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Prediction of breast cancer using volatile biomarkers in the breath

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Summary

We evaluated a breath test for volatile organic compounds (VOCs) as a predictor of breast cancer. Breath VOCs were assayed in 51 asymptomatic women with abnormal mammograms and biopsy-proven breast cancer, and 42 age-matched healthy women. A fuzzy logic model predicted breast cancer with accuracy superior to previously reported findings. Following random assignment to a training set (64) or a prediction set (29), a model was constructed in the training set employing five breath VOCs that predicted breast cancer in the prediction set with 93.8% sensitivity and 84.6% specificity. The same model predicted no breast cancer in 16/50 (32.0%) women with abnormal mammograms and no cancer on biopsy. A two-minute breath test could potentially provide a safe, accurate and painless screening test for breast cancer, but prospective validation studies are required.

There is a clinical need for a screening test for breast cancer that is accurate, safe and painless. We have previously reported a two-minute breath test for volatile organic compounds (VOCs) that identified distinctive markers of oxidative stress in breast cancer 1, as well as in other conditions including lung cancer 2, heart transplant rejection 3 and diabetes mellitus 4. We report here a re-analysis of data from the breast cancer study employing fuzzy logic, in order to determine if breath VOC analysis can predict the disease.

Human subjects

The clinical study has been described 1. We studied two groups of asymptomatic women with abnormal mammograms, 51 with and 50 without histologic evidence of breast cancer in a breast biopsy, and a third group of 42 age-matched healthy women with no history of breast cancer. Women were eligible to participate if they were 18 years of age or older and had no history of previously diagnosed cancer of any site. All gave written informed consent to participate, and the institutional review boards of participating institutions approved the research.

Breath collection and assay

The method has been described 5. Following an overnight fast, VOCs in 1.0 l breath and 1.0 l room air were captured on separate sorbent traps using a breath collection apparatus. Samples were analyzed by automated thermal desorption, gas chromatography and mass spectroscopy. A subtraction chromatogram was constructed for each woman by determining the abundance of VOCs in alveolar breath minus their abundance in ambient room air.

Statistical methods – Training set

Using fuzzy logic software (Interrelation Miner, SystAim, Zürich, Switzerland), women with breast cancer and age-matched healthy volunteers were randomly assigned to a training set or a prediction set in a ratio of approximately 2:1. A breath VOC was included in the

Contributions of authors: Michael Phillips MD, FACP was the principal investigator who coordinated the study.
Renee N. Cataneo MA analyzed all breath samples in the laboratory.
Beth Ann Ditkoff MD recruited patients with abnormal mammograms for the study.
Peter Fisher MD and C. Stephan Kwon MD reviewed the microscopic pathology of the breast biopsies.
Joel Greenberg BS and Olaf Tietje PhD analyzed the data.
Ratnasiri Gunawardena MD collected breath samples from subjects.
Cynthia Wong MD recruited normal controls and patients with abnormal mammograms for the study.

analysis if it was present in the subtraction chromatogram of at least 40% subjects of the training data set, and its Goodman Kruskal λ value was above 0.35. Fuzzy functions were constructed for the candidate breath VOCs in order to create one set of parameters (typicality matrix) for controls and another set of parameters (typicality matrix) for breast cancer patients.

Prediction set

The typicality matrices were employed to generate two numerical values from the breath VOCs: T_{neg}, the fuzzy membership function for no disease, and T_{pos}, the fuzzy membership function for breast cancer. The value of $T_{pos}-T_{neg}$ was employed as a predictor of breast cancer, and the accuracy of prediction was displayed in a receiver operating characteristic (ROC) curve. The predictive model was tested in the women with abnormal mammograms and no histologic evidence of breast cancer in a breast biopsy.

Results

Five breath biomarkers of breast cancer were identified: 2-propanol, 2,3-dihydro-1-phenyl-4(1H)-quinazolinone, 1-phenyl-ethanone, heptanal, and isopropyl myristate. The training and prediction ROC curves are shown in Figure 1. The breath test predicted breast cancer with 93.8% sensitivity and 84.6% specificity. The same model predicted no breast cancer in 16/50 (32.0%) of the women with abnormal mammograms and no cancer on biopsy.

Discussion

A combination of five VOCs in breath predicted the presence or absence of breast cancer. The biological significance of the breath VOCs identified as biomarkers of breast cancer is unclear, though heptanal has been previously reported as a cancer biomarker 6, analogues of 2,3-dihydro-1-phenyl-4(1H)-quinazolinone have anti-tumor activity 7, and analogues of 1-phenyl-ethanone (acetophenone) exhibit potent anti-invasive activity against human MCF-7/6 mammary carcinoma cells 8.

When women with breast cancer were compared to normal volunteers, a fuzzy logic model employing five breath VOCs predicted breast cancer with 93.8% sensitivity and 84.6% specificity. This was superior to the discriminant analysis model we previously reported that employed eight breath VOCs and predicted breast cancer with 88.2% sensitivity and 73.8% specificity 1.

These findings suggest that the breath testing might potentially be valuable in clinical practice because its accuracy was superior to the reported sensitivity and specificity of breast cancer detection by radiologists reading mammograms when blinded to the diagnosis 9. The predictive model would probably identify women who are likely to have abnormal mammograms, but not all of them would have breast cancer. In our sample of

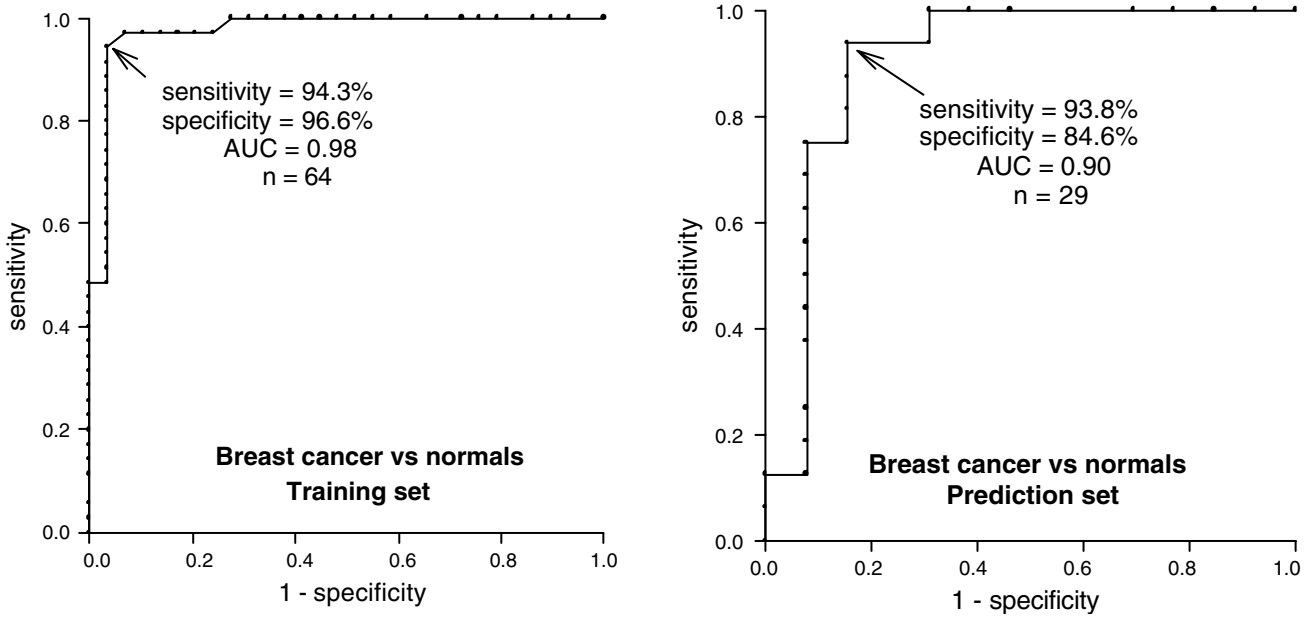

Figure 1. Breath test results – training and prediction sets. In the training set, women with breast cancer were compared to healthy volunteers, and fuzzy functions were derived from their breath VOCs the parameters of which create typicality matrices for each group. In the prediction set, these matrices were employed to generate two numerical values for each subject: T_{neg}, the typicality for no disease, and T_{pos}, the typicality for breast cancer. The value of $T_{pos}-T_{neg}$ was employed as a predictor of breast cancer, and its accuracy is shown in the receiver operating characteristic (ROC) curve and its area under curve (AUC). The arrow indicates where the sum of sensitivity plus specificity was maximal. A predictive test that is no better than chance alone results in an ROC curve that is a 45° straight line commencing from the origin, with AUC = 0.5. If a test is perfectly accurate, without any false positive or false negative predictions, the resulting ROC curve is a right angle with its apex at the top left of the panel, and AUC = 1.0. A test that predicts disease with AUC > = 0.9 is usually considered highly accurate.

women whose mammograms were sufficiently suspicious to require biopsy, the breath test predicted breast cancer in 68% where no cancer was detected. The significance of these apparent false positives is unknown, and further studies are required to determine whether abnormal biochemical changes in breath VOCs constitutes a risk factor for future development of breast cancer.

Fuzzy logic is a powerful computational tool for multivariate analysis of data that employs ranges of values and their relationships to one another e.g. the combination of two different breath VOCs might be identified as a marker of disease if one is present in abnormally high concentration when the other is abnormally low. Fuzzy logic resembles human decision making in clinical medicine by deriving precise solutions from approximate data, and has been employed to accurately predict disorders as diverse as lung cancer 10 and risk of suicide 11.

We conclude that fuzzy logic analysis of volatile biomarkers in breath accurately predicted breast cancer in asymptomatic women. A two-minute breath test could potentially provide a safe, accurate and painless screening test for breast cancer. However, these are preliminary findings that should be interpreted with caution. This was a relatively small case control study, and the findings require validation in a larger and more highly powered prospective trial before breath testing can be employed to complement or replace current screening methods.

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