Increased Sensitivity and Specificity of Screening Mammography Combined With an Ancillary Breath Test for Biomarkers

Michael Phillips
mphillips@menssanaresearch.com

Menssana Research Inc
Therese B Bevers
University of Texas, MD Anderson Cancer Center
Linda Hovanessian Larsen
Keck Medical Center of University of Southern California
Nadine Pappas
Saint Michael's Medical Center
Sonali Pathak
Menssana Research Inc

Research Article

Keywords:

Posted Date: August 14th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-7160489/v1

Additional Declarations: Competing interest reported. Michael Phillips is President and CEO of Menssana Research, Inc. All other authors declare that they have no conflict of interest.

Abstract

Background

When two tests for a disease are employed together, the resulting combination can be more sensitive or specific than either test employed alone, provided that they employ different biological mechanisms. We determined changes in sensitivity and specificity of screening mammography when combined with a breath test for biomarkers of breast lesions.

Methods

We analyzed volatile organic compounds (VOCs) in the breath of women having screening mammography, using gas chromatography with mass spectrometry (GC MS) and surface acoustic wave detection (GC SAW). We compared breath VOCs in women with normal screening mammograms to those whose mammograms were subsequently associated with biopsy-proven breast cancer. Multiple Monte Carlo simulations identified breath VOC biomarkers that predicted the likelihood of a structural breast lesion. Sensitivity and specificity of mammography and breath VOC biomarkers were determined as predictors of breast lesions when employed separately and in combination.

Results

Mammography employed alone detected breast lesions with 79% sensitivity and 88.9% specificity; breath biomarkers were 86.5% sensitive and 66.7% specific. When the results of the two tests agreed (i.e. the mammogram and the breath test were both positive or both negative), the specificity of the combination rose to 96.3%, indicating an increased probability of a true positive or a true negative mammogram result. When the results of the two tests disagreed (i.e. the mammogram was positive and the breath test was negative, or vice versa), the sensitivity of the combination rose to 97.2%, indicating an increased probability of a false positive or a false negative mammogram result.

Conclusions

Mammography combined with a breath test for VOC biomarkers predicted breast lesions with greater sensitivity and specificity than mammography employed alone.

INTRODUCTION

Mammography is widely employed as a as a screening test for breast cancer but it is susceptible to false positive and false negative findings [1]. It is also susceptible to errors arising from interobserver variation when two observers reading the same mammogram reach different conclusions[2,3].

In order to minimize these problems, the sensitivity and specificity of mammography could potentially be improved by combining it with an ancillary nondependent test for structural abnormalities in breast tissue. This approach is employed in other conditions in which a test for abnormal anatomy is combined with a test for abnormal function. Examples include prostate cancer screening with MRI combined with PSA biomarkers[4], and colon cancer screening with colonoscopy combined with fecal occult blood testing[5]. By employing two diagnostic tests for the same condition, it is possible to achieve a combined test that has greater sensitivity and specificity than either test employed alone, provided that the two tests employ different biological mechanisms [6-8]. Weinstein et al analyzed the mathematical basis of combination diagnostic testing, and showed that two tests employed in combination can deliver either greater sensitivity or specificity than either test employed alone[8]. They proposed an AND/OR rule to combine two tests so that they are optimized to deliver increased specificity or sensitivity (Figure 1).

Breath biomarkers can identify altered metabolic pathways associated with structural abnormalities in breast tissue. Every exhaled breath contains more than 2,000 different volatile organic compounds (VOCs) mostly in parts per billion (ppb) concentrations[9]. Previous studies have reported altered abundance of breath VOCs in a variety of diseases, and the Food & Drug Administration (FDA) has cleared or approved breath tests for conditions including infections (Helicobacter pylori, COVID-19), carbohydrate malabsorption, asthma and airways inflammation. Researchers have employed breath VOC tests using gas chromatography (GC), mass spectrometry (MS), electronic noses, and sniffing dogs, we have reported breath biomarkers for the detection of lung cancer[10], tuberculosis[11], and radiation exposure[12].

We have previously reported studies of a rapid point-of-care breath test employing GC with surface acoustic wave detection (GC SAW) that detected breath VOC biomarkers associated with structural abnormalities in breast tissue[13-15]. Since breath testing and radiologic imaging employ different biological mechanisms, a test for breath VOC biomarkers could provide a rational ancillary test in combination with mammography. Breath tests have several practical advantages: they are simple to use and cost-effective; they are also inherently painless, non-invasive, objective, and safe. We report here an evaluation of combining mammography with an ancillary breath test for VOC biomarkers, and its resulting sensitivity and specificity of the combination for detecting structural abnormalities in breast tissue.

RESEARCH METHODS

Sensitivity and specificity of screening mammography: Values were obtained from the Food & Drug Administration (FDA) publication Mammography Quality Standards Act; Amendments to Part 900 Regulations Docket No. FDA-2013-N-0134[16]. According to FDA: “Results were shown from estimating annual values for screening mammography in the U.S.” and “Because data on sensitivity are difficult to obtain and estimates vary, calculations are presented using both a high and low estimate of sensitivity”. The high values were sensitivity = 79.0%, specificity = 88.9% and the low values were sensitivity = 66.0%, specificity = 88.9%. In this study, the high values were employed for analysis of data.

Sensitivity and specificity of breath VOC biomarkers: We previously reported studies of breath VOCs using GC MS and GC SAW in 783 women: normal mammograms (473), abnormal mammograms (171), and biopsy-proven breast cancer (139) [13–15]. Since the clinical designs were not identical in all studies, we selected a typical subset that distinguished between normal and abnormal screening mammograms with 86.5% sensitivity, 66.7% specificity, and 83% accuracy[14]. Structural abnormalities in breast tissue were identified by imaging with mammography augmented with MRI and sonography where indicated. Prior to screening mammography, all subjects donated a breath sample by inflating an ultra-clean collection balloon. Breath VOCs were analyzed with GC SAW and the resulting chromatograms were decomposed into a series of time-related segments, each of 0.8 sec duration, comprising the mass response of the SAW detector. Chromatographic peak segments were compared in two groups of women, normals and breast cancer, and ranked as candidate biomarkers according to their C-statistic values i.e. the area under curve (AUC) of the receiver operating characteristic (ROC) curve. Biomarkers with statistically significant C-statistic values were identified with multiple Monte Carlo simulations. These biomarkers were entered into a multivariate predictive algorithm in order to generate a numerical value, the discriminant function (df), which varied with the likelihood of a structural breast abnormality.

Combination diagnostic testing. The method of Weinstein et al was employed to determine the sensitivity and specificity of mammography and breath VOC biomarkers employed alone or in combination using the AND/OR rule. The method is summarized in Fig. 1.

RESULTS

Impact of ancillary testing on mammography performance. Figures 2 and 3 show the sensitivity and specificity of mammography and breath VOC biomarkers used alone and in combination for detection of a structural lesion in the breast. When the results of the two tests agreed (i.e. mammography and the breath test were both positive or both negative), the specificity of the combination was 96.3%, an increase from 88.9% with mammography employed alone. This finding indicated an increased probability of a true positive or a true negative mammogram result. Conversely, when the results of the two tests disagreed (i.e. mammography was positive and the breath test was negative, or vice versa), the sensitivity of the combination rose to 97.2%, compared to 79% with mammography alone, indicating an increased probability of a false positive or a false negative mammogram result.

DISCUSSION

The main finding of this study was that screening mammography combined with an ancillary breath test for VOC biomarkers detected breast lesions with greater sensitivity and specificity than mammography employed alone.

The potential clinical outcomes arising from increased sensitivity and specificity of screening mammography are described in Figure 4. There were four possible outcomes when the two tests were employed in combination: the results either agreed (mammography and the breath test were both positive or both negative), or they disagreed (mammography was positive and the breath test was negative, or vice versa). When the results agreed, the specificity of the combination was 96.3%, compared to 89.9% for mammography employed alone. When the results disagreed, the sensitivity of the combination was 97.2%, compared to 79% for mammography employed alone. All outcomes were accompanied by a specific recommended plan of action.

In clinical practice, a breath test for VOC biomarkers can be performed either in the laboratory or at the point-of-care. The procedure is safe, painless, rapid, and cost-effective. An ancillary breath test could potentially benefit physicians by improving the diagnostic accuracy of screening mammography. Women and the healthcare system might also benefit from a reduced number of needless procedures.

Declarations

Acknowledgements: Schmitt & Associates, Newark, NJ, and Michael Phillips analyzed the data. Breath test studies were funded by NIH NCI Grant Number: 5R44CA203019 – 02. ClinicalTrials.gov Identifier: NCT02888366.

Conflict of Interest: Michael Phillips is President and CEO of Menssana Research, Inc. All other authors declare that they have no conflict of interest.

Animals: No animals were involved.

Ethics, reporting, and human subjects: Clinical studies were reviewed and approved by an Institutional Review Board (IRB) at all participating sites. Written informed consent was obtained from all individual participants. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Author Contribution

MP wrote the main manuscript text. SP analyzed breath samples. TBB, LHL and NP supervised clinical studies. All authors reviewed the manuscript."

Data Availability

Data is provided within the manuscript

References

1. Nelson HD, O'Meara ES, Kerlikowske K, Balch S, Miglioretti D. Factors Associated With Rates of False-Positive and False-Negative Results From Digital Mammography Screening: An Analysis of Registry Data. Ann Intern Med 2016;164(4):226-35. DOI: 10.7326/M15-0971.

2. Duijm LE, Louwman MW, Groenewoud JH, van de Poll-Franse LV, Fracheboud J, Coebergh JW. Inter-observer variability in mammography screening and effect of type and number of readers on screening outcome. Br J Cancer 2009;100(6):901-7. DOI: 10.1038/sj.bjc.6604954.
3. Koyama Y, Nakashima K, Orihara S, et al. Inter- and intra-observer variability of qualitative visual breast-composition assessment in mammography among Japanese physicians: a first multi-institutional observer performance study in Japan. Breast Cancer 2024;31(4):671-683. DOI: 10.1007/s12282-024-01580-8.
4. Hugosson J, Månsson M, Wallström J, et al. Prostate Cancer Screening with PSA and MRI Followed by Targeted Biopsy Only. N Engl J Med 2022;387(23):2126-2137. (In eng). DOI: 10.1056/NEJMoa2209454.
5. Wiwanitkit V. Colonoscopy with and without occult blood test pre-screening: which is more cost effective for implementation for screening for colon cancer? Asian Pac J Cancer Prev 2010;11(3):823-4. (In eng).
6. Borowiak D, Reed JF, 3rd. Utility of combining two diagnostic tests. Comput Methods Programs Biomed 1991;35(3):171-5. DOI: 10.1016/0169-2607(91)90119-e.
7. Tang ML. On simultaneous assessment of sensitivity and specificity when combining two diagnostic tests. Stat Med 2004;23(23):3593-605. DOI: 10.1002/sim.1906.
8. Weinstein S, Obuchowski NA, Lieber ML. Clinical evaluation of diagnostic tests. AJR Am J Roentgenol 2005;184(1):14-9. DOI: 10.2214/ajr.184.1.01840014.
9. Phillips M, Cataneo RN, Chaturvedi A, et al. Detection of an extended human volatome with comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry. PLoS ONE 2013;8(9):e75274. DOI: 10.1371/journal.pone.0075274.
10. Phillips M, Cataneo RN, Cummin AR, et al. Detection of lung cancer with volatile markers in the breath. Chest 2003;123(6):2115-23. (http://www.ncbi.nlm.nih.gov/pubmed/12796197).
11. Phillips M, Basa-Dalay V, Bothamley G, et al. Breath biomarkers of active pulmonary tuberculosis. Tuberculosis (Edinb) 2010;90(2):145-51. (Multicenter Study Research Support, N.I.H., Extramural) (In eng). DOI: 10.1016/j.tube.2010.01.003.
12. Phillips M, Byrnes R, Cataneo RN, et al. Detection of volatile biomarkers of therapeutic radiation in breath. J Breath Res 2013;7(3):036002. DOI: 10.1088/1752-7155/7/3/036002.
13. Phillips M, Bevers TB, Larsen LH, Pappas N, Pathak S. Rapid point-of-care breath test predicts breast cancer and abnormal mammograms in symptomatic women. J Breath Res 2024;18(4). DOI: 10.1088/1752-7163/ad7a20.
14. Phillips M, Beatty JD, Cataneo RN, et al. Rapid point-of-care breath test for biomarkers of breast cancer and abnormal mammograms. PLoS ONE 2014;9(3):e90226. DOI: 10.1371/journal.pone.0090226.
15. Phillips M, Cataneo RN, Cruz-Ramos JA, et al. Prediction of breast cancer risk with volatile biomarkers in breath. Breast Cancer Res Treat 2018;170(2):343-350. DOI: 10.1007/s10549-018-4764-4.

16. Food&DrugAdministration. Mammography Quality Standards Act; Amendments to Part 900 Regulations Docket No. FDA-2013-N-0134.

Figures

	sensitivity	***Specificity***
The AND rule: *Use if Test A AND Test B are both positive; their combined result is read as positive.* *Conversely, if Test A AND Test B are both negative, then their combined result is read as negative.* ***Outcome:*** *The combination is more specific than either Test A or Test B used alone, but less sensitive.*	A_{sens} X B_{sens} 	A_{spec} + B_{spec} – (A_{spec} X B_{spec}) 
The OR rule: *Use if Test A OR Test B is positive while the other is negative (or vice versa).* ***Outcome:*** *The combination is more sensitive than either Test A or Test B used alone, but less specific.*	A_{sens} + B_{sens} - (A_{sens} X B_{sens}) 	A_{spec} X B_{spec} 

Figure 1

The AND/OR rules for combining Test A with Test B *(adapted from Weinstein et al[8]).* The subscripts "spec" and "sens" indicate sensitivity and specificity of Test A and Test B respectively.

Tests used alone	***Sensitivity***	***Specificity***
Screening mammography	***79%***	***88.9%***
Breath test	***86.5%***	***66.7%***
Tests used in combination		
Scenario: *Test results agree:* • *Mammography AND breath test are both positive* • *Mammography AND breath test are both negative* ***Action:*** *Use the AND rule* ***Outcome:*** *Specificity increased; sensitivity decreased*	***68.9%***	***96.3%***
Scenario: *Test results disagree* • *Mammography OR breath test positive, the other negative* • *Mammography OR breath test negative, the other positive* ***Action:*** *Use the OR rule* ***Outcome:*** *Sensitivity increased; specificity decreased*	***97.2%***	***59.3%***

Figure 2

Sensitivity and specificity of mammography and breath VOC biomarkers used alone and in combination for detection of a structural lesion in the breast: Sources of values of sensitivity and specificity of screening mammography and breath VOC biomarkers are described in the methods section. Combined values were determined using the AND/OR equations shown in Figure 1.

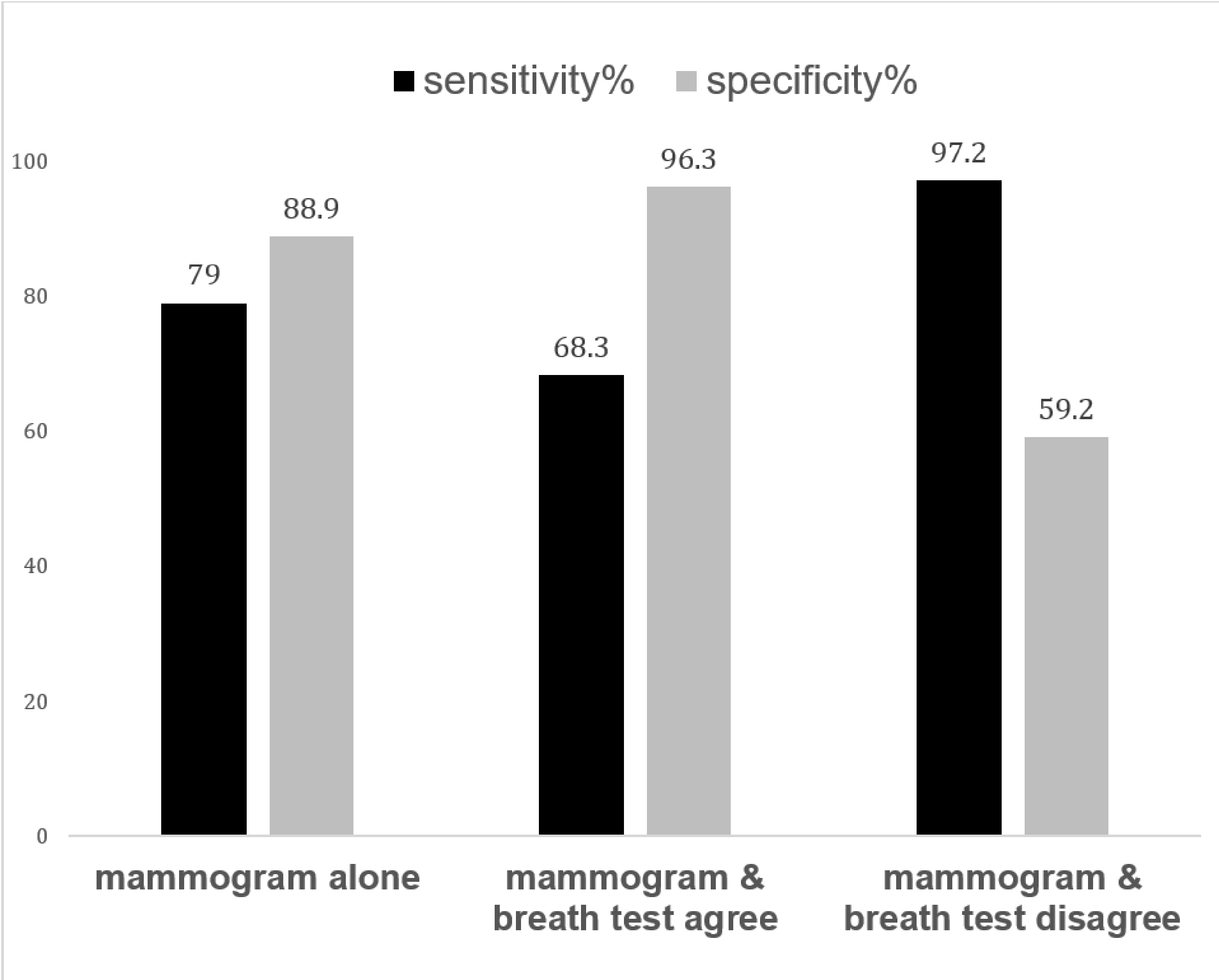

Figure 3

Sensitivity and specificity of mammography alone versus mammography combined with ancillary breath test. Values were determined by application of the AND/OR rule***,*** so that outcomes varied according to whether the results of the two tests agreed or disagreed.

Outcome			
1. Agreement *Mammogram positive* *Breath test positive*	***Combination compared to mammogram alone*** *Increased specificity* *89.9% → 96.3%*	***Interpretation*** *Increased probability of a TRUE POSITIVE mammogram*	***Recommendation*** Further evaluation of the lesion e.g. breast biopsy
2. Agreement *Mammogram negative* *Breath test negative*		*Increased probability of a TRUE NEGATIVE mammogram*	Reassure patient.
3. Disagreement *Mammogram positive* *Breath test negative*	*Increased sensitivity* *79% → 97.2%*	*Increased probability of a FALSE POSITIVE mammogram*	Review mammograms
4. Disagreement *Mammogram negative* *Breath test positive*		*Increased probability of a FALSE NEGATIVE mammogram*	Additional imaging e.g. sonogram, MRI

Figure 4

Clinical applications of the mammogram-breath test combination.

The four possible outcomes of the mammogram-breath test combination are shown. Possible interpretations include increased probability that the mammogram result was a true positive, true negative, false positive or a false negative.