage Invasive Ductal Carcinoma (IDC) BC patients with positive estrogen and progesterone receptors status as promising diagnostic biomarkers.

Methods

1. Plasma was isolated by centrifugation of blood from 41 Lebanese IDC BC patients and 32 healthy controls
2. Total RNA was extracted using Norgen kit and quantified using the Nanodrop.
3. cDNA of specific miRNA was synthesized using the TaqMan MicroRNA Reverse Transcription Kit.
4. Finally, the expression levels of miRNAs of interest were measured using RT-qPCR.
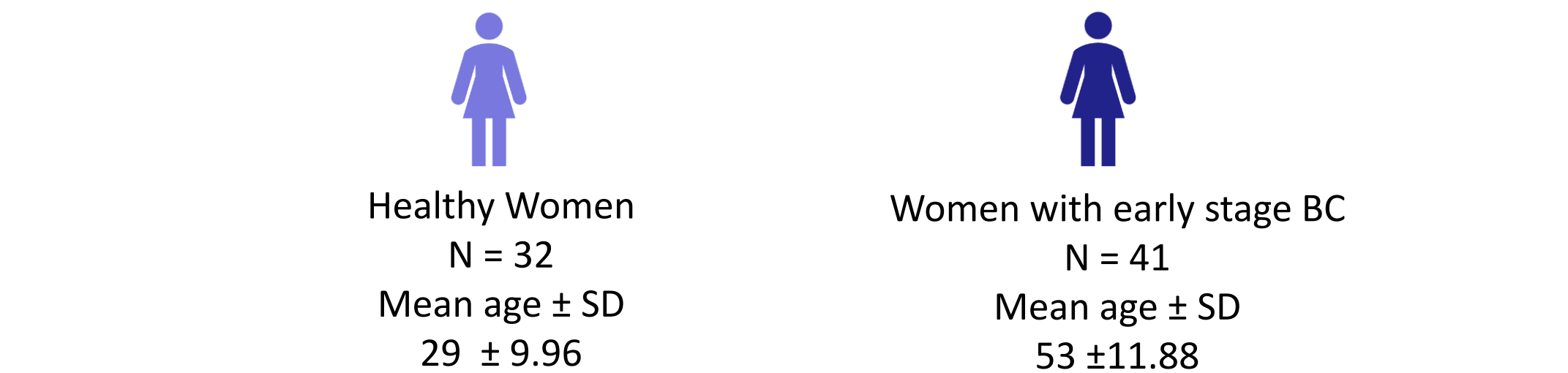5. Statistical analysis and ROC curve analysis.

Results

Figure 2. Sample size and age (mean ± standard deviation) of participated healthy subjects and BC patients.

Healthy Women
N = 32
Mean age ± SD
29 ± 9.96

Women with early stage BC
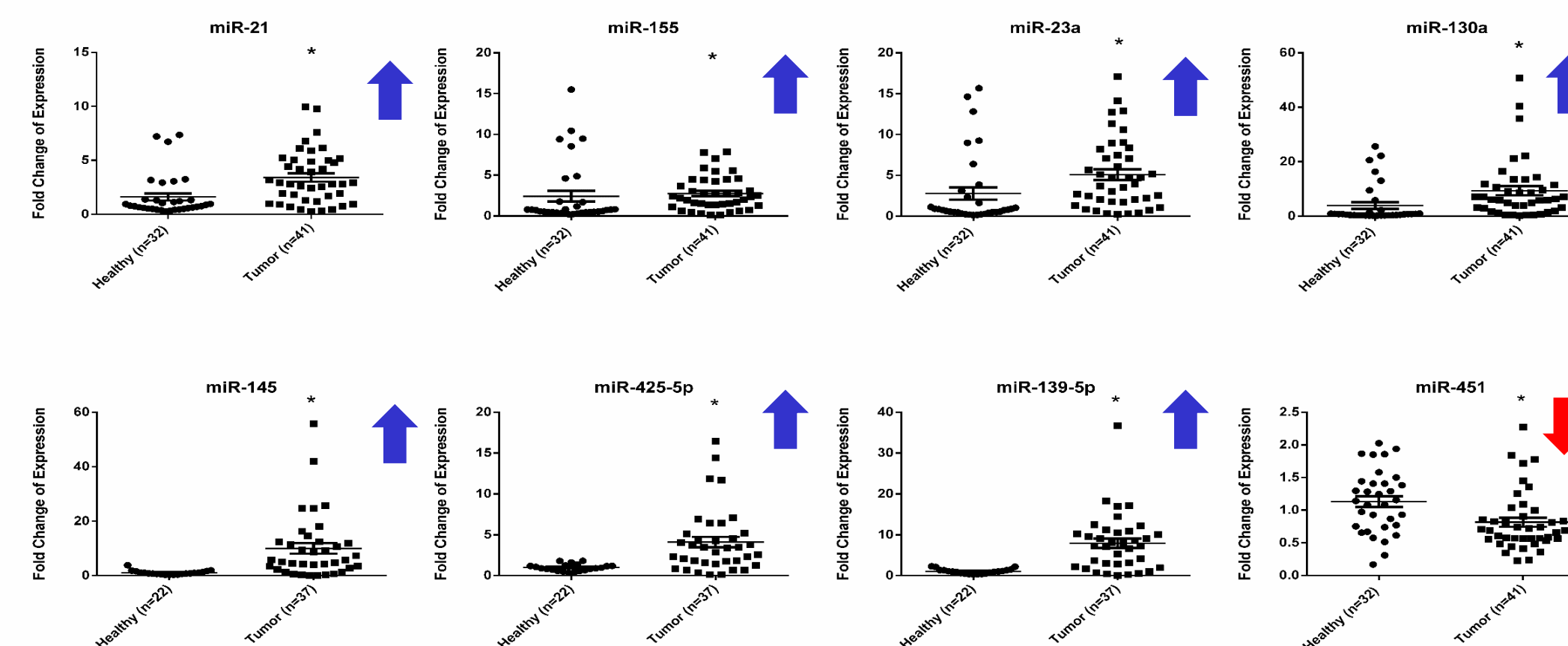N = 41
Mean age ± SD
53 ±11.88

Figure 3. Expression of miRNA in plasma samples of BC patients and healthy female subjects. The plot represents the mean (middle line) and the standard error of mean (error bars). * denotes p-value < 0.05 according to Wilcoxon's signed-rank test.
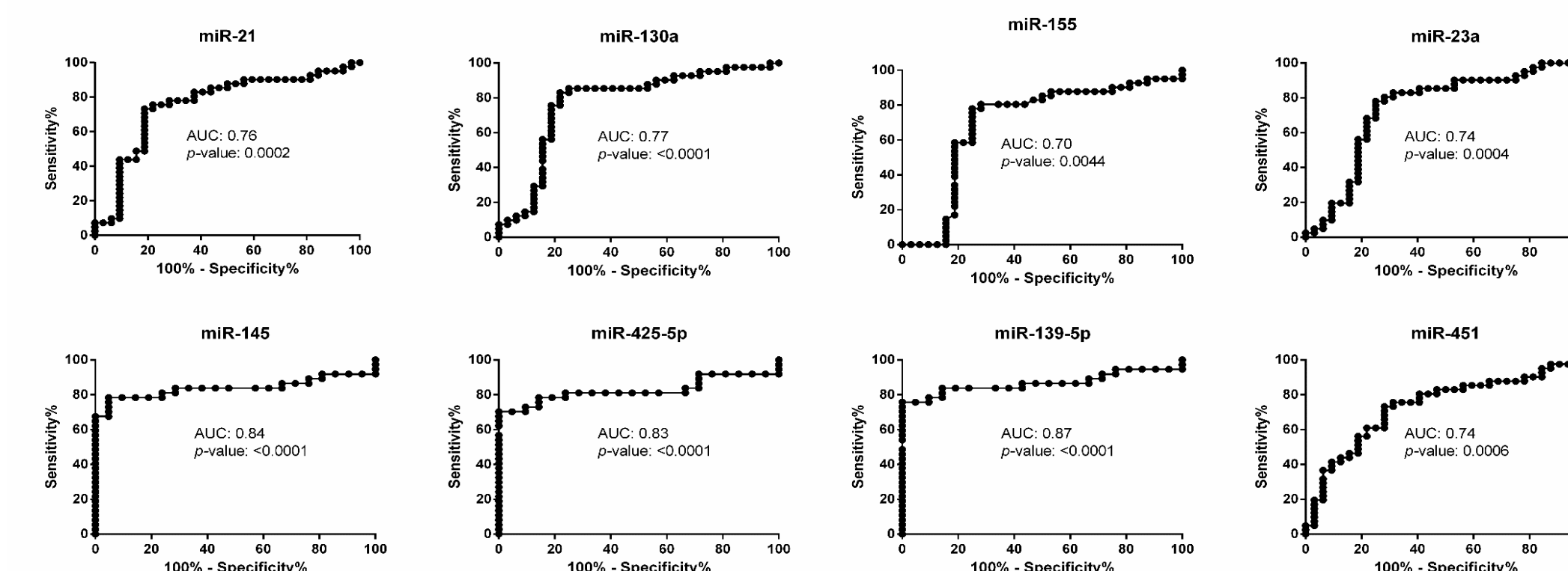

Figure 4. Diagnostic accuracy of deregulated miRNA. ROC curve analysis was done to separate between BC patients and healthy controls through individual miRNA. T= 41 and N=32 (for miR-21, miR-130a, miR-155, miR-23a & miR-451), T=37 and N=22 (for miR-145, miR-425-5p & miR-139-5p).

miRNA	AUC	p-value	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	DA (%)	95% CI
Top 2 miRNA	0.87	<0.0001	0.54	78%	100%	78%	100%	86%	0.765-0.969
Top 3 miRNA	0.87	<0.0001	0.59	78%	100%	78%	100%	86%	0.769-0.968
Top 4 miRNA	0.90	<0.0001	0.63	81%	100%	81%	100%	88%	0.816-.983
Top 5 miRNA	0.91	<0.0001	0.64	78%	100%	78%	100%	86%	0.840-0.987
Top 6 miRNA	0.92	<0.0001	0.67	78%	100%	78%	100%	86%	0.853-0.990
Top 7 miRNA	0.94	<0.0001	0.75	78%	100%	78%	100%	86%	0.878-0.993
Top 8 miRNA	0.93	<0.0001	0.76	76%	100%	76%	100%	84%	0.868-0.990

Table 1. Diagnostic parameters (sensitivity, specificity, PPV, NPV, and Diagnostic accuracy) to evaluate the early stage BC diagnostic ability of combination of miRNA. The combinations were based on the descending DA of the miRNA. AUC; area under the curve, PPV; positive predicted value, NPV; negative predicted value, DA; diagnostic accuracy, CI; confidence interval.

Conclusions

- 7 miRNA are significantly upregulated, while miR-451 is significantly downregulated and 4 miRNA show non-significant mode of dysregulation (miR-125b, miR-100, miR-182, and miR-195) in the plasma of early stage BC patients compared to healthy controls.
- According to ROC curves, miR-139-5p, miR-145, and miR-425-5p recorded the highest AUC, showing the highest accuracy in diagnosing early stage BC cases.
- The combined diagnostic potential of miR-145, miR-425-5p, miR-139-5p and miR-130a recorded the highest sensitivity, absolute specificity, and highest diagnostic ability (81%, 100%, & 88%, respectively) and have the best diagnostic ability.

Importance of the study

- Detection of BC in its early stages (before metastasis) provides better chances for treatment, increase survival, and improves quality of life.
- Although mammography is the "gold standard" screening method for BC, it is associated with pain, anxiety, radiation exposure, and restricted by dense breasts and in Lebanon, not all women are eligible to do mammography screening.
- Circulating microRNAs could serve as potential minimally-invasive biomarkers to diagnose early stages BC cases among Lebanese women.

References: 1. Bray, F., et al., Global cancer statistics 2018. 68(6): p. 394-424.