

BRCA detection rate in an Italian cohort of luminal early-onset and triple-negative breast cancer patients without family history: when biology overcomes genealogy.

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Abstract

BACKGROUND: NCCN Guidelines recommend BRCA genetic testing in individuals with a probability >5% of being a carrier. This analysis aimed to evaluate the rate of BRCA mutations in triple-negative breast cancer (TNBC) patients diagnosed ≤60 years and luminal-like breast cancer (BC) patients diagnosed ≤35 without breast and/or ovarian family history. METHODS: 159 TNBC patients diagnosed ≤60 years and 109 luminal-like BC patients diagnosed ≤35 years without family history were retrospectively identified. Mutation prevalence and clinical-pathological characteristics associated with mutational status were evaluated. RESULTS: In TNBC patients, BRCA mutation prevalence was 22.6% (21.4% BRCA1 and 1.2% BRCA2). BRCA1-related TNBC patients were younger ($p < 0.001$). Mutation prevalence was 64.2% ≤30 years, 31.8% in patients aged 31-40, 16.1% for those aged 41-50 and 7.9% for those between 51 and 60 years of age. A total of 40% of patients with estrogen receptor (ER) 1-9% were BRCA1 carriers. BRCA detection rate in early-onset BCs was 6.4% (1.8% BRCA1 and 4.6% BRCA2). Mutation prevalence was 0% 0-25 years, 9% 26-30 years and 6% 31-35 years. BRCA2-positive luminal-like early-onset BCs were more likely associated with low progesterone receptor (PR) expression ($p = 0.049$). CONCLUSIONS: BRCA genetic testing is recommended in TNBC diagnosed ≤ 60 years, regardless of cancer family history, histotype and by using immunohistochemical staining <10% of nuclei for both ER and PR as a cut-off. In luminal-like early-onset BC patients, a lower BRCA detection rate was observed, suggesting a role for other predisposing genes.

Introduction

First- and second-degree relatives of breast cancer (BC) patients present an increased risk for this malignancy. In particular, these individuals may have an increased susceptibility to cancer as a result of gene mutations present in parental germ-line cells. Tumors developing in these families are classified as hereditary cancers, and the most frequently involved predisposition genes include BRCA1 and BRCA2. Overall, BRCA1/2 positive families present an increased incidence of breast, ovarian, prostate and pancreatic cancers.¹⁻⁴ Assessment of an individual's risk for hereditary tumors is therefore based on a thorough evaluation of personal and family cancer history.

The identification of a mutation in BRCA genes plays a crucial role in the management of hereditary cancer prevention, diagnosis and treatment.^{2,5-10} Nonetheless, because of the high costs associated with genetic analyses, especially in those countries where BRCA testing is offered by the National Health Service, BRCA testing has been restricted to BC patients having an a priori high risk of being carriers or candidates to approved targeted treatment strategies (i.e. PARP inhibitors¹¹). In particular, according to the National Institute for Health and Care Excellence (NICE) in the UK, BRCA testing should be offered to BC patients with a probability of mutation ≥ 10%.¹² On the other hand, according to the recent update of NCCN Guidelines,¹³ BRCA genetic testing is clinically recommended in individuals with a probability > 5% based on prior probability models (e.g. Tyrer-Cuzik, BRCAPro etc).

Several International Oncology Associations, such as ESMO, ASCO, NCCN, etc., provide guidelines for BRCA testing based on clinical-pathological characteristics of tumors and cancer family history. On these grounds, in individuals potentially meeting established criteria for hereditary cancer syndrome, genetic testing is performed beginning from an appropriate examination of family history. Indeed, effective pre-test counseling includes the development of an expanded pedigree that collects the health status of individuals diagnosed with cancer and first-, second- and third-degree relatives on both maternal and paternal sides. Nevertheless, several factors may limit the informativeness of the pedigree, such as small family size, a small number of individuals from the susceptible gender for sex-limited cancers, reduced penetrance, early deaths in family members, prophylactic surgeries that remove an organ due to subsequent cancer risk, adoptions, and inaccurate or incomplete information on family members.^{14,15} Consequently, other factors should be considered during genetic counseling, including biology and age at diagnosis of the tumors developed by the counseled patient. In particular, the Italian Association of Medical Oncology (AIOM) guidelines include personal history of triple-negative BC (TNBC) diagnosed \leq 60 years and personal history of early onset breast cancer (EOBC) diagnosed \leq 35 years, regardless of family history,¹⁶ among the BRCA testing criteria.

With the aim to evaluate the weight of clinical-pathological characteristics compared to tumor family history, we evaluated the prevalence of BRCA germ-line mutations in an Italian cohort of TNBC and luminal-like EOBC patients without breast/ovarian cancer family history.

Materials And Methods

Study Population and design

Since 1995, the Modena Family Cancer Clinic (MFCC) has taken charge of women with a family history of BC and/or ovarian cancer (OC). According to the Modena Criteria^{17,18} and, more recently, using the Tyrer-Cuzick model,¹⁹ women are classified in risk categories and are included in personalized surveillance programs. Moreover, individuals who meet the AIOM Criteria for genetic testing can undergo the BRCA test. According to the result, they may access risk-reducing surgeries,²⁰ chemoprevention studies⁹ or more intensive surveillance programs.^{6,7} During pre-test counseling, family and personal history of cancer is collected and a family pedigree is drawn including third-degree relatives on both maternal and paternal sides. Finally, after post-test counseling, a copy of all patient documents and reports are stored in the MFCC archive.

For the purpose of our study, we retrospectively identified pedigrees for patients with TNBC diagnosed \leq 60 years and luminal-like EOBC diagnosed \leq 35 with neither breast nor ovarian family history. The MFCC began to test all EOBC patients in 1998, whereas TNBC patients younger than 40 years have been tested since 2006. Only in 2016 has the indication to genetic testing been extended to 60 years at TNBC diagnosis.

ER, PR and HER2 expression was determined according to the national pathology guidelines, which closely adhere to international standards. For the purpose of our study, triple negativity was defined as immunohistochemical staining of less than 10% of nuclei for both ER and PR, and an immunohistochemical result (DAKO score) of 0 or 1+ for HER2/neu. This means that in our analyses ER low positive tumors were included in the group of triple negative tumors. With regard to the luminal-like group, on the other hand, we included tumors with immunohistochemical staining of at least 10% of nuclei for ER. The systematic evaluation of HER2 status became available at our Institution only in 2006, when Trastuzumab was approved in the adjuvant setting.

BRCA testing procedures

Before 2014, the genetic testing of BRCA1 and BRCA2 genes at our institution was carried out by direct Sanger sequencing, whilst it was performed using Next Generation Sequencing (NGS) after 2014. With both methods, the molecular test was performed on genomic DNA, isolated from fresh peripheral blood samples and encompassing the entire coding region as well as adjacent intronic splice-site consensus sequences of BRCA genes. The NGS workflow benefited from the use of the Ion AmpliSeq™ technology, which was initially handled with a semi-automated procedure, and subsequently with a fully automated procedure for multiplex PCR-based library preparation and sequencing on the Ion Torrent platforms (Thermo Scientific). Sanger sequencing was routinely performed to validate candidate mutations, as long as Multiplex Ligation Probe Amplification (MLPA, MRC-Holland) was carried out to detect copy number variations. Sequences alignment, base calling, variant filtering and annotation process relied on the Torrent Software Suite (Thermo Scientific) and a custom designed bioinformatic pipeline, as described elsewhere.^{21,22} Mutations were classified according to the International Agency for Research on Cancer (IARC) system and considered pathogenic or likely pathogenic (class 4 or 5) based on literature evidence, multifactorial likelihood and functional analyses from the ENIGMA consortium that comprise genetic data of the GC-HBOC database.^{23,24} For the purpose of this study, variants of unknown significance (class 3) were considered as clinically negative results.

Statistical analysis

Mutation prevalence and clinical-pathological characteristics were evaluated for each sub-group of patients. Patient characteristics and the distribution of each parameter across sub-groups were reported as absolute and percentage frequencies. A comparison between groups was made by means of Fischer's exact test. Statistical analyses were conducted using IBM SPSS Statistics for Windows Version 23.0 (IBM Corporation, Armonk, NY, USA). P-values < 0.05 were considered significant.

Results

Triple-negative Breast Cancer

Overall, 159 TNBC patients diagnosed ≤ 60 years were identified in our archives (Table 1). The prevalence of germ-line BRCA mutation was 36/159 (22.6%). Thirty-four patients presented a BRCA1 mutation

(21.4%), whereas 2 patients were BRCA2 carriers (1.2%). BRCA1-positive TNBC patients were diagnosed at a younger age (37 years) than non-carriers (44 years) or BRCA2 carriers (45 years) ($p < 0.001$). Mutation prevalence in TNBC patients was 9/14 (64.2%) in the age group ≤ 30 years, 14/44 (31.8%) in 31–40 years, 10/62 (16.1%) in 41–50 years, 3/38 (7.9%) in 51–60 years (Fig. 1). As expected, most of TNBCs present a high proliferation rate and ductal histotype. Only one invasive lobular carcinoma was recorded and was categorized as BRCA1-associated. Moreover, 3 metaplastic carcinomas, 2 medullary, 1 sarcomatoid and 1 papillary tumor were diagnosed, of which one medullary and one papillary were BRCA1 associated. Ten out of 159 patients presented ER and/or PR between 1% and 9%, and 4 of these (40%) were BRCA1 carriers. No significant differences were observed between carriers and non-carriers in clinical and pathological characteristics such as ki-67 ($p = 0.462$), the presence of bilateral or second primary BC ($p = 0.088$), histotype ($p = 0.301$) and hormone receptor expression ($p = 0.226$).

Early-Onset Luminal-like Breast Cancer

A total of 109 luminal-like EOBC patients with no BC/OC family history undergoing BRCA-genetic testing were identified in our archives (Table 2). BRCA detection rate among EOBCs was 7/109 (6.4%). Two patients presented a BRCA1 mutation (1.8%), whereas 5 patients were BRCA2 carriers (4.6%). The mutation prevalence was 0/5 (0%) in the age group 0–25 years, 2/22 (9%) between 26 and 30 years, and 5/82 (6%) in the age group 31–35 years (Fig. 1). Most patients were diagnosed with an invasive ductal carcinoma (in particular, 91.3% of the BRCA negative patients and all BRCA positive patients). One tubular, one papillary and one mucinous carcinoma were also diagnosed, all of them BRCA mutation negative. BRCA2-positive luminal-like EOBCs were more likely associated to low PR expression ($p = 0.049$). On the other hand, no significant difference in age at diagnosis ($p = 0.353$), ki-67 ($p = 0.712$), presence of bilateral or second primary BC ($p = 0.117$), histotype and HER2 expression ($p = 0.112$) was observed.

Discussion

According to the recommendations of all International Oncology Associations, BRCA genetic testing criteria are based on clinical-pathological characteristics of personal tumor and cancer family history. Pre-test counseling is performed beginning from a thorough examination of family history. It is common thought that hereditary cancers should develop in the context of a family seriously affected by the same disease. Nevertheless, several factors may limit the informativeness of the pedigree, such as inaccurate or incomplete information on family members, while our patient's cancer could be the first in the family. Indeed, a prospective study of 306 women diagnosed with breast cancer at < 50 years of age, with no first- or second-degree relatives with breast or ovarian cancer, showed that those individuals with limited family history may have an underestimated probability of BRCA mutation, based on models relying exclusively on family history.²⁵

Previous research helped define the clinical-pathological characteristics of BRCA-related tumors. In detail, BRCA1-associated tumors are poorly differentiated infiltrating carcinomas, more frequently ER and PR-

negative and p53-positive.^{26,27} On the other hand, BRCA2-associated BC tends to be of higher grade than sporadic age-matched controls.²⁸ Overall, BRCA-associated BCs are diagnosed at a young age and show a low frequency of HER2 expression.^{27,29} On these grounds, International Guidelines included the presence of young age at diagnosis and TN profile, regardless of family history, in the BRCA genetic testing criteria. Nonetheless, the cost-effectiveness of testing individuals with no tumor family history is still debated, especially in those countries where BRCA testing is offered by the National Health Service. Our study aimed to evaluate the rate of BRCA mutations in patients selected according to the biology and age of diagnosis of their tumors, in the absence of cancer family history.

Our analyses highlighted a mutation rate greater than 10% in the overall population of TNBC diagnosed \leq 60 years (22.6%). In detail, BRCA testing was deemed cost-effective according to the NICE Guidelines up to 50 years, while in the subgroup of patients diagnosed between 51 and 60 years, detection rate was 7.9%, still cost-effective according to the last NCCN Guidelines. These results show differences from the previous literature. According to the literature, 15–30% of unselected TNBCs had confirmed BRCA mutations.^{30–32} Conversely, mutation prevalence decreased to 6–15% in patients without breast/ovarian cancer family history.^{31,33} This discrepancy could be explained by the fact that 37.1% of our patients were diagnosed \leq 40 and 76.1% \leq 50. Young age at diagnosis could therefore have increased the rate of BRCA detection compared to previous publications. According to the literature, the BRCA1 patients in our study were diagnosed at a younger age than non-carriers. On the other hand, the high rate of young TNBC patients in our population could be explained by the fact that TNBC is more common in young patients. Until 2016, moreover, we only tested patients diagnosed \leq 40 years.

Interestingly, despite the cut-off for hormone-receptor negativity defined by the ASCO-CAP Guidelines,³⁴ 40% of the patients with ER between 1 and 9% (ER low positive) were observed to be BRCA1-positive at genetic testing. Previous analyses have already highlighted that tumors with ER < 10% clinically behave as ER < 1% tumors.³⁵ Along with the results presented in this paper, these data indicate that for clinical purposes, tumors with ER < 10% and HER2/neu 0 or 1+ should be considered as TNBC. Furthermore, as reported in other analyses,²⁷ 3 out of 34 (8.8%) BRCA1-related tumors presented a more rare non-ductal histotype (in our study one medullary, one papillary and one lobular). Finally, no differences were observed between carriers and non-carriers in proliferation rate and the presence of bilateral tumors or second primary BC. Since 2013, patients at our Family Cancer Clinic have been offered rapid genetic counseling and testing at BC diagnosis. This strategy was demonstrated to improve the rate of risk-reducing bilateral mastectomy at the time of BC surgery,²⁰ enabling us to reduce the risk of contralateral tumor in BRCA carriers.

In the second part of our analysis, we evaluated 109 patients diagnosed with luminal-like BC at \leq 35 years. To our knowledge, this is the first study to evaluate the rate of BRCA mutation in luminal-like EOBC patients with no family history. This analysis is even more valuable in light of the EMBRACA trial results, in which talazoparib provided a significant PFS improvement over standard chemotherapy in patients with BRCA-related luminal-like advanced breast cancer.³⁶ Overall and in each age subgroup (\leq

25, 26–30 and 31–35), BRCA detection rate was less than 10% (6.4%, 0%, 9% and 6%, respectively). Interestingly, all the patients diagnosed with EOBC under 26 years were observed to be negative at genetic testing, possibly underlining the need to evaluate other predisposing factors. No difference in proliferation rate was observed between carriers and non-carriers, contrary to what is reported in the literature.²⁸ On the other hand, we found that BRCA2-positive luminal-like EOBCs were more likely associated to low PR expression, even if this type of evidence was only marginally significant due to the small sample available. Finally, as expected, most of hereditary luminal-like EOBCs were BRCA2-associated and HER2/neu negative, and all of them were accounted for as ductal carcinoma.

In conclusion, according to the last NCCN Guidelines, these results confirm the recommendation to test for BRCA genes TNBC ≤ 60 years, regardless of tumor histotype and by using immunohistochemical staining of less than 10% of nuclei for both ER and/PR as a cut-off. In luminal-like EOBC patients with no family history, on the other hand, a lower BRCA detection rate was observed yet overall > 5%, suggesting a role for other predisposing genes in this subset of patients.

Table 1
Characteristics of triple-negative breast cancer patients.

TNBC	Negative	BRCA1	BRCA2	p-value	
Number of patients (159)	123	34	2	*	**
Mean age at diagnosis (y)	44,78 (24–59) SD 9,07	36,97 (26–54) SD 8,74	45,00 (39–51) SD 8,48	0.004	< 0.001
Age group (y)				< 0.001	< 0.001
<= 30	5 (4,0%) 31 (25,2%)	9 (26,5%) 13 (38,3%)	0 1 (50%)		
31–40	52 (42,3%)	10 (29,4%)	0		
41–50	35 (28,5%)	2 (5,9%)	1 (50%)		
51–60					
Ki 67 (%)	11 (10,1%) <=20 >20 unknown	1 (3,3%) 29 (96,7%) 4	0 1 (100%) 1	0.508	0.462
Bilaterality					
Yes	5 (4,2%) 114 (95,7%)	4 (12,9%) 27 (87,1%)	0 2 (100%)	0.193	0.088
No	4	3	0		
unknown					
Histotype					
ductal	103 (95,4%) 0	28 (90,3%) 1 (3,2%)	2 (100%) 0	0.466	0.301
lobular	5 (4,6%)	2 (6,5%)	0		
others	15	3	0		
unknown					
RO					
negative	116 (95,1%) 6 (4,9%)	30 (88,2%) 4 (11,8%)	2 (100%) 0	0.321	0.226
1–9%	1	0	0		
unknown					

* Comparison Negative vs BRCA1 vs BRCA2

** Comparison Negative vs BRCA1

Table 2
Characteristics of luminal-like early onset breast cancer patients.

EOBC	Negative	BRCA1	BRCA2	p-value	
Number of patients (109)	102	2	5	*	**
Mean age at diagnosis (y)	31.95 (23–35) SD 2.85	34.0 (33–35) SD 2.93	30.6 (27–33) SD 2.89	0.488	0.353
Age group (y)	5 (4.9%) <= 25 20 (19.6%) 26–30 77 (75.5%) 31–35	0 0 2 (100%)	0 2 (40%) 3 (60%)	0.688	1.00
Ki 67 (%)	35 (44.9%) <=20 43 (55.1%) > 20 24 unknown	0 1 (100%) 1	2 (50%) 2 (50%) 1	0.920	0.712
Bilaterality	5 (5.3%) Yes 90 (94.7%) No 7 unknown	1 (50%) 1 (50%) 0	0 5 (100%) 0	0.249	0.117
Histotype	74 (91.3%) ductal 3 (3.7%) lobular 4 (4.9%) others 21 unknown	2 (100%) 0 0 0 0	4 (100%) 0 0 0 1	1.00	1.00
PR	29 (33.7%) <=20 57 (66.3%) > 20 23 unknown	0 0 2	2 (40%) 3 (60%) 0	0.105	0.049
HER2	54 (69.2%) negative 24 (30.8%) positive 24 unknown	0 0 2	5 (100%) 0 0	0.052	0.112

* Comparison Negative vs BRCA1 vs BRCA2

** Comparison Negative vs BRCA1

Abbreviations

TNBC: triple-negative breast cancer; BC: breast cancer; ER: estrogen receptor; PR: progesterone receptor; NICE: National Institute for Health and Care Excellence; AIOM: Italian Association of Medical Oncology;

EOBC: early onset breast cancer; MFCC: Modena Family Cancer Clinic; OC: ovarian cancer; NGS: Next Generation Sequencing.

Declarations

Ethics approval and consent to participate

Ethics approval was obtained from the Ethical Committee of Modena (#3387). All participants signed informed consent forms.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AT, EM and CP designed the study; EM, MV and IM identified patients; SP, EB and GG acquired written informed consent from the patients; LM, FD and ET (Tenedini) contributed to the interpretation and analysis of the data; AT and EM wrote the article; ET (Tagliafico), GT and LC revised the manuscript and made the final approval. All authors have read and approved the final version for publication.

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Figures

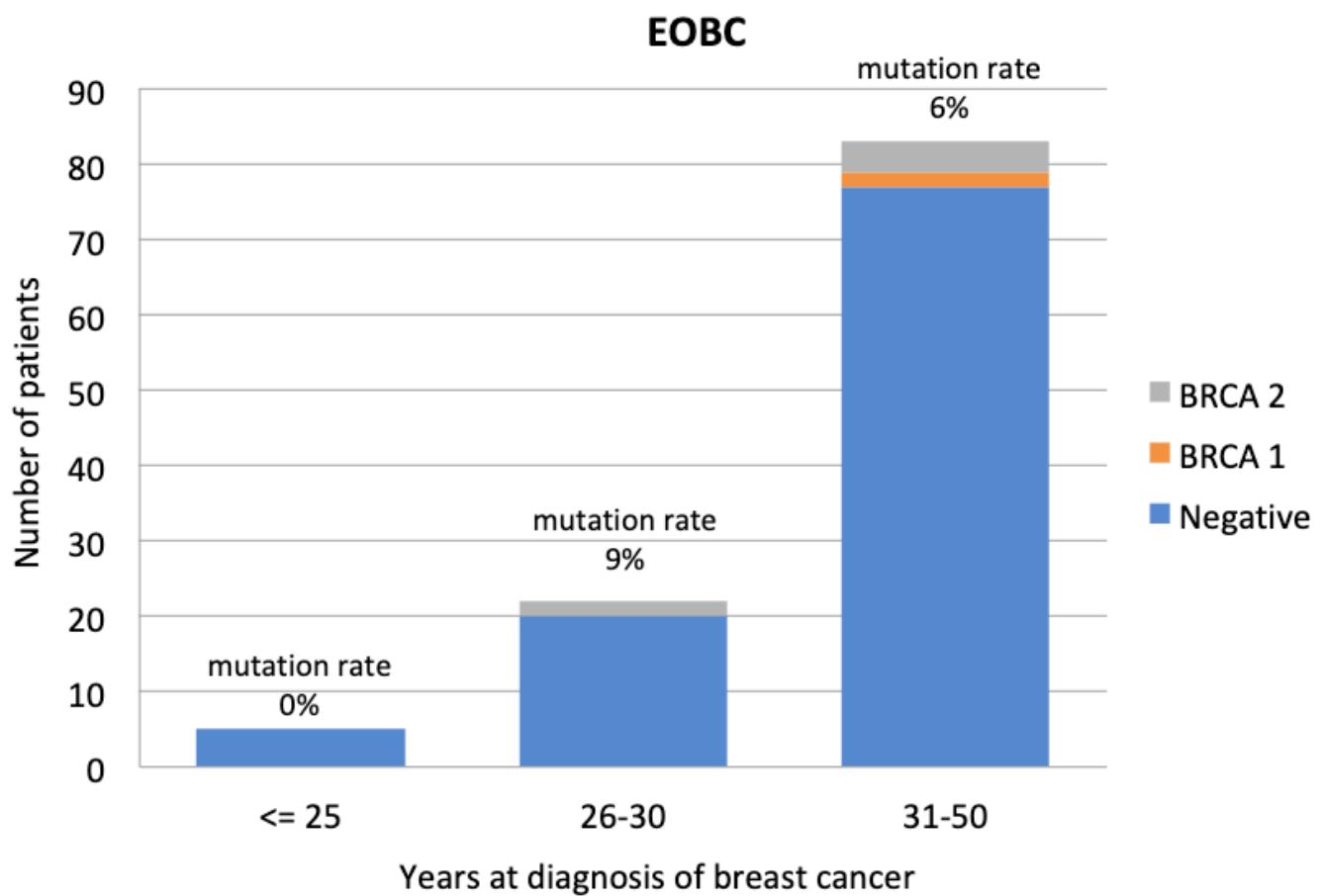


Figure 1

BRCA detection rate in triple-negative breast cancer patients diagnosed ≤ 60 years.

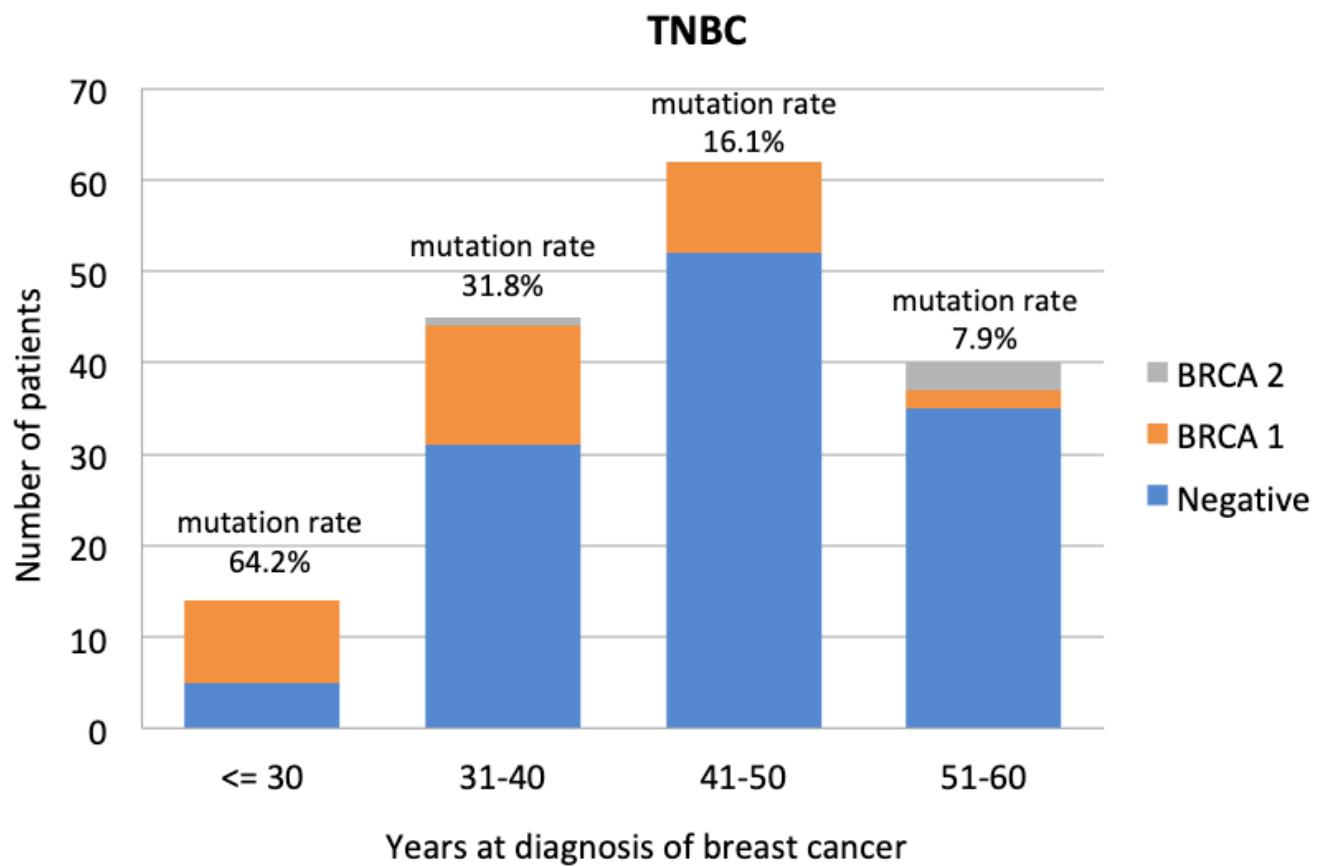


Figure 2

BRCA detection rate in luminal-like early-onset breast cancer patients (diagnosed ≤ 35 years).