INVESTIGATING THE RELATIONSHIP BETWEEN MAMMOGRAPHIC BREAST DENSITY AND TRIPLE NEGATIVE BREAST CANCER IN NOVA SCOTIA, CANADA

by

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ABSTRACT

The study objectives were to estimate the association between mammographic breast density (MBD) and triple negative breast cancers (TNBC); as well as to estimate the discriminatory ability of MBD, alone and with clinical risk factors, in the screening population. This case-control study consisted of 121 TNBC cases with a full-field digital mammography (FFDM) screen in 2009-2015 in Nova Scotia. The 6807 controls were women with a prior negative FFDM screening mammogram episode. Odds ratios and areas under curves were reported for models generated using two measures of MBD, percent and BI-RADS categories (5^{th} ed.), both separately and in combination. Aside from the two forms of MBD, other variables included self-reported risk factors (menopausal status, hormone replacement therapy use, parity, family history), biopsy history, and derived breast volume. A significant positive association was found between MBD and TNBC in this screening population. The addition of clinical factors to density improved the discriminatory ability of the prediction models.

LIST OF ABBREVIATIONS

ACR	American College of Radiology
AUROC	Area under the receiver operator characteristic curve
BI-RADS	Breast Imaging Reporting and Data System
BIS	Breast information system
BMI	Body mass index
CC	Craniocaudal
CI	Confidence interval
ER	Estrogen receptors
FDA	Food and Drug Administration
FFDM	Full field digital mammography
FISH	Fluorescence In-Situ Hybridization
HER2	Human epidermal growth factor receptor
HRT	Hormone replacement therapy
IHC	Immunohistochemistry
MBD	Mammographic breast density
NSBSP	Nova Scotia Breast Screening Program
OR	Odds ratio
PR	Progesterone receptors
Ref	Referent
ROC	Receiver operator characteristic
SFM	Screen-film mammography
SNP	Single nucleotide polymorphisms
TDLU	terminal duct lobular units
TNBC	Triple negative breast cancer

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CHAPTER 1 INTRODUCTION

As the most common type of cancer in women[Canadian Cancer Statistics 2019], breast cancer causes short and long term burden at the individual and societal level. In response to this burden, Canada and many other countries have implemented organized breast screening programs, with mammography as the frontline primary screening modality. Given concerns about over- and under-screening, there have been discussions regarding risk assessment and personalized screening guidelines to target individuals at higher risk within the average risk population. Mammographic breast density (MBD) is measured on a mammogram and alongside clinical traditional risk factors, plays a significant role in breast cancer risk assessments.

Breast cancer is a heterogenous disease comprised of distinct subtypes, which are heterogeneous with respect to survival. The subtype with the worst prognosis is triple negative breast cancer (TNBC). As there is known heterogeneity in risk factors for distinct subtypes, it is important to understand the relationship between these subtypes and mammographic breast density. This research will explore both association with and prediction of, triple negative breast cancers using various measures of breast density by way of a case-control study within the screened population of women in Nova Scotia.

1.1 BREAST CANCER EPIDEMIOLOGY

Breast cancer occurs when the cells in the breast rapidly reproduce, causing tumours and alterations of the intended functions of the cells. In Canada, breast cancer has the second highest incidence rate among all cancers, and is the most common cancer in females, equating to 1 out of 8 women developing it in their lifetime.[Canadian Cancer Statistics 2019] Specifically, Nova Scotia is projected to have the second highest age-standardized incidence rate of the Canadian provinces, reaching 129.4 cases per 100,000 in 2019.[Canadian Cancer Statistics 2019] In women, breast cancer is responsible for 25% of all types of cancer and breast cancer mortality is the second highest when compared to other cancers.[Canadian Cancer Statistics 2019]

Breast cancer is associated with a range of factors that increase risk, including: older age, family history of breast cancer, post-menopausal status, low parity, hormone replacement therapy (HRT) and oral contraceptive use, early menarche, late menopause, increased breast density, and increased alcohol consumption.[Sun 2017]

1.2 TRIPLE NEGATIVE BREAST CANCERS

There are has various subtypes of breast cancer based on hormone receptor status, which vary in prognostic outcomes and available treatment options.[Eriksson 2012, Onitilo 2009] One method for determining breast cancer subtype is immunohistochemistry (IHC) classification. Tumour biopsies undergo IHC staining and negative or positive status of the hormone receptors are reported based on the applicable clinical guidelines. The North American guidelines, published by the American Society of Clinical Oncology/College of American Pathologists, define positive estrogen receptors (ER) and progesterone receptors (PR) status as ≥1% of tumour cells expressing positive nuclear staining, and negative status as a result of <1% expression.[Hammond 2010, Effi 2017] Human Epidermal Growth Factor Receptor (HER2) receptor status is determined as either positive, negative or equivocal: ≥10% expression is positive, <10% is negative or equivocal, depending on visual assessment. Equivocal tumours undergo Fluorescence In-Situ Hybridization (FISH), to determine positive or negative status.[Wolff 2013]

The subtype with the worst overall and disease-free survival is TNBC,[Onitilo 2009] which was first defined in 2006 as having no expression of ER and PR, as well as no overexpression of the HER2.[Bryan 2006] Fifteen to twenty percent of breast cancers are considered TNBC.[Bae 2016] Typically, TNBC are diagnosed at a later stage (increased size and spread), and are associated with a higher tumour grade, and increased growth rate. [Boyle 2012] These circumstances combined with the limited responsiveness to interventions, explains why TNBC is associated with a worse prognosis than other breast cancer subtypes.[Bae 2016, Baré 2015] Given this worse prognosis, the identification of women at high risk of TNBC, could allow for intervention prior to disease onset or advancement. These interventions could increase lifestyle factors management (such as decreasing BMI, and/or alcohol intake), and altering screening protocols, such as shorter intervals

between screening events, or screening with different imaging modalities. Early detection of these tumors could reduce this high disease burden associated with TNBC.

1.2.1 TNBC and established breast cancer risk factors

As there is heterogeneity in the pathology of breast cancer subtypes, there may also be heterogeneity in the associated risk factors. Including all breast cancer subtypes as one group in regression analyses could nullify or weaken the magnitude of the observed relationships, as there may be opposing associations between subtypes. Many classical breast cancer risk factors are related to hormone exposure, which makes a strong rationale for studying these risk factors' relationships with breast cancer with respect to hormone receptor level. Furthermore, understanding how the relationships vary for TNBC specifically could significantly aid in primary and secondary prevention of TNBC.

Much research investigating risk factors of TNBC utilizes the Luminal A subtype (ER+ and/or PR+, HER2−)[Shin 2017] as a comparison group, while other research uses non-cancer, healthy control groups. Individuals with TNBC are frequently younger, when compared to other subtypes,[Boyle 2012] and in particular, the Luminal A subtype.[Ma 2017] Increased parity is more common in TNBC, compared to subtypes with positive status of hormone receptors,[Yang 2011] and has been found to be associated with TNBC, relative to a non-cancer control group.[Phipps 2011] At diagnosis, increased risk of TNBC was associated with increased body mass index (BMI).[Boyle 2012, Sun 2017] When compared to other breast cancer subtypes, TNBC was significantly associated with obesity - although after stratifying the results by menopausal status, this association was only significant in pre-menopausal women.[Pierobon 2013] When breast cancer cases were compared with controls with benign breast disease, family history of breast cancer was reported to be associated with an elevated risk of breast cancer, but the results showed there was no difference between TNBC and non-TNBC cases.[Zhou 2013] Pre-menopausal status is more common in TNBC, compared to the Luminal A subtype.[Ma 2017] A study conducted by Phipps et al. (2008) reported no statistically significant association between age at menopause and the risk of TNBC.[Phipps 2008] A 2014 review of reproductive risk factors indicated that there is no evidence supporting a significant association between TNBC and age at

menopause.[Anderson 2014] There is conflicting evidence regarding the association between TNBC and oral contraceptive use.[Boyle 2012] In addition, no significant association has been found between HRT use and TNBC, when comparing to the Luminal A subtype.[Gierach 2010] Many classical breast cancer risk factors are related to TNBC risk specifically while others show significant heterogeneity in the associations observed with TNBC versus non-TNBC. The relationship between MBD and TNBC will be reviewed in detail in a later section.

1.3 MAMMOGRAPHIC BREAST DENSITY

Breast density refers to the degree of fibroglandular tissue content, such as epithelial and stromal cells,[Destounis 2017] compared to the non-dense adipose tissues.[Jeffers, 2017] Since the varying x-ray attenuation properties alter the appearance of the mammographic image,[Boyd 2010, Tamimi 2007] dense tissue is radiopaque and appears white,[Boyd 2010, Tamimi 2007, Krishnan, 2016] while adipose tissue is radiolucent and is therefore not visible (i.e., appears dark on the mammogram).[Boyd 2010, Tamimi 2007] Due to the visual similarities, high density can mask the presence of a tumour,[Krishnan, 2016] decreasing the sensitivity of screening.[Destounis 2017] Density is not only prominent for its masking properties, but also for its association with breast cancer risk. Epithelial cells are thought to be the cells in which breast cancer originates [Hinck 2014] and also, paired with stromal cells, make up dense tissue. The concentration of these cells and their ability to proliferate influences the risk of breast cancer, while altering the amount of density viewed on a mammogram.[Boyd 2011]

Breast density was first described as a breast cancer risk factor in 1976. [Wolfe 1976] Women with greater than 75% dense tissue are at approximately 4–5 times greater risk of breast cancer, than women with a low percentage of dense tissue.[Boyd 2005] It has been argued that 16-30% of breast cancers are attributable to breast density.[Destounis 2017] In addition, it is estimated that a shift from high to low breast density categories, can reduce risk by ~26–39%.[Engmann 2017, Antoni 2013] It is accepted that MBD plays a significant role in determining the risk of breast cancer, but the causal pathway remains unknown, and it's not clear if the magnitude of the relationship is heterogenous for specific breast cancer subtypes. Investigating the subtypes could

lead to a better understanding of the pathways between MBD and breast cancer risk,[Shin 2017] as the causal pathway has not yet been determined.[Antoni 2013]

There is a complex interaction between MBD and other established breast cancer risk factors. Increased BMI and hormonal exposures, such as increased number of births and post-menopausal status are associated with decreased MBD.[Li 2005] Furthermore, as age increases, the breast tissue increases in adiposity, and decreases in glandular tissue, thus decreasing in density.[Boyd 2010, Boyd 2011] Higher MBD is associated with first degree family history,[Ziv 2003] as well as HRT use.[Li 2005]

1.3.1 Mammographic breast density measurements

Using MBD to identify individuals at high risk of breast cancer, or TNBC requires a valid and reliable measurement method is essential.[Destounis 2017] It has been shown that the association between MBD and risk varies depending on the measurement of density.[Jeffers, 2017] The historical variability of the measurement process and scale used for MBD is speculated to cause issues with the interpretability while reviewing and synthesizing the literature on MBD and breast cancer risk.

Traditionally, the standard method for measuring MBD has been visual assessment. With visual assessment, inter-rater agreement between radiologists and intra-rater agreement within radiologists has been shown to vary widely.[Ciatto 2005, Spayne 2012] In recent years there has been a global shift from screen-film mammography (SFM) to full-field digital mammography (FFDM). FFDM can not only better distinguish contrasting tissue densities altering the appearance and distribution of MBD,[Faridah 2008] but the introduction of FFDM has also allowed for a shift away from visual assessment of MBD to computer algorithms and software tools that were not achievable with SFM.[Destounis 2017] Since this shift, a number semi- and fully-automated software tools have become available for measuring MBD. Fully-automated software removes the operator-dependency that semi-automated systems have, and can remove the intra- and inter-rater variability known to be associated with the visual assessment of MBD.[Holland 2017, Edwards 2017] FDA approved, commercially available, fully-automated density measures, such as

Quantra™, VolparaDensity™, and densitas densityai™ have been shown to agree well with radiologists visual assessment of MBD, with kappa statistics ranging between 0.68 (95%CI: 0.77, 0.83) and 0.87 (0.87, 0.87).[FDA 2020, Lee 2015, Ekpo 2016] Fully-automated software also allows for feasible MBD assessments in population level health care delivery, such as screening programs.[Destounis 2017, Pollán 2013]

Much of the scientific evidence on breast density is largely based on SFM and visual assessments, therefore the advancements in technology and shifts toward digital mammography and automated assessments of MBD creates inconsistencies in the literature, and these inconsistencies increase the difficulty in the interpretation of previous work on current findings.[Destounis 2017]

Breast density is measured by way of both quantitative and qualitative measures. The original method for measuring MBD was a visual analog scale rating the percentage of radiopaque area compared to the total breast area. The Breast Imaging Reporting and Data System (BI-RADS) 4th and 5th editions are density reporting guidelines developed by the American College of Radiology (ACR) beginning in 2003, to reduce the variability and subjectivity within visual estimation of MBD. The BI-RADS 4th edition categories are as follows: Category A: “The breasts are almost entirely fatty (less than 25% glandular)”; Category B: “There are scattered areas of fibroglandular densities (25-50% glandular)”; Category C: “The breasts are heterogeneously dense, which could obscure detection of small masses (50-75% glandular)”; Category D: “The breasts are extremely dense. This may lower the sensitivity of mammography (greater than 75% glandular)”.[Spak 2017]

In 2013, the 5th edition of the BI-RADS density classification was developed (Figure 1). The transition to the fifth edition BI-RADS removed the quantitative percent density ranges associated with the categories, and slightly modifying the wording in category C and category D. This emphasizes the importance of the qualitative descriptions of the dense regions and the possible likelihood of missing cancer.[Ekpo 2016]

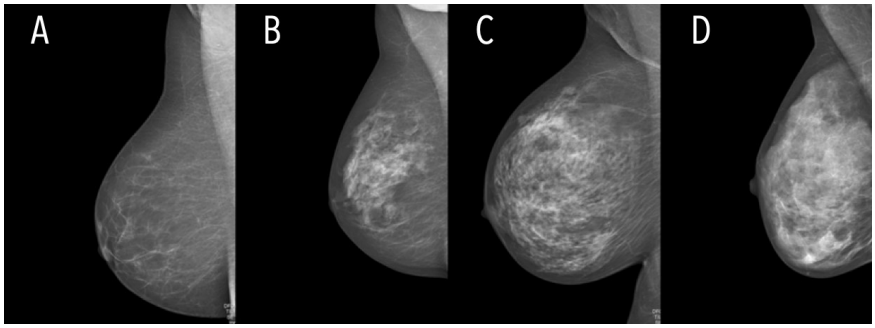

Figure 1.1. Example mammogram images of BI-RADS 5th edition mammographic density categories: (A) Breast is almost entirely fatty; (B)There are scattered areas of fibroglandular densities; (C) The breasts are heterogeneously dense, which may obscure detection of small masses; and (D) The breasts are extremely dense, which lowers the sensitivity of mammography.

With the various measurement methods and forms of MBD found within the literature, and the conflicting results, it is unclear if the relationships are sensitive to the nature of the MBD measure.

1.6 BREAST CANCER RISK PREDICTION

Risk prediction models are slowly becoming common practice in breast cancer screening programs. Just recently, an official position statement published by The American Society of Breast Surgeons recommends risk-based screening guidelines and acknowledges breast density as one of the key factors for determining the need for supplemental imaging.[breastsurgeons.org, 2019] This position statement demonstrates the need for further investigation into risk prediction models.

The validity of a predictive test, or model is the ability of the test to distinguish between who will develop the disease and who will not. Validity or accuracy is typically evaluated using discrimination measures, [Kramer 2007] with most common measure of discrimination being the area under the receiver operator characteristic curve (AUROC). The AUROC is a measure of how well the model classifies the subjects into the appropriate disease categories. [Zou 2007] A receiver operator characteristic (ROC) curve is a curve generated by plotting pairs of sensitivity and 1-specificity from a prediction model.[Zou 2007] When the AUROC is 0.5, the model does not

predict the outcome any better than random chance, and if the AUROC is equal to one, then the model predicts the outcome perfectly.[Zou 2007] The change in the AUROC can be used to evaluate the change in predictive performance after the addition of a new variable. There are distinct methods for comparing AUROC values. One of the first methods was proposed by Hanley et al (1983), computing a p-value to reject or accept the null hypothesis that the AUROC of two curves are equal.

The Gail Model was one of the first breast cancer risk prediction models developed.[Gail 1989] This Gail model, otherwise known as the Breast Cancer Risk Assessment Tool (BCRAT), along with other traditional clinically adopted breast cancer risk models to follow, including the International Breast Cancer Intervention Study (IBIS) Breast Cancer Risk Assessment Tool (otherwise known as the Tyrer-Cuzick model), as well as the Rosner & Colditz model developed from the 'Nurses' Health Study', did not contain MBD as a covariate.[Gail 1989, Tyrer 2004, Rosner 1996] The literature reports moderate discriminatory accuracy in the traditional risk factor models without MBD, with c-statistics ranging from 0.56 to 0.6[Boyle 2004, Gail 2007, Louro 2019]

As the relationship between MBD and breast cancer became more apparent in the literature, MBD was starting to be recognized as a value predictor in breast cancer risk prediction models.[Eriksson 2017, Tice 2005, Tice 2008, Chen 2006, Barlow 2006, Cuzick 2016] It has been demonstrated that the addition of density to previously validated and clinically adopted breast cancer risk models, improves predictive performance. Brentnall et al. (2015) reported an increase in the AUROC from 0.55 to 0.59 of the Gail model, and an increase in the AUROC from 0.57 to 0.61 in Tyrer-Cuzick v6 model. In this same study, a univariate model of density alone had an AUROC of 0.59.[Brentnall 2015] In 2016, a study in Nova Scotia using an average risk population, by Abdolell et al., demonstrated that adding MBD to a breast cancer risk model with clinical risk factors increased the AUROC from 0.53 to 0.63, and that density alone produced an AUROC of 0.62.[Abdolell 2016] MBD has more recently been incorporated into the Tyrer-Cuzick model version 8.[Cuzick 2016]

The measures of MBD used in risk models vary. One study conducted by Abdolell et al. in 2017,[Abdolell 2017] compared the predictive performance of a risk model using different scales of MBD, and explored the loss of predictive power with smaller number of categories of MBD; including in a breast cancer risk model as a continuous measurement (1% increments), transformed into a Boyd's 6-category scale [0; <10%; 10-25%; 25-50%; 50-75%; >75%][Pollán 2013] and a 2-category scale [<50%; ≥50%] yields decreasing discriminatory accuracy of the risk model.[Abdolell 2017] When the form of MBD is altered, the individual risk estimates can be altered, which could be problematic for implementing the prediction model into clinical practice.

It is now established that density is an important consideration for estimating breast cancer risk. This is demonstrated by the United States Food and Drug Administration's (FDA) breast density notification law. This law requires all women undergoing mammography and health care providers to be notified of their breast density, with specific language recommended to provide women with an understanding of how breast density impacts their risk, and their mammogram's sensitivity to detect a cancer. [21 CFR Part 900, MQSA] In Canada, British Columbia was the first province to mandate the reporting of MBD to the patients and their health care providers in 2018.[BC Gov 2018]

1.7 STUDY SETTING - BREAST SCREENING IN NOVA SCOTIA

The Nova Scotia Breast Screening Program (NSBSP) is a provincial screening program, established in 1991. In 2016, 54.6% of the target population aged 50-69 in Nova Scotia participated in the NSBSP; this equates to 43544 screens, which detected 222 cancers.[NSBSP 2017] The NSBSP requires accreditation of machines and radiologists, mandatory program evaluations and regular evidence-based reporting intervals. Breast imaging in Nova Scotia employs standardized FFDM equipment from one vendor, following a province-wide transition from film of all fixed sites completed in May 2010.[NSBSP 2012] All screening data and any associated diagnostic follow-up information are contained in the Breast Information System (BIS) managed by the NSBSP. In Nova Scotia, it is routine practice to collect receptor status, determined by IHC stains of tumour biopsies following the clinical guidelines published by the American Society of Clinical

Oncology/College of American Pathologists[Hammond 2010, Wolff 2013] and this information is recorded in the BIS.

CHAPTER 2 LITERATURE REVIEW AND RATIONALE

2.1 MBD AS A RISK FACTOR OF TNBC

Studies investigating the associations between MBD and subtypes of breast cancer have primarily utilized ER status to define the populations.[Edwards 2017] A meta-analysis that examined these results prior to June 2012, concluded that MBD is associated with the risk of breast cancer in general, as well as, the varying subtypes. The investigators indicate that MBD is associated with risk at comparable strengths for both ER positive and ER negative breast cancer.[Antoni 2013]

The existing evidence regarding MBD and TNBC consists of 6 observational comparative studies, many of which have reported variable results.[Edwards 2017] Some studies compared TNBC with non-cancer, and other studies present case-only analyses,[Edwards 2017, Sartor 2015] in which TNBC is compared with Luminal A cancers, the most common breast cancer subtype. Three larger studies incorporate both case-control and case-only analyses.[Razzaghi 2013, Ma 2009, Holm 2017] Of these six primary studies, five utilized secondary data available from a parent case-control or cohort study, for example, data from the Women's Contraceptive and Reproductive Experiences Study,[Ma 2009] or Malmo Diet and Cancer Study.[Sartor 2015] Two studies took place in the United States, [Ma 2009, Razzaghi 2013] two in Sweden,[Sartor 2015, Holm 2017] one in South Korea,[Shin 2017] and one study did not specify the location or much about the source population.[Edwards 2017] The details of the studies using logistic regression are reported in table format in Table 2.1.

The two more recent studies assessed MBD using digital mammography,[Shin 2017, Edwards 2017] while the other studies included both digital and film mammograms[Sartor 2015], or digitized film mammograms.[Ma 2009, Holm 2017] MBD was either collected retrospectively from reports,[Edwards 2017] or prospectively collected[Shin 2017, Holm 2017] using qualitative visual assessments[Sartor 2015], semi-automated density assessment[Shin 2017, Ma 2009], or fully automated tools[Holm 2017]. Furthermore, each of the six studies measures and reports MBD differently – quartiles based on the distribution of percent MBD of control group[Shin 2017]; BI-RADS 4th edition density classification – ACR version and varying modifications[Edwards 2017,

Razzaghi 2013, Sartor 2015]; categories based on arbitrary thresholds of percent MBD[Ma 2009]; 1 Standard deviation in absolute MBD.[Holm 2017]

Of the four analyses using non-breast cancer controls as comparators, two studies found a positive statistically significant association between MBD and TNBC.[Ma 2009, Holm 2017] Both studies measuring MBD using software, and on digitized film mammograms. In the Los Angeles study, it was reported that women with MBD ≥60% have a 2.96-fold increased odds of TNBC than women with MBD <10% (OR = 2.96 (95%CI: 1.21, 7.23))[Ma 2009]. Furthermore, in the Swedish population, that a per 1 SD increase in absolute density, increased the odds of TNBC by a factor of 1.58. (OR = 1.58 (95%CI: 1.34, 1.87)[Holm 2017].

The five analyses using Luminal A Subtype as the comparator found no statistically significant association between MBD and TNBC risk.[Sartor 2015, Edwards 2017, Razzaghi 2013, Ma 2009, Holm 2017] This suggests that the association between MBD and breast cancer risk is not heterogeneous by subtype. It is unknown if the non-statistically significant findings are a result of the low numbers of TNBC cases, and large numbers of covariates including in the logistic regression modelling. One of these 4 studies, Razzaghi et al. (2013), report no significant association between MBD and all breast cancer subtypes combined,[Razzaghi 2013] which contradicts the existing evidence. The results from all six studies are reported in Table 2.2.

Varying results were found when comparing differing methods of detection, interval and screen-detected cancers. Sartor et al. [Sartor 2015] found that within the interval cancers, a significant positive association was reported between MBD and the risk of TNBC, when compared to Luminal A cancers – an increase in 1% MBD increases the odds of interval detected triple negative breast cancer by a factors of 2.44 (95%CI: 1.01, 5.89). This result was not observed in the screen-detected cancers.[Sartor 2015]

Table 2.1. Primary research studies evaluating the association between mammographic breast density and triple negative breast cancer

	Edwards 2017	Shin 2017	Sartor 2015	Razzaghi 2013	Ma 2009	Holm 2017
Study Design	Case-Only	Case-Control	Case-Only	Case-Control & Case-Only	Case-Control & Case-Only	Case-Control & Case-Only
TNBC Cases, n	86	68	41	84	106	95
Non-BrCa, n	N/A	1241	N/A	528	376	14814
Luminal A, n	233	N/A	303	181	184	1240
Source Population	A case-control study, location unknown	Samsung Medical Center, South Korea	Malmo Diet and Cancer Study, Sweden	Carolina Breast Cancer Study, North Carolina	Women's Contraceptive and Reproductive Experiences Study, Los Angeles County	KARolinska MAmmography Project (KARMA) and Libro-1study, Sweden
Matched Variables	N/A	Age; menopausal status	N/A	Age; race	Age; ethnicity	Frequency
Mammography Type	Digital mammography	Full-field digital mammography	Analog and digital images	Unknown	Digitized mammogram images	Digitized analog images
MBD Measure	BI-RADS 4	Quartiles based on distribution in control group	Fat involuted (BIRADS1); moderately dense (BIRADS 2+3); dense (BI-RADS 4)	Qualitative BIRADS 4th edition Scoring	Categories of percentage MBD <10, 10-29, 30-59, ≥60	1 Standard deviation in absolute MBD
MBD Collection	Abstracted from imaging reports	Prospective; Single blinded observer Cumulus Thresholding	Qualitative; abstracted from radiology report	Abstracted from the Mammography Registry	Prospective; Researcher using validated computer-assisted software	Prospective; Automated measure which mimics cumulus
TNBC Collection	IHC; abstracted Pathology reports,	IHC; abstracted from electronic medical records	Prospective IHC	Prospective IHC and medical records	Prospective IHC	IHC; abstracted from register
Adjustment Variables	Age, race, hx of LCIS, 1st- or 2nd degree BrCa family hx	BMI, age at menarche, parity, ever-smoker, alcohol consumption, physical exercise, 1st degree BrCa family hx, hx of benign breast disease, HRT use	Age at diagnosis, mode of detection, BMI at baseline, HRT use at baseline	Age, race, BMI, menopausal status, BrCa family hx, age at menarche, use of hormone therapy, and parity, age at first full-term pregnancy	Age, 1st degree BrCa family hx, BMI, age at menarche, parity, age at 1st full-term pregnancy, menopausal status + hormone therapy use, race, laterality of mammogram	Age, education level, parity, BMI, if born in Sweden or not.

Mammographic Breast Density (MBD); Triple Negative Breast Cancer (TNBC); Immunohistochemical (IHC); Body Mass Index (BMI); Hormone Replacement Therapy (HRT); Breast Cancer (BrCa); Lobular carcinoma in situ (LCIS); History (hx); Breast Imaging and Reporting Data System (BIRADS)

Table 2.2. Cited Odd Ratios measuring the relationship between mammographic breast density and triple negative breast cancer

	Odds Ratio (95% CI)
TNBC vs Controls	
Shin, 2017	
Q1 (< 8.997960 percent dense area)	1.0 (Ref)
Q2 (8.997960–17.55 percent dense area)	1.64 (0.59, 4.57)
Q3 (17.547183–26.27 percent dense area)	2.22 (0.83, 5.93)
Q4 (≥26.273696)	1.70 (0.61, 4.70)
Holm, 2017	
Per 1 standard deviation increase in absolute MBD	1.58 (1.34, 1.87)
Razzaghi, 2013	
Almost entirely fatty	1.0 (Ref)
Scattered fibroglandular densities	5.96 (0.70, 50.64)
Heterogeneously dense	5.83 (0.68, 50.04)
Extremely dense	7.13 (0.74, 68.90)
Ma, 2009	
<10 percent dense area	1.0 (Ref)
10-29 percent dense area	1.09 (0.52, 2.29)
30-59 percent dense area	1.81 (0.91, 3.63)
≥60 percent dense area	2.96 (1.21, 7.23)
TNBC vs Luminal A	
Edwards, 2017	
BIRADS 4th edition density category 1	0.73 (0.35, 1.50)
Category 2	1.0 (Ref)
Category 3	1.16 (0.65, 2.07)
Category 4	1.54 (0.57, 4.16)
Holm, 2017	
Per 1 standard deviation increase in absolute MBD	0.89 (0.71, 1.13)
Sartor, 2015	
Per increase in BI-RADS 4th edition density category	1.64 (0.94, 2.86)
Razzaghi, 2013	
Almost entirely fatty	1.0 (Ref)
Scattered fibroglandular densities	3.05 (0.25, 36.68)
Heterogeneously dense	2.62 (0.22, 31.62)
Extremely dense	3.57 (0.26, 49.11)
Ma, 2009	
<10 percent dense area	1.0 (Ref)
10-29 percent dense area	0.74 (0.30, 1.83)
30-59 percent dense area	0.98 (0.42, 2.28)
≥60 percent dense area	1.38 (0.47, 4.01)

Triple Negative Breast Cancer (TNBC); Confidence Interval (CI); Referent (Ref); Breast Imaging and Reporting Data System (BIRADS)

Due to the inconsistencies in the methods and results of the studies investigating the association between MBD and TNBC, the limited evidence gathered from digital mammograms, the small number of TNBCs included in the studies, and the variation in the collection methods of the exposure variable MBD, there is need for a large population-based study that prospectively collects MBD using validated fully-automated software.

2.2 MBD AS A PREDICTOR OF TNBC

Much of the literature is focused on how MBD can predict breast cancer generally, but there is limited evidence investigating how MBD can predict the distinct subtypes of breast cancer. There is one study that evaluated the ability of IBIS, a previously-validated and clinically adopted breast cancer risk model, to discriminate between non-breast cancer controls and 64 TNBC cases in a hospital-based screening population.[McCarthy, 2020] Investigating the changes in the AUROC between different subtypes of breast cancer, there was a slight decrease observed in the AUROC when attempting to classify the TNBC cases, compared to the AUROC values observed for receptor positive cases. The AUROC for the TCv8 model was 0.63 (95%CI: 0.60 to 0.66) in the ER+/PR+/HER2- group; the AUROC was 0.57 (95%CI: 0.47 to 0.66) in the HER2+ group; lastly, the AUROC was 0.52 (95%CI: 0.45 to 0.61) in the TNBC group.

There is one study that developed and cross-validated a TNBC risk prediction models. This is a case-only analysis, with 134 TNBC and 893 with other forms of invasive breast cancer. Using a combination of single nucleotide polymorphisms (SNPs), as well as age and BMI as clinical risk factors, the authors reported an AUROC of 0.625. Among all of the SNPs, as well as, BMI, age was the strongest single predictor available. This paper did not include breast density in any of their models.[Häberle, 2017]

Although the IBIS model was evaluated on the TNBC population, it was not specifically developed for the TNBC population. There are no published risk prediction models for TNBC using MBD,in the screening population.

2.3 FILLING THE LITERATURE GAP

The body of literature on MBD and breast cancer is growing and the web-like complexities in the relationship are becoming familiar, but there is little evidence on the relationship between MBD and TNBC. The literature is limited in many ways: small samples sizes, and inconsistencies in: the form of the density measure (e.g., percent dense), as well as how it was measured (e.g., fully automated assessment); and use of clinical risk factors. By increasing the number of subjects, focusing on the current digital mammography period, investigating multiple density measures, controlling for many possible confounding variables, as well as incorporating a component of both association and prediction in the methods, this present study will contribute substantially to the limited evidence and support a more relevant, specific understanding of risk prediction within subtypes of breast cancer.

CHAPTER 3 RESEARCH OBJECTIVES

3.1 OBJECTIVE 1: THE ASSOCIATION BETWEEN MBD AND TNBC

The first objective of this study was to estimate the association between mammographic breast density (in the form of percent and BI-RADS 5th edition density) and triple negative breast cancers in the general screening population, adjusting for clinical risk factors.

3.2 OBJECTIVE 2: DISCRIMINATORY ABILITY OF DENSITY AND CLINICAL RISK FACTORS

The second study objective was to estimate the ability of mammographic breast density (again, in the form of percent and BI-RADS 5th edition density) to discriminate between triple negative breast cancer cases and controls, alone and with clinical risk factors, in the general screening population.

CHAPTER 4 METHODOLOGY

4.1 STUDY DESIGN AND SAMPLE

The study sample was derived from a nested case-control study entitled, *Toward Personalized Breast Cancer Risk Assessment: Breast Density, Pathology and Clinical Risk Factors*, which included all breast cancers (in situ and invasive) diagnosed in Nova Scotia among women who underwent a digital breast screening mammogram between January 2009 and December 2015. Cases were either diagnosed with breast cancer as a result of an abnormal digital screening mammogram, or within 24 months of a negative digital mammographic screening episode. Negative-screened controls were randomly selected and frequency matched to breast cancer cases on age at screen, as well as year of screen to control for any variability in practice over time. Included in the parent case-control study were 2,328 cases and 7,046 controls, with information on clinical factors and MBD.

4.2 DATA COLLECTION

4.2.1 Outcome: Triple negative breast cancer

The subset used in this study consisted of only those cases diagnosed with TNBC and all negative screen controls. The outcome of interest is TNBC, defined as cases that are ER negative (<1% expression), PR negative (<1% expression), and HER2 negative (<10% expression, or as determined by FISH). Expression levels were routinely determined by immunohistochemistry and assessed by pathologists, and captured in the provincial Breast Imaging System. Any data missing from the Breast Imaging System was extracted from archived pathology reports. The cases (n = 121) were all TNBCs that were screen-detected or interval breast cancers diagnosed by FFDM in Nova Scotia in women who underwent a digital breast screening mammogram from 2009 to 2015. The controls (n = 6,807) were women who underwent a digital breast screening mammogram from 2009 to 2015 and had negative results. There were at minimum 3 controls for each case of a given age and screen year.

4.2.2 Risk factor of interest: Mammographic breast density

The exposure of interest was MBD. MBD was measured using a fully-automated density measurement software, densitas density**ai**™ Version 3.0 (*Densitas, Inc).* The density**ai**™ algorithm measures MBD on individual FFDM 'for-presentation' images and has previously demonstrated excellent agreement with 6 radiologists' independent visual assessments (kappa 0.81-0.90).[Abdolell 2016] This software has been used in previously developed risk models, and has been shown to have comparable performance to risk models including other forms of MBD.[Astley 2018, Abdolell 2016]

MBD was expressed as two distinct measures. The proportion of radiopaque area, otherwise referred to as the percent MBD, was measured at 1% intervals and ranges from 0-100%. MBD will also be classified using the previously described qualitative BI-RADS 5th edition. The density**ai**™ software uses three factors in the determination of the BI-RADS 5th edition classification: (1) level of density; (2) compactness of the dense tissue; and (3) dispersion pattern of the dense tissue.

For the TNBC cases, density was measured from the craniocaudal (CC) view from the contralateral side of the breast cancer abnormality, if data on side is available. For the non-breast cancer controls, density will be measured from the CC view, randomly from the left or right side, ensuring the same distribution of right/left as the cases. Sensitivity analyses were performed and determine there is no difference in breast density according to the side (right/left) or image type (CC/MLO).

4.2.3 Clinical risk factors

In addition to age at screen (continuous; measured in years), clinical risk factors were self-reported at the time of the screen and included parity (continuous); HRT use at the time of screen (yes/no); first degree family history (yes/no); menopausal status at time of screen (pre-/post-menopausal); and personal history of needle core biopsy (yes/no), referred to hereafter as biopsy history.

It has been speculated that BMI confounds the association of MBD and subtypes of breast cancer.[shin 2017] As BMI was not reported in the original dataset, total breast area and

compression thickness from the density measurement software were used to calculate total breast volume (continuous; measured in cm^3), which will be used as a proxy for BMI. Various breast size measurements, such as total beast volume[Duffy 2018], as well as total mammographic area[Stuedal 2008], have been reported to have a strong correlation with BMI, and act as a viable surrogate for BMI in estimating the effect of MBD on the risk of breast cancer.[Duffy 2018]

4.3 STATISTICAL ANALYSIS

R Studio (version 1.2.1335) was used to perform all analyses.

Descriptive statistics were calculated separately for cases and controls. Means and standard deviations are reported for the continuous variables. Frequencies and percentages are reported for the discrete variables. The Pearson chi-square test was utilized to compare the TNBC cases and the negative-screened controls with respect to each of the discrete variables, and t-tests were used to compare the continuous variables between groups. All analyses were evaluated using a significance level of 95% ($\alpha = 0.05$).

4.3.1 Objective 1: The associative relationship

Simple logistic regression was used to produce crude odds ratios for each variable and associated 95% confidence intervals. Using the e^{bx10} equation, crude and adjusted odds ratios for age and breast volume were displayed in 10 year, and 200 cm^3 increments, respectively. Furthermore, crude and adjusted odds ratios for percent density were displayed in increments of 10%. Table 4.1 describes the six regression models produced, using the two measures of MBD: BI-RADS 5th edition and percent MBD. The Lowess curve of continuous percent MBD was plotted to visualize linearity of the association between MBD and the odds of TNBC. Regression diagnostics were performed to test the validity of the model assumptions. Multiple logistic regression was used to evaluate confounding; adjusted odds ratios and 95% confidence intervals were reported. Confounding was define using a 10% change when comparing the crude and adjusted odds ratios.

4.3.2 Objective 2: The predictive relationship

The discrimination of the logistic regression models described in table 4.1 was assessed using AUROC (otherwise known as the c-statistic), and their 95% confidence intervals (CI). The ROC curves will be presented and compared between models. Simple logistic regression models of individual clinical risk factors were compared using AUROC. Lastly, using a backwards stepwise approach based on the AUROC values generated from models with individual clinical risk factors, the AUROC values were compared to investigate how the discriminatory accuracy varies when predictors are dropped from the full model. A statistical comparison of the AUROC values to the fully saturated model was performed using the methods described by Hanley et al.[Hanley 1983] The null hypothesis was that the AUROC of two curves being compared were equal.

Table 4.1. Simple and multiple logistic regression models used to assess the association between mammographic breast density and triple negative breast cancer in the screening population.

$y \sim x_1 + x_2 + x_3 + x_4 + x_5 + x_6$		
Model 1 x_1 = Percent MBD	**Model 3** x_1 = BI-RADS 5	**Model 5** x_1 = Percent MBD x_2 = BI-RADS 5
Model 2 x_1 = Percent MBD x_2 = Age x_3 = Menopause x_4 = Biopsy history X_5 = Breast volume x_6 = Family history x_7 = Parity x_8 = HRT use	**Model 4** x_1 = BI-RADS 5 x_2 = Age x_3 = Menopause x_4 = Biopsy history X_5 = Breast volume x_6 = Family history x_7 = Parity x_8 = HRT use	**Model 6** x_1 = Percent MBD x_2 = BI-RADS 5 x_3 = Age x_4 = Menopause x_5 = Biopsy history x_6 = Breast volume x_7 = Family history x_8 = Parity x_9 = HRT use

4.4 ETHICAL CONSIDERATIONS

This study was approved by the Nova Scotia Health Authority Research Ethics Board.

CHAPTER 5 RESULTS

5.1 CHARACTERISTICS OF THE STUDY POPULATION

121 TNBC cases and 6807 non-breast cancer controls were included in this study. 6 TNBC cases were excluded from the analyses, as were 239 negative-screen controls, for reasons of unknown density and use of hormone replacement therapy (Figure 5.1).

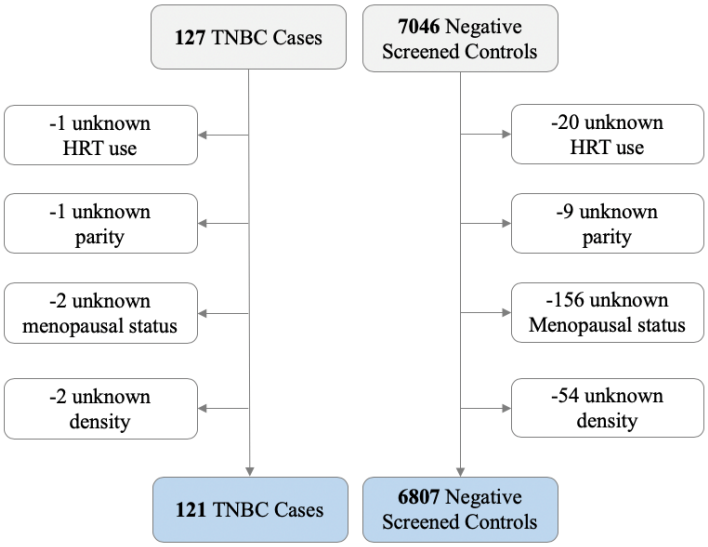

Figure 5.1. Frequencies of subjects excluded for the lack of complete data.

The characteristics of subjects included in the analysis are described in Table 5.1. Pearson chi-square test and two-sample t-tests were used to compare clinical risk factors between cases and controls. HRT use at the time of screen was found to be more common among the controls than cases (p =0.012).

Table 5.1. Odds Ratios (95% CI) of clinical risk factors for triple negative breast cancer cases relative to controls in the Nova Scotia screening population, 2009-2015

Clinical Risk Factors	**TNBC Cases (*n*=121)**	**Controls (*n* = 6807)**	***p***	**Crude OR (95% CI)**
Age, mean (SD)	58.3 (8.2)	59.4 (8.4)	0.150	0.98 (0.96, 1.00)
Breast Volume (cm^3), mean (SD)	946.6 (557)	854.9 (514)	0.074	1.00 (1.00, 1.00)
Pre-menopausal, n (%)	24 (19.8%)	1391 (20.4%)	0.961	0.96 (0.60, 1.49)
HRT Use, n (%)	6 (4.96%)	896 (13.2%)	0.012	0.34 (0.13, 0.72)
Parity, median	2.0 (0,7)	2.0 (0,10)	0.536	1.05 (0.91, 1.20)
Biopsy History, n (%)	13 (10.7%)	512 (7.52%)	0.248	1.48 (0.79, 2.55)
1st Degree Family History, n (%)	38 (31.4%)	1646 (24.2%)	0.084	1.44 (0.96, 2.10)

Hormone Replacement Therapy (HRT); Standard deviation (SD); *p*-value (*p*)

Investigating the crude associations between the clinical risk factors and the odds of triple negative breast cancer (Table 5.1), revealed that among the clinical risk factors, HRT use at the time of screen was the variable with the strongest crude association with triple negative breast cancers. Women using HRT at the time of screen had a lower risk of TNBC – the odds of triple negative breast cancer were 66% lower in women who reported using HRT at the time of screen, compared to women who have not used HRT use in the past (OR: 0.34, 95%CI: 0.13, 0.72) (Table 5.1).

Focusing on the exposure of interest, TNBC cases also differed from non-breast cancer controls with respect to MBD, both percent MBD ($p = 0.02$) and BI-RADS 5th edition density ($p = 0.001$) (Table 5.2). Stratified by cases (A) and controls (B), the distribution of MBD is illustrated in Figure 5.2.

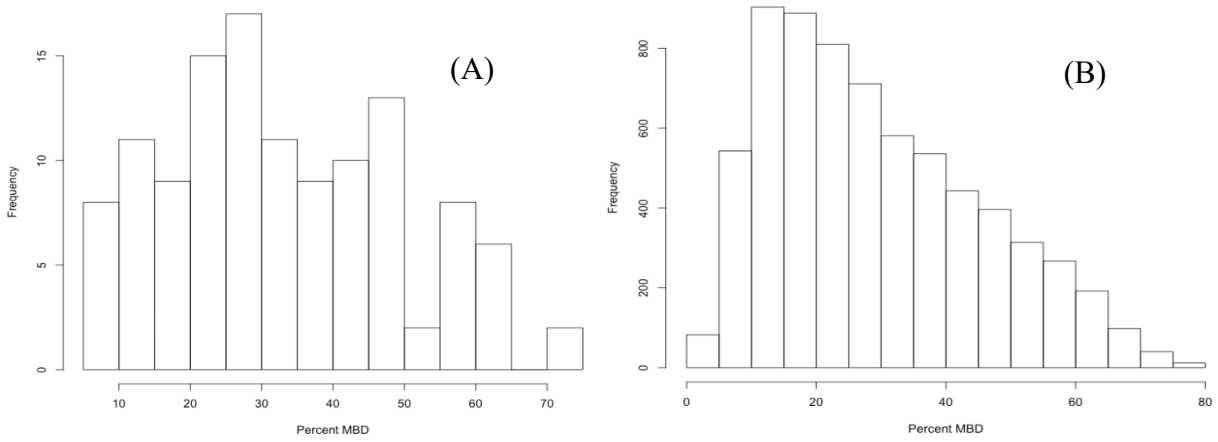

Figure 5.2. Distribution of percent mammographic breast density observed in triple negative breast cancer cases (A) and controls (B) in the Nova Scotia screening population, 2009-2015.

Further investigating the distribution of MBD, Figure 5.3 illustrates the distribution of percent density within the BI-RADS 5th edition categories of the cases and the controls. These plots indicate that there is a positive relationship between percent MBD and BI-RADS 5th edition density. An individual at 40% density could fall into category B, C, or D, or an individual with 20% dense tissue can be classified as a A, B, or C category BI-RADS 5th edition density scale. This demonstrates that BI-RADS 5th edition density classification may depend on factors other than quantitative percent MBD, such as the dispersion of the dense tissues, the level of risk of masking, or difficulty in interpreting the mammogram.

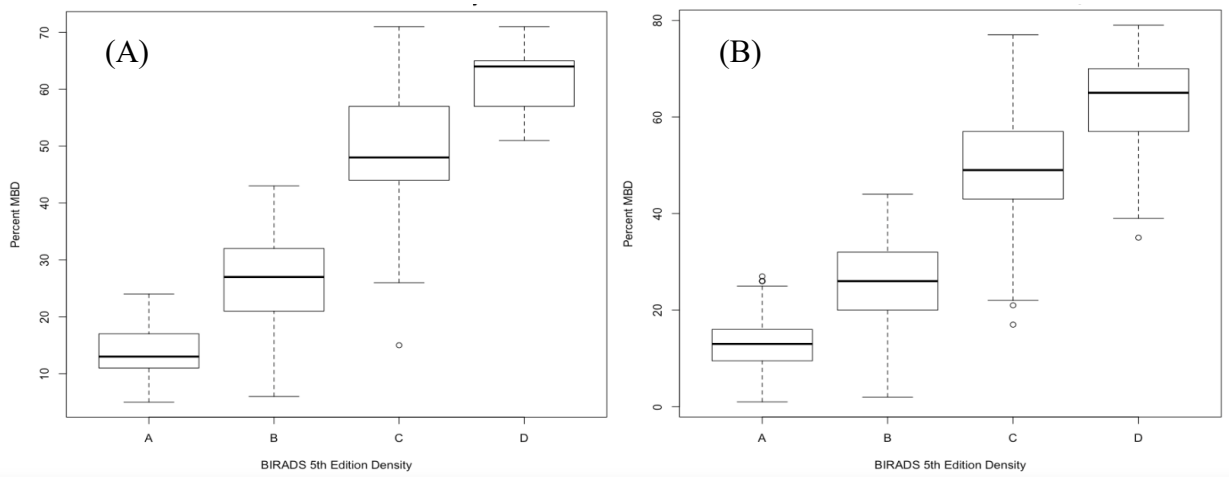

Figure 5.3. Distribution (box plots) of percent mammographic breast density and BI-RADS 5th edition density in triple negative breast cancer cases (A) and controls (B) in the Nova Scotia screening population, 2009-2015

There is a higher concentration of individuals in the middle of the density range, relative to the extremities in both the cases and the controls. Examining percent density by 5-year age intervals (Figure 5.3) revealed expected trends; percent MBD decreases with increasing age.

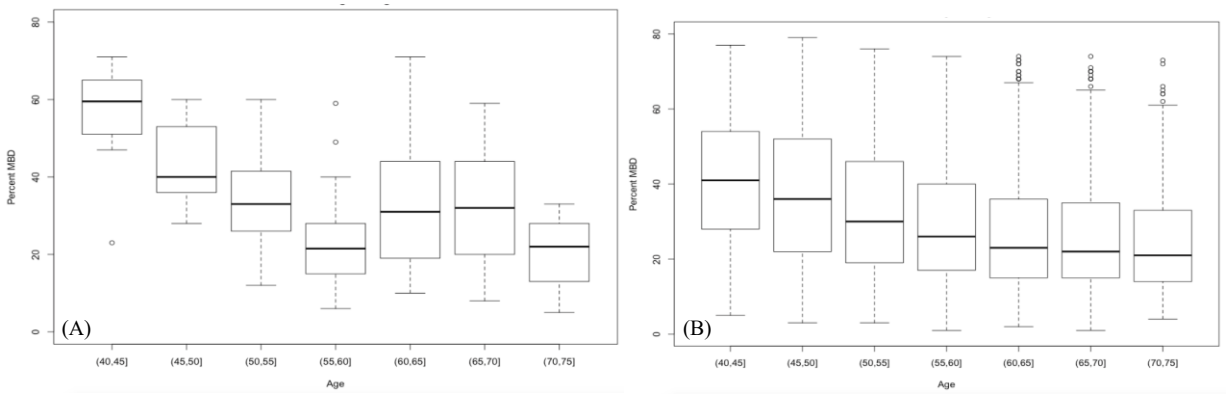

Figure 5.4. Distribution (box plots) of percent mammographic breast density by 5-year age groups in triple negative breast cancer cases (A) and controls (B) in the Nova Scotia screening program, 2009-2015

Given that one form of the main exposure variable was continuous, first the linearity of the percent density measure was examined by plotting the log odds of TNBC versus percent MBD (Figure 5.5).

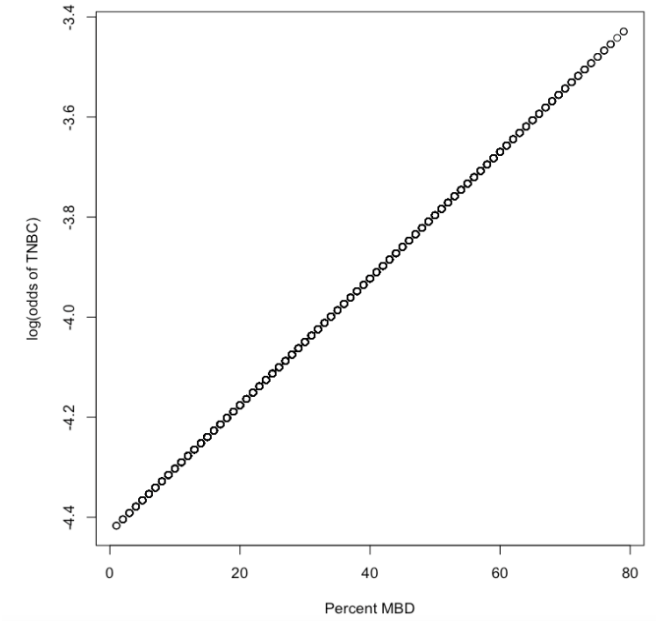

Figure 5.5. Percent mammographic breast density versus plotted against the log odds of triple negative breast cancer, in the Nova Scotia screening population, 2009-2015

This Lowess curve demonstrated that percent mammographic breast density was linearly related to the log odds produced by the simple logistic regression, satisfying the condition of linearity, and therefore confirming that inferences can be made from the logistic regression modelling performed within these analyses.

5.2 THE ASSOCIATION BETWEEN MBD AND TNBC (OBJECTIVE 1)

5.2.1 Assessment of confounding in the Association between MBD and TNBC

The potential confounding effects of the clinical risk factors were examined prior to building any adjusted models. The relationship between MBD and the odds of TNBC is modelled and controlled by the individual risk factors. Menopausal status and breast volume separately were determined to confound the association between MBD and TNBC (Table 5.2).

Table 5.2. Odds Ratios (95% CI) describing the association between mammographic breast density and triple negative breast cancer, crude and adjusted for clinical risk factors, in the Nova Scotia screening population, 2009-2015

	Crude Density	Adjustment variable						
		Menopausal Status	**1° Family Hx**	**Biopsy Hx**	**Age**	**Parity**	**Breast Volume**	**HRT Use**
1 - Percent MBD								
% MBD	1.01	1.01 (0%)	1.01 (0%)	1.01 (0%)	1.01 (0%)	1.01 (0%)	1.01 (0.99%)	1.01 (0%)
2 - BI-RADS 5								
Category A	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Category B	2.12	2.17 (2.36%)	2.13 (0.47%)	2.11 (-0.47%)	2.10 (-0.94%)	2.13 (0.47%)	2.29 (8.02%)	2.15 (1.42%)
Category C	2.41	2.55 (5.81%)	2.41 (0%)	2.38 (-1.24%)	2.33 (-3.32%)	2.48 (2.90%)	**3.05 (26.6%)**	2.50 (3.73%)
Category D	6.48	**7.47 (15.3%)**	6.53 (0.77%)	6.59 (1.70%)	6.09 (-6.02%)	6.78 (4.63%)	**9.58 (47.8%)**	6.73 (3.86%)
3 - Percent MBD + BI-RADS 5								
% MBD	0.99	0.99 (0%)	0.99 (0%)	0.99 (0%)	0.99 (0%)	0.99 (0%)	1.00 (1.01%)	0.99 (0%)
Category A	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Category B	2.32	2.33 (0.43%)	2.33 (0.43%)	2.31 (-0.43%)	2.32 (0%)	2.31 (-0.43%)	2.28 (-1.72%)	2.31 (-0.43%)
Category C	3.13	3.16 (0.96%)	3.15 (0.64%)	3.09 (-1.28%)	3.11 (-0.64%)	3.14 (0.32%)	3.04 (-2.9%)	3.09 (-1.28%)
Category D	9.25	9.96 (7.68%)	9.39 (1.5%)	9.42 (1.84%)	8.98 (-2.92%)	9.36 (1.19%)	9.54 (3.14%)	8.98 (-2.92%)

BI-RADS 5th edition density (BI-RADS5); mammographic breast density (MBD); hormone replacement therapy (HRT); history (Hx)

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Adjusting by menopausal status passed the confounding threshold with a percent change of 15.3% in BI-RADS 5th edition density category D. Adjusting by breast volume, also altered the ORs with a percent change of 26.6% change in BI-RADS 5th edition density category C and 47.8% change in BI-RADS 5th edition density category D. Negative confounding in the relationship between BI-RADS 5th edition density and TNBC was demonstrated with menopausal status and breast volume suggesting that when either variable is removed, the observed relationship between BI-RADS 5th edition density and the risk of TNBC is stronger

5.2.2 Quantifying the strength of the association between MBD and TNBC

Crude and adjusted odds ratios measuring the association between MBD and TNBC are reported below in Table 5.3. When using simple logistic regression, there was a significant positive association observed between both forms of MBD and TNBC. The odds of TNBC increased by a factor of 1.01 for every 1 percent increase in MBD. There was a modest increase in the odds ratio (1.02; 95% CI: 1.01, 1.03) after controlling for the other clinical risk factors. When both measures of density were incorporated into the model, the association between percent MBD and TNBC was weakened, and loses statistical significance. Examining the qualitative measure, the crude odds of TNBC in women classified as BI-RADS category-D density was 6.48-times the odds of women classified as BI-RADS category-A density. The fully adjusted odds (model incorporating clinical risk factors and percent density) of TNBC in subjects classified as BI-RADS category-D density was 11.1-times the odds of subjects classified as BI-RADS category-A density (Table 5.3).

Table 5.3. Odds Ratios (95% CI) describing the association between mammographic breast density and triple negative breast cancer, crude and adjusted for mammographic breast density and clinical risk factors in the Nova Scotia screening population, 2009-2015

	TNBC (*n*=121)	**Controls (*n* = 6807)**	***p*-val**	**Odds ratios (95% CI)**			
				Crude	**MBD+MBD**	**MBD + RF**	**MBD + MBD + RF**
Percent MBD, median (IQR)	31 (25)	27 (24)	0.02	1.01 (1.00, 1.02)	0.99 (0.97, 1.01)	1.02 (1.01, 1.03)	1.00 (0.98, 1.02)
BI-RADS 5, n (%)			<0.001				
Category A	15 (12.4%)	1672 (24.5%)		1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)
Category B	60 (49.6%)	3152 (46.3%)		2.12 (1.23, 3.88)	2.32 (1.27, 4.48)	2.28 (1.32, 4.20)	2.19 (1.19,4.23)
Category C	41 (33.9%)	1897 (27.9%)		2.40 (1.35, 4.49)	3.13 (1.18, 8.27)	3.13 (1.70, 6.04)	2.90 (1.09,7.73)
Category D	5 (4.1%)	86 (1.3%)		6.48 (2.07, 17.2)	9.25 (1.99, 38.95)	10.8 (3.25, 31.0)	11.1 (2.35,47.7)

BI-RADS 5th edition density (BI-RADS5); Mammographic Breast Density (MBD); Clinical Risk Factors including age at the time of screen, parity, HRT use at the time of screen, first degree family history, menopausal status, biopsy history, total breast volume (RF); p-value (p-val)

A significant positive association between MBD as measured using BI-RADS5 and TNBC was observed in the general screening population of Nova Scotia - with classification into a higher density category, comes an increased risk of TNBC.

5.3 THE PREDICTIVE RELATIONSHIP (OBJECTIVE 2)

Various models with different combinations of clinical risk factors and measures of MBD were assessed using AUROC curves, illustrating the discriminatory ability of the models to distinguish between TNBC cases and non-cancer controls.

5.3.2 Individual Clinical Risk Factors

The results from simple logistic regression models of each individual clinical risk factor are reported in Table 5.4, and illustrated by the receiver operator characteristic curves in Figure 5.7. Also presented is the performance of the multiple logistic regression model with all risk factors including age, parity, menopause, HRT, family history, breast volume, biopsy history, and excluding density.

Table 5.4. Comparison (AUROC and 95% CI) of clinical risk factor predictive models for triple negative breast cancer in the Nova Scotia screening population, 2009-2015

Predictor	AUROC (95% CI)
Menopausal Status	0.503 (0.467, 0.539)
Parity	0.509 (0.460-0.558)
Biopsy History	0.516 (0.488-0.544)
1° Family History	0.536 (0.494, 0.578)
Age	0.537 (0.487, 0.587)
HRT Use	0.541 (0.521, 0.561)
Breast Volume	0.551 (0.497, 0.605)
Combined	0.625 (0.577-0.673)

Hormone replacement therapy (HRT)

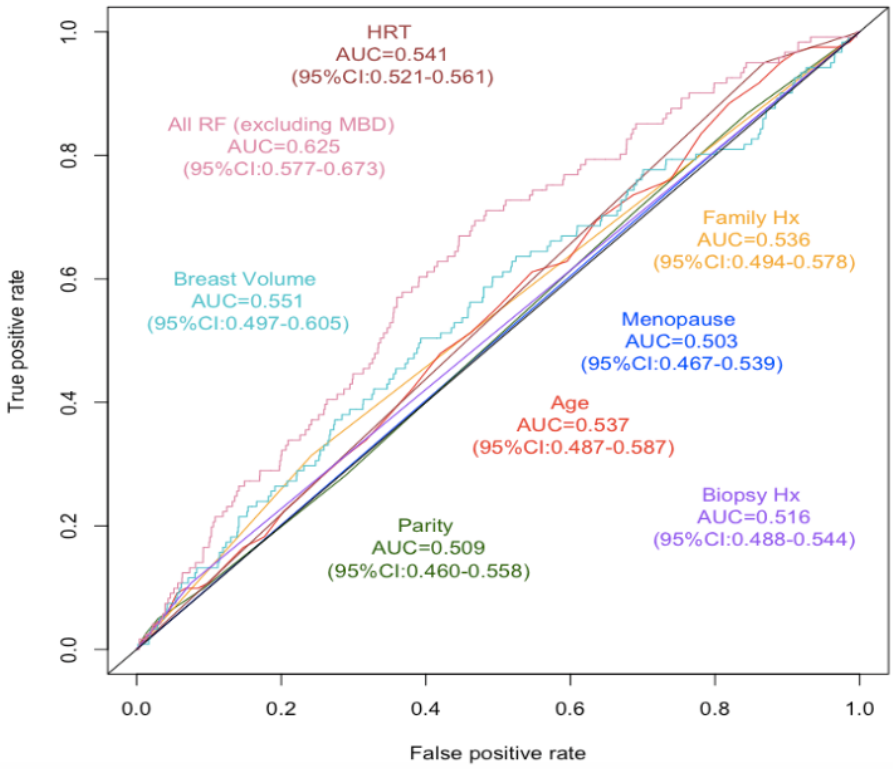

Figure 5.6. Comparison (Receiver Operator Characteristic Curves) of clinical risk factor predictive models for triple negative breast cancer in the Nova Scotia screening population, 2009-2015

5.3.1 Density Alone and with Clinical Risk Factors

The AUROC of each of the 6 models listed in Table 4.1 and utilized for examining odds ratios in objective 2, are reported Table 5.5 and Figure 5.7.

Table 5.5. Comparison (AUROC and 95% CI) of mammographic breast-density predictive models, adjusted for clinical risk factors, for triple negative breast cancer in the Nova Scotia screening population, 2009-2015

	a – MBD only	b – MBD + clinical risk factors
1 - Percent MBD	0.567 (0.517, 0.617)	0.654 (0.609, 0.700)
2 - BI-RADS 5	0.580 (0.535, 0.626)	0.670 (0.624, 0.715)
3 - Percent MBD + BI-RADS 5	0.581 (0.530, 0.632)	0.670 (0.625, 0.715)

Mammographic Breast Density (MBD)

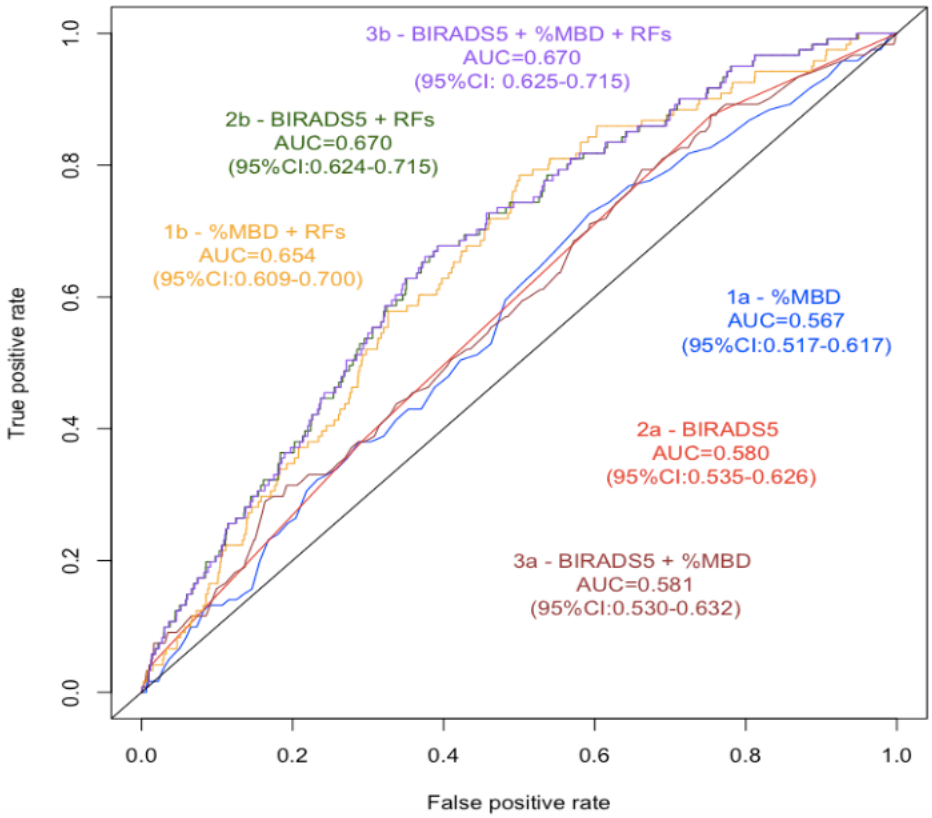

Figure 5.7. Comparison (Receiver Operating Characteristic Curve) of mammographic breast density predictive models, adjusted for clinical risk factors, for triple negative breast cancer in the Nova Scotia screening population, 2009-2015

A comparison of the simple and multivariable risk models with different measures of density revealed that MBD on its own contributed to discriminating between TNBC cases and controls (percent MBD: AUROC 0.567; 95%CI 0.517, 0.617; BI-RADS 5: AUROC 0.580; 95%CI 0.535, 0.626). In all three adjusted models, the discriminatory accuracy was augmented with the addition of clinical risk factors. For example, the AUROC of the model including percent MBD and other risk factors increased from 0.567 to 0.654 (95%CI: 0.609, 0.700). There was little to no difference in the predictive performance of the single MBD models with varying measures of MBD. Compared to MBD alone, the combined model with both the BI-RADS 5th edition density, and percent MBD led to an increased predictive performance. MBD had the highest discriminatory ability for TNBC compared to the rest of the individual risk factors.

5.3.3 The Parsimonious Model

Using the ranked order of predictive performance, the individual covariates were removed from the full model in a backwards stepwise process. Table 5.9 reports the resulting AUROC values and *P*-values highlighting the models with a significantly different area than that of the fully saturated model.

Table 5.6. Comparison (AUROC and 95% CI) of predictive models for triple negative breast cancer, using backwards elimination, in the Nova Scotia screening population, 2009-2015

Model	AUROC for the Differing Density Measures					
	Percent MBD	***p***	**BIRADS5**	***p***	**Percent + BIRADS5**	***p***
m1 (*full model)	0.655 (0.609-0.700)	--	0.670 (0.624-0.715)	--	0.670 (0.625-0.715)	--
m2 (m1 -menopause)	0.647 (0.601-0.694)	0.401	0.662 (0.614-0.709)	0.343	0.662 (0.614-0.709)	0.334
m3 (m2 -parity)	0.645 (0.597-0.692)	0.371	0.659 (0.613-0.706)	0.288	0.659 (0.613-0.706)	0.272
m4 (m3 - biopsy history)	0.641 (0.593-0.688)	0.265	0.657 (0.611-0.703)	0.274	0.657 (0.611-0.703)	0.264
m5 (m4 -family history)	0.636 (0.588-0.683)	0.208	0.652 (0.606-0.699)	0.219	0.652 (0.606-0.699)	0.226
m6 (m5 -age)	0.636 (0.589-0.683)	0.223	0.653 (0.606-0.699)	0.243	0.653 (0.607-0.699)	0.257
m7 (m6 -HRT use)	0.609 (0.561-0.658)	0.032	0.631 (0.582-0.680)	0.047	0.631 (0.582-0.681)	0.046
m8 (m7 - breast volume)	0.567 (0.517, 0.617)	0.002	0.580 (0.535, 0.626)	0.000	0.581 (0.530, 0.632)	0.000

*full model (m1) includes: density, age, parity, menopausal, HRT, family history, breast volume, biopsy history

Figure 5.8 illustrates the varying ROC plots for the investigation into the BI-RADS 5th edition density model.

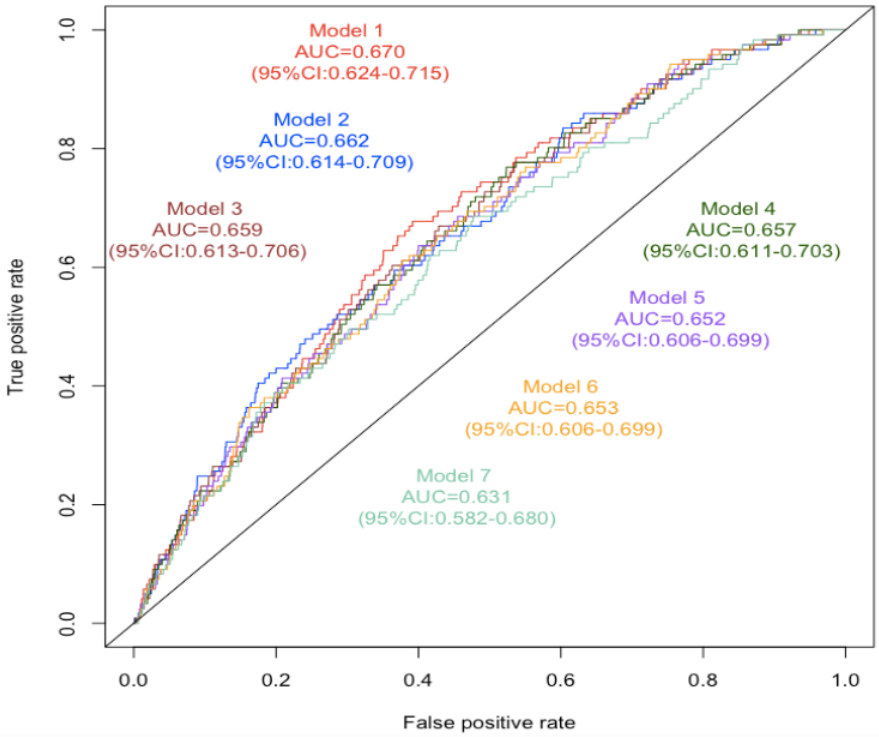

Figure 5.8. Comparison (Receiver Operating Characteristic Curve) of predictive models for negative breast cancer, using backwards elimination, in the Nova Scotia screening population, 2009-2015

When removing the predictor with the lowest discriminatory accuracy in each model, the AUROC decreased from 0.670 (95% 0.624, 0.715) with the BI-RADS 5th edition density fully-adjusted model to 0.580 (95% CI:0.535, 0.626) with the single-factor model (Table 5.9; Figure 5.8).

As predictors were removed from the full model, the difference in the AUROC reached statistical significance with the removal of the 5th predictor, HRT use at the time of screen, across all three adjusted models (Table 5.9). After dropping HRT use from the BI-RADS 5th ed. model, an AUROC of 0.6731; 95%CI:0.582-0.680 was observed, which was significantly different from the AUROC of the full model 0.670 (95% 0.624, 0.715), concluding that the two models were different in their predictive ability (p = 0.047).

CHAPTER 6 DISCUSSION

6.1 THE ASSOCIATION BETWEEN MBD AND RISK OF TNBC IN AN ORGANIZED BREAST SCREENING PROGRAM

There was a positive association observed between MBD and the risk of TNBC in the general screening population of Nova Scotia (Table 5.3). This is consistent with one of the first publications that investigated this relationship.[Ma 2009] Although at a lower magnitude, the authors reported an association observed in their highest density category, ≥60% density (OR 3.01, 95%CI: 1.29, 7.02). This relationship has been demonstrated in other analyses, but most published results have a small number of cases causing inadequate statistical power and wide confidence intervals.[Shin 2017, Razzaghi 2013] The varying definitions of the density measures, including categories based on (1) quartiles of the percent density distribution;[Shin 2017] (2) percent density cut points;[Ma 2009, Holm 2017] and, qualitative BI-RADS 4th edition scoring,[Razzaghi 2013] causes difficulty in synthesizing the results.

The trends observed in the MBD measure in the study population are similar to those described in the literature. Figure 5.3 supports the commonly reported trend that there are lower frequencies in the extremities of the range of MBD. There have been previous reports by the ACR using BI-RADS 4 data from over 3 million analog screening mammograms in the United States between the years of 1996 and 2008, demonstrating that approximately 10% of individuals fall within the lowest density category (I), and approximately 10% fall within the highest density category (IV). The other approximately 80% of individuals distribute relatively evenly between the two middle categories (II and III).[Sickles 2013] The expected relationship between MBD and age was also observed (Figure 5.3). Despite the slight variation in the plot of the TNBC, which may be an artifact of the low sample size of TNBC, these distributions are consistent with the established understanding that as age increases, fibroglandular tissue in the breast decreases as breast tissue content increases in fat.[Boyd 2010, Boyd 2011]

The positive association between MBD and TNBC risk was observed for both forms of MBD measurement in the present study (Table 5.3). Although there were no published studies found

using the BI-RADS 5th edition density categories for a direct comparison, the reported odds ratios were of greater magnitude compared to those of other published 4-category scales.[Ma 2009; Shin 2017; Razzaghi 2013] The observed association between TNBC and MBD density strengthens after adjusting for the other clinical risk factors (Table 5.3). This demonstrate that the true association between MBD and TNBC may have been underestimated due to the effects of the clinical risk factors, as the observed associations were weaker in strength when not controlling for clinical risk factors.

The newest edition of the BI-RADS density scale no longer includes the quantitative percent density ranges defining the categories that were used in the previous edition and now uses the qualitative physical appearance combined with the possibility of missing a cancer. In the present study, the independent association of both qualitative and quantitative measures of MBD were examined. Because the BI-RADS 5th edition density scale and the percent MBD measures were still strongly associated with both in the logistic regression models, only the BI-RADS 5th edition density measure maintained an independent association with TNBC, whereas the percent density measure did not appear to have an independent effect. It may be possible that after removing the effect of quantitative breast area coverage (percent or absolute dense area) from the observed relationship, it may be capturing some of the masking capabilities of the dense tissue. The largest percent change occurs in the BI-RADS 5th edition categories C (30.0%) and D (43.4%) (Table 5.3), which is consistent with the suggestion that when the amount of density is held constant, the observed spike in the strength of the association could be caused by the categories C and D being dependent on the other factors, such as the level of risk of masking (category C), or difficulty in interpreting the mammogram (category D). To our knowledge, this is the first study to combine two measures of density in one TNBC risk model.

The results indicate the risk of TNBC is not just affected by the quantitative amount of MBD but also by the dispersion of the dense tissue and its implications on potentially missing cancers. This was demonstrated in a 2005 study, in which Torres-Mejia et al investigated models incorporating both qualitative Wolf categories and quantitative measures of MBD, area and percent area, measured using a computer-assisted method. The findings revealed that the measures of MBD

were independently associated with breast cancer risk as the risk estimates remained elevated when both forms were in the model [Torres-Mejia 2005].

With the two measures of MBD, one simply measuring the quantitative percentage of dense tissue relative to breast tissue, and the other measuring the qualitative amount of dense tissue and the spatial properties that affect its tumor-masking ability, it is understood that there should be a positive relationship between percent MBD and the increasing level of the BI-RADS 5th edition categories.

In an attempt to disentangle if the association between MBD and the TNBC subtype was different from that with breast cancer as a whole, we examined the literature. In published case-case analyses, and comparisons of case-control analyses, there is little evidence that the association is heterogenous by subtype.[Ma 2009] Published case-case analyses demonstrate inconsistent results for very similar reasons as presented above in the case-control analyses.[Shin 2017]

Menstrual cycles and pregnancies alter hormone exposure and trigger many cycles of growth and involution throughout the lifetime of women affecting breast tissue composition.[Hinck 2014] It has been speculated that hormone-related risk factors could be responsible for the observed association between MBD and breast cancer overall, as hormone and growth factor exposure has the ability to increase quantities of dense tissue, as well as boost proliferative activity.[Razzaghi 2013] Therefore, it may be that MBD is in the direct path of these hormone-related factors, and therefore mediates the association between hormonal risk factors and ER+/ PR+ breast cancer risk. This confounding relationship may impact the association between MBD and TNBC compared to other breast cancer subtypes such as luminal A, due to the fact that TNBC tumors do not express the common hormone receptors that Luminal A and other hormone-receptor positive tumors do. These speculations were inconclusive due to low levels of statistical power and no heterogeneity found in the associations of varying subtypes.[Ma 2009, Razzaghi 2013]

Contradictory to this above noted pathway, the opposite has been hypothesized: there should be a stronger association between MBD and TNBC, as opposed to luminal A and other ER+/PR+ tumors. The rationale behind this statement is related to the terminal duct lobular units

(TDLUs),[Guo 2017, Shin 2017, Razzaghi 2013] within which many of the breast cancer precursors develop.[Razzaghi 2013, Ma 2009] Triple negative (ER-/PR-/HER-2/neu-) and basal-like (ER-/PR-/HER-2/neu- and cytokeratin 5/6+ and/or HER-1+) breast cancers have been found to be more strongly associated with decreased involution of TDLUs in surrounding breast parenchyma, than the other breast cancer subtypes.[Guo 2017] Furthermore, the involution of TDLUs has an inverse association with MBD; in other words, higher MBD is associated with lower involution of TDLUs.[Ghosh 2010] Therefore, it has been hypothesized in previous research that these relationships cause the observed association between MBD and TNBC to be stronger than that of other breast cancer subtypes.[Guo 2017, Razzaghi 2013, Ma 2009]

In our investigations of the three-way relationships between the clinical risk factors, our exposure of interest and our outcome of interest, it was noted that there may be negative confounding effects by two risk factors, menopausal status and breast volume (which is the surrogate measure for BMI). Both have been noted as risk factors for TNBC and associated with MBD. Therefore, it was determined that menopausal status and breast volume should be controlled by this to appropriately interpret the true relationship between TNBC and MBD. This is not the first study to suggest a negative confounding with clinical risk factors, specifically BMI and menopausal status. Shin et al. (2017) concluded that BMI should be considered in any attempts to model this relationship as it was shown to have negative confounding effects. They also demonstrated that BMI effects the risk of TNBC differently depending on menopausal status.[Shin 2017]

Although a statistically insignificant finding, Ma et al. (2009) also examined a possible effect modification exerted by menopausal status.[Ma 2009] MBD has been described as an indicator of non-hormonal and hormonal exposure and impact on breast tissue.[Razzaghi 2013] Additionally, based on the literature, it is clear that menopause is an important event in a women's life that seems to alter her exposure and response to hormones, and other exposures. Menopausal status has been shown to exert effect modification in certain risk factor relationships with MBD, as well as risk of TNBC. For example, hormone therapy has been shown to be associated with MBD in post-menopausal women.[Ma 2009] Furthermore, increased breast density has a higher risk of ER-negative tumors, than ER-positive tumors, specifically in pre-menopausal women.[Shieh 2019]

6.2 THE ABILITY OF MBD TO PREDICT THE RISK OF TNBC OF WOMEN PARTICIPATING IN AN ORGANIZED BREAST SCREENING PROGRAM

Due to the inconsistencies and limited statistical power found in the results of the published literature, another method was used to evaluate the relationship found using logistic regression modelling. MBD was found to be a significant predictor in a predictive model discriminating between TNBC cases and non-cancer controls (Table 5.6). The predictive performance of the model did not vary significantly with the form of breast density measure.

It was hypothesized that there may be added value to MBD to predict TNBC if we combined the two measures of density as separate predictors in one risk prediction model. In other words, it was believed that the risk model may perform better if not just having the percentage (i.e., 26%) but also a description of the physical appearance of the density (i.e., category (B) there are scattered areas of fibroglandular densities; or category (C) the breasts are heterogeneously dense, which may obscure small masses). However, the performance of BI-RADS 5th edition density did not significantly improve with the addition of MBD density measure (with clinical risk factors, *p*-val=0.79). This may be due to the strong positive relationship observed between percent MBD and the increasing BI-RADS categories.

There is currently no literature to which these results can be directly compared, as the only TNBC prediction model is a case-case analysis using SNPs, age and BMI.[Häberle 2017] As many clinical risk factors have distinct relationships with the risk of TNBC compared to other subtypes, and on top of that, they also have varying relationships with MBD, it is expected that MBD would also differentially impact the risk of distinct subtypes. In the Häberle et al. (2017) case-case analysis, age was reported as the strongest predictor of TNBC in the breast cancer population, when compared to BMI and numerous SNPs.[Häberle 2017] This increased discriminatory accuracy was not observed in the general breast screening population of Nova Scotia, when age was compared to other clinical risk factors: HRT use, breast volume, and biopsy history. The TNBC risk prediction model with clinical risk factors (age and BMI) published by Häberle et al. in 2017 reported an AUROC of 0.618. In our study that focused on predicting TNBC in the general screening

population, a model with similar predictors, age and breast volume, excluding density, produced an AUROC of 0.573.

In the present study, the combination of clinical risk factors (age, menopause, HRT, family history, breast volume, biopsy history) had a better discriminatory ability (AUROC 0.625; 95% CI:0.577, 0.673) than percent MBD (AUROC 0.567; 95%CI: 0.517, 0.617). The discriminatory accuracy, as measured by the AUROC was augmented with the addition of clinical risk factors. This was not the case in early research on overall breast cancer, showing that density contributes equal or more value to predicting breast cancer than the clinical risk factors alone. As in the Brentnall et al. (2015) study previously discussed, the investigators reported an increase in the AUROC from 0.55 to 0.59 of the Gail model, and from 0.57 to 0.61 in Tyrer-Cuzick v6 model, with the addition of percent density to clinical risk factors.[Brentnall 2015] This was also demonstrated in the average risk screening population in Nova Scotia; the addition of MBD to a breast cancer risk model with clinical risk factors increased the AUROC from 0.53 to 0.63.[Abdolell 2016] It is well established that MBD contributes significantly to breast cancer risk prediction models, with traditional clinically-validated models reaching an AUROC of approximately 0.6. Just recently, a group from MIT published a paper about their novel breast cancer risk prediction model that surpassed any cited AUROCs in the breast cancer literature, achieving an AUROC of 0.82 (95%CI: 0.80, 0.85) using a deep learning approach.[Yala 2019] The highest AUROC in this study of the TNBC population was the model containing all risk factors, reaching an AUROC of 0.670 (95%CI: 0.625, 0.715), which is higher than the Gail and TCv6 models (AUROC, 0.59 and 0.61 respectively) that predict risk of all breast cancer subtypes combined.

Comparing with the only risk model evaluated in the TNBC population (Tyrer-Cuzick v8), which included many clinical and family history variables as covariates, including MBD, the discriminatory ability found in this study was higher than that of the Tyrer-Cuzick v8 (AUROC 0.52; 95%CI: 0.45 to 0.61). This is expected, as this model was built on the TNBC population, and not only evaluated on the TNBC population.

6.3 STUDY STRENGTHS

With the accelerated shift from film to digital mammography in clinical use, and the shift from quantitative percent ranges to the qualitative descriptions, there are implications on appearance of MBD. A strength of this study is that it builds on the limited digital mammography evidence by only including full-field digital mammograms.

A further advantage to this study is that in the province of Nova Scotia, mammography machines and procedures are standardized, and therefore mammographic images are taken with similar quality. The outcome of TNBC was defined as in standard clinical practice and the procedures for determining receptors using IHC is also standardized throughout the province. The clinical risk factors are all routinely collected, including density, again in the exact same manner throughout Nova Scotia. Although BMI was not available from the clinical record, image-derived total breast volume, which was measured in a standard way, was considered as a surrogate in the risk models. There was no apparent systematic error introduced in this study that could cause an under or over-estimation of the observed relationships.

The collection of the exposure variable using a fully-automated software that uses "for-presentation" views of the mammogram, allows for (1) a reliable and manageable data collection process, and (2) using "for-presentation" views more closely resembles the density as the radiologists view it, and thus may more closely mimics the relationship observed in clinical practice. Examining relationships in non-controlled pragmatic setting is the way to achieve the closest possible truth.

6.4 STUDY LIMITATIONS

With the retrospective and observational design of this research, the analyses are limited to previously collected risk factor data. It may be possible that there are influential variables not routinely collected in clinical practice. In attempt to weaken the impact of this limitation, we are using a surrogate measure for a potential key variable, BMI. Without the ability to test varying risk factors, it is unknown if this is a limitation of this work.

To draw conclusions on causality, the exposure must precede the outcome. The available risk factors in this data set were collected at the time of the diagnosis information (the screening exam). For example, HRT use at the time of screen, it is unknown if the HRT use is antecedent to the onset of the breast cancer. This limitation affects the main exposure variable, as breast density is collected at the same time as the screening mammogram. This could affect missed answers, and cancers that were diagnosed via screening pathways. Although, the conduction of this study utilized self-reported data for the women's clinical history, the exposure of interest is not self-reported, and there is no rationale to support that the degree of any biases are dependent on the exposure status.

This study population was restricted to women with a digital screening mammogram, which may have impacted the observed results, in the association and predictive relationship. It is possible that a large portion of the TNBC population may have been missed as cases were restricted to those over age 40 and who also had a mammogram as TNBC cases specifically are younger in age [Boyle 2012] and MBD is typically increased at a younger age, this may be underestimating the odds ratios and the AUROC.

Lastly, these models were not validated internally or externally during this study, nor calibrated. It cannot be confirmed if the results persist in subpopulations of Nova Scotia, or if the results are generalizable to other populations. There was no opportunity to perform any external validation nor cross validation due to the small number of events.

CHAPTER 7 CONCLUSION

7.1 IMPLICATIONS FOR PUBLIC HEALTH AND POLICY MAKERS

Due to the large burden on individual, as well as population health, breast cancer is an important public health concern. Public health aims to increase awareness of potential risk factors, and promote strategies for prevention and early detection, such as breast cancer screening. Due to the later stage and higher grade of TNBC at diagnosis, targeting an effective screening strategy for earlier diagnosis and treatment could make a large impact on prognosis for this subtype.

Health care delivery is shifting toward personalized medicine, including screening protocols. Women at high risk of TNBC could be screened more frequently or using a different modality than mammography, as it is known that their cancer stage and tumor grade progress more quickly than other subtypes. Elucidating the relationship between TNBC and tissue properties such as breast density is the first step in determining how to translate that relationship into a positive clinical impact that could include varying screening protocols to improve early detection.

Screening programs may benefit from leveraging the relationship between MBD and TNBC. Using non-breast cancer cases from the general screening population for the control group in the TNBC risk prediction models has clinical value in screening, as the rate of interval cancer is a quality indicator in a screening program, and TNBC are one of the most difficult breast cancer subtypes as they are more commonly detected as interval cancers.

Using few clinical risk factors, the models were able to predict the risk of TNBC within the screening population with a similar discriminatory ability as most other published risk models. As breast volume and MBD is automatically generated from the mammography images in Nova Scotia, there are only two questions, any breast biopsy history and current HRT use, that would have to be asked to the patient, or extracted from electronic medical records, to complete a personalized risk estimate for the patient. As all of the clinical and image variables used in the prediction model are either collected routinely by the province-wide screening program, or directly fed to the data capture system in Nova Scotia called the Breast Imaging System, situates Nova

Scotia in a position to become one of the leaders in the early detection of poor prognosis tumors, such as TNBC. This research helps affirm the potential of building a validated and feasible TNBC risk prediction model that could facilitate screening guideline alterations (i.e., shorter screening intervals or alternative modalities) for high risk patients.

This study did not only further clarify the relationship between TNBC and a potentially clinically relevant risk factor, but we were able to see the potential value behind breast density and other risk factors in predicting TNBC cases in the screening population.

7.2 IMPLICATIONS FOR FUTURE RESEARCH

With inconsistencies in published results, possibly due to the small study sizes, this subject would call for a meta-analysis for a comprehensive interpretation of the published data, but this is not feasible given the proportion of non-comparable studies. A larger sample of TNBC may provide further insight into the true connection between MBD and the risk of TNBC.

Clinical impact can only occur once there is a consensus formed on how MBD relates to TNBC in context of other subtypes and sub-populations (i.e., pre-menopausal women). Prior to affecting any routine clinical decision making (i.e., implementing a risk model to triage women at high risk of TNBC for more frequent screening exams), extensive research is required to validate, calibrate and translate these preliminary findings into models adopted in clinical settings.

Shorter term research includes investigating how these results differ by breast cancer subtype; identifying additional predictors that may improve the TNBC risk model performance; and lastly, further investigate how breast density could be utilized in a manner that impacts the screening outcomes for TNBC population.

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