Annals of Medicine and Surgery 77 (2022) 103625

Contents lists available at ScienceDirect

Annals of Medicine and Surgery

journal homepage: www.elsevier.com/locate/amsu

Review

Nipple aspirate fluid and its use for the early detection of breast cancer

Natasha Jiwa [a,*], Ahmed Ezzat [a], Josephine Holt [c], Dhuleep S. Wijayatilake [d], Zoltan Takats [b], Daniel Richard Leff [a]

[a] Department of Surgery and Cancer, St Marys Hospital, Praed Street, London, W2 1NY, UK
[b] Imperial College London, South Kensington, Campus Exhibition Road, London, SW7 2AZ, UK
[c] Royal Free NHS Trust, London, NW3 2QG, UK
[d] Queens Hospital, Romford, Essex, RM7 0AG, UK

ARTICLE INFO

Keywords:
Breast cancer
Biomarkers
Metabolomics
Early detection
Nipple aspirate fluid
Mass spectrometry

ABSTRACT

Nipple aspirate fluid is the physiological biofluid lining ductal epithelial cells. Historically, cytology of nipple fluid has been the gold standard diagnostic method for assessment of ductal fluid in patients with symptomatic nipple discharge. The role of biomarker discovery in nipple aspirate fluid for assessment of asymptomatic and high-risk patients is highly attractive but evaluation to date is limited by poor diagnostic accuracy. However, the emergence of new technologies capable of identifying metabolites that have been previously thought unidentifiable within such small volumes of fluid, has enabled testing of nipple biofluid to be re-examined. This review evaluates the use of new technologies to evaluate the components of nipple fluid and their potential to serve as biomarkers in screening.

1. What is nipple aspirate fluid?

Bodily fluids provide a unique window into the biological processes occurring within organ systems. For example, fluids from certain organs e.g. blood, urine and cerebrospinal fluid, enable various disease processes to be diagnosed [1]. Nipple aspirate fluid (NAF) is the clear liquid produced by the lining of the nipple ductal epithelial cells (See Fig. 1). It is well known that the majority of breast cancers arise from the epithelial lining of the terminal ducts – invasive ductal carcinomas [2]. NAF therefore mirrors biological processes occurring in the tumour microenvironment [3], or in high risk individuals, in the lead up to cancer [4]. On the contrary, only a small proportion of women with breast cancer present with nipple discharge as a primary symptom and it comprises 5% of all attendances to the breast clinic [5]. Nipple discharge can be a presenting feature of several benign diseases, as well as physiological processes such as breast feeding [6]. Therefore, if mammary duct biofluid is to be proven to be of value, then in the majority of women a system is required to obtain a nipple fluid sample.

NAF can be expressed in women using a multitude of techniques: manual compression [7,8]; modified breast pumps (manual and automated) [9] and pharmacological agents (oxytocin) [10,11]. Moreover, this biofluid contains a number of micronutrients (tocophenols, cholesterols, carotenes); hormones (oestrodiol, estrone, progesterone, testosterone); microRNA, microbes [12] and other proteins [8,13]. Despite the ongoing challenges in acquiring NAF, particularly from post-menopausal women [14], its potential use for cytology, genomic expression and metabolomic profiling makes it a powerful substrate for biomarker analysis in the early detection of breast cancer.

2. The importance of NAF

Early breast cancers no matter how small diagnosed on mammography currently require treatment in the form of breast conserving surgery followed by adjuvant therapy. Nipple aspirate fluid (NAF) is the local biofluid bathing the ductal system and is thought to represent the micro-environment of a developing beast neoplasm [14,15]. The hope is that the assessment of NAF may enable far earlier detection than is currently possible with conventional diagnostic systems and may guide novel pathways for surveillance, particularly in younger women where mammography plays a limited diagnostic role.

With early detection of breast cancer remaining a challenge, the National Health Service Breast Screening Program (NHSBSP) aims to reduce breast cancer mortality by 20%, partly through earlier detection of Stage 1 and 2 disease [16]. Current validated breast cancer risk stratification models such as the Gail and Tyrer-Cuzick models include gynaecological, obstetric and family history to predict the 5

* Corresponding author.
E-mail address: natasha.jiwa04@imperial.ac.uk (N. Jiwa).

https://doi.org/10.1016/j.amsu.2022.103625
Received 15 March 2022; Received in revised form 10 April 2022; Accepted 11 April 2022
Available online 18 April 2022

year/life-time breast cancer risk and 10-year/lifetime breast cancer risk, respectively [17]. A study in 2005 [18] followed up 8000 breast-cancer free patients utilising the augmented Gail's model with NAF cytology to determine risk of breast cancer. Authors concluded that adding NAF to the Gail model statistically improved accuracy within women in the top third of the Gail model [18].

Combining this evidence with the advent of new advances in NAF biofluid marker discovery, along with NAF's ability to distinguish benign from malignant disease, therein lies an opportunity for reconsideration of NAF utility, not only as screening tool, but potentially as a bedside tool to augment traditional triple assessment or even surveillance of post-operative patients in remission.

Emerging techniques have placed diagnostic emphasis on monitoring young and high-risk patients. This cantake the form of ductoscopy, ductal lavage and the direct acquisition of nipple aspiration fluid (NAF) [19]. This review therefore explores the plethora of potential applications of nipple biofluid testing and early work undertaken thusfar, as well as promising directions for the future.

3. Methods of collecting nipple aspirate fluid

When considering the optimal technique for acquiring bodily fluids such as nipple aspirate fluid, there are several key attributes to consider (see Table 1). Over the years, several methods have been trialled for the successful acquisition of nipple fluid. From manual palpation techniques, topical treatments to automated pumps, each technique carries its own advantages and disadvantages (see Table 2). By way of example, Proctor et al. [9] reported a production rate of 38% when the automated pump HALO was used as the sole collection method of fluid [9] and slightly higher in a study by Deladisma et al. [20], (51%, 40 out of 78 patients). Using manual compression techniques or a handheld pump increases yield to 40–80% [7,21]. Producibility is quoted as high as 99.7% when used in conjunction with the ForeCyte Aspirator [22].

Table 1
Summary of the key attributes required for the acquisition of nipple aspirate fluid.

Key Attributes of a Nipple Fluid Yielding Technique	
Minimal side effect profile	• Pain/complications not only increases the chances of patients being unable to tolerate the procedure, but may also be likely to affect the chances of blood cells being in the sample collected, skewing results [23].
Quick [24]	• Extraction methods take anywhere between 5 and 15 min.
Easy	• Increasing the chances of being undertaken in various settings [24]
Aseptic	• Therefore, minimising the chances of nipple skin flora contaminating the samples).
Cheap	• Therefore reproducible on a large scale [24].
Readily Available	• Thus optimising likelihood of compliance/ability to self-acquire etc [24].

4. Methods of analyzing NAF

Over the years, with advances in technology and explosion of the field of metabolomics, options for the interrogation of miniscule volumes of bodily fluids have expanded to encompass a multiplicity of new approaches (see Fig. 2). Here we discuss past, current and newly developing methods for diagnosis of nipple aspirate fluid.

5. Cytology

Cytology has been utilised for tissue diagnosis in breast cancer for over 70 years [33] and the potential of regular nipple aspirate fluid smears as a screening tool has been evaluated by several groups, not only in terms of safety [25], but also reproducibility [34] and diagnostic accuracy [35]. A recent meta-analysis by our group in 2020 [35], concluded that the diagnostic accuracy of nipple fluid cytology is limited due to poor sensitivity secondary to a lack of cellular material. Therefore, emerging technologies aim to surpass cytopathology as a

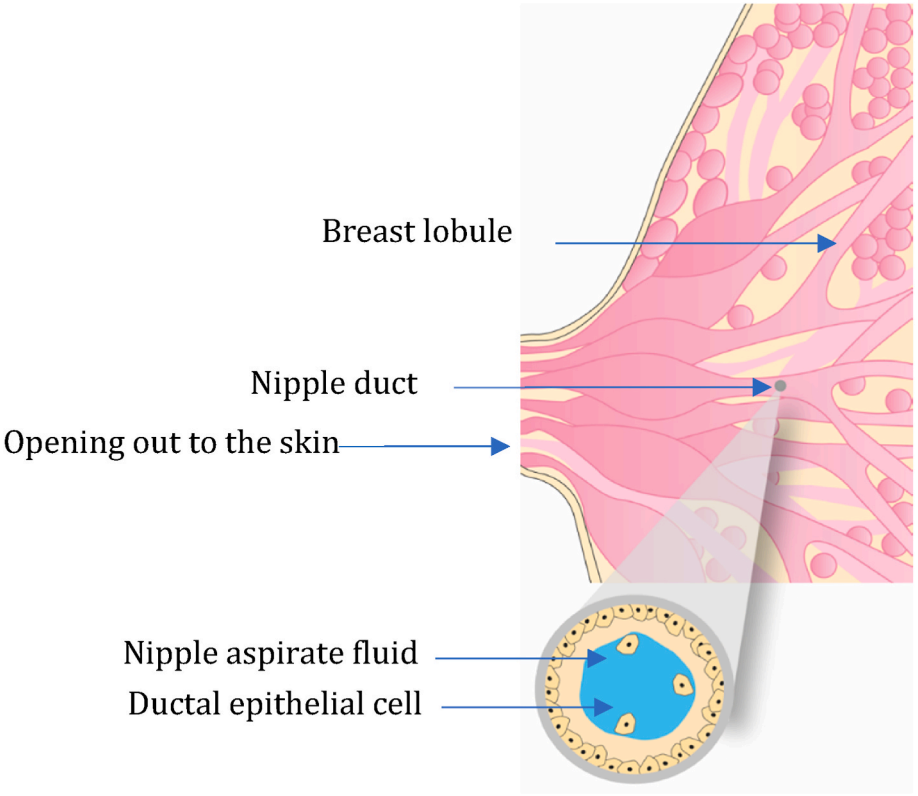
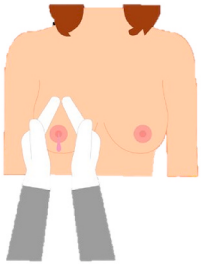
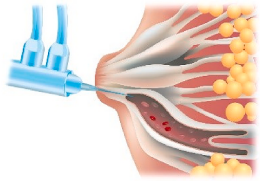
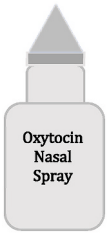
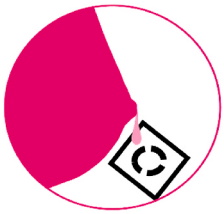


Fig. 1. Anatomical representation of ductal system and nipple aspirate fluid.

Table 2
Summary of methods of collection of nipple aspirate fluid.

Method of Collection	Advantages	Disadvantages
Automated Pump 	Allows for quick and easy collection, no user variability [25].	• 38% success rate • Does have a side effects profile [25] including pain, redness, surface skin lacerations which may limit its use commercially.
Hand-held Manual Pump 	Quick, cheap, easy, readily available, could be performed at home [26,27]. Less likely to produce adverse side effects as manually controlled.	• User dependent • May not provide the same negative pressure as automated pumps.
Manual Palpation 	Quick, free, easy, no equipment required, can be carried out by either healthcare professional or the subject (8)	• 50% success rate [8] • Mostly trialed under general anaesthetic [7]
Ductal Lavage 	High cellular yield [28,29]	• Expensive, invasive, time-consuming. Requires an expert and use of either local or general. anaesthetic [18,30,31]. • Not readily available and therefore not commonly used.
Nasal Oxytocin Oxytocin Nasal Spra 	Easy to administer, no side effects reported [10] can increase yield up to 98% [11].	• Licensed drug, should be administered in a medical setting. • Used as an adjunct to one of the methods above rather than a standalone test [10].
Guthrie Cards 	]Cheap, easy to use, reliable, pain-free [32].	• Used preferentially when NAF is easily obtainable. • Used as an adjunct to the methods above rather than a standalone test [32].

diagnostic and screening tool, whilst considering it's other and advantages, namely reproducibility, cost effectiveness and turnaround time.

6. Genes

Genetic testing plays an important role in assessing risk in breast cancer patients. It is therefore unsurprising that this has been extrapolated to nipple aspirate fluid. A decade ago, Antill et al. [36], assessed the hypermethylation of p13, RASSF1A, twist and RARβ using qualitative, real-time PCR assay [36]. P16 methylation was shown to be a potential predictor of BRCA1 mutation status and therefore may have a role in predicting future breast cancer risk. It may also be applicable to diagnosis as shown by De Groot et al., in 2016 [37], where methylation levels of 13 genes where measured in nipple fluid samples from breasts of healthy women, and from the affected and contralateral breasts of breast cancer patients. They illustrated that cancerous nipple fluid contains increased levels of methylation of tumour suppressor genes, with an AUC of 0.64 (95% CI 0.54–0.74, $p < 0.01$), therefore carrying future potential to serve as a biomarker for early breast cancer detection [34,36].

7. Proteins

A plethora of studies have been conducted to assimilate the nipple aspirate proteome, thus identifying targets for biomarkers for breast cancer detection [7,8,13,38,39]. The scientific basis behind the interrogation of the protein profile of nipple fluid is explained by the concept that NAF carries proteins from cancerous ducts, which may therefore identify protein patterns that are consistent with a developing tumour [40]. By way of example, Alexander et al. [38], identified candidate markers using matrix-assisted laser desorption ionization time-of-flight (ESI Q-TOF) proteomic analysis and validated the markers identified using quantitative, high-throughput ELISA analysis. In their cohort of 89 patients, GCDFP-15 levels were lower and AAG levels correlated with presence and stage of breast cancer disease [38]. In addition, He et al. [39], identified a 8 protein markers which collectively gave a 89% specificity and 76% accuracy for distinguishing between cancer and non-cancer [39]. Further work on the proteome was conducted by Pavlou et al., in 2010 [13], utilising liquid chromatography mass spectrometry (LC-MS) to generate the most extensive nipple aspirate fluid proteome at the time. Over 800 unique proteins were successfully identified, of which, more than 50% of which were extracellular or plasma membrane proteins [13].

In 2015, Delmonico et al. [32], used Guthrie cards to process NAF proteins from 80 patients over a 3 year period. Following collection, proteins were separated using gel electrophoresis, excised for destaining, subjected to enzymatic digestion and then analysed on an ESI-Q-TOF mass spectrometer. Immunoglobulins, Zn-α2-glicoprotein, apoliprotein D and prolactin inducible protein were among those proteins identified and the method was deemed feasible for the collection of NAF for proteomic analysis.

Building on earlier work on proteins, in 2017, Shaheed et al. [7] employed manual expression techniques to acquire NAF samples for proteomic analysis with 2D LC-MS separation. They revealed an average of 1374 proteins per sample, identifying 332 new proteins from previously seen by Pavlou et al. in NAF [7,13]. Further work must be conducted in this area before testing can feasibly be used for symptomatic differentiation. Currently, work is limited by the strong similarity in the complement of proteins in matched pairs – potentially due to transport through cross-lymphatic drainage. This leans towards the theory that a patient with a diagnosis of cancer will have a metabolomic profile reflecting cancer changes throughout the body and therefore, their contralateral breast will also reflect this change. However, the argument against cross-lymphatic drainage is demonstrated by the fact that the lymphatic system is known to drain towards the axilla on the ipsilateral side, as evidenced by the fact that patients do not commonly get bilateral breast cancer. Moreover, similarities in the protein profile of the breast are likely to be representative of the overall metabolomic change and be relevant to lipids and genetic differences alike. Irrespective of this, advances have, been made with identification of the multiplicity of proteins in the fluid, which had not previously been identified. Acceleration in discovery and validation of the proteins found will lie in improving success with expression and collection of nipple aspirate fluid, as well as

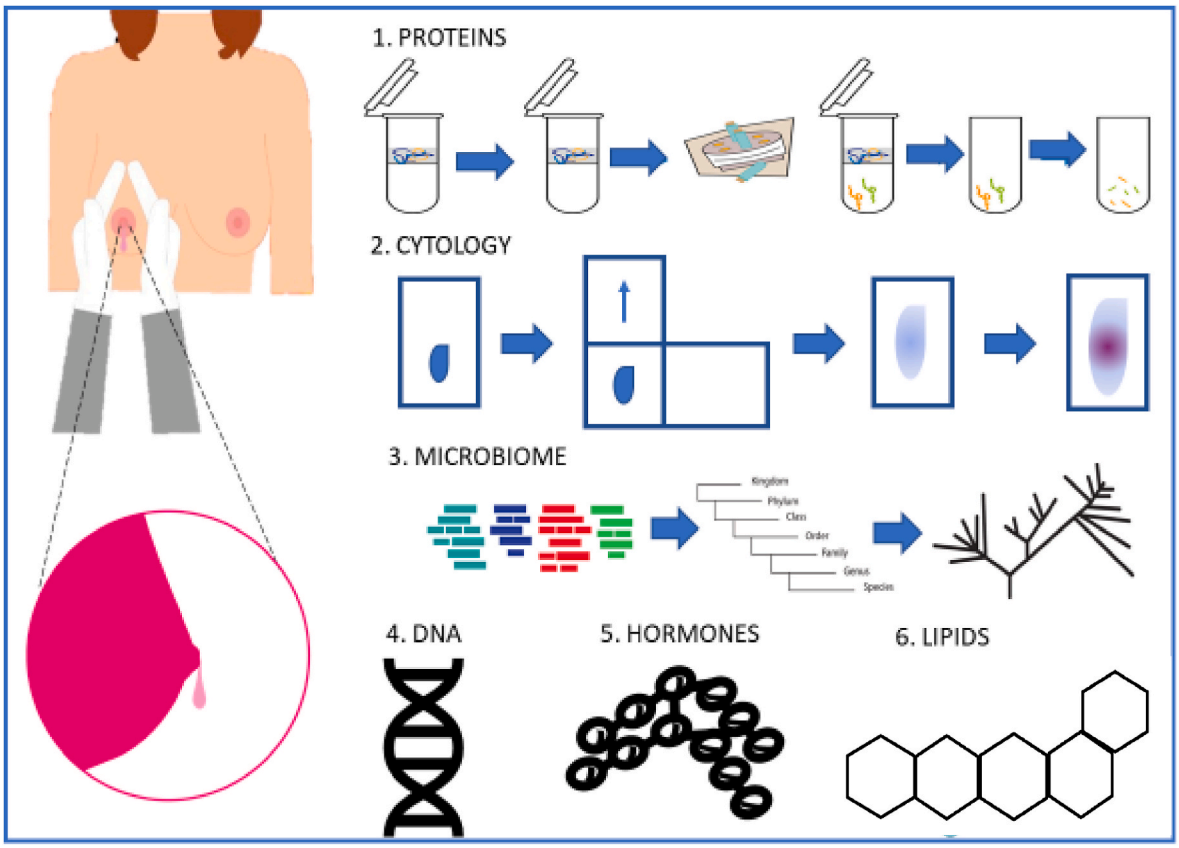


Fig. 2. Summary of key advances in the diagnostic capabilities of nipple aspirate fluid.

employing a more expansive study of larger patient cohorts, including healthy volunteers.

8. Hormones/tumour markers

The long-term endogenous and exogenous exposure of oestrogen is a strongly associated risk factor in the aetiology of breast cancer, but what diagnostic and preventative role do hormones play in NAF? The stability and subtle variation of hormonal levels in NAF can be accurately measured [41]. Therefore, it is logical to assume that they may be more direct determinants to breast cancer risk than potentially even circulating hormones [42]. In 2004, Wang et al. [43] identified a panel of tumour markers including CA125, CA15-3, CEA and malignant TSGF, evaluating their expression both within serum and nipple fluid. The results demonstrate that levels of the four biomarkers were significantly higher in nipple fluid than serum and that there was a positive correlation between Ki-67, tumour grade, clinical stage, lymph node metastasis and tumour recurrence [43]. Their hypothesis that a panel rather than an individual biomarker reflects the heterogeneous nature of breast cancer may provide a basis for future tumour marker detection for both diagnosis and prognosis of breast cancer. Similarly, hormones may also play a role in predicting breast cancer risk. NAF oestradiol is more stable over time than serum concentrations [41], and so is potentially a more reliable indicator of breast cancer risk than serum oestradiol. In a case-control study, Chatterton et al. [44] measured hormone concentration in the at-risk but unaffected contralateral breast of incident breast cancer cases (considered a high risk group) compared to screening mammography controls. Higher NAF (but not serum) DHEA concentrations were associated with breast cancer cases [44], particularly among oestrogen receptor (ER)-positive cases indicating a potentially important role of this steroid in breast cancer risk. However, there is limited follow up work or validation studies utilising tumour-markers in complex biofluids such and NAF for the early detection of breast cancer. Research groups have favoured collection from blood/serum, which may reflect NAF's limitations in terms of quantity of fluid acquired as well as difficulty in obtaining it in all women [45,46].

9. Microbiome

From numerous other cancer models (gastric, cervical, hepatocellular etc) it is apparent that the microbiome plays a central role in the development of cancer [47,48]. The breast microbiome, however, has been relatively understudied. It does not feature in the Human Microbiome Project, likely due to the fact that nipple fluid has traditionally been thought of as a sterile biofluid [49]. To date, studies have investigated both the difference between the microbial composition of breast tissue in cancer and normal tissue [50–52], as well as nipple fluid between cancer and healthy volunteers. The interrogation of NAF in particular, has eluded to a higher incidence of various genii of micro-bacteria in breast cancer patients [12]. In 2016, Chan et al. [12] demonstrated a relatively higher abundance of the genus Alistapes in breast cancer patients, without a difference in areolar skin samples [12]. Moreover, microbes associated with breast cancer were found to share enzymatic activity of beta-gluronidase which is thought to promote breast cancer [12]. This is the first report of bacterial DNA in human breast ductal fluid, with demonstrable differences between the microbiomes of cancer and normal NAF. The metataxome of nipple fluid is in the primitive stages of its discovery, along with the impact it has on tumour/host cells [12,52,53]. Further work in this area will need to focus on patients who have not previously undergone breast cancer surgery (therefore demonstrating whether the NAF microbiome can be utilised as a screening tool), comparing the NAF microbiome of both the cancer and non-cancer breasts of the same patient, as well as looking at whether these findings are both reproducible and demonstrable on

larger cohorts of patients.

10. Lipids

Lipids can be split into 8 major categories according to the National Institute of Health [54]. They play an important role in regulating physiological activities and the use of lipid species as a biomarker is driven by the essential role of metabolism in carcinoma [55]. Phospholipids including phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), phosphatidylinositols (PIs), sphingomyelin (SMs) and ceramides are increased in breast cancer tissues [56] and SMs are upregulated in ER negative tumours [54]. The products of lipid metabolism and the lipidomic profile of tissue are being used in the intelligent-knife work spanning across breast, colorectal and even neurosurgical tissue diagnostics [57–60]. A recent study by St John et al. [56], identified 63 phospholipids and 6 triglyceride species which are responsible for 24 spectral differences between breast cancer and normal tissue types, with a 93.4% sensitivity and 94.9% specificity. MS/MS undertaken on 18 significant peaks identified a 0.5 log2 fold increase in these lipids compared to normal breast tissue and identified them all as glycerophospholipds, the most common being PEs. This work continues to expand its diagnostic capabilities in being able to identify DCIS tumours specifically from invasive ductal cancers. However, in terms of progress in nipple aspirate fluid lipidomics, Matos Do Canto et al. [61] identified up to 83 ions with a significant fold change. The metabolites identified included endogenous metabolites such as amino acid derivatives, products of lipid metabolism, glycerophopholipids and phosphatidylserine [61], some of which mirror findings in breast tissue [56]. This illustrates the initial feasibility of conducting a comprehensive lipidomic profiling of breast tumours using mammary ductal fluid, albeit from lavage samples. The prospect of conducting this work using direct expression techniques on tiny quantities of fluid (2–10 μL) is yet to be investigated and may be the key to developing a non-invasive bedside test that will act as an adjunct to the early detection of breast cancer in high-risk groups. Barriers to overcome this will lie in the ability to optimise expression, collection and processing of tiny quantities of fluid, as well as successfully demonstrating its reproducibility.

11. Conclusion

Methods for early breast cancer detection enable lesions to be treated at the earliest possible time-point, increasing survival, and improving outcomes. Nipple biofluid has great potential for use to develop a biomarker for early detection and has been understudied in the diagnosis of breast cancer. Reasons for this may include difficulty in extraction of NAF, low yield rates, and of course, the challenges of processing a single droplet of fluid. However, despite being present in such small quantities, nipple biofluid is a rich source of metabolomic information and future work in this area may be the key to unravelling breast cancer's complexity and unlocking a biomarker test than may be transferable from the laboratory to the bedside.

Provenance and peer review

Not commissioned, externally peer reviewed.

Funding

The research was funded by the NIHR Imperial Biomedical Research Centre (BRC). No commercial interest, no external financial or material support.

Ethical approval

N/A.

Research Registration Unique Identifying Number (UIN)

Name of the registry:
Unique Identifying number or registration ID:
Hyperlink to your specific registration (must be publicly accessible and will be checked): N/A

Author contribution

N. Jiwa and D. R. Leff synthesised the idea for this manuscript. N Jiwa A Ezzat, J Holt and D S Wijayatilake BSc created the manuscript and D. R. Leff and Z Takats reviewed and edited the manuscript.

Guarantor

N. Jiwa is the guarantor and accepts full responsibility for the work and the conduct of the review.

Declaration of competing interest

N. Jiwa is a PhD candidate investigating Nipple Biofluid in the Early Detection of Breast Cancer, under the supervision of D.R. Leff and Z. Takats. There are no other declarations of interest.

References

[1] J. Loo, W. Yan, P. Ramachandran, DJJodr Wong, in: Comparative human salivary and plasma proteomes 89, 2010, pp. 1016–1023, 10.
[2] J.D. Strehl, D.L. Wachter, P.A. Fasching, M.W. Beckmann, A. Hartmann, Invasive breast cancer: recognition of molecular subtypes, Breast Care 6 (4) (2011) 258–264.
[3] S.A. Khan, R.T. Chatterton Jr., Cellular and hormonal content of breast nipple aspirate fluid in relation to the risk of breast cancer, Biomarkers Med. 2 (5) (2008) 479–493.
[4] Z. Djuric, G. Chen, J. Ren, R. Venkatramanamoorthy, C.Y. Covington, O. Kucuk, et al., Effects of high fruit-vegetable and/or low-fat intervention on breast nipple aspirate fluid micronutrient levels, Cancer Epidemiol. Biomarkers Prev. 16 (7) (2007) 1393-9.
[5] M.H.J.T. Seltzer, Breast Complaints, Biopsies, and Cancer Correlated with Age in 10,000 Consecutive New Surgical Referrals, vol. 10, 2004, pp. 111–117, 2.
[6] J.E. Lang, Kuerer HMJCc, Breast Ductal Secretions: Clinical Features, Potential Uses, and Possible Applications, vol. 14, 2007, pp. 350–359, 4.
[7] Shaheed Su, C. Tait, K. Kyriacou, J. Mullarkey, W. Burrill, L.H. Patterson, et al., Nipple Aspirate Fluid—A Liquid Biopsy for Diagnosing Breast Health, vol. 11, 2017, 1700015, 9-10.
[8] S.U. Shaheed, C. Tait, K. Kyriacou, R. Linforth, M. Salhab, C. Sutton, Evaluation of nipple aspirate fluid as a diagnostic tool for early detection of breast cancer, Clin 15 (2018) 3.
[9] K.A. Proctor, L.R. Rowe, J.S. Bentz, Cytologic features of nipple aspirate fluid using an automated non-invasive collection device: a prospective observational study, BMC Wom. Health 5 (2005) 10.
[10] K. Suijkerbuijk, E. Van der Wall, P. Van Diest, Oxytocin: bringing magic into nipple aspiration, Ann. Oncol. 18 (10) (2007) 1743–1744.
[11] K.P. Suijkerbuijk, E. Van Der Wall, H. Meijrink, X. Pan, I.H.B. Rinkes, M.G. Ausems, et al., Successful oxytocin-assisted nipple aspiration in women at increased risk for breast cancer, Fam. Cancer 9 (3) (2010) 321–325.
[12] A.A. Chan, M. Bashir, M.N. Rivas, K. Duvall, P.A. Sieling, T.R. Pieber, et al., Characterization of the microbiome of nipple aspirate fluid of breast cancer survivors, Sci. Rep. 6 (2016).
[13] M.P. Pavlou, V. Kulasingam, E.R. Sauter, B. Kliethermes, Diamandis EPJCc, Nipple Aspirate Fluid Proteome of Healthy Females and Patients with Breast Cancer, vol. 56, 2010, pp. 848–855, 5.
[14] N.L. Petrakis, Physiologic, biochemical, and cytologic aspects of nipple aspirate fluid, Breast Cancer Res. Treat. 8 (1) (1986) 7–19.
[15] F. Mannello, New horizon for breast cancer biomarker discoveries: what might the liquid biopsy of nipple aspirate fluid hold? Proteonomics Clin. Appl. 11 (9–10) (2017), 1700060.
[16] W.H. Organization, Essential Public Health Functions, Health Systems and Health Security: Developing Conceptual Clarity and a WHO Roadmap for Action, 2018.
[17] A.R. Brentnall, J. Cuzick, Risk models for breast cancer and their validation, Stat. Sci.: Rev. J. Inst. Math. Statis. 35 (1) (2020) 14.
[18] J.A. Tice, R. Miike, K. Adduci, N.L. Petrakis, E. King, M.R. Wrensch, Nipple aspirate fluid cytology and the Gail model for breast cancer risk assessment in a screening population, Cancer Epidemiol. Biomarkers Prev. 14 (2) (2005) 324–328.
[19] J.E. Lang, H.M. Kuerer, Breast ductal secretions: clinical features, potential uses, and possible applications, Cancer Control 14 (4) (2007) 350–359.

[20] A. Deladisma, E. Seeley, L. Tafra, D. Ellsworth, R. Ellsworth, K. Sawyer, et al., Identification of breast cancer protein biomarkers in nipple aspirate fluid, Ann. Surg Oncol. 1 (2012) 13–14.

[21] J. Bentz, The role of nipple-aspirate fluid cytology in breast-cancer risk screening, MLO Med. Lab. Obs. 41 (3) (2009) 2–7, 8, 10.

[22] J.W. Kylstra, M.H. Kalnoski, T. Vo, M.L. Lee, S.C. Chen, S.C. Quay, Proliferative breast disease identified by nipple aspirate fluid cytopathology has the laterality and asymmetry characteristics of breast cancer, supporting the thesis it is a cancer precursor, in: Cancer Research Conference: 38th Annual CTRC AACR San Antonio Breast Cancer Symposium San Antonio, vol. 76, TX United States Conference Publication, 2016, 4 SUPPL. 1.

[23] C.E. Kistler, T.M. Hess, K. Howard, M.P. Pignone, T.M. Crutchfield, S.T. Hawley, et al., Older adults' preferences for colorectal cancer-screening test attributes and test choice, Patient Prefer. Adherence 9 (2015) 1005.

[24] M. Thompson, B. Weigl, A. Fitzpatrick, N. Ide, More than just accuracy: a novel method to incorporate multiple test attributes in evaluating diagnostic tests including point of care tests, IEEE J. Transl. Eng. Health Med. 4 (2016) 1–8.

[25] K.A. Proctor, L.R. Rowe, Bentz JSJBwsh, Cytologic Features of Nipple Aspirate Fluid Using an Automated Non-invasive Collection Device: a Prospective Observational Study, vol. 5, 2005, p. 10, 1.

[26] E. Sauter, E. Ross, M. Daly, A. Klein-Szanto, P. Engstrom, A. Sorling, et al., Nipple Aspirate Fluid: a Promising Non-invasive Method to Identify Cellular Markers of Breast Cancer Risk, vol. 76, 1997, p. 494, 4.

[27] N. Jiwa, Z. Takats, D.R. Leff, C. Sutton, Breast Health Screening: a UK-wide Questionnaire. BMJ Nutrition, Prevention & Health, 2021 bmjnph-2021-000266.

[28] D. Twelves, A. Nerurkar, P. Osin, A. Ward, C.M. Isacke, G.P.J.E. Gui, The Feasibility of Nipple Aspiration and Duct Lavage to Evaluate the Breast Duct Epithelium of Women with Increased Breast Cancer Risk, vol. 49, 2013, pp. 65–71, 1.

[29] W.C. Dooley, B.-M. Ljung, U. Veronesi, M. Cazzaniga, R.M. Elledge, J. A. O'Shaughnessy, et al., Ductal Lavage for Detection of Cellular Atypia in Women at High Risk for Breast Cancer, vol. 93, 2001, pp. 1624–1632, 21.

[30] K.-W. Shen, J. Wu, J.-S. Lu, Q.-X. Han, Z.-Z. Shen, M. Nguyen, et al., Fiberoptic ductoscopy for breast cancer patients with nipple discharge, Surg. Endosc. 15 (11) (2001) 1340–1345.

[31] K. Visvanathan, D. Santor, S. Ali, A. Brewster, A. Arnold, D. Armstrong, et al., The reliability of nipple aspirate and ductal lavage in women at increased risk for breast cancer—a potential tool for breast cancer risk assessment and biomarker evaluation, Can. Epidemiol. Prevent. Biomark. 16 (5) (2007) 950–955.

[32] L. Delmonico, V.R. Areias, R.C. Pinto, C.D.S. Matos, M.F.F. Rosa, C.M. De Azevedo, et al., Protein identification from dried nipple aspirate fluid on Guthrie cards using mass spectrometry, Mol. Med. Rep. 12 (1) (2015) 159–164.

[33] G.N. Papanicolaou, D.G. Holmquist, G.M. Bader, E.A. Falk, Exfoliative cytology of the human mammary gland and its value in the diagnosis of cancer and other diseases of the breast, Cancer 11 (2) (1958) 377–409.

[34] J. De Groot, C. Moelans, S. Elias, A. Hennink, B. Verolme, K. Suijkerbuijk, et al., Repeated nipple fluid aspiration: compliance and feasibility results from a prospective multicenter study, PLoS One 10 (5) (2015), e0127895.

[35] N.G.R. Jiwa, H. Chauhan, H. Ashrafian, S. Kumar, C. Wright, Z. Takats, D. Leff, Diagnostic accuracy of nipple aspirate fluid cytology in asymptomatic patients: a meta-analysis and systematic review of the literature, Ann. Surg Oncol. 28 (7) (2020) 3751–3760, https://doi.org/10.1245/s10434-020-0913-9.

[36] Y.C. Antill, G. Mitchell, S.A. Johnson, L. Devereux, A. Milner, J. Di Iulio, et al., Gene Methylation in Breast Ductal Fluid from BRCA1 and BRCA2 Mutation Carriers, vol. 19, 2010, pp. 265–274, 1.

[37] J.S. de Groot, C.B. Moelans, S.G. Elias, M.J. Fackler, R. van Domselaar, K. P. Suijkerbuijk, et al., DNA Promoter Hypermethylation in Nipple Fluid: a Potential Tool for Early Breast Cancer Detection, vol. 7, 2016, 24778, 17.

[38] H. Alexander, A.L. Stegner, C. Wagner-Mann, G.C. Du Bois, S. Alexander, E.R.J. C. Sauter, Proteomic Analysis to Identify Breast Cancer Biomarkers in Nipple Aspirate Fluid, vol. 10, 2004, pp. 7500–7510, 22.

[39] J. He, J. Gornbein, D. Shen, M. Lu, L.E. Rovai, H. Shau, et al., Detection of Breast Cancer Biomarkers in Nipple Aspirate Fluid by SELDI-TOF and Their Identification by Combined Liquid Chromatography-Tandem Mass Spectrometry, vol. 30, 2007, pp. 145–154, 1.

[40] M. Debald, M. Wolfgarten, G. Walgenbach-Brünagel, W. Kuhn, M.J.E.J. Braun, Non-invasive Proteomics—Thinking about Personalized Breast Cancer Screening and Treatment, vol. 1, 2010, pp. 413–420, 3.

[41] R.T. Chatterton, A.S. Geiger, S.A. Khan, I.B. Helenowski, B.D. Jovanovic, P. H. Gann, Variation in estradiol, estradiol precursors, and estrogen-related products in nipple aspirate fluid from normal premenopausal women, Canc. Epidemiol. Prev. Biomark. 13 (6) (2004) 928–935.

[42] R.T. Chatterton Jr., A.S. Geiger, E.T. Mateo, I.B. Helenowski, P.H. Gann, Comparison of hormone levels in nipple aspirate fluid of pre-and postmenopausal women: effect of oral contraceptives and hormone replacement, J. Clin. Endocrinol. Metab. 90 (3) (2005) 1686–1691.

[43] G. Wang, Y. Qin, J. Zhang, J. Zhao, Ya Liang, Z. Zhang, et al., Nipple Discharge of CA15-3, CA125, CEA and TSGF as a New Biomarker Panel for Breast Cancer, vol. 15, 2014, pp. 9546–9565, 6.

[44] R.T. Chatterton, R.E. Heinz, A.J. Fought, D. Ivancic, C. Shappell, S. Allu, et al., Nipple aspirate fluid hormone concentrations and breast cancer risk, Horm. Canc. 7 (2) (2016) 127–136.

[45] E. Seregni, A. Coli, N. Mazzucca, Circulating tumour markers in breast cancer, Eur. J. Nucl. Med. Mol. Imag. 31 (1) (2004) S15–S22.

[46] J.D. Wulfkuhle, L.A. Liotta, E.F. Petricoin, Proteomic applications for the early detection of cancer, Nat. Rev. Cancer 3 (4) (2003) 267–275.

[47] H. Kuper, H.O. Adami, Trichopoulos DJJoim, Infections as a Major Preventable Cause of Human Cancer, vol. 248, 2000, pp. 171–183, 3.

[48] L.E. Wroblewski, R.M. Peek Jr., K.T. Wilson, Helicobacter pylori and gastric cancer: factors that modulate disease risk, Clin. Microbiol. Rev. 23 (4) (2010) 713–739.

[49] P.J. Turnbaugh, R.E. Ley, M. Hamady, C.M. Fraser-Liggett, R. Knight, J.I.J. N. Gordon, in: The human microbiome project 449, 2007, p. 804, 7164.

[50] T.J. Hieken, J. Chen, T.L. Hoskin, M. Walther-Antonio, S. Johnson, S. Ramaker, et al., The Microbiome of Aseptically Collected Human Breast Tissue in Benign and Malignant Disease, vol. 6, 2016, 30751.

[51] C. Urbaniak, J. Cummins, M. Brackstone, J.M. Macklaim, G.B. Gloor, C.K. Baban, et al., Microb. Human Breast Tiss 80 (10) (2014) 3007–3014.

[52] C. Urbaniak, G.B. Gloor, M. Brackstone, L. Scott, M. Tangney, G.J.A. Reid, et al., The Microbiota of Breast Tissue and its Association with Tumours, 2016. AEM. 01235-16.

[53] C. Xuan, J.M. Shamonki, A. Chung, M.L. DiNome, M. Chung, P.A. Sieling, et al., Microbial Dysbiosis Is Associated with Human Breast Cancer, vol. 9, 2014, e83744, 1.

[54] E. Fahy, S. Subramaniam, R.C. Murphy, M. Nishijima, C.R. Raetz, T. Shimizu, et al., Update of the LIPID MAPS Comprehensive Classification System for Lipids, vol. 50, 2009, pp. S9–S14. Supplement.

[55] J. Baumann, C. Sevinsky, D.S. Conklin, Lipid biology of breast cancer, Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1831 (10) (2013) 1509–1517.

[56] E.R. St John, R. Al-Khudairi, J. Balog, M. Rossi, L. Gildea, A. Speller, et al., Rapid evaporative ionisation mass spectrometry towards real time intraoperative oncological margin status determination in breast conserving surgery, in: Cancer Research Conference: 38th Annual CTRC AACR San Antonio Breast Cancer Symposium San Antonio, vol. 76, TX United States Conference Publication, 2016, 4 SUPPL. 1.

[57] M. Hilvo, C. Denkert, L. Lehtinen, B. Muller, S. Brockmoller, T. Seppanen-Laakso, et al., Novel Theranostic Opportunities Offered by Characterization of Altered Membrane Lipid Metabolism in Breast Cancer Progression, 2011 canres. 3894.2010.

[58] E.R. St John, J. Balog, J.S. McKenzie, M. Rossi, A. Covington, L. Muirhead, et al., Rapid Evaporative Ionisation Mass Spectrometry of Electrosurgical Vapours for the Identification of Breast Pathology: towards an Intelligent Knife for Breast Cancer Surgery, vol. 19, 2017, p. 59, 1.

[59] J. Alexander, L. Gildea, J. Balog, A. Speller, J. McKenzie, L. Muirhead, et al., A novel methodology for in vivo endoscopic phenotyping of colorectal cancer based on real-time analysis of the mucosal lipidome: a prospective observational study of the iKnife, Surg. Endosc. 31 (3) (2017) 1361–1370.

[60] B. Vaqas, S.J. Cameron, J.L. Alexander, K.S. O'Neill, J.M. Kinross, Z. Takats, The iKnife: Development and Clinical Applications of Rapid Evaporative Ionization Mass Spectrometry. The Handbook of Metabolic Phenotyping, Elsevier, 2019, pp. 219–236.

[61] L. Matos Do Canto, C. Marian, R.S. Varghese, J. Ahn, P.A. Da Cunha, S. Willey, et al., Metabolomic Profiling of Breast Tumors Using Ductal Fluid, vol. 49, 2016, pp. 2245–2254, 6.