Using Organ Specific and Circulatory Biofluids to Screen Individuals at High Risk for Breast Cancer Presents Unique Challenges and Opportunities

Edward R. Sauter

ABSTRACT

Intraductal assessment of the breast holds the potential to provide useful information regarding breast cancer risk assessment, early diagnosis, and/or response to therapy. Intraductal assessment can be through imaging (ductography), direct visualization (mammary ductoscopy), or evaluation of the intraductal fluid collected. The most common nonradiologic approaches to intraductal assessment that provide intraductal fluid for evaluation include breast nipple aspiration fluid (NAF), spontaneous nipple discharge (SND), mammary ductoscopy, and ductal lavage. The first two approaches are entirely noninvasive while the latter are considered minimally invasive. Nipple aspiration is performed both on women with and without evidence of possible disease in the breast. On the other hand, unilateral SND suggests the presence of a lesion in the incident breast, while bilateral SND is most often physiologic. The focus of the report by Patuleia and colleagues is on challenges, lessons learned, and recommended solutions in the identification of women with increased breast cancer risk who are more likely to develop *in situ* or invasive breast cancer based on sequential collection and subsequent analysis of biofluids (NAF and serum). The lessons learned that are discussed can also be applied to other types of biofluid studies for cancer early detection and response to treatment.

See related article by Patuleia et al., p. 441

The authors provide insights on 11 challenges that they encountered in the establishment of risk assessment biofluid studies at two centers in the Netherlands using NAF (https://www.trialregister.nl/trial/8661) and serum (https://www.trialregister.nl/trial/8661) in women at elevated risk for breast cancer (1). At sequential time points up to and including the development of a breast event, defined as *in situ* or invasive breast cancer, the plan is to compare miRNA expression levels in both NAF and blood, and protein levels in blood.

Topics That The Report Addresses Well

The authors accomplish the stated goal of the report to ". . .provide an overview of these challenges and its possible solutions to support future researchers that intend to design similar cohort studies."

Sequential biofluid collection

An important reason to consider sequential biofluid collection and analysis in their cohort as a means to assess risk, as discussed by the authors, is the postponement of screening by means of mammography and MRI during pregnancy and lactation, as well as the low positive predictive value and high false positive rates of breast ultrasound. Whether to include high risk women who are pregnant or lactating in the cohort is an important topic for many reasons, not the least of which is the potential for delayed diagnosis, less accurate breast imaging and lower survival after treatment compared with nonpregnant/lactating women of similar age and risk characteristics (2).

Division of Cancer Prevention, NCI, Rockville, Maryland.

Corresponding Author: Edward R. Sauter, National Cancer Institute, Rockville, MD 20850. Phone 240–276–7657; E-mail: edward.sauter@nih.gov

Cancer Epidemiol Biomarkers Prev 2021;30:429–31

doi: 10.1158/1055-9965.EPI-20-1486

Establishment of a biobank

The authors rightly outline the importance of a establishing a large biobank, expecting enrollment time to last more than five years before sample evaluation with attendant startup and maintenance costs (and therefore needed funding) involved, and close tabs needs to be kept on dropouts as well as samples not collected or not evaluable for other reasons to determine when sample evaluation can begin. They point out that keeping participants regularly informed of study progress is an important lesson learned to minimize dropouts, and for participants to inform the study team if a new breast related event develops, so that breast cancers and precancers, especially those diagnosed at a time other than at a planned screening visit, are not missed.

Which cohorts to include in a high risk study

Deciding on which high risk groups to include in the cohort are discussed, balancing the benefits of broad inclusion with the potential dilutional effects of evaluating diverse groups. They rightly discuss the potential downside of including subjects with a history of breast or another cancer whose future risk, as well as risk assessment biomarkers, may be altered by the treatment of incident disease.

What information and samples to collect when designing a study in a high-risk cohort

Determining what information to collect at study start is also very important. While deciding once the study is ongoing that additional information should be collected is common, minimizing these additions is important because the added information will not be available from subjects already enrolled, and baseline comparisons not possible. As is pointed out, having standard operating procedures, including providing to all enrollment sites sample collection containers, diluents, and consistent diluent volume into which a sample may be placed, as well as hiring a data manager who has access to an online data management system before or soon after study start are also keys to success.

The report does not provide detail on biomarker analysis, including detailed results of biomarkers analyzed. The authors do, however,

suggest that investigators conduct biomarker analysis on a biofluid sample set before picking the biomarker(s) for further study in NAF and/or blood.

What factors to consider when choosing a biomarker

The authors discuss important factors to consider when choosing a biomarker for biofluid analysis, especially when the sample is limited. For instance, NAF samples are often 50 μL or less. As such, most or all of the entire sample may be needed for a single experiment. It is therefore important to use "a trustworthy, familiar technique that has already been optimized." It is also useful to determine the minimum sample volume required to reliably obtain a signal for a given biomarker. In addition to reliable detection, the biomarkers chosen for analysis should, whenever possible, be based on promising prior findings in the same or another biofluid. It is nonetheless possible that once the biomarker is chosen, new findings that are less promising may lead investigators to alter their biomarker choice(s).

Additional Considerations

NAF color

NAF can range in color from clear to milky to yellow to green to brown to red. Clear and red NAF may indicate a higher risk of breast cancer (3). The authors suggest to label NAF as bloody or nonbloody, but I believe that more detail regarding color is desirable. Red NAF may be come from at least two sources, inside the breast ducts or on the nipple surface. Blood contamination from the nipple surface is not associated with risk, whereas intraductal NAF may indicate an intraductal papilloma, *in situ*, or invasive breast cancer (3).

NAF producers versus nonproducers

Despite its importance, this topic is not well addressed. One of the primary criticisms of using NAF to evaluate risk, especially when successful collection of sequential samples is required to compare changes in a given breast, is collection success, both at baseline and at each subsequent visit. Indeed, the authors use oxytocin nasal spray in an attempt to optimize NAF collection in women who participated in their study to increase collection success.

Reports of collection success vary widely, ranging from <50 to 90+% (4, 5). The definition of NAF collection success is often not clearly defined, but usually implies a visible NAF sample from one or both breasts. Real success for long term cohort studies, as well as studies that use biofluids for clinical trials to monitor medication or other intervention effects, implies successful collection of a NAF sample from each breast at each visit which is sufficient to detect the biomarkers of interest (should they be present) and that the NAF sample is not diluted with milk or blood. Some investigators, including those in this report, have used oxytocin nasal spray to increase NAF collection success. As such, more detail on NAF collection success should be provided, and the choice of NAF biomarkers should include those that are most likely to be reliably detected in all NAF samples collected, should the biomarker be present in the sample. This implies that the biomarker is generally present in high concentration in NAF, since NAF volume is limited. The authors indicate that their success rate of bilateral NAF harvesting is 65.8%. It is not clear if this means that bilateral NAF was collected at every visit for 65.8% of participants, whether each sample was adequate for biomarker analysis, or that bilateral NAF was collected at least once in this percent of participants. The more time points at which samples are available, the greater the ability to identify useful biomarkers.

We assessed our success in women from whom sequential NAF samples were collected and considered two factors regarding NAF yield. The first was the ability to obtain a sample. Of 113 aspiration visits made, on only one occasion was fluid not obtained (6). The ability to obtain a NAF samples did not decrease over time. The second factor considered was whether NAF volume decreased over time. There was not a significant decline in fluid volume over time in either pre- or postmenopausal participants, considering all aspirations, the first versus the second aspiration in each subject, the first versus the third aspiration, the first versus the last aspiration, the second versus the third aspiration, or the second versus the last aspiration. Age did not significantly influence NAF volume.

What "events" should prompt evaluation of biomarkers from biofluid samples collected

The authors define events as the development of *in situ* or invasive breast cancer, at which time the last biomarker samples are collected. While it is clear that the development of breast cancer is the most important event in a cohort of individuals at increased breast cancer risk, there are other events which should be considered. The diagnosis of atypical hyperplasia (ductal or lobular) on needle biopsy frequently prompts an excisional biopsy, which may or may not identify more advanced disease. Either way, the surgical intervention alters the locoregional NAF milieu, ductal scarring occurs, and future NAF collection from the incident breast may have biomarker changes independent of disease considerations within the breast ducts and lobules. Indeed, any event prompting surgery, such as the identification of a mass leading to surgical intervention, even if the lesion which prompted surgery is found to be benign, should raise the question of whether future NAF collection provides a valid comparison with prior NAF samples, at least in the short term. The effects of needle biopsy are also likely to influence the locoregional NAF milieu at least in the short term, although perhaps not as significantly due to the less invasive nature of the intervention.

Another important event is breast irradiation. While this is most often due to the treatment of breast cancer, it has also occurred in women being treated for Hodgkin lymphoma. It has been our experience that breast irradiation significantly decreases the investigator's ability to collect NAF, if successful the NAF volume is rarely more than 10 μL, and the NAF sample generally lacks epithelial cells.

Collection of NAF during pregnancy and lactation

The collection of NAF during pregnancy and lactation raises at least potential concerns. The authors explain that NAF is highly diluted with milk in lactating women, which can alter biomarker results. Their strategy was to collect NAF starting three months after lactation ceased. As they note, milk components have been reported to exist in NAF up to 24 months after breastfeeding ends (7), so samples collected sequentially starting less than 24 months after breastfeeding ceases compared with later samples may have biomarker changes due to the influence of milk rather than risk increasing changes in the breast epithelium. There is also the low but potential risk of inducing uterine contractions among investigators who administer oxytocin nasal spray to women of childbearing potential to increase NAF collection success (8).

In summary, this report provides a useful discussion of challenges, lessons learned, and possible solutions among investigators wishing to conduct studies with biofluids such as NAF and serum. While

information provided focuses on subjects at increased risk of breast cancer who are followed from enrollment until a breast event, the challenges regarding study design and funding, biomarker choice, sample collection, storage, and analysis are also largely applicable to the development of biofluid studies for cancer early detection and response to treatment studies. Biofluids have untapped potential for translational evaluation and clinical care. The value of biofluid analysis will continue to increase as our ability to collect evaluable samples is optimized and technology allows us to identify useful biomarkers with increasingly small concentrations of these biomarkers.

Authors' Disclosures

E.R. Sauter reports a patent for Breast Cancer Diagnosis Using Nipple Discharge issued to US2012/022754. No other disclosures were reported.

Disclaimer

The opinions expressed by the author are his own and this material should not be interpreted as representing the official viewpoint of the U.S. Department of Health and Human Services, the NIH, or the NCI.

Received October 14, 2020; revised December 18, 2020; accepted December 18, 2020; published first March 5, 2021.

References

1. Patuleia SIS, Hagenaars SC, Moelans CB, Ausems MGEM, van Gils CH, Tollenaar RAEM, et al. Lessons learned from setting up a prospective, longitudinal, multicenter study with women at high risk for breast cancer. Cancer Epidemiol Biomarkers Prev 2021;30:441–9.
2. Borges VF, Lyons TR, Germain D, Schedin P. Postpartum involution and cancer: an opportunity for targeted breast cancer prevention and treatments? Cancer Res 2020;80:1790–8.
3. Sauter ER, Winn JN, Dale PS, Wagner-Mann C. Nipple aspirate fluid color is associated with breast cancer. Cancer Detect Prev 2006;30: 322–8.
4. Sauter ER, Klein-Szanto A, Macgibbon B, Ehya H. Nipple aspirate fluid and ductoscopy to detect breast cancer. Diagn Cytopathol 2010; 38:244–51.
5. Zhao YS, Pang D, Wang F, Xue YW, Gao DN, Li H, et al. Nipple aspirate fluid collection, related factors and relationship between carcinoembryonic antigen in nipple aspirate fluid and breast diseases in women in Harbin, PRC. Cancer Epidemiol Biomarkers Prev 2009;18:732–8.
6. Sauter ER, Babb J, Daly M, Engstrom PF, Ehya H, Malick J, et al. Prostate-specific antigen production in the female breast: association with progesterone. Cancer Epidemiol Biomarkers Prev 1998;7:315–20.
7. Petrakis NL, Lowenstein JM, Wiencke JK, Lee MM, Wrensch MR, King EB, et al. Gross cystic disease fluid protein in nipple aspirates of breast fluid of Asian and non-Asian women. Cancer Epidemiol Biomarkers Prev 1993;2:573–9.
8. Hohmann M, Künzel W, Kirschbaum M. [The uterine contraction stress test with oxytocin nasal spray in the diagnosis of hypoxemia]. Z Geburtshilfe Perinatol 1986;190:210–4.