Journal of Oncology

BRCA Mutations In Cancer: Implications For Tumor Biology, Surveillance And Treatment

Lead Guest Editor: Angela Toss
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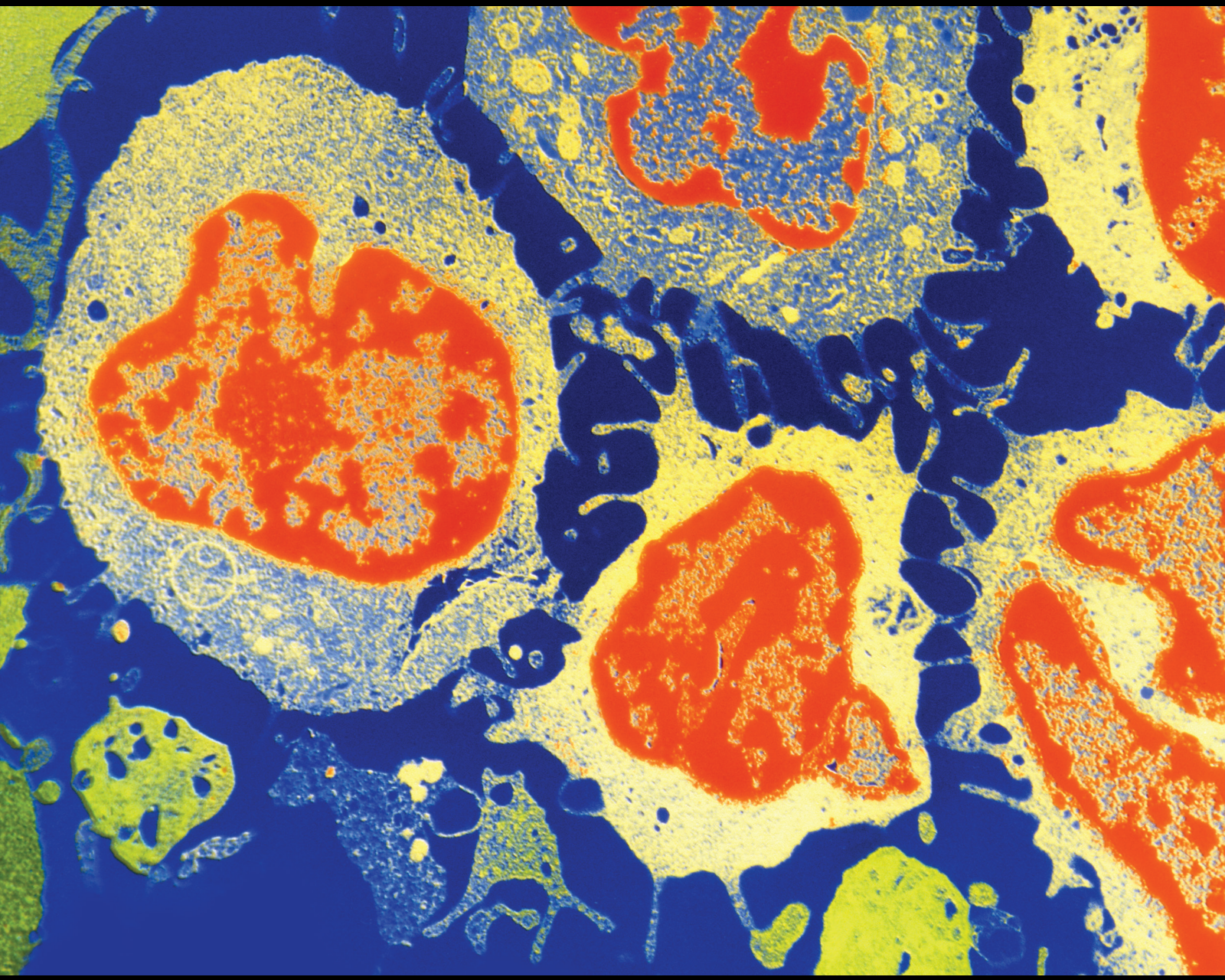

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Corrigendum

Corrigendum to "Mucinous Histology, *BRCA1/2* Mutations, and Elevated Tumor Mutational Burden in Colorectal Cancer"

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In the article titled "Mucinous Histology, BRCA1/2 Mutations, and Elevated Tumor Mutational Burden in Colorectal Cancer" [1], some information was omitted in the Acknowledgments section, which should be corrected as follows:

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Review Article

BRCA Mutations in Prostate Cancer: Prognostic and Predictive Implications

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Despite chemotherapy and novel androgen-receptor signalling inhibitors (ARSi) have been approved during the last decades, metastatic castration-resistant prostate cancer (mCRPC) remains a lethal disease with poor clinical outcomes. Several studies found that germline or acquired DNA damage repair (DDR) defects affect a high percentage of mCRPC patients. Among DDR defects, BRCA mutations show relevant clinical implications. BRCA mutations are associated with adverse clinical features in primary tumors and with poor outcomes in patients with mCRPC. In addition, BRCA mutations predict good response to poly-ADP ribose polymerase (PARP) inhibitors, such as olaparib, rucaparib, and niraparib. However, concerns still remain on the role of extensive mutational testing in prostate cancer patients, given the implications for patients and for their progeny. The present comprehensive review attempts to provide an overview of BRCA mutations in prostate cancer, focusing on their prognostic and predictive roles.

1. Introduction

Prostate cancer (PCa) is the second most common neoplasm in men worldwide and the second leading cause of cancer deaths in Western countries [1]. In the USA, 165,000 new cases and 29,000 deaths are estimated annually due to PCa [2]. Despite a median overall survival (OS) of 42.1 months, the failure-free survival (FFS) was only 11.2 months in patients with metastatic hormone-sensitive PCa enrolled in the control arm of the STAMPEDE trial [3]. Moreover, PCa patients live most of their natural history of disease in the castration-resistant setting, and in the last decade, the approval of six novel treatments for the management of metastatic castration-resistant prostate cancers (mCRPC), spanning from chemotherapy agents (docetaxel and cabazitaxel), androgen-receptor signalling inhibitors (ARSi, i.e., enzalutamide and abiraterone), vaccines (sipuleucel-T), and bone-seeking radiopharmaceuticals (radium-223), has dramatically changed the management of mCRPC [4]. Despite meaningful advances in PCa care, the clinical outcome of mCRPC patients is still poor, and the median OS is unsatisfactory, ranging approximately between 18 and 36 months [4]. A better understanding of the molecular characterization of mCRPC patients is an urgent medical need in order to better define diagnosis and prognosis and to deliver appropriate treatment. PCa is one of the most heritable human tumors [5]; the integrative analysis of advanced prostate cancer has revealed that approximately 90% of mCRPC patients harbor clinically actionable germline and somatic alterations [6]. In this scenario, DNA damage repair defects (DDR) represent 25% of these alterations, with BRCA2 mutations representing the most

frequent event [6–8]. Inherited mutations in BRCA genes are associated with an increased risk of developing breast, ovarian, prostate, and other cancers [7, 8]. DDR genes are involved in the mechanisms of genomic stability, repairing DNA aberrations during the cell cycle, ensuring a correct mitotic cell division, and distribution of the genomic material to the daughter cells [9]. In order to solve threats generated by DNA damage, cells have developed several processes of DNA-damage response that detect DNA lesions, signal their presence, and promote the repair [10]. If the extent of DNA damage is beyond repair capacity, alternative signalling pathways lead to apoptosis of potentially dangerous mutated cells [11]. Several DNA repair pathways are involved to cope with different DNA lesions, and they usually occur by a common general program [12]. BRCA1/2 is a protein encoded by the major oncogene responsible for the susceptibility of breast and ovarian cancers and plays a key role in the system of the homologous recombination (HR), working simultaneously with several enzymes to protect the genome from double DNA strand breaks [13]. BRCA2 mutations are a strong negative prognostic factor associated with short metastasis-free survival (MFS) and cancer-specific survival (CSS) in patients with mCRPC [14]. Moreover, BRCA mutations can predict response to poly-ADP ribose polymerase (PARP) inhibitors and to platinum salts [15, 16]. The following attempts to provide a comprehensive review of the literature on BRCA mutations in patients suffering from PCa, highlighting their prevalence and prognostic and predictive role, as well as their implications for hereditary cancer and genetic counselling.

1.1. Prevalence of BRCA Mutations in Prostate Cancers. The incidence of germline mutation in DDR genes among men with metastatic PCa varies between 11% and 33%, and it is significantly higher compared to the incidence in men with localized PCa [15, 17]. In a landmark study, Pritchard and colleagues showed that 11% of 692 patients with metastatic PCa harbored inherited mutations in 16 DDR genes [17]. The most frequent aberration was BRCA2 (5.3%) followed by ATM (1.6%), CHEK2 (1.9%), BRCA1 (0.9%), and RAD51 (0.4%). Mutation frequency did not differ based on PCa family history or age at diagnosis [17]. In a multi-institutional integrative clinical sequencing analysis, 23% of 150 mCRPC biopsies were found to be positive for DDR aberrations. BRCA2 was mutated in 13% of samples followed by ATM (7.3%), MSH2 (2%), BRCA1, FANCA, MLH1, and RAD51 (0.3%) [15].

Several studies showed a different genomic landscape in mCRPC compared to localized PCa [6, 18]. In a large retrospective study, Robinson et al. analyzed 680 primary tumors and 333 mCRPC biopsies [6]. The authors identified germline and/or somatic DDR defects in 10% of primary tumors and 27% of metastatic samples. The different molecular profile between localized PCa and metastatic lesions might be a direct consequence of tumor evolution under the selective pressure of ARSi or chemotherapy. However, small subpopulations of variant clones might be already present in primary tumors and might expand during the development of metastatic disease.

In this regard, Mateo and colleagues profiled 470 treatment-naïve PCa biopsies from patients who developed lethal mCRPC; of these, 61 patients had matched samples of primary tumors and metastatic lesions [19]. DDR gene aberrations (BRCA2 7%; CDK12 5%; and ATM 4%), TP53 (27%), and PTEN (12%) were commonly detected. Interestingly, while AR, TP53, and RB1 mutations were more commonly found in mCRPC lesions compared to primary tumors, DDR mutations had similar prevalence in primary and mCRPC settings [19]. These findings suggested that the use of prostate biopsy might be useful to profile patients for DDR mutations, avoiding rebiopsies of metastatic lesions that are potentially dangerous and time-consuming. Moreover, these data supported the testing for DDR defects in earlier stages of PCa as many of these alterations are already present during the initial phases of PCa development. However, given the limits of the study by Mateo and colleagues, further studies are needed. In fact, the retrospective design of this study did not take into account different treatments received in the mCRPC setting and heterogeneity in primary tumors that might have resulted in different profile between primary and mCRPC lesions.

1.2. Clinical Implications of BRCA Mutations in Prostate Cancers. PCa is a clinically heterogeneous disease. Patients commonly show variable responses to treatments that result in different clinical outcomes. This clinical variability may reflect molecular heterogeneity. Therefore, molecular profiling could have a meaningful translational relevance, allowing to distinguish PCa with indolent behaviour from those with a lethal course. Several studies explored the prognostic role of BRCA mutations in localized PCa and in mCRPC patients treated with standard therapies [20]. In a large retrospective study, BRCA1/2 mutations correlated with higher Gleason score, nodal involvement, metastatic disease at diagnosis, and T3/4 stage [14]. Moreover, BRCA2 was an independent prognostic factor that was associated with poorer outcomes. In localized PCa, the 5-year CSS and MFS were significantly shorter in BRCA2 carriers than in noncarriers (82% vs. 96%; 77% vs. 93%, respectively) [14]. Given conflicting results reported in retrospective studies, it is currently uncertain whether BRCA2 mutation may affect the clinical outcome of mCRPC patients treated with standard treatments [21, 22]. Annala and colleagues retrospectively analyzed 319 charts of mCRPC patients, including 22 germline DDR (gDDR) carriers (16 BRCA2-mutated). Interestingly, gDDR carriers had a significant shorter progression-free survival (PFS) than noncarriers (3.3 vs. 6.2 months, $p = 0.01$) when treated with first-line ARSi [21].

Antonarakis et al. evaluated the clinical significance of gDDR mutations in 172 mCRPC receiving first-line ARSi. Notably, in contrast to what was reported by Annala et al. [21], ATM-BRCA1/2 carriers had a trend towards longer PFS than noncarriers (15 vs. 10.8 months, $p = 0.090$) [23]. Conversely, Mateo et al. found no difference in PFS on first-

line ARSi (8.3 months in both groups) between gDDR carriers ($n = 330$) and noncarriers ($n = 60$) [22].

PROREPAIR-B was the first prospective trial designed to elucidate the prognostic impact of BRCA1/2, ATM, and PALB2 on CSS of mCRPC patients. All patients enrolled in this trial have not been treated with platinum or PARP inhibitors. Although the study failed to reach the primary endpoint of improved CSS between gDDR carriers ($n = 68$) and noncarriers ($n = 351$) (23.3 vs. 33.2 months; $p = 0.264$), germline BRCA2 mutation (gBRCA2) was confirmed to be an independent prognostic factor that negatively affected CSS (17.4 months in gBRCA2 vs. 33.2 months in non-mutated patients; $p = 0.027$) [24]. In a non-preplanned subgroup analysis of PROREPAIR-B, gBRCA2 mutation was also predicted for shorter CSS in mCRPC patients treated with the sequence docetaxel-ARSi compared to noncarriers (median 28.4 vs. 10.7 months, $p = 0.0003$) [24]. In contrast, CSS of gBRCA2 carriers did not differ from that of noncarriers in patients treated with the sequence ARSi-docetaxel (31.2 vs. 24 months, $p = 0.901$) [24]. This finding suggests that the choice of first-line therapy may affect the outcome of gBRCA2 patients, and these results may explain the aforementioned conflicting results from the three retrospective series [21, 22]. The multicenter and ambispective BRCA2MEN study is currently planned to validate the role of BRCA2 as a predictive biomarker to select the first-line therapy (ARSi vs. taxane) in patients with mCRPC.

1.3. Targeting BRCA Mutations in Prostate Cancer

1.3.1. Platinum Agents. Platinum-based chemotherapy, alkylating DNA, induces genomic strand breaks that may be translated in a synthetic lethality in tumor cells with DDR mutation. Carboplatin is a standard treatment for BRCA1/2 patients in breast [25, 26]. and ovarian cancer [27]. Satraplatin provided a significant reduction in the risk of progression or death (HR 0.67; 95% CI, 0.57 to 0.77; $p < 0.001$) in a randomized phase 3 trial that enrolled unselected mCRPC patients who had progressed to prior taxane [28]. However, this benefit did not translate in OS advantage compared to placebo (HR = 0.98; 95% CI, 0.84 to 1.15; $p = 0.80$).

Retrospective series and case reports also described the potential efficacy of platinum-based chemotherapy in mCRPC patients harboring gBRCA2 mutations [29, 30]. A retrospective study carried out at Dana-Farber Institute assessed the activity of carboplatin AUC 3–5 and docetaxel 60–75 mg/mq in 141 mCRPC patients who were previously progressed to standard therapies [16]. The combo significantly improved the rate of PSA decline in 6 out of 8 *BRCA2* carriers compared to 23 out of 133 noncarriers ($p = 0.001$), and improved OS was also observed (18.9 in *BRCA2* carriers vs. 9.5 months in non-carriers) [16].

Several ongoing trials are evaluating the efficacy of platinum-based chemotherapy in mCRPC patients selected for DDR mutations [31–33].

1.3.2. PARP Inhibitors. DDR defects cause the accumulation of genomic mutations in cancer cells, eventually leading to their proliferation, immortalization, and acquisition of an aggressive phenotype [34].

In vitro models showed that BRCA1- and BRCA2-defective cells are sensitive to PARP inhibitors, whereas BRCA1- and BRCA2-proficient cells are resistant [34].

ADP-ribosylation is involved in several cellular processes, including cell growth and differentiation, apoptosis, and transcriptional regulation. However, ADP-ribosylation has a significant role in DNA repair and genome stability, promoting double-strand break repair via homologous recombination [35]. The blockade of PARP1 through the use of PARP inhibitors or alkylating agents causes accumulation of DNA damages in DDR-defective tumor cells, resulting in a synthetic lethality (Figure 1) [36]. Several PARP inhibitors have been developed and are under investigation in clinical research for mCRPC patients (see Table 1) [37]. Olaparib was the first PARP inhibitor that showed significant activity in patients with mCRPC who had progressed to standard treatments. In a phase II trial, 50 heavily pretreated, molecularly unselected, mCRPC patients received olaparib 400 mg twice a day until progression or unacceptable toxicities [15]. The primary composite endpoint was the objective response rate (ORR), defined according to RECIST 1.1 criteria or as a reduction of at least 50% in PSA levels or a confirmed reduction in the circulating tumor-cell count from 5 or more cells to less than 5 cells per 7.5 ml of blood [15]. The prevalence of gDDR alterations was 33%. In the whole population, 16 out of 49 evaluable patients had a response (33%; 95% CI, 20 to 48). Among patients with gDDR, 88% had a response to olaparib [15]. Moreover, olaparib significantly improved PFS (median 9.8 vs. 2.7 months; $p < 0.001$) and OS (median 13.8 months vs. 7.5 months $p = 0.05$) of gDDR-mutated mCRPC patients compared to biomarker-negative patients [15]. In the randomized phase II TOPARP-B trial, 92 heavily pretreated mCRPC patients, selected for the presence of gDDR mutations, were randomized 1 : 1 to receive olaparib 400 mg twice daily or olaparib 300 mg twice daily [38]. The primary endpoint was defined as the presence of one of the following outcomes: radiological ORR assessed by RECIST 1.1 criteria, PSA response ≥50%, or circulating tumor-cell count conversion (from ≥5 cells per 7.5 mL blood at baseline to <5 cells per 7.5 mL blood). The primary endpoint was met. Composite response was achieved in 25 out of 46 patients receiving olaparib 400 mg (54.3%; 95% CI, 39.0–69.1) and in 18 out of 46 patients enrolled in olaparib 300 mg arm (39.1%; 25.1–54.6). The composite response was lower in patients treated with olaparib 300 mg, not reaching the prespecified criteria for success. However, almost 30% of patients treated with a higher dose of olaparib discontinued the treatment or reduced the schedule due to the development of grade 3-4 adverse events. Moreover, this trial showed that BRCA2-mutated patients had the greatest benefit from olaparib compared to those harboring CDK12 or ATM mutations. This trial suggested that the type of DDR mutation and olaparib dose had predictive implications. However, the type of mutation was not a stratification criterion for

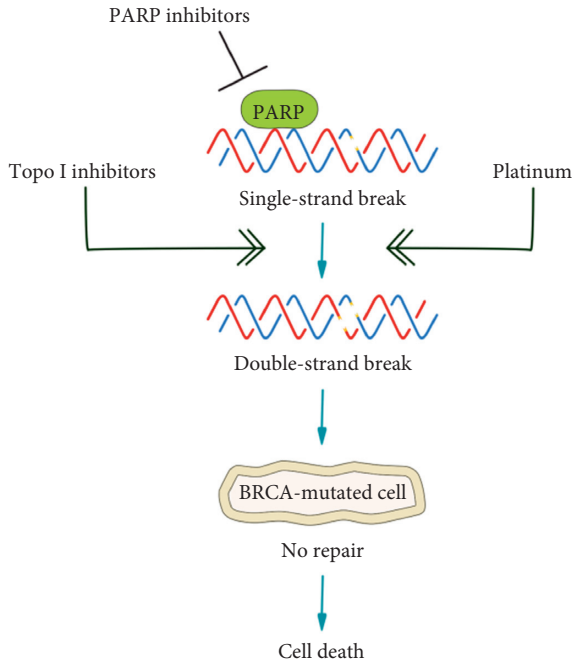

Figure 1: Mechanism of action of PARPi, platinum salts, and topoisomerase I inhibitors in the BRCA-mutated cell.

randomization; therefore, an allocation bias might have affected the results. The olaparib 300 mg arm was enriched of CDK12 patients, and this unbalance may have caused a lower benefit in this group of patients [38].

The role of DDR defects in predicting response to PARP inhibitors was more consistently demonstrated in the phase III PROFOUND trial, which randomized 387 mCRPC patients who were progressing to prior ARSi. Patients were allocated in two cohorts based on the presence of specific DDR defects (cohort A including BRCA1/2 or ATM and cohort B including other DDR defects). Olaparib 300 mg twice daily and second-line ARSi were administered in a 2 : 1 ratio [39]. The primary endpoint was radiological PFS (rPFS). Patients in cohort A treated with olaparib reported a median rPFS of 7.4 months compared to 3.55 months in those in the same cohort treated with ARSi (HR 0.34 (95% CI, 0.25–0.47), $p < 0.0001$). The PFS benefit was consistent throughout all subgroups within the prespecified subgroup analysis. Similar to that observed in the TOPARP-B trial [38], BRCA2-mutant patients had a better benefit from olaparib than patients harboring CDK12 or ATM mutations. The interim OS analysis also favored the olaparib arm (HR 0.64, 0.43–0.97), despite more than 80% of patients in the control group did crossover after disease progression. The ORR was 33% and 2.3% for experimental and control groups, respectively. Based on these findings, the US Food and Drug Administration (FDA) granted approval for olaparib in mCRPC patients with germline or somatic deleterious homologous recombination repair gene mutations who had progressed to prior ARSi on May 19, 2020.

The predictive value of DDR mutations was also confirmed in the preliminary findings from two phase II trials, TRITON-2 [40, 41] and GALAHAD [42], which investigated the activity of two other PARP inhibitors in patients with DDR-deficient mCRPC. In TRITON-2, mCRPC patients who had previously progressed to at least one ARSi and a taxane-based chemotherapy were screened for germline or somatic alterations in DDR genes. A total of 190 patients were treated with rucaparib 600 mg twice daily; the vast majority (98 pts) had BRCA1/2 alterations; and the remaining patients had alterations in ATM (57 pts), CDK12 (14), CHECK2 (7), and other genes (14 patients). ORR was 43.9% for patients with BRCA alterations, 9.5% for ATM, and 0% for the others. A similar pattern was observed for PSA response [41, 42]. On the basis of the preliminary results of the TRITON-2 trial, FDA announced on 15 May 2020 the accelerated approval of rucaparib for BRCA1/2 mCRPC patients progressing to prior ARSi or taxane.

In the GALAHAD trial, 165 patients with mCRPC and DDR defects progressing to at least one prior ARSi and taxane-based chemotherapy received niraparib 300 mg once daily. DDR positivity was defined by biallelic alterations in BRCA1/2, ATM, CHECK2, and other genes identified in plasma or tissue. ORR was the primary endpoint of the study. Patients who carried biallelic BRCA mutations achieved higher ORR (41% vs. 9%) and rPFS (8.2 months vs. 5.3) compared to those who did not harbor BRCA alterations [42]. It should be highlighted that the PROFOUND and the TRITON-2 trials evaluated mono- and biallelic alterations in DDR genes in tumor tissue and tumor tissue or plasma, respectively. Conversely, the GALAHAD trial required biallelic alterations in plasma samples to confirm eligibility. It is currently unknown whether the type and origin of BRCA mutations (germline vs. somatic and monoallelic vs. biallelic) could affect the response to treatment with PARP inhibitors.

1.4. Relevance of Germline Testing and Genetic Counselling. The high prevalence of DDR mutations and the clinical implications for their prognostic and predictive role have progressively led the international guidelines to implement recommendations for genetic and germline testing. The Philadelphia consensus conference recommends to test all patients with metastatic PCa, both in hormone-sensitive and castration-resistant settings, and in all patients with a significant family history of PCa or of tumors in the hereditary breast and ovarian cancer (HBOC) syndrome or Lynch syndrome spectrum. In metastatic PCa, both germline testing and somatic testing can be performed, and large gene panels can be used; however, the test should prioritize genes with more relevant clinical implications such as BRCA2, BRCA1, and mismatch repair (MMR). Furthermore, when somatic mutations are identified in BRCA2 or BRCA1, germline evaluation should also be performed due to the implications for all related family members. For patients with nonmetastatic PCa, the Philadelphia consensus suggests to use reflex testing, which consists of initial testing of priority genes followed by expanded testing, with a particular focus on BRCA2 results to personalize the strategies of active surveillance [43]. The US National Comprehensive Cancer Network (NCCN) guidelines recommend genetic

Table 1: Ongoing clinical trials assessing the role of PARPi in mCRPC.

Clinical trial	Phase	Study drug	Strategy	Primary endpoint
NCT02861573	I	Olaparib	Pembrolizumab + olaparib in postdocetaxel setting	RR (PSA50)
NCT03874884	I	Olaparib	Olaparib + 177Lu-PSMA in mCRPC	DLTs, MTD, RP2D
NCT03205176	I	Olaparib	Olaparib ± AZD5153 (BRD4/BET bromodomain inhibitor) in mCRPC	DLT
NCT02484404	I/II	Olaparib	Olaparib ± ceridanib ± MEDI4736 (anti-PD-1) in mCRPC	RP2D, AE
NCT03317392	I/II	Olaparib	Ra223 ± olaparib in mCRPC patients with bone metastases	MTD, rPFS
NCT03787680	II	Olaparib	Olaparib + ATR inhibitor (AZD6738) in second-line setting	RR
NCT03012321	II	Olaparib	Olaparib ± abiraterone/prednisone in first-line setting	PFS
NCT03434158	II	Olaparib	Olaparib for patients who are responding after docetaxel chemotherapy	rPFS
NCT03263650	II	Olaparib	Olaparib for patients who are responding after cabazitaxel plus carboplatin	PFS
NCT03516812	II	Olaparib	Olaparib + testosterone enanthate in postabiraterone/enzalutamide setting	RR (PSA50)
NCT02893917	II	Olaparib	Olaparib ± cediranib in second-line setting	rPFS
NCT03732820	III	Olaparib	Abiraterone/prednisone ± olaparib in first-line setting	rPFS
NCT03834519	III	Olaparib	Olaparib plus pembrolizumab versus abiraterone acetate or enzalutamide after chemotherapy and ARSi	OS and PFS
NCT03076203	I	Niraparib	Niraparib + radium-223	MTD
NCT03431350	I/II	Niraparib	Niraparib + abiraterone/prednisone or JNJ-63723283 in post-ARSi setting	AE, ORR
NCT02854436	II	Niraparib	Niraparib in postdocetaxel and post-ARSi settings	ORR
NCT03748641	III	Niraparib	Abiraterone/prednisone ± niraparib in first-line setting	rPFS
NCT04179396	I	Rucaparib	Rucaparib + abiraterone or enzalutamide in mCRPC	PK, AE
NCT03840200	I	Rucaparib	Rucaparib + ipatasertib in mCRPC after ARSi	AE, DLTs, PSA response
NCT04253262	I/II	Rucaparib	Rucaparib + copanlisib (PI3K inhibitor) in mCRPC progressing after ARSi	MTD, ORR
NCT03840200	I/II	Rucaparib	Rucaparib + ipatasertib after ARSi	AE, DLT, RR (PSA)
NCT03572478	I/II	Rucaparib	Rucaparib vs. rucaparib + nivolumab vs. nivolumab	DLT
NCT02952534	II	Rucaparib	Rucaparib in postdocetaxel and post-ARSi settings	ORR
NCT03338790	II	Rucaparib	Nivolumab + rucaparib or docetaxel or enzalutamide	ORR
NCT03442556	II	Rucaparib	Rucaparib for patients who are responding after docetaxel plus carboplatin	rPFS
NCT02975934	III	Rucaparib	Rucaparib vs. abiraterone/enzalutamide/docetaxel in second-line setting	rPFS
NCT04019327	I/II	Talazoparib	Talazoparib + temozolomide in mCRPC without DNA damage repair mutation after at least one ARSi	AE, ORR
NCT04052204	I/II	Talazoparib	Talazoparib + avelumab + bempegaldesleukin in mCRPC	DLT, ORR
NCT03330405	II	Talazoparib	Avelumab plus talazoparib in advanced solid tumors	DLT, ORR
NCT03148795	II	Talazoparib	Talazoparib in postdocetaxel and postabiraterone/enzalutamide settings	ORR
NCT03395197	III	Talazoparib	Enzalutamide ± talazaparib in first-line setting	rPFS
NCT04182516	I	NMS-03305293	NMS-03305293 (PARP inhibitor) in mCRPC	First cycle DLTs

RR: response rate; PSA50: decline in PSA level ≥50% than baseline; MTD: maximum tolerated dose; rPFS: radiological progression-free survival; PFS: progression-free survival; OS: overall survival; AE: adverse events; ORR: objective response rate; DLT: dose-limiting toxicities; MTD: maximum tolerated dose; RP2D: recommended phase II dose; and PK: pharmacokinetic.

testing (somatic and/or germline) for patients with high, very-risk, regional, and metastatic PCa or with a significant family history for cancer [44]. The recently published European Society for Medical Oncology (ESMO) guidelines recommend germline screening for all patients with mPCa and to consider genetic testing in patients with localized PCa and a family history suggestive for hereditary cancer predisposition [45]. Multidisciplinary discussion and integration with genetic services are fundamental to decide when and whether a genetic test should be performed and to select the appropriate therapeutic and diagnostic strategies. The IMPACT study is evaluating a screening strategy in men with gBRCA1/2 in order to define how to manage the population at a higher risk of PCa development in the presence of the BRCA2 mutation [46]. Annual prostate-specific antigen (PSA) tests and a biopsy for PSA >3 ng/ml are performed. Preliminary results revealed a higher incidence of PCa in gBRCA2 mutation carriers (3.3% vs. 2.6% in gBRCA1 mutation carriers, <2% for controls). Final results are awaited to be aware of the optimal screening strategies for this population.

2. Conclusions

Despite the development of several treatment options for mCRPC patients, metastatic PCa remains a lethal disease with poor prognosis [4]. Molecular characterization of mCRPC patients should be routinely integrated into the clinics in order to select patients who are more likely to respond to targeted agents and to minimize toxicities from unnecessary therapies. Furthermore, the emerging role of BRCA2 underlines the growing importance of genetic counselling and the multidisciplinary approach in the management of PCa patients. Recent evidence highlights

that gBRCA2 is an independent prognostic factor associated with shorter CSS in mCRPC patients, and the type of first-line treatment might affect the outcome of gBRCA2 carriers [24]. Moreover, it has been demonstrated that gBRCA2 is a strong predictor of response to PARP inhibitors. The role of PARP inhibitors in non-BRCA DDR mCRPC patients remains less clear, and further studies should investigate this specific issue.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Review Article

Secondary Prevention in Hereditary Breast and/or Ovarian Cancer Syndromes Other Than BRCA

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BRCA1- and BRCA2-associated hereditary breast and ovarian cancer syndromes are among the best-known and most extensively studied hereditary cancer syndromes. Nevertheless, many patients who proved negative at BRCA genetic testing bring pathogenic mutations in other suppressor genes and oncogenes associated with hereditary breast and/or ovarian cancers. These genes include *TP53* in Li–Fraumeni syndrome, *PTEN* in Cowden syndrome, mismatch repair (*MMR*) genes in Lynch syndrome, *CDH1* in diffuse gastric cancer syndrome, *STK11* in Peutz–Jeghers syndrome, and *NF1* in neurofibromatosis type 1 syndrome. To these, several other genes can be added that act jointly with *BRCA1* and *BRCA2* in the double-strand break repair system, such as *PALB2*, *ATM*, *CHEK2*, *NBN*, *BRIP1*, *RAD51C*, and *RAD51D*. Management of primary and secondary cancer prevention in these hereditary cancer syndromes is crucial. In particular, secondary prevention by screening aims to discover precancerous lesions or cancers at their initial stages because early detection could allow for effective treatment and a full recovery. The present review aims to summarize the available literature and suggest proper screening strategies for hereditary breast and/or ovarian cancer syndromes other than BRCA.

1. Introduction

In hereditary cancer syndromes (HCSs), inherited mutations lead to an increased risk of developing certain tumors, frequently at an earlier age than in the rest of the population [1]. Elevated cancer risk is usually due to a mutation in a single gene involved in cell cycle regulation or in DNA damage repair mechanisms (Figure 1). The most widely known HCSs include hereditary breast and ovarian cancer syndromes due to mutations in the *BRCA1/2* genes [2, 3], Li–Fraumeni syndrome due to mutations in *TP53* [4], Cowden syndrome due to mutations in *PTEN* [5], Lynch syndrome, in which mutations in the DNA mismatch repair system are involved [6, 7], diffuse gastric cancer syndrome caused by *CDH1* gene mutation [8], Peutz–Jeghers syndrome caused by mutations in the *STK11* [9] gene, and neurofibromatosis type 1 syndrome caused by *NF1* mutations [10]. Additionally, pathogenic alterations in *PALB2* [11], *ATM* [12], *CHEK2* [13], and *NBN* [14] are correlated with an increased risk for breast cancer and/or other cancers, whereas other genes such as *BRIP1*, *RAD51C*, and *RAD51D* are associated with an increased ovarian cancer risk [15].

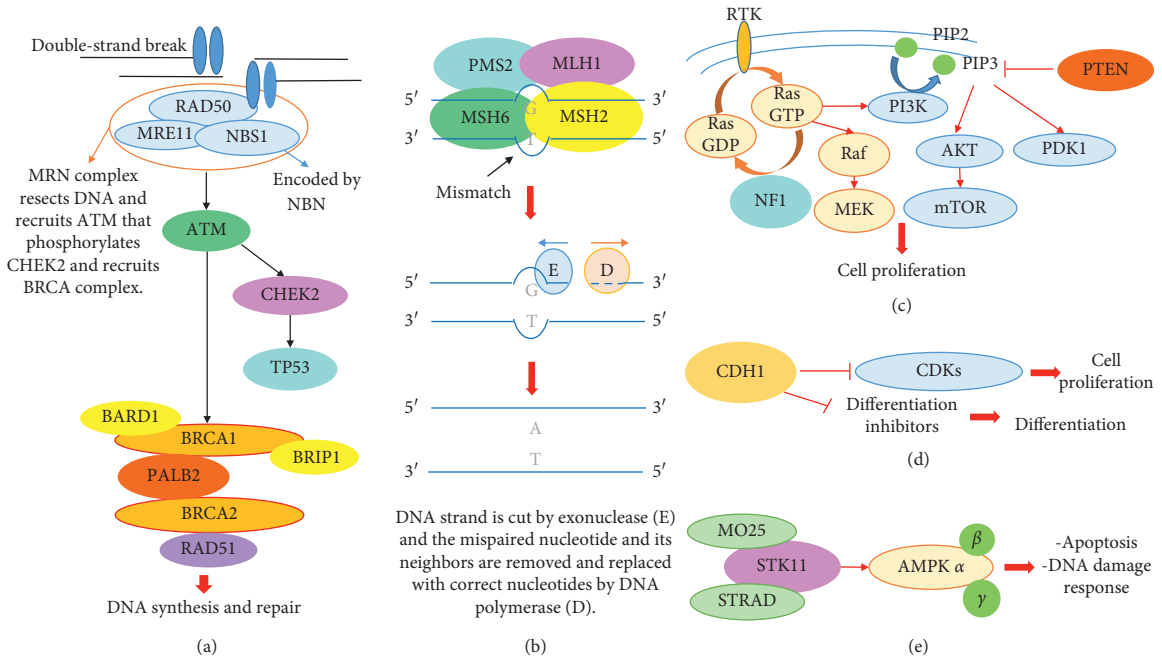

Figure 1: Molecular pathways involved in hereditary cancer risk. Susceptibility genes described in the text are reported in bold. G: guanine, T: thymine, A: adenine, E: exonuclease, D: DNA polymerase, RTK: receptor tyrosine kinase, PIP2: phosphatidylinositol 4,5-bisphosphate, PIP3: phosphatidylinositol (3,4,5)-trisphosphate, GDP: guanosine diphosphate, GTP: guanosine triphosphate, CDKs; cyclin-dependent kinases, and AMPK: 5′ adenosine monophosphate-activated protein kinase. (a) Homologus recombination (HR), (b) mismatch repair (MMR), (c) PTEN and NF1 pathways, (d) CDH1 pathway, and (e) STK11 pathway.

Management of cancer prevention is crucial in HCSs. Cancer prevention can be divided into primary and secondary strategies [16–23]. The aim of the primary prevention is to avoid cancer development by strategies including health counselling and education, environmental controls, prophylactic surgery, and chemoprevention. Secondary prevention by screening aims to discover precancerous lesions or cancers at their initial stages because early detection could allow for an effective treatment and full recovery. Strategies of primary and secondary cancer prevention are well established in the setting of *BRCA*-associated breast and ovarian cancer. For all other syndromes, on the other hand, the most appropriate screening protocol is still debated.

This review aims to summarize the available literature and suggest proper screening strategies for hereditary breast and/or ovarian cancer syndromes other than those associated with *BRCA* mutations.

2. Li–Fraumeni Syndrome

Li–Fraumeni syndrome is a rare autosomal dominant cancer predisposition syndrome that involves a germline mutation of the tumor protein 53 (*TP53* gene) [4]. The estimated prevalence of pathogenetic germline *TP53* mutations ranges from 1/10,000 to 1/25,000 in the UK and is estimated at 1/20,000 in the US [24]. The lifetime cancer risk in individuals with Li–Fraumeni syndrome is ≥70% for men and ≥90% for women [25]. Five cancer types account for the majority of Li–Fraumeni tumors: adrenocortical carcinomas, breast cancer, central nervous system tumors, osteosarcomas, and soft-tissue sarcomas [26]. Individuals with Li–Fraumeni syndrome are also at an increased risk of developing hematologic tumors (leukaemia and lymphomas), gastrointestinal cancers, gynecological tumors, and melanoma [4].

Surveillance recommendations for individuals with Li–Fraumeni syndrome are primarily based on the "Toronto protocol" [27]. For breast cancer, screening recommendations advise starting with clinical breast examination once in every 6–12 months from the age of 20. Annual breast MRI screening with contrast is suggested from 20 to 75 years of age. Given the increased sensitivity to ionizing radiation and the increased risk for radiation-induced malignancies in patients with germline pathogenic *TP53* variants, there are concerns about the safety of repeated mammograms. There is no consensus in the literature, but in light of the limited additional sensitivity of mammography when MRI and alternating whole-body diffusion-weighted MRI are used, risks seem to outweigh benefits [28–30]. In case of family history of breast cancer diagnosed earlier than 20 years of age, breast MRI might start five years prior to the earliest age of diagnosis. Although there are no data regarding risk-reduction surgery in women with Li–Fraumeni syndrome, the option of risk-reducing bilateral mastectomy should be considered and discussed with female patients [6, 28]. Concerning gastrointestinal cancer, colonoscopy and upper endoscopy should be performed once in every 2–5 years starting from 25 years of age or five years prior to the earliest case of colorectal cancer in the family. Moreover, annual

dermatologic examination is recommended from 18 years of age due to increased skin cancer risk, although less well-defined.

As regards many of the other cancers associated with Li–Fraumeni syndrome, early symptom-based detection is quite difficult. General recommendations include complete physical examination (including blood pressure evaluation, full neurologic exams, assessment of growth, sudden weight gain or loss, Cushingoid appearance, or signs of virilization in children) once in every 3–4 months until the age of 18 and then once in every six months. Annual whole-body diffusion-weighted MRI could allow for early detection of adrenocortical carcinomas and sarcomas, based on the results of multiple international trials [27, 31, 32]. As far as the central nervous system is concerned, the Toronto protocol with modifications [27] recommends annual brain MRI: first, MRI with contrast and then without contrast if previous MRI is normal and no new abnormality has been detected, in order to minimize the potential for gadolinium accumulation in the basal ganglia in individuals undergoing multiple enhanced MRIs [28]. Periodic blood tests can be considered in those at increased risk for myelodysplastic syndrome or leukaemia due to prior cancer treatments [28].

3. Cowden Syndrome

Cowden syndrome is the most prevalent PTEN hamartoma tumor syndrome associated with multiple hamartomatous and/or cancerous lesions in the skin, mucous membranes, thyroid, breast, endometrium, kidney, and brain [33]. Affected individuals usually have macrocephaly, trichilemmomas, and papillomatous papules, and the syndrome becomes apparent by the late 20s [5]. The estimated incidence of Cowden syndrome is 1/200,000, but it is likely to be underestimated due to the difficulties of making a clinical diagnosis of the disease [34]. Cowden syndrome is an autosomal dominant disorder due to germline *PTEN* mutation in 80% of cases [35].

The lifetime risk of developing breast cancer is 85%, with an average age at diagnosis between 38 and 46 years [5]. NCCN guidelines [6] recommend clinical breast examination once in every six months beginning at 25 years of age and annual mammogram and breast MRI screening with contrast starting at 30–35 years of age. However, screening should start 5–10 years prior to the earliest case of breast cancer in the family. Although there are no data regarding risk-reduction surgery in women with Cowden syndrome, the option of risk-reducing bilateral mastectomy should be considered.

The lifetime risk for thyroid cancer (usually follicular, rarely papillary) is approximately 35% [36]. Annual thyroid ultrasound from the time of diagnosis, including childhood, should be performed according to NCCN recommendations [6].

The risk for endometrial cancer may be close to 28% [36]. There are no data on screening for endometrial cancer. Routine transvaginal ultrasound has low sensitivity and specificity, especially in premenopausal women, whereas endometrial biopsy is highly sensitive and specific, but invasive. Therefore, screening with endometrial biopsy once in every 1–2 years may be considered, while hysterectomy should be discussed on a case-by-case basis, according to NCCN guidelines [6].

Half as many individuals with Cowden syndrome have adenomatous or hyperplastic colorectal polyps associated with early-onset (<50 years of age) colorectal cancer in 13% of patients [37]. Routine colonoscopy should be performed from the age of 35 once in every five years or more frequently, if the patient is symptomatic or polyps are found. However, screening should start 5–10 years before the age of the earliest case of colorectal cancer in the family.

Renal carcinoma may be present in up to 30% of patients. Melanoma skin cancer is also increased in patients with Cowden disease and may occur in 5% of patients [38]. Yearly to biennial renal imaging (preferably through CT or MRI) beginning at the age of 40 is recommended to screen renal cell carcinoma, while yearly dermatologic evaluation could help to detect early melanoma.

Brain tumors as well as vascular malformations occasionally affect individuals with Cowden syndrome. Cerebellar dysplastic gangliocytoma (Lhermitte-Duclos disease), a rare central nervous system tumor, can also be found in Cowden syndrome. However, the risk of developing these conditions is not well defined [39]. In the presence of neurological symptoms, especially in children, assessment of psychomotor abilities and brain MRI should be performed [6].

4. Lynch Syndrome

Lynch syndrome is caused by a germline mutation in one of four DNA mismatch repair genes (*MLH1*, *MSH2*, *MSH6*, or *PMS2*) [40] or deletions in the *EPCAM* gene resulting in *MSH2* silencing [41]. The estimated population frequency is 1 : 370 to 1 : 2,000 in Western populations [42]. Lynch syndrome is characterized by an increased lifetime risk for colorectal cancer (48–57% vs. 4.5%), endometrial cancer (43–57% vs. 2.7%), and other cancers including stomach (up to 13%), ovary (up to 24%), small bowel, hepatobiliary tract, urinary tract, brain, and skin [43].

Guidelines for cancer screening in patients with Lynch syndrome have been proposed by several groups including the American College of Gastroenterology, United States Multi-Society Task Force on Colorectal Cancer [44], European Hereditary Tumor Group [45], American Society of Clinical Oncology [46], and National Comprehensive Cancer Network [6].

Colonoscopy is recommended once in every 1–2 years starting from 20 to 25 years of age or 2–5 years before than the youngest diagnosis age in the family. Moreover, chromoendoscopy is a promising technique that could facilitate the detection of lesions and flat adenomas [47].

Regarding gynaecologic cancers, lifetime risk varies according to mutated gene and patient's age [48]. Transvaginal ultrasound and serum CA-125 testing were shown to be neither sufficiently sensitive nor specific to warrant a routine recommendation for early detection of endometrial and ovarian cancers. However, they may be required at

clinicians' discretion in assessing tumor risk on a case-by-case basis [6]. Annual endometrial biopsy can be used as a screening tool for endometrial cancer because of its high sensitivity and sensibility [6]. Total hysterectomy is an option that may be considered to reduce the risk of endometrial cancer in women with Lynch syndrome; likewise, bilateral salpingo-oophorectomy may reduce the incidence of ovarian cancer [49]. Since there is no effective screening for gynaecologic cancers, women should be educated on relevant symptoms such as abnormal uterine bleeding, pelvic or abdominal pain, bloating, dyspepsia, or increased urinary frequency or urgency.

Regarding gastric, duodenal, and more distant small bowel cancer, there is insufficient evidence to recommend surveillance [50], except for individuals with relevant family history of these tumors [51]. Besides, esophagogastroduodenoscopy extended to the duodenum or into the jejunum once in every 3–5 years starting from 40 years of age should be considered in case of mutation in *MLH1*, *MSH2,* or *EPCAM* [45]. Considering that infection with *Helicobacter pylori* is a cause of gastric cancer, testing and treating for this bacterium is suggested [46].

There is no clear evidence to support screening for urinary tract cancer, except for individuals with a family history of urothelial cancer or *MSH2* mutation who may benefit from annual urinalysis beginning at 30–35 years of age [6]. The International Cancer of the Pancreas Screening (CAPS) Consortium recommends screening for pancreatic cancer in patients with Lynch syndrome and one first-degree relative with pancreatic cancer [52]. Nonetheless, no protocol for pancreatic cancer screening has been established yet. The NCCN panel therefore recommends MRI and endoscopic ultrasonography as screening modalities to be performed at high-volume centres with multidisciplinary teams and preferably in a research protocol [6]. By reason of the increased risk for brain cancer, in addition, annual physical and neurologic examination from 25 to 30 years of age may be considered, although no data support this practice [6].

Some studies have shown that mutations in *MLH1* and *MSH2*, and less frequently in *PMS2* and *MSH6*, could be associated with increased breast cancer risk [53–55]. Nevertheless, no specific recommendations for breast screening in women with Lynch syndrome have been made available so far, beyond those offered to the average risk population [6]. Finally, a study suggested an increased risk for prostate cancer in men with Lynch syndrome [56]. However, there is no sufficient evidence to recommend different prostate cancer screening from the rest of the population [6].

5. Diffuse Gastric Cancer Syndrome

Hereditary diffuse gastric cancer is a cancer susceptibility syndrome defined by the early onset of diffuse gastric cancer with or without lobular breast cancer. It is mainly caused by germline mutations in the epithelial cadherin (*CDH1*) gene. A most serious problem is that genetic diagnosis remains unknown in up to 60% of patients [57]. The risk for symptomatic gastric cancer, occurring by the age of 80, ranged between 67 and 70% in men and 56 and 83% in women, whereas the risk for breast cancer among women, especially the lobular phenotype, amounted to 52% [8].

Prophylactic total gastrectomy is strongly recommended between 18 and 40 years of age [58]. Screening by esophagogastroduodenoscopy with multiple random biopsy once in every 6–12 months should be reserved to patients who cannot undergo prophylactic total gastrectomy, since upper endoscopy may not detect early precursor lesions [59].

In women, finally, annual mammogram with consideration of breast MRI with contrast beginning at the age of 30 (or prior to that, with a family history of breast cancer before the age of 25) is recommended by NCCN guidelines [6]. However, given the high lifetime risk and the low sensitivity of mammography for lobular breast cancer, the added value of MRI over mammography seems high in this situation [60]. Risk-reducing mastectomy may be discussed with these carriers, depending on family history [6].

6. Peutz–Jeghers Syndrome

Germline pathogenic alterations in *STK11* are associated with Peutz–Jeghers syndrome. This is an autosomal dominant disorder characterized by hamartomatous gastrointestinal polyps, mucocutaneous pigmentation, and an increased risk of colorectal, gastric, pancreatic, gallbladder, small bowel, gynaecologic (uterus, cervix, and ovary), breast, testicular, and lung cancers [9].

Regarding the risk of colorectal, gastric, and small bowel cancers, colonoscopy, upper endoscopy, and capsule endoscopy should be recommended once in every 2–3 years, starting from the late teens [61]. Moreover, the American College of Gastroenterology recommends magnetic resonance cholangiopancreatography with contrast or endoscopic ultrasound once in every 1–2 years from 30 years of age, in order to detect early pancreatic cancer [62].

For breast cancer, annual mammogram and breast MRI screening with contrast should be recommended from 25 years of age. In women, transvaginal ultrasound, serum CA-125, and pelvic exam with Pap smear should be proposed annually beginning at 18 years of age. No data on the benefit of risk-reducing mastectomy are available, so that this procedure may be considered based on family history [6].

In males, annual testicular exam and, subsequently, ultrasound in case of symptomaticity or abnormality on exam are suggested from birth to the teen years [62].

7. Neurofibromatosis Type 1

Pathogenic variants of *NF1* cause neurofibromatosis type 1. This is an autosomal dominant HCS associated with increased risk for nervous system tumors (especially malignant peripheral nerve sheath tumors), gastrointestinal stromal tumors, and breast cancer [10].

The American Academy of Paediatrics and the American College of Medical Genetics and Genomics have published guidelines for children and adult surveillance [63, 64]. Annual physical examination, annual ophthalmologic examination in children (less frequently in adults), regular

developmental assessment in children, regular blood pressure monitoring, and MRI for followup of clinically suspected intracranial tumors and other internal tumors are recommended. Additionally, annual mammography, possibly associated with breast MRI, is suggested between 30 and 50 years of age [65]. After 50 years of age, breast cancer risk in women with *NF1* mutation becomes similar to that of the rest of the population. Breast MRI could therefore be discontinued [66], while mammography can be performed at longer intervals. No data on the benefit of risk-reducing mastectomy are available, so that this procedure may be considered based on family history [6].

8. Other Breast and Ovarian Cancer Predisposition Genes

In addition to the known high-penetrance pathogenic variants of *BRCA1/2*, mutations in other intermediate or low-penetrant genes can increase the risk of breast and/or ovarian cancer. According to a retrospective analysis, these mutations account for 7.4% of patients who met the NCCN criteria for *BRCA1/2* mutation test [6]. The most common are *PALB2*, *ATM,* and *CHEK2* [67].

8.1. PALB2. It is estimated that 0.6%–3% of patients with breast cancer harbour a mutation in *PALB2* (partner and localizer of *BRCA2*) [11, 15, 68]. Moreover, women carrying pathogenetic variants of *PALB2* have a 35% lifetime risk to develop breast cancer by 70 years of age. The higher the number of relatives affected, the higher the risk [69]. Breast ultrasound and MRI are recommended yearly from 25 to 29 years of age, alternating once in every six months. Annual mammogram and breast MRI screening are alternatively recommended once in every six months, starting at 30 until 65 years of age [6].

Some studies highlight a possible association between *PALB2* mutations and ovarian cancer. Recently, *PALB2* has also been reported to be a new pancreatic cancer susceptibility gene [70]. However, the associated risks are unclear and not well-estimated. Furthermore, no effective screening method is available for ovarian or pancreatic cancer. Screening and/or risk-reducing surgery should be individualized based on familial history [71].

8.2. ATM, CHEK2, NBN, and BARD1. Individuals carrying heterozygous pathogenic variants in *ATM* have a 33% cumulative lifetime risk for breast cancer by 80 years of age [12]. Mammogram with consideration of breast MRI is recommended yearly from 40 years of age [6]. No data are available on the benefit of risk-reducing mastectomy, so that this procedure may be considered based on family history [6]. *ATM* heterozygous pathogenic variants have been reported in some cases of familial ovarian [15], pancreatic [72], and prostate [73] cancer. Screening for pancreatic and ovarian cancers in carriers of *ATM* pathogenic variants is not recommended in the absence of familial antecedents, while men should be encouraged to participate in prostate cancer screening [6]. Homozygous or compound heterozygous *ATM* mutations cause ataxia telangiectasia, a syndrome characterized by progressive cerebellar ataxia, oculomotor apraxia, immunodeficiency, and general increased risk of malignancies [74].

The rate of *CHEK2* germline mutation is higher in Northern European countries than in Mediterranean ones. Certain mutations in the *CHEK2* gene (c.1100delC and I157T) are associated with increased breast cancer risk, with a cumulative lifetime risk ranging from 28% to 37% depending on family history [13, 75]. Mammogram and breast MRI once a year start at 40 years of age [6]. No data are available on the benefit of risk-reducing mastectomy, so that this procedure may be considered based on family history [6]. Within families carrying pathogenic *CHEK2* variants, there is also an increased risk of other malignancies including colon, prostate, kidney, bladder, and thyroid cancers [76], with the vast majority of data for the c1100delC variant. Colonoscopy once in every five years, beginning from 40 years of age or 10 years earlier than the age of diagnosis for any first-degree relative with colorectal cancer, is recommended in individuals carrying *CHEK2* mutations [6]. Currently, there are no specific medical management guidelines to address the possible risk of developing prostate, kidney, bladder, and thyroid cancer in these individuals.

Individuals with slavic founder heterozygous *NBN* mutation 675del5 have an increased risk of developing numerous types of cancer, including breast (up to 30% at 80 years of age) and ovarian cancer. Moreover, an unestimated increased risk of prostate cancer at 80 years of age is also apparent in men [77]. The presence of biallelic hypomorphic *NBN* mutations leads to the Nijmegen breakage syndrome, a rare autosomal recessive syndrome of chromosomal instability mainly characterized by microcephaly at birth, combined immunodeficiency, and predisposition to malignancies. Approximately 40% of the affected patients develop a malignancy before the age of 21 [14]. In slavic mutation carriers, breast MRI is recommended yearly from 40 years of age, whereas no recommendations are provided for ovarian and prostate cancer screening [6]. No data are available on the benefit of risk-reducing mastectomy, so that this procedure may be considered based on family history [6].

Deleterious *BARD1* germline variants are significantly associated with early-onset breast cancer, according to recent studies [78, 79]. On the grounds of these data, intensified breast cancer screening programs should be offered to women carrying pathogenic *BARD1* gene variants. However, the starting age and the frequency of mammogram and/or breast MRI have not been established yet.

8.3. BRIP1, RAD51C, and RAD51D. Mutations in *BRIP1*, *RAD51C,* or *RAD51D* are associated with an increased risk of developing ovarian cancer. The prevalence rate of *BRIP1*, *RAD51C,* or *RAD51D* pathogenic variants is about 1% in women with ovarian cancer [15]. Nevertheless, there are no data supporting screening for ovarian cancer. Transvaginal ultrasound and serum CA-125 testing have not been shown to be sufficiently sensitive or specific, even in the setting of

Table 1: Summary of the recommendations for each predisposition gene.

Predisposition genes	Cancer risk	Lifetime risk	Surveillance
High-penetrance genes for breast and/or ovarian cancer			
TP53	Adrenocortical gland	6–13% [25]	Ultrasound of abdomen and pelvis: every 3–4 mos, birth to age 18 yrs [27]
	Breast	54% [25]	Clinical breast examination: every 6–12 mos, age ≥ 20 yrs Breast MRI screening with contrast (with or without mammogram): annually, age 20–75 yrs [27]*
	Central nervous system	6–19% [25]	Neurologic exam: annually, all ages Brain MRI: annually [27]
	Sarcomas	5–22% [25]	Whole-body MRI: annually, all ages Ultrasound of abdomen and pelvis: annually, age ≥18 yrs [27]
	Hematologic tumors	NA	Periodic blood test if increased risk for myelodysplastic syndrome or leukaemia [28]
	Gastrointestinal system	NA	Upper endoscopy and colonoscopy: every 2–5 yrs, age ≥25 yrs [27]
	Skin	NA	Dermatologic exam: annually, age ≥18 yrs [27]
PTEN	Breast	85% [5]	Clinical breast examination: every 6 mos, age ≥ 25 yrs Mammogram and breast MRI with contrast: annually, age 30–75 yrs [6]*
	Thyroid	35% [36]	Ultrasound of thyroid: annually, all ages [6]
	Endometrium	28% [36]	Endometrial biopsy: every 1–2 yrs [6]*
	Colon and rectum	9% [36]	Colonoscopy: every 5 yrs, age ≥ 35 yrs [6]
	Kidney	30% [36]	CT or MRI of abdomen: every 1–2 yrs, age ≥ 40 yrs [6]
	Melanoma	5% [38]	Dermatologic exam: annually, age ≥18 yrs [38]
CDH1	Stomach	56–83% [8]	Upper endoscopy: every 6–12 mos, age ≥ 18 yrs [59]*
	Breast	52% [8]	Mammogram and breast MRI with contrast: annually, age ≥ 30 yrs [6]*
STK11	Colon and rectum	39% [9]	Colonoscopy: every 2–3 yrs, age ≥ 18 yrs [61]
	Stomach	29% [9]	Upper endoscopy: every 2–3 yrs, age ≥ 18 yrs [61]
	Small bowel	13% [9]	Capsule endoscopy: every 2–3 yrs, age ≥ 18 yrs [61]
	Pancreas	11–36% [9]	MR cholangiopancreatography with contrast or endoscopic ultrasound: every 1–2 yrs, age ≥ 30 yrs [62]
	Breast	32–54% [9]	Clinical breast examination: every 6 mos, age ≥ 20 yrs Mammogram and breast MRI with contrast: annually, age ≥ 25 yrs [6]*
	Ovary, cervix, and uterus	9–21% [9]	Transvaginal ultrasound, serum CA 125, pelvic exam with pap smear: annually, age ≥ 18 yrs [6]
	Testis	9% [9]	Testicular exam: annually, until 18 yrs [62]
	Lung	7–17% [9]	Not recommended
Low-/moderate-penetrance genes for breast and/or ovarian cancer			
PALB2	Breast	35% [69]	Mammogram and breast MRI with contrast: annually, age ≥ 30 yrs [6]*
	Ovary, pancreas	NA	Not recommended
CHEK2	Breast	28–37% [13, 75]	Mammogram and breast MRI with contrast: annually, age ≥ 40 yrs [6]*
	Colon	NA	Colonoscopy: every 5 yrs, age ≥ 40 yrs [6]
	Prostate, kidney, bladder, and thyroid	NA	Not recommended
NBN (*675del5*)	Breast	Up to 30% [77]	Breast MRI with contrast: annually, age ≥ 40 yrs [6]*
	Ovary and prostate	NA	Not recommended

Table 1: Continued.

Predisposition genes	Cancer risk	Lifetime risk	Surveillance
MLH1, MSH2, MSH6, PMS2, EPCAM	Colon and rectum	48–57% [43]	Colonoscopy: every 1-2 yrs, age ≥ 20–25 yrs [6]
	Endometrium	43–57% [43]	Not recommended*
	Ovary	Up to 24% [43]	Not recommended*
	Stomach, small bowel	4–13% [43]	Upper endoscopy: every 3–5 yrs, age ≥ 40 yrs if relevant family history or mutation in *MLH1*, *MSH2* or *EPCAM* [45, 51]
	Hepatobiliary tract	Up to 4% [43]	In research protocol [6]
	Urinary tract	Up to 25% [43]	Urinalysis: annually, age ≥ 30–35 yrs if relevant family history or *MSH2* mutation [6]
	Brain	1–4% [43]	Physical and neurologic examination: annually, age ≥ 25–30 yrs [6]
	Breast (*MSH2*, *MLH1*, *PMS2,* or *MSH6*)	11–18% [53–55]	Not recommended
	Prostate	NA	Not recommended
ATM	Breast	33% [12]	Mammogram with consideration of breast MRI with contrast: annually, age ≥ 40 yrs [6]*
	Ovary, prostate, and pancreas	NA	Not recommended
BRIP1, RAD51C, RAD51D	Ovary	Up to 10% [15]	Not recommended*
NF1	Nervous system	8–16% [64]	Physical and eye examination: annually, every age [63, 64]
	Breast	17% [64]	Mammogram and breast MRI with contrast: annually, age 30–50 yrs [65]*
BARD1	Breast	NA	Not recommended

NA = not available, *Risk-reducing surgery can be considered based on type of mutation and family history.

women at high risk of ovarian cancer due to an inherited mutation [6]. NCCN guidelines recommend that risk-reducing salpingo-oophorectomy should be considered beginning at 45–50 years of age [6]. At present, *BRIP1*, *RAD51C,* or *RAD51D* are not associated with an increased risk for breast cancer [7].

9. Conclusions

In the last years, a large number of large case-control studies have shown the correlation between mutations in some genes and an increased risk of developing breast and/or ovarian cancer, explaining tumor recurrence in those families where mutations in *BRCA1/2* were not found. However, the lifetime risk in case of low-penetrant genes has not been defined yet and additional prospective studies are needed to establish a more customised screening program for carriers. Summary of the recommendations for each predisposition gene discussed in the present review is reported in Table 1.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Review Article

Cancer Surveillance in Healthy Carriers of Germline Pathogenic Variants in *BRCA1/2*: A Review of Secondary Prevention Guidelines

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Germline pathogenic alterations in the breast cancer susceptibility genes 1 (*BRCA1*) and 2 (*BRCA2*) are the most prevalent causes of hereditary breast and ovarian cancer. The increasing trend in proportion of cancer patients undergoing genetic testing, followed by predictive testing in families of new index patients, results in a significant increase of healthy germline *BRCA1/2* mutation carriers who are at increased risk for breast, ovarian, and other *BRCA*-related cancers. This review aims to give an overview of available screening guidelines for female and male carriers of pathogenic or likely pathogenic germline *BRCA1/2* variants per cancer type, incorporating malignancies that are more or less recently well correlated with *BRCA1/2*. We selected guidelines from national/international organizations and/or professional associations that were published or updated between January 1, 2015, and February 1, 2020. In total, 12 guidelines were included. This review reveals several significant discordances between the different guidelines. Optimal surveillance strategies depend on accurate age-specific cancer risk estimates, which are not reliably available for all *BRCA*-related cancers. Up-to-date national or international consensus guidelines are of utmost importance to harmonize counseling and proposed surveillance strategies for *BRCA1/2* carriers.

1. Introduction

Germline pathogenic alterations in the breast cancer susceptibility genes 1 (*BRCA1*) and 2 (*BRCA2*) are the most prevalent causes of hereditary breast and ovarian cancer (HBOC). Family studies and segregation analyses have estimated carrier rates of pathogenic and likely pathogenic *BRCA1* or *BRCA2* alterations in a mixed western population between 1 in 200 and 1 in 1500 persons with most estimates towards the lower end of the range [1, 2]. In some populations like Ashkenazi Jews, founder effects are observed with carrier frequencies up to 1% or more [3]. Better knowledge of the implications of *BRCA* alterations in cancer treatment led to higher awareness among patients and physicians. Together with improved availability of genetic testing, this has led to lower testing thresholds and more germline diagnostic tests, resulting in an increase of cancer patients with known germline pathogenic variants in *BRCA1/2*. Predictive testing in families of new index patients leads to a further increase of healthy carriers with germline alterations correlated with *BRCA1*/2 and other monogenetic causes of HBOC [4].

There are several implications for carriers of (likely) pathogenic variants in *BRCA1/2*. Besides the increased cancer risks and the identified prognostic and predictive implications in *BRCA*-related breast, ovarian, pancreatic, and prostate cancers, the autosomal dominant inheritance pattern has important implications for the children and relatives of mutation carriers [5, 6]. Known female and male carriers of pathogenic or likely pathogenic variants who plan to conceive should be counseled about options of prenatal and preimplantation genetic diagnosis [7].

The elevated cancer risks extend beyond breast and ovarian cancer. There is clear evidence for an increased risk for prostate and pancreatic cancer. The risk for other cancers such as stomach, colorectal, and endometrial cancer and melanoma might also be elevated to some extent, and some guidelines give recommendations for these possible associations, while for other reported supposed correlations, none of the guidelines give specific recommendations (e.g., cervical cancer) [8, 9]. There are important uncertainties and differences in strength of evidence and differential effects for *BRCA1* and *BRCA2* with regard to these and other possible additional cancer risks. Lifetime risks have not been reliably estimated for all these correlations (Table 1). Given the burden of cancer risks and surveillance for germline carriers of a hereditary cancer syndrome, appropriate counseling about primary and secondary prevention strategies is a crucial factor in the care for these individuals. Several national and international guidelines and algorithms for surveillance of *BRCA*-related cancers exist. With this review, we aim to give an overview and comparison of available screening guidelines for *BRCA*-related cancers for female and male carriers of pathogenic or likely pathogenic germline *BRCA1/2* variants per cancer type, incorporating malignancies where a correlation with *BRCA1/2* is more or less recently well demonstrated.

2. Methods

We selected articles for our review by Medline search and additional web-based search of the national and international organizations and/or professional associations for guidelines that reported recommendations on secondary prevention in female and/or male carriers of pathogenic or likely pathogenic germline *BRCA1/2* variants. Only guidelines published or updated between January 1, 2015, and February 1, 2020, were eligible for inclusion in this review. The review is limited to recommendations available in English, French, or Dutch. Guidelines that did not provide clear information about the starting age of surveillance or about the recommended screening modality were excluded. We retrieved 12 guidelines that met our criteria.

3. Cancer Surveillance Guidelines in Germline *BRCA1/2* Mutation Carriers

3.1. Breast Cancer. Germline pathogenic variants in *BRCA1/2* are highly penetrant for breast cancer. The incidence of breast cancer in female *BRCA1/2* carriers increases rapidly in early adulthood. The breast cancer risk increases between 30 and 40 years in *BRCA1*, but the higher penetrance of *BRCA2* at later ages has been confirmed reaching an absolute cumulative risk between 60 and 80% at age 80 years for both *BRCA1* and *BRCA2*. The risk of contralateral breast cancer is estimated at 40% for *BRCA1* carriers and 25% for *BRCA2* carriers at 20 years after the first breast cancer diagnosis [10].

The high lifetime risk of breast cancer in female *BRCA* carriers makes the discussion of primary prevention strategies (lifestyle modifications, chemoprevention, and risk-reducing surgery) important. Regarding chemoprevention, only limited data exist on the preventive benefit of tamoxifen in *BRCA1/2* mutation carriers. In addition, there is some concern about the safety of tamoxifen regarding endometrial cancer risk. Moreover, there is discordance as to whether *BRCA1* carriers, who are more prone to estrogen receptor negative breast cancer, benefit as much from this chemoprevention approach as *BRCA2* carriers [22, 23]. Several trials investigating new chemoprevention approaches in *BRCA* carriers are ongoing [24].

Risk-reducing mastectomy (RRM) has been shown to be a very effective breast cancer primary prevention option [25, 26]. Breast cancer after RRM in *BRCA* carriers has been reported, but the absolute risk is very low and none of the guidelines propose imaging surveillance after RRM [25, 27]. A cohort study has shown improved overall and breast cancer-specific mortality rates in *BRCA1* mutation carriers, while for *BRCA2*, survival rates were not significantly different after a median follow-up for 10.3 years [28]. In clinical practice, for the vast majority of women opting for RRM, mortality reduction is not the dominant driver in the decision process [29]. The option of RRM should be discussed with female carriers of (likely) pathogenic germline mutations in *BRCA1/2*. However, risk-reducing surgery should never be recommended as the only option to address the high breast cancer risk, and the advantages and

Table 1: Overview of lifetime cancer risks in carriers of germline *BRCA1/2* (likely) pathogenic variants.

Type of malignancy	Lifetime risk of malignancy		
	General population (%)	*BRCA1* (%)	*BRCA2* (%)
Breast, female [10, 11]	12	72	69
Breast, male [11–13]	0.1	1.2	6.8–8.4
Ovarian [10, 11]	1-2	44	17
Pancreatic‡ [14–16]	0.5	1–3	2–7
Prostate‡ [11, 17, 18]	6 (by age 65)	8.6 (by age 65)	15 (by age 65)
Colorectal‡ [11, 19]	4-5	Possibly elevated	¥
Endometrial‡ [11, 20]	3	Possibly elevated	¥
Melanoma‡ [11, 21]	2-3	¥	Possibly elevated

‡Lifetime risks not estimated, extrapolated from odds ratios/standardized incidence ratios. ¥Insufficient or inconsistent data about possible association with increased risk.

disadvantages of this option and other primary or secondary prevention strategies should be extensively discussed.

Although male breast cancer is a rare disease in the general population, with a lifetime risk of 0.1% accounting for less than 1% of all cancers in men and about 1% of all breast cancers, the cumulative incidence is significantly increased in male *BRCA1/2* carriers and is estimated at 1% in *BRCA1* carriers and 7-8% in *BRCA2* carriers [12, 13, 30].

Breast cancer screening in germline mutation carriers is correlated with an increased rate of stage 0 or stage 1 breast cancer, and there is limited data about survival benefit [31, 32]. There are several guidelines and recommendations for breast cancer surveillance in germline *BRCA* mutation carriers. A schematic overview of guidelines for female carriers is shown in Figure 1. The majority of guidelines address screening approaches for female and male carriers and discuss recommendations on breast awareness, clinical examination, mammography, and magnetic resonance imaging (MRI), but guidance on the use of digital breast tomosynthesis (DBT) and ultrasound is often not specified. There is concern that the exposure to diagnostic radiation at young age may be associated with an increased risk of breast cancer in *BRCA* carriers [33]. Moreover, the decision as to whether or not to undergo a RRM is often not made at the recommended starting age for breast cancer screening. Therefore, the starting age of mammography in female carriers is an important aspect of the surveillance guidelines. Some guidelines advise annual screening procedures, while the concern about interval cancers in these high-risk patients leads to semiannual alternating schedules in other recommendations [31]. The option of DBT is mentioned in some guidelines based on the superior sensitivity and specificity compared to standard mammography; however, there is no data on the use in *BRCA* mutation carriers who undergo MRI screening [34]. In a recent study among 1444 average-risk women aged 40–70 with heterogeneously dense or extremely dense breast, the invasive cancer detection rate was significantly higher for MRI compared to DBT, and no invasive cancer was identified by DBT alone [35].

3.1.1. European Society for Medical Oncology Guidelines. The clinical practice guidelines for cancer prevention and screening in *BRCA* mutation carriers from the European Society for Medical Oncology (ESMO) were published in 2016 [36]. For female carriers of pathogenic *BRCA* variants, breast awareness and clinical breast examination are recommended every 6–12 months from the age of 25 or 10 years before the youngest breast cancer diagnosis in the family, whichever occurs first. Annual MRI is recommended from the age of 25, with the addition of annual mammography from the age of 30. The decision to introduce mammography before the age of 40 should take into consideration the increased breast density at younger ages and the availability of annual screening MRI. In women ≤30 years, breast ultrasound can be considered in case MRI is unavailable. Ultrasound can also be considered in addition to mammography at all ages and as an alternative when MRI is not available. Upper age limit or other conditions where screening should be discontinued are not described for female carriers. After RRM, routine surveillance is not recommended but should be considered in patients who have undergone nipple-sparing mastectomy. Male carriers should be advised to undergo annual clinical breast examination by a physician from age 30 onwards. Routine annual breast imaging among male carriers is not recommended.

3.1.2. National Comprehensive Cancer Network Guidelines. The last version from the clinical practice guidelines in genetic high-risk assessment for breast, ovarian, and pancreatic cancer of the National Comprehensive Cancer Network (NCCN) dates from December 2019 [37]. With regard to breast cancer surveillance in women, breast awareness is recommended starting at age 18 and clinical breast exam every 6–12 months from age 25. Between age 25 and 29, annual breast MRI with contrast is recommended. Starting age should be individualized based on family history if a breast cancer was diagnosed in a relative before age 30. When MRI is unavailable, annual mammogram with consideration of DBT is recommended. Between ages 30 and75, both annual MRI with contrast and annual mammogram with consideration of DBT are recommended. In carriers >75 years, management should be considered on an individual basis. Criteria for high-quality breast MRI include availability of experienced breast MRI radiologists, a dedicated breast coil, the ability to perform MRI-guided biopsies, and regional availability. Breast MRI

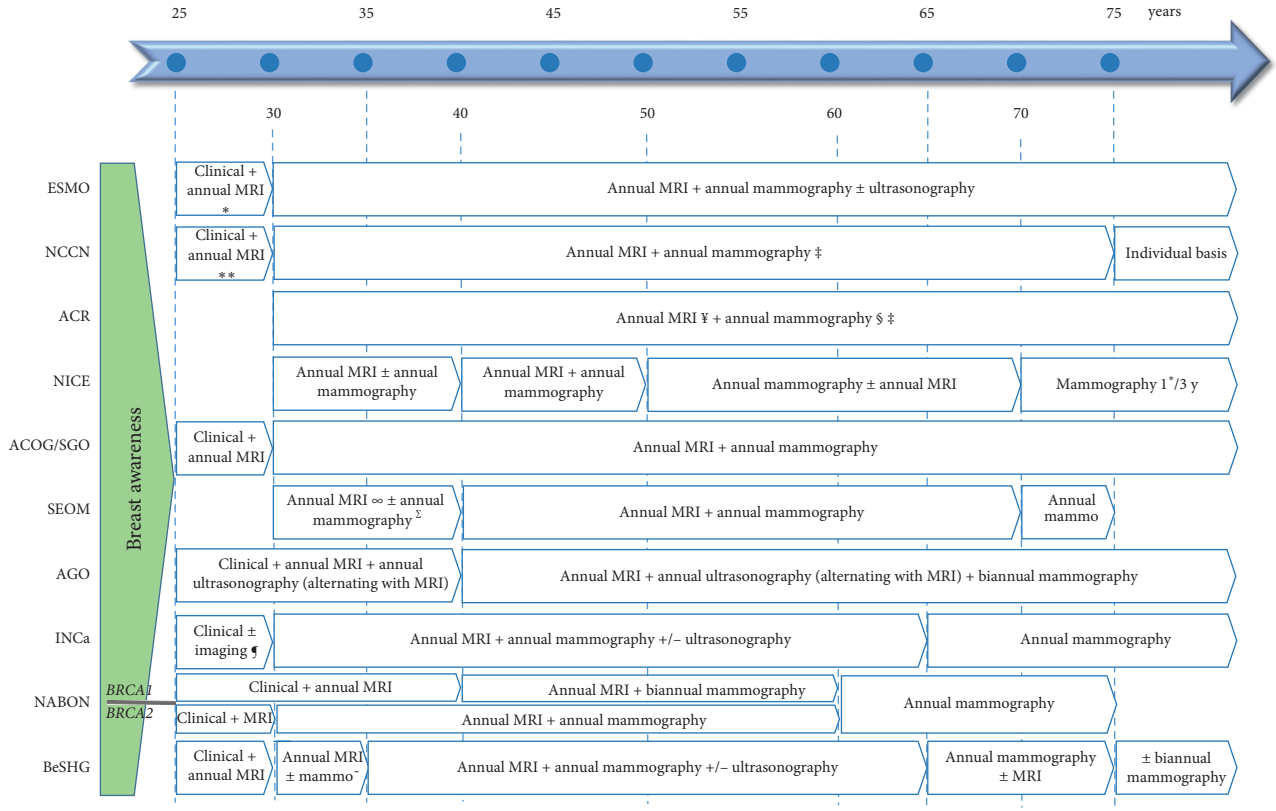

Figure 1: Schematic overview of surveillance guidelines for breast cancer in asymptomatic female carriers of (likely) pathogenic *BRCA1/2* variants. MRI: magnetic resonance imaging; ESMO: European Society for Medical Oncology; NCCN: National Comprehensive Cancer Network; ACR: American College of Radiology; NICE: National Institute for Health and Care Excellence; ACOG: American College of Obstetricians and Gynecologists; SGO: Society of Gynecologic Oncology; SEOM: Sociedad Espanola de Oncologia Médica; AGO: Arbeitsgemeinschaft Gynäkologische Onkologie; INCa: Institut National du Cancer; NABON: Nationaal Borstkanker Overleg Nederland; BeSHG: Belgian Society of Human Genetics. *Or starting 10 years earlier than youngest breast cancer diagnosis in the family. **Or individualized based on family history if a breast cancer diagnosis is present before age 30. ¥Or starting 5–10 years earlier than the youngest breast cancer diagnosis in the family. §Starting 10 years before the youngest breast cancer diagnosis in the family, but not before 30. ‡Considering breast tomography. ∞Or starting earlier if there is a family history of breast cancer before 30 years. ΣDiscussing delaying mammography until 40 years with *BRCA1* carriers who undergo annual MRI screening. ¶Considering imaging in case of early breast cancer diagnosis in the family. -Mammography at age 30, annual mammography from 30 onwards in case of microcalcifications.

is preferably performed on days 7–15 of a menstrual cycle in premenopausal women.

Male carriers of (likely) pathogenic variants in *BRCA* are recommended to undergo annual clinical breast examination and undergo training in self-examination with monthly practice starting from age 35 onwards. Regularly scheduled mammography is not recommended in male *BRCA* carriers.

3.1.3. American College of Radiology. The publication of the Appropriateness Criteria® for breast cancer screening from the American College of Radiology (ACR) dates from 2017 [34]. Recommendations are limited to the radiological imaging procedures, and guidelines for breast cancer screening in women with a *BRCA* gene mutation are similar to the recommendations for women with a history of chest irradiation between 10 and 30 years of age and women with ≥20% lifetime breast cancer risk. Annual mammography is recommended starting 10 years earlier than the affected relative at the time of diagnosis, but not before 30 years. The superior sensitivity and specificity of DBT over planar mammography are described, and the advantages seem to be most pronounced in women with higher breast density, in women under age 50, and in carriers with spiculated masses and asymmetries. Since in the majority of situations standard two-dimensional images are obtained in addition to the DBT images, the radiation dose is increased compared to standard mammography. However, virtual planar images created from the tomographic data set could replace the need for a 2D correlative view in the near future. Surveillance with annual breast MRI (with and without contrast) is recommended in addition to mammography. For the starting age of MRI screening in *BRCA* carriers, ACR refers to the American Cancer Society Guidelines for breast screening with MRI as an adjunct to mammography. The recommended starting age is 30 years for the majority of women, or 5 to 10 years before the earliest breast cancer diagnosis in the family. The starting age should be based on shared decision making, considering individual preferences and

circumstances. Screening with breast MRI should be continued as long as the woman is in good health.

3.1.4. National Institute for Health and Care Excellence Guidelines. The clinical guidelines on familial breast cancer by the National Institute for Health and Care Excellence (NICE) were originally published in 2013, but the online version was verified as up-to-date in November 2019 [38]. All carriers should be informed about breast awareness. Annual mammography should be considered in female carriers aged 30–39 and recommended aged 40–69, while patients ≥70 years should be offered mammography every three years as part of the population screening program. Mammographic surveillance should never be offered for patients <30 years.

Annual MRI surveillance should be offered to female carriers aged 30–49 years and can be considered between 50 and 69 years in case of dense breast pattern but should not be offered to *BRCA* carriers <30 years.

The NICE guidelines state that ultrasound surveillance should not be routinely offered but could be considered when MRI is not possible or when results of mammography or MRI are difficult to interpret. No recommendations are made for male carriers.

The guidelines on breast cancer screening from the London Cancer Alliance (published in 2013 and updated in 2016, [39]) and the Institute of Cancer Research protocol for *BRCA* mutation carriers (2015, [40]) are concordant with the NICE guidelines. The latter specifies that no breast surveillance is recommended for male carriers.

3.1.5. American College of Obstetricians and Gynecologists/ Society of Gynecologic Oncology. The HBOC clinical management guidelines from the committee on practice bulletins from the American College of Obstetricians and Gynecologists (ACOG) and the committee on genetics from the Society of Gynecologic Oncology (SGO) were last reviewed in 2017 [41]. For woman aged 25–29, recommended surveillance consists of clinical breast examination every 6–12 months in combination with annual radiographic screening (preferably MRI with contrast). For women ≥30 years, annual mammography and annual MRI with contrast are recommended, often alternating every 6 months. There are no specific statements regarding the use of ultrasonography, or about age limits or male carriers, in these guidelines.

3.1.6. Spanish Society of Medical Oncology. The clinical guidelines in HBOC of the hereditary cancer working group from the Spanish Society of Medical Oncology (Sociedad Espanola de Oncologia Médica, SEOM) were revised in 2019 [42]. Annual breast MRI should be proposed between 30 and 70 years, or earlier in case of family history of breast cancer before 30 years. Addition of annual mammogram should be considered from 30 years onwards and recommended between 40 and 75 years. Delaying mammography until 40 years should be discussed for *BRCA1* carriers who undergo annual MRI screening.

When MRI is unavailable, screening with mammography and ultrasound is advised between 30 and 75 years. For male *BRCA* carriers, the SEOM guidelines advise that screening mammography should be considered only in the presence of gynecomastia.

3.1.7. German Society for Gynecological Oncology. The proposed surveillance program of the German Society for Gynecological Oncology (Arbeitsgemeinschaft Gynäkologische Onkologie, AGO) is available in the latest version of the AGO breast guidelines which were last revised in 2019 [43]. Clinical breast examination is recommended semiannually for female carriers from age 25 onwards. Starting age for annual breast MRI is 25 years. Annual ultrasonography is recommended in interval between the MRI examinations from age 25 onwards. Biannual mammography is recommended starting at age 40. In upper age limit, other conditions where screening should be discontinued and recommendations for male carriers are not described.

3.1.8. French National Cancer Institute Guidelines. The guidelines on early breast and ovarian cancer detection and risk-reducing strategies for female *BRCA* carriers from the French National Cancer Institute (Institut National du Cancer, INCa) were published in 2017 [44]. In female carriers <30 years of age, annual clinical breast exam is recommended, with the addition of imaging only in case of early familial antecedents. Between age 30 and 65, annual synchronous MRI and mammogram are recommended with the addition of ultrasonography on indication, six-monthly alternating with a clinical breast exam. Specific guidance on imaging technique (e.g., single oblique incidence in conjunction with breast MRI) and radiologist requirements are described. For female carriers above age 65, annual mammography (double incidence) is recommended. Regarding the upper age limit, comorbidities and life expectancy have to be considered.

3.1.9. National Breast Cancer Council Netherlands. The breast cancer surveillance guidance for *BRCA* mutation carriers from the Dutch national breast cancer guidelines (Nationaal Borstkanker Overleg Nederland, NABON) were last revised in 2017 [45]. Annual clinical breast examination is recommended between 25 and 75 years. Interestingly, regarding breast imaging guidelines, a differentiation between *BRCA1* and *BRCA2* is made. For *BRCA1* carriers, only annual breast MRI is advised between 25 and 40 years. Between age 40 and 60, annual MRI and biannual mammogram is recommended. For *BRCA2* carriers, annual breast MRI is recommended from age 25 onwards, with the addition of annual mammogram starting at age 30. Between age 60 and age 75 annual mammogram is recommended, where in case of high breast density annual imaging with alternating MRI and mammogram should be considered, both in *BRCA1* and *BRCA2* carriers.

3.1.10. Belgian Society of Human Genetics. The Belgian guidelines for managing hereditary breast and ovarian cancer were developed in 2019 within the working group oncogenetics from the College of Genetics and Rare disease and the Belgian Society of Human Genetics (BeSHG) and are endorsed by the hereditary cancer task force of the Belgian society of Medical Oncology (BSMO) [46]. For female *BRCA* carriers, clinical breast examination is recommended every 6 months from age 25 onwards. Between age 25–35, annual breast MRI is advised. At age 30 a first baseline mammogram is recommended. In case microcalcifications are present as a possible reflection of in situ carcinoma, yearly mammogram (+/− ultrasound when indicated by the radiologist) should be recommended from age 30 onwards in situations where no treatment is indicated yet, whereas in the absence of these findings annual mammogram can be considered from 30 onwards, but is only routinely recommended from age 35. Between 35 and 65 years, both breast MRI and mammogram (+/− ultrasound) are recommended, alternating every 6 months. Between age 65 and 75, annual mammography is recommended, and MRI should only be considered in case of residual dense breast tissue or other findings on breast imaging where added value of MRI could be expected. For women >75 years, a biannual mammogram should be considered. With regard to male breast cancer, routine screening is not recommended for *BRCA1*, while for *BRCA2* annual clinical exam can be considered starting from age 40 onwards.

3.2. Ovarian, Fallopian Tube, and Primary Peritoneal Cancer. Carriers of a pathogenic *BRCA* mutation are at high risk for epithelial ovarian, fallopian tube and primary peritoneal cancer, with a cumulative risk at 80 years of 44% for *BRCA1* and 17% for *BRCA2* [10, 47]. Ovarian cancer incidence increases slowly from approximately 35 years onwards in patients with *BRCA1*-and from around 50 years onwards in *BRCA2*-mutations. In contrast to breast cancer where both prophylactic mastectomy and medical surveillance are reasonable, outcomes of epithelial ovarian cancer are poor and there are major limitations regarding early detection. Risk-reducing salpingo-oophorectomy (RRSO) provides an important reduction in ovarian and breast cancer risks and related mortality; however, the latter is less clearly demonstrated for *BRCA2* [48–50]. Therefore, all female carriers with (likely) pathogenic *BRCA* variants should be recommended to undergo risk-reducing surgery of the fallopian tubes and ovaries after completion of childbearing [37]. With regard to the timing of surgery, quality of life and age-adjusted ovarian cancer risk should be considered. In *BRCA1* carriers, RRSO is usually advised between the age of 35 to 40, after completion of childbearing. Because later onset of disease in BRCA2 mutation carriers, RRSO can be advised between the age of 40–50, however some guidelines still use the 35 lower age limit for RRSO recommendation for *BRCA2* [37, 46, 51]. Although there is some evidence regarding the safety of interval salpingectomy (with retention of the ovaries) as initial procedure with the goal to decline or delay menopause initiation, more data are needed before this can be routinely recommended. Clinical trials investigating the safety of this procedure are currently ongoing (e.g., NCT02321228) [52]. Due to this strong recommendation for risk-reducing surgery, ovarian cancer surveillance only is applicable in patients who refuse or have not yet undergone RRSO. Primary peritoneal carcinoma after RRSO has been reported mainly in *BRCA1* carriers but remains rare. Moreover this stays a controversial entity since this could possibly reflect a metastatic lesion arising from serous tubal intraepithelial carcinoma (STIC), which is a precursor lesion of high-grade serous ovarian cancer [53, 54]. Therefore, the risk of primary peritoneal carcinoma in *BRCA* carriers is not discussed in the majority of secondary prevention guidelines. Adequate pathological examination of RRSO specimens by the 'standardized sectioning and extensively examining the fimbriated end' protocol (SEE-FIM) is necessary in order to detect the presence of precancerous lesions in the fallopian tube, e.g., serous tubal intraepithelial carcinomas [55]. These lesions warrant further staging, as they were correlated with metastatic potential in sporadic ovarian cancer [54].

3.2.1. European Society for Medical Oncology Guidelines. The clinical practice guidelines for cancer prevention and screening in *BRCA* mutation carriers from ESMO emphasize the technical limitations for early detection of ovarian cancer and that there are no data proving that screening for ovarian cancer in *BRCA* carriers reduces mortality [36]. There are some promising results with serial CA125 screening, but sufficient data are unavailable [56]. Before RRSO, six-monthly transvaginal ultrasound and serial measures of serum CA125 could be considered from the age of 30. The limited data on this approach should be communicated with the patient. After RRSO, surveillance for the residual risk of peritoneal carcinoma is not recommended.

3.2.2. National Comprehensive Cancer Network Guidelines. The NCCN clinical practice guidelines in genetic high-risk assessment for breast, ovarian and pancreatic cancer state that transvaginal ultrasound combined with serum CA125 measures for ovarian cancer screening may be considered in *BRCA* mutation carriers who have not underwent elective RRSO starting at age 30 to 35. The benefit of this screening is uncertain.

3.2.3. American College of Obstetricians and Gynecologists/ Society of Gynecologic Oncology. The HBOC clinical management guidelines from the ACOG/SGO do not generally recommend routine ovarian cancer screening with measurement of serum CA125 or transvaginal ultrasonography [41]. These screening procedures have not proven to decrease mortality rate or increase survival rate associated with ovarian cancer-specific mortality. Transvaginal ultrasonography or CA125 measures are reasonable options for short-term surveillance in women at high risk of ovarian cancer, starting at age 30–35 years and continuing until they opt for RRSO.

3.2.4. American College of Radiology. The publication of the Appropriateness Criteria® for ovarian cancer screening from the American College of Radiology (ACR) dates from 2017 [57]. They state that transvaginal, transabdominal and color Doppler of the ovaries may be appropriate in premenopausal and postmenopausal *BRCA* carriers, and specify that other imaging techniques are usually not appropriate.

3.2.5. Spanish Society of Medical Oncology. The SEOM clinical guidelines in HBOC advise considering six-monthly transvaginal ultrasound and CA125 measures from the age of 30 in *BRCA1*/2 mutation carriers until the age of RRSO, as well as for those who have not elected RRSO [42].

3.2.6. French National Cancer Institute Guidelines. The French guidelines by INCa recommend annual clinical pelvic examination as screening for ovarian cancer in *BRCA1* and *BRCA2* mutation carriers [44]. Starting age is not specified.

3.2.7. National Breast and Ovarian Cancer Council Netherlands. The breast cancer surveillance guidance for *BRCA* mutation carriers from the NABON specifies that screening for ovarian cancer in *BRCA1* and *BRCA2* mutation carriers is not recommended [45].

The familial and hereditary ovarian cancer guidelines from the Dutch cancer center (Integraal Kankercentrum Nederland, IKNL) advise counseling carriers on the absence of data that supports effectivity of ovarian screening and recommend not offering ovarian screening to *BRCA* carriers [58].

3.2.8. Belgian Society of Human Genetics. The Belgian guidelines for managing HBOC do not recommend screening for ovarian cancer in *BRCA1* or *BRCA2* mutation carriers [46]. A tailored screening program could be offered from age 40, when the patient refuses RRSO.

3.2.9. Institute of Cancer Research. While the NICE guidelines do not mention the option of ovarian cancer screening, the ICR BRCA mutation carrier guidelines specify that ovarian surveillance is not recommended [40].

3.3. Pancreatic Cancer. The risk of pancreatic adenocarcinoma is increased in *BRCA2* mutation carriers, while data for *BRCA1* are conflicting [9, 14, 59]. Screening in high-risk patients, like *BRCA* mutation carriers with familial antecedents, might be beneficial given the high mortality rate of pancreatic adenocarcinoma. Data suggest that screening is able to detect earlier stages of pancreatic cancer that are still curable, in comparison to people who are diagnosed with symptomatic disease [60]. Also in pancreatic cancer, *BRCA* pathogenic variants have therapeutic implications [6]. Given the well documented correlation between smoking and pancreatic adenocarcinoma, additional counseling for smoking cessation in this regard seems to be an important primary prevention strategy in *BRCA* mutation carriers [61, 62].

3.3.1. European Society for Medical Oncology Guidelines. The ESMO clinical practice guidelines for cancer prevention and screening in *BRCA* mutation carriers state that annual screening for pancreatic cancer may be considered in *BRCA2* mutation carriers [36]. People should be informed about the very limited available data for this approach. There is no consensus about when screening should be initiated, but it is reasonable to start at age 50 or 10 years before the earliest diagnosed case of pancreatic cancer in the family. Screening consists of endoscopic ultrasound (EUS) or MRI/magnetic resonance cholangiopancreatography (MRCP). Trials evaluating the efficacy of screening techniques for pancreatic cancers should be strongly encouraged for *BRCA* carriers.

3.3.2. National Comprehensive Cancer Network Guidelines. The NCCN clinical practice guideline in genetic high-risk assessment for breast, ovarian, and pancreatic cancer does not recommend pancreatic cancer screening for *BRCA1* and *BRCA2* mutation carriers in the absence of a close family history of exocrine pancreatic cancer [37]. Pancreatic cancer screening can be considered for individuals with exocrine pancreatic cancer in one or more first- or second-degree relatives from the same side of the family as the identified pathogenic/likely pathogenic *BRCA1/2* mutation. Screening starts at the age of 50, or 10 years younger than the earliest exocrine pancreatic cancer diagnosis in the family. Screening recommendations include annual contrast-enhanced MRI/MRCP and/or EUS, with consideration of shorter screening interval when worrisome abnormalities are found. The majority of small cystic lesions found on screening will not warrant a biopsy or surgical resection. Before attending screening, people should be informed about the potential limitations to screening, including cost, high incidence of pancreatic abnormalities, and uncertainties about the potential benefits. It is recommended that pancreatic cancer screening should be performed in experienced high-volume centers under research conditions.

3.3.3. Spanish Society of Medical Oncology. The SEOM clinical guidelines in HBOC propose to consider pancreatic cancer surveillance with EUS and MRI in carriers with FDG with pancreatic cancer from the age of 50, or 10 years before the youngest diagnosis in the family [42].

3.3.4. Belgian Society of Human Genetics. The BeSHG guidelines propose to discuss the arguments in favor of and against pancreatic cancer screening with *BRCA1* carriers if they have ≥1 first-degree relative with pancreatic cancer and with *BRCA2* carriers if they have ≥1 first-degree or ≥2 second-degree relatives with pancreatic cancer [46]. This should preferably be performed in the context of a clinical trial. Regarding the starting age and screening modality, they refer to the recommendation from the International Cancer of the Pancreas Screening Consortium [59]. Recommended

starting age for *BRCA* carriers with familial antecedents as described above is 50 years, unless there is a first-degree relative with pancreatic cancer onset <50 years. Surveillance for pancreatic cancer should consist of MRI/MRCP and an EUS combined with fasting blood glucose and/or HbA1c. Annual blood sugar tests and imaging are recommended; however, there is no consensus as to whether and how to alternate MRI/MRCP and EUS. Serum CA 19-9 is not routinely recommended.

3.4. Prostate Cancer. The link between an elevated risk of prostate cancer and germline *BRCA* pathogenic variants has been well established, with the strongest association for *BRCA2* [63, 64]. Prostate cancer in germline *BRCA2* carriers appears to occur at an earlier age; has a more aggressive phenotype, a higher risk of nodal involvement, and distant metastasis; and is associated with a poor survival outcome in comparison to noncarriers. In advanced castration-resistant prostate cancer, *BRCA* status can have therapeutic implications regarding the use of platinum agents and PARP inhibitors [65, 66].

3.4.1. European Society for Medical Oncology Guidelines. The ESMO clinical practice guidelines state that annual screening for prostate cancer may be considered from age 40 onwards, particularly for *BRCA2* mutation carriers [36]. The optimal duration of screening is not specified but should be tailored to an individual's family history of prostate cancer.

3.4.2. National Comprehensive Cancer Network Guidelines. The NCCN clinical practice guidelines in genetic high-risk assessment for breast, ovarian, and pancreatic cancer refer to the NCCN prostate cancer early detection guidelines for prostate cancer screening in *BRCA* carriers [37, 67]. Prostate cancer screening in *BRCA2* mutation carriers is recommended starting at the age of 40, whereas in *BRCA1* mutation, carriers screening should be considered from the age of 40 onwards. Shared decision making is recommended. In men older than 75 years, prostate cancer screening should be considered in selected patients only. The NCCN prostate cancer early detection guidelines specify yearly screening for PSA. Digital rectal examination (DRE) should not be used as a stand-alone test but may be considered as baseline test and as follow-up exam as it may identify high-grade cancers associated with low serum PSA values. It should be performed in carriers with an elevated serum PSA. Referral for biopsy should be considered if DRE is very suspicious.

3.4.3. Spanish Society of Medical Oncology. The hereditary cancer working group from SEOM recommends prostate cancer screening with annual serum PSA measurements in male *BRCA2* carriers starting at age 40, while this screening approach can also be offered to *BRCA1* carriers [42].

3.4.4. Belgian Society of Human Genetics. The Belgian Society of Human Genetics recommends annual prostate cancer screening with serum PSA and DRE for male *BRCA1* and *BRCA2* mutation carriers from the age of 40 onwards [46].

3.5. Colorectal and Gastric Cancer. Data about a possible relationship between gastric and colorectal cancer (CRC) and germline *BRCA* pathogenic variants are conflicting. A large prospective study on 7015 women with *BRCA* alterations showed a significant increased risk for CRC in women younger than 50 years with a *BRCA1* mutation, but not in older *BRCA1* carriers or in *BRCA2* carriers [68, 69]. A systematic review and meta-analysis confirmed the differential effect between *BRCA1* and *BRCA2* (odds ratio [OR] 1.49 [95% CI 1.19–1.85] for *BRCA1*, not significant [OR 1.1; 95% CI 0.77–1.58] for *BRCA2*), but could not validate the age effect [19]. Regarding a possible relationship with gastric cancer, there is only weak evidence for a correlation with germline *BRCA* mutations and gastric cancer; anecdotal findings have not been confirmed in larger series [8, 70, 71]. These recent findings warrant increased attention to familial CRC and possibly gastric cancer antecedents and the need for individualized surveillance in *BRCA* carriers. The majority of guidelines do not mention the possible increased risk for digestive tract cancer.

3.5.1. European Society for Medical Oncology Guidelines. The ESMO clinical practice guidelines state that the association between *BRCA* mutation carriers and an elevated risk of colorectal and gastric cancer is weak [36]. Therefore, screening is generally not indicated. Recommendations should be tailored to an individual's familial history.

3.5.2. Spanish Society of Medical Oncology. The clinical guidelines in HBOC of the hereditary cancer working group from SEOM point towards the controversial results on the association of *BRCA1/2-mutations* and colorectal cancer and towards the possible differences between *BRCA1* and *BRCA2,* but latest version of these guidelines does not mention colorectal cancer surveillance, while specific recommendations for *BRCA1* were reported in the previous version [42, 51].

3.5.3. Belgian Society of Human Genetics. The BeSHG guidelines for managing HBOC indicate that *BRCA1* mutation carriers have an increased risk of early-onset colorectal cancer (diagnosis <50 years), but the increase is small. Screening for colorectal cancer is not recommended for this elevated risk besides the national population screening program independent of *BRCA* status which offers biannual fecal blood test between 50 and 74 years of age in absence of familial history [46]. Also here, a possible correlation with elevated gastric cancer risk is not mentioned.

3.6. Endometrial Cancer. Some data suggest a slightly increased risk of endometrial cancer in *BRCA* carriers, with more evidence for a correlation with *BRCA1* and then with

BRCA2; however, the risk is not clearly defined. Several reports showed that tamoxifen use for previous breast cancer is an important confounding factor in the earlier observed correlations between endometrial cancer and germline *BRCA* mutations [72, 73]. A prospective cohort study analyzing the risk of endometrial cancer after RRSO in 1083 *BRCA* carriers showed no significant increase of endometrial cancer overall, but a higher than expected risk of serous endometrial carcinoma in *BRCA1* mutation carriers (however, only 4 cases were described in 453 *BRCA1* carriers after a median follow-up of 5.1 years), while the risk for endometrioid endometrial cancer or uterine sarcoma was not increased in this study [20]. Another cohort study on 828 carriers could not confirm the correlation with serous endometrial cancer. Overall, no significant correlation with endometrial cancer was demonstrated, but there was a possible trend for the endometrioid subtype [74]. Based on these findings, some guidelines advise discussing these risk uncertainties and the risks and benefits of concurrent hysterectomy at the time of RRSO in female *BRCA1* carriers [37]. However, the majority of guidelines do not recommend considering hysterectomy for the presumed increased risk of endometrial cancer. In female *BRCA* carriers who have opted for breast surveillance instead of risk-reducing mastectomy, there is more data on safety with regard to breast cancer risk of estrogen-only hormonal substitution compared to combined estrogen-progesterone substitution after RRSO [75]. With regard to endometrial cancer risk, however, estrogen-only substitution is not a safe option when no hysterectomy has been performed, making this an additional factor to be considered in these discussions [76].

3.6.1. European Society for Medical Oncology Guidelines. The ESMO clinical practice guidelines for cancer prevention and screening in *BRCA* mutation carriers report that the association between *BRCA1*/*BRCA2* mutations and an elevated risk of endometrial cancer remains weak [36]. They conclude that screening for and prevention of endometrial cancer are generally not indicated. Recommendations should be tailored to an individual's familial history.

3.6.2. National Comprehensive Cancer Network Guidelines. The NCCN guidelines state that there is limited data suggesting there might be a slightly increased risk of serous endometrial cancer among women with a *BRCA1* pathogenic or likely pathogenic variant [37]. The clinical significance is unclear. There is no guidance with regard to screening or prevention. Further evaluation of the risk of serous endometrial cancer in the *BRCA* population needs to be undertaken.

3.6.3. Belgian Society of Human Genetics. Surveillance and prevention of endometrial cancer in *BRCA1* mutation carriers are not advised by the Belgian Society of Human Genetics, because the cumulative risk of serous endometrial cancer is less than 5% at 70 years of age. The risk in *BRCA2* mutation carriers is described as equal to a population without germline *BRCA* pathogenic variants.

3.7. Melanoma. Literature suggests a possible association between germline *BRCA2* pathogenic variants and an elevated risk for melanoma. This possible link has been suggested for both cutaneous and ocular melanoma in *BRCA2*, but data are conflicting and mainly based on small studies at risk for sampling bias [9, 15, 21, 77]. Overall there seems to be insufficient evidence for a clear correlation between skin and uveal melanoma and germline *BRCA* pathogenic variants. However, increased awareness of familial history and preventive measurements in *BRCA* carriers seems reasonable.

3.7.1. European Society for Medical Oncology Guidelines. The ESMO guidelines demonstrate that there is no evidence-based data with regard to screening for melanoma [36]. They advise considering annual skin and eye examination as screening for melanoma in all *BRCA2* carriers. Screening should be tailored to the individual's family history.

3.7.2. National Comprehensive Cancer Network Guidelines. The NCCN guidelines of genetic high-risk assessment for breast, ovarian, and pancreatic cancer state that no specific screening guidelines exist for melanoma, but general melanoma risk management with education regarding clinical signs, minimizing UV exposure, and annual full-body skin examination with the addition of an eye exam should be considered for both *BRCA1* and *BRCA2* mutation carriers with a pathogenic or likely pathogenic mutation [37]. An individualized screening approach based on personal and family history of cancer may be provided.

3.7.3. Spanish Society of Medical Oncology. In the SEOM clinical guidelines in HBOC, screening for melanoma with a skin and eye examination should be considered according to personal and familial risk factors [42]. It is not specified if this applies to *BRCA1* and/or *BRCA2* mutation carriers.

4. Discussion

This review demonstrates that there are major differences in national and international guidelines on early detection of and screening for *BRCA*-related cancers in *BRCA* carriers. These differences are triggered by temporal evolution in risk assessments, discordances in literature and interpretation, assessment of the advantages and disadvantages of screening, cost-benefit analyses, and absence of high levels of evidence. As the case for cancer screening in the general population, different thresholds and risk/benefit analyses are used by different societies publishing guidelines for HBOC. More harmonized guidelines could be relevant from a clinical perspective, but this is hard to implement at a global level for the reasons stated above. However, harmonization efforts by translation of international guidelines into the local context in regional or national guidelines can be of high

value to avoid differences in counseling and risk management advice.

In general, guidelines are more concordant for *BRCA*-related cancers in situations where the age-specific risks for this cancer type are more extensively studied, while there is more discordance in other *BRCA*-related cancers. However, also in breast cancer, there are differences in screening modalities, thresholds, and frequency and duration of screening. The majority of guidelines recommend starting imaging surveillance in female carriers from age 25 onwards and also consider screening for all untested first-degree relatives of *BRCA* carriers [34, 36, 37]. Only occasionally, a differentiation between *BRCA1* and *BRCA2* carriers is made with a trend to start later or decrease mammography frequency in *BRCA1* compared to *BRCA2* carriers, probably based on the possible higher likelihood of microcalcifications as a reflection of in situ carcinoma in ER-positive breast cancers which are enriched in *BRCA2* [42, 45]. However, this differential correlation with in situ carcinoma has not been confirmed [78]. The age of onset of mammographic surveillance varies significantly between the different guidelines as described above. There seems to be a potential of adding digital breast tomosynthesis to the imaging surveillance for breast cancer in some women given the higher sensitivity and specificity compared to routine mammogram and possibly decreasing false positive findings of standard mammography, and some guidelines already describe this option [34, 37]. Individualization of starting age based on family history is recommended in the majority of guidelines. In the concept of shared decision making, patient preference is a very important consideration in the discussion of breast cancer surveillance strategies and risk-reducing options. There are still a lot of open questions regarding optimal breast cancer screening in *BRCA* carriers, e.g., recommended surveillance when MRI is not possible/unavailable, optimal age to discontinue surveillance, value of ultrasonography, and value of alternating versus concomitant imaging when 2 modalities are combined. These and other questions stress the importance of ongoing and future studies.

In contrast to RRSO, there is no evidence that screening for ovarian cancer in *BRCA* carriers reduces mortality. RRSO should be recommended for all *BRCA* mutation carriers, with important differences in age recommendations for RRSO between different guidelines. Some guidelines consider screening for ovarian cancer in people refusing RRSO. Others also consider surveillance in *BRCA* carriers before RRSO is performed. If this is considered, it is of utmost importance that patients are informed that there is no proven benefit of screening with serial CA125 measurements and transvaginal ultrasonography.

All the four guidelines that covered pancreatic carcinoma considered screening only in the presence of a positive familial history and after proper counseling of advantages and disadvantages of pancreatic cancer screening. There is no consensus as to whether it should be proposed to *BRCA2* carriers only or both, or about the screening modality or the age when screening should start. Because it is unknown if pancreatic cancer screening impacts overall survival, it is preferred to perform pancreatic cancer screening in the context of clinical trials and in high-volume centers.

The link between *BRCA* mutation carriers and prostate cancer has been well established. Screening is recommended by the NCCN, SEOM, and BeSHG guidelines, while the ESMO guidelines consider it [36, 37, 46, 51]. Differentiation is made between *BRCA1* and *BRCA2* carriers based on the higher penetrance of prostate cancer in male *BRCA2* carriers [67]. Most guidelines recommend PSA and DRE as screening methods, but optimal duration is not properly addressed.

Because there is less evidence about an association between *BRCA* pathogenic variants and colorectal, endometrial, skin, and gastric cancer, the majority of guidelines do not recommend systematic screening. Raised awareness and careful incorporation of familial history to individualize primary and secondary prevention for these cancer types seem appropriate. Further investigation of these cancer risks in *BRCA* carriers and evaluation of surveillance methods in clinical trials are warranted.

5. Conclusion

There are major differences between available guidelines for cancer surveillance in germline *BRCA* mutation carriers. Optimal surveillance strategies depend on accurate age-specific cancer risk estimates, which are reliably estimated for breast and ovarian cancer but not for other *BRCA*-related cancers. Up-to-date national or international consensus guidelines are of utmost importance to harmonize counseling and proposed surveillance strategies for *BRCA1/2* carriers. Improving awareness of carriers and primary care physicians together with shared decision making is a key aspect of cancer surveillance in *BRCA* carriers. Possible benefits of screening and risk-reducing strategies should always be discussed in combination with possible risks and limitations of these surveillance strategies.

Conflicts of Interest

RdP receives honoraria for advisory board for Astra Zeneca. ML has the advisory role for Roche and receives speaker honoraria from Theramex, Takeda, Roche, and Lilly. AT has the advisory role for Lilly and Roche. CVO, MK, and RP authorize the advisory role for the Belgian Society of Human Genetics guidelines. WE receives honoraria for consulting and speaker fees from Janssen, Astellas, and Bayer. He is a senior clinical researcher of FWO Flanders. HW receives consulting fees and honoraria from Abbvie, Amgen, Ariez International, Astra Zeneca, Biocartes, Celldex Therapeutics, DNA Prime, Janssen-CILAG, Lilly, Novartis, ORION Corporation, Pfizer, The Planning Shop, PUMA Biotechnology, Roche, Sirtex, TRM Oncology, and Vifor Pharma. He also receives travel support from Roche, Pfizer, Nippon Travel Agency, Congress Care, DNA Prime, and Global Teamwork. PN receives honoraria for consultancy and/or advisory roles from Pfizer, Novartis, Eli Lilly, and Roche. He receives research funding from Kom op Tegen Kanker. RdP, ED, GVB, and EL are the co-authors of the Belgian Society of

Human Genetics guidelines. KP has the advisory/consultancy role for Astra Zeneca, Eli Lilly, Novartis, Pfizer, Pierre Fabre, Roche, and Vifor Pharma. He also receives speaker fees for Eli Lilly, Mundi Pharma, Novartis, Pfizer, and Roche and receives research funding from Sanofi. He obtains travel support from Astra Zeneca, Novartis, Pfizer, Pharma Mar, and Roche. The other authors declare that there are no conflicts of interest.

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Research Article

A Novel Nomogram including AJCC Stages Could Better Predict Survival for NSCLC Patients Who Underwent Surgery: A Large Population-Based Study

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Objective. In this study, we aimed to establish a novel nomogram model which was better than the current American Joint Committee on Cancer (AJCC) stage to predict survival for non-small-cell lung cancer (NSCLC) patients who underwent surgery. *Patients and Methods*. 19617 patients with initially diagnosed NSCLC were screened from Surveillance Epidemiology and End Results (SEER) database between 2010 and 2015. These patients were randomly divided into two groups including the training cohort and the validation cohort. The Cox proportional hazard model was used to analyze the influence of different variables on overall survival (OS). Then, using R software version 3.4.3, we constructed a nomogram and a risk classification system combined with some clinical parameters. We visualized the regression equation by nomogram after obtaining the regression coefficient in multivariate analysis. The concordance index (C-index) and calibration curve were used to perform the validation of nomogram. Receiver operating characteristic (ROC) curves were used to evaluate the clinical utility of the nomogram. *Results*. Univariate and multivariate analyses demonstrated that seven factors including age, sex, stage, histology, surgery, and positive lymph nodes (all, $P < 0.001$) were independent predictors of OS. Among them, stage (C-index = 0.615), positive lymph nodes (C-index = 0.574), histology (C-index = 0.566), age (C-index = 0.563), and sex (C-index = 0.562) had a relatively strong ability to predict the OS. Based on these factors, we established and validated the predictive model by nomogram. The calibration curves showed good consistency between the actual OS and predicted OS. And the decision curves showed great clinical usefulness of the nomogram. Then, we built a risk classification system and divided NSCLC patients into two groups including high-risk group and low-risk group. The Kaplan–Meier curves revealed that OS in the two groups was accurately differentiated in the training cohort ($P < 0.001$). And then, we validated this result in the validation cohort which also showed that patients in the high-risk group had worse survival than those in the low-risk group. *Conclusion*. The results proved that the nomogram model had better performance to predict survival for NSCLC patients who underwent surgery than AJCC stage. These tools may be helpful for clinicians to evaluate prognostic indicators of patients undergoing operation.

1. Introduction

NSCLC accounts for about 85% of all lung cancer, which remains the leading cause of cancer-related death in the world [1, 2]. In recent years, with the wide application of high-resolution spiral computed tomography (CT) screening technology, the detection rate of early lung cancer has increased significantly [3]. Surgery treatment is the first choice for patients diagnosed with early NSCLC, including stage I, stage II, and partial stage III cases. [4] The current treatment options for NSCLC mainly depend on the eighth edition of the American Joint Committee on Cancer TNM staging. However, patients' survival rate varies greatly at the same stage [5–7]. The 5-year survival rates range from 60% of stage I to about 30% of stage IIIA [8,9]. And patients with the same stage showed different rates of survival. It is of great significance in guiding clinical treatment to find independent prognostic factors. Previous studies [5–7] have reported that some factors may significantly promote the survival prediction of patients, such as age, race, sex, stage, and histology.

Nomogram is a convenient tool to predict and quantify risk for patients' prognosis by incorporating and validating some relevant factors. In some other types of tumors, nomograms that calculate numerical probability of clinical events, such as cancer-specific survival (CSS) and OS, have shown more precise prediction than the traditional TNM staging systems. At present, AJCC TNM staging is the main criterion to guide the treatment and prognosis of NSCLC patients. However, the staging could not be good to predict the survival for these patients. Other variables including age, sex, and histology may be significant independent prognostic factors for NSCLC patients. Therefore, the combination of AJCC staging and these variables may be better to predict the outcomes and it would be better in clinical guidance.

Therefore, in the present study, we built and validated the nomogram combined with several clinical variables to predict prognosis for patients with NSCLC who underwent surgery.

2. Materials and Methods

2.1. Data Source. The SEER Program (http://www.seer.cancer.gov) consists of 9 Regs Research Data in the United States [10]. Information for patients with stages I–III NSCLC between 2010 and 2015 was extracted from the SEER database. According to the AJCC criteria, we selected a total of 19617 patients diagnosed with NSCLC using the SEER*Stat 8.3.5 software. The inclusion criteria for recruiting patients were as follows: NSCLC patients, only one malignant primary lesion, available clinical information, and active follow-up. The exclusion criteria were patients with benign tumor. In addition, patients containing any missing information on extracted data were all excluded.

2.2. Ethics Statement. Our study was constructed in accordance with the Helsinki Declaration. This study was also approved by the ethics committee of the Shandong Cancer Hospital. This study did not involve any personal information, and therefore, informed patient consent was not required.

2.3. Statistical Analysis. These eligible patients were randomly divided into the training cohort (70%, $n = 13732$) and the validation cohort (30%, $n = 5885$) to establish and validate the nomogram. The OS was defined as the time from diagnosis to death due to any reason. The data in training cohort were used to develop the prediction model and construct nomogram and risk classification system. Furthermore, the data of the validation cohort were used to make a validation.

Univariate and multivariate analyses were used to determine independent prognostic variables. And then, based on these variables contained in the final model, we built the nomogram and the risk classification system. The C-index was used to determine discrimination ability of the nomogram, and each parameter and ROC curves were used to evaluate the clinical utility of the nomogram. The calibration for 1-, 3-, and 5-year OS was evaluated using a calibration curve by comparing the predicted survival and the observed survival. Furthermore, based on the total score of each patient in the validation cohort, the risk classification system was established and all patients were divided into low-risk and high-risk prognosis groups. The OS was estimated using the Kaplan–Meier method and compared by the log-rank test.

All statistical analyses were made using R software version 3.4.3 (R Foundation) and Statistical Product Service Solutions (SPSS) 22.0 software package. All statistical P values were 2-sided, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Patients Characteristics. A total of 19617 patients initially diagnosed with NSCLC from the SEER database were included for analysis. All enrolled patients were randomly divided into the training cohort (13732, 70%) and the validation cohort (5885, 30%). According to age, all patients were divided into four groups including <60 years old ($n = 4203$, 21.4%), 60–69 years old ($n = 7054$, 36.0%), 70–79 years old ($n = 6588$, 33.6%), and >80 years old ($n = 1772$, 33.6%). In the total cohort, training cohort, and validation cohort, the proportion of patients aged 60–69 (36.0%, 36.1% and 35.6, respectively) was the largest. The majority of cases were white ($n = 16312$, 83.2%). Male and female patients accounted for the same proportion (50% vs. 50%).

According to the AJCC stage, patients of stage T1 were the largest in the total cohort, training cohort, and validation cohort (58.8%, 58.6%, and 59.4 respectively), followed by the T2 stage (23.3%, 23.5%, and 22.9%, respectively). And patients with stage T3 was the least in the total cohort, training cohort, and validation cohort (17.9%, 17.9%, and 17.7%, respectively). 12278 (62.6%) patients had adenocarcinoma

and 7336 (37.4%) had squamous. 5.6% of patients underwent complete surgical resection, and 94.4% of patients underwent partial surgical resection. Of these patients, only 24.5% patients had positive lymph nodes. Baseline clinicopathological characteristics of all patients in the training cohort and the validation cohort are shown in Table 1.

3.2. Independent Prognostic Factors in Predicting OS. Univariate and multivariate Cox proportional hazards regression models were used to assess each factor's ability in predicting OS. In univariate analysis, we found that age ($P < 0.001$), race ($P < 0.001$), sex ($P = 0.03$), stage ($P < 0.001$), histology ($P < 0.001$), surgery ($P < 0.001$), and positive lymph nodes ($P < 0.001$) were associated with OS in patients with stages I–III NSCLC. Among them, stage (C-index = 0.615), positive lymph nodes (C-index = 0.574), histology (C-index = 0.566), age (C-index = 0.563), and sex (C-index = 0.562) had superior discrimination power in predicting OS compared with other variables. Multivariate analysis further analyzed the factors of a $P < 0.05$ in univariate analysis. In the multivariate analysis, we found that age ($P < 0.001$), other races ($P < 0.001$), sex ($P < 0.001$), stage ($P < 0.001$), histology ($P < 0.001$), surgery ($P < 0.001$), and positive lymph nodes ($P < 0.001$) were independent prognostic factors and were incorporated into the predictive model. Univariate and multivariate analyses of each factor's ability in predicting OS are shown in Table 2.

3.3. Building and Validating the Predictive Nomogram. We built a novel nomogram that included the significant and independent prognostic factors (Figure 1). Each factor had a score on the point scale. We can draw a straight line to determine the estimated probability of prognosis at each time point by adding up the total score and locating it on the total point scale. And then, the validation cohort was used to verify the novel nomogram. In the validation cohort, we compared the OS rate predicted by the nomogram with observed 1-, 3-, and 5-year OS rates.

In a well-calibrated model, the prediction will fall on a 45-degree diagonal line. From Figure 2, the calibration curves revealed good consistency between the actual observation and the nomogram prediction for 1-, 3-, and 5-year survival rates. Figure 2(a) shows good consistency between the actual 1-year overall survival and predicted 1-year overall survival. And the ROC curve revealed that the area under the curve (AUC) is 0.701. Figure 2(b) shows good consistency between the actual 3-year overall survival and predicted 3-year overall survival. And the ROC curve revealed that the AUC is 0.687. Figure 2(c) shows good consistency between the actual 5-year overall survival and predicted 5-year overall survival. And the ROC curve revealed that the AUC is 0.669.

In addition, decision curves exhibited great positive net benefits in the predictive model among almost all of the threshold probabilities at different time points, indicating the favorable potential clinical effect of the predictive model (Figures 3(a) and 3(b)).

3.4. Risk Classification System. According to the total scores of every patient, we also developed a risk classification system in the training cohort generated by nomogram. All patients in the training cohort and validation cohort were divided into the high-risk and low-risk groups. The Kaplan–Meier curve was used to draw the OS curves for the high-risk group and low-risk group in the training cohort and validation cohort. In the training cohort, the Kaplan–Meier curves revealed that patients' survival in the low-risk group was better than that in the high-risk group ($P < 0.001$) (Figure 4(a)). Then, we validated it in the validation cohort. Similarly, patients in the low-risk group had better survival than those in the high-risk group ($P < 0.001$) (Figure 4(b)).

4. Discussion

In this study, we established and developed a nomogram and a risk classification to predict the OS of patients with stages I–III NSCLC after surgery using the data originated from SEER database. A total of 19167 patients were included, and seven significant prognosis factors including age, race, sex, stage, histology, surgery, and positive nodes were identified. And these predictive factors could be easily obtained from clinical practice. Then, we established the validation of model and used different statistical methods to demonstrate its great performance.

Over time, the prospects for lung cancer patients and treatment have changed. Lung lobectomy is often considered the best treatment option for stages I, II, and partial III NSCLC patients [7,8,11]. Recurrence and metastasis have become important factors affecting the 5-year survival rate of patients with lung cancer after operation. So, it is very important to predict factors of survival after surgery in NSCLC patients. Furthermore, NSCLC has significant heterogeneity in individual survival, and it is inaccurate to use the TNM staging system to predict survival. Although several prognostic models have been reported previously [6,12], a relevant nomogram was rarely developed to predict prognostic variables for patients NSCLC after surgery.

Some research studies [13–18] reported that a nomogram could predict the prognosis of NSCLC patients. However, most studies focused on patients with early or advanced NSCLC. Nonetheless, both research studies had a small sample size which may inhibit their generalization.

Liang et al. [19] showed that the C-index for the established nomogram to predict OS was 0.71 in the primary cohort and 0.67 in the IASLC cohort. Sun et al. [13] showed that the C-index of the nomogram was 0.638 which exhibited a sufficient level of discrimination. However, in our study, the C-index of the nomogram is higher than that of other previous models. In addition to a nomogram, we also developed a risk classification system and the risk classification divided the whole NSCLC patients into two distinct prognostic groups which could supplement the nomogram in our study.

In univariable and subsequent multivariable analysis, we identified age, race, sex, stage, histology, surgery types, and positive lymph nodes as independent prognostic factors.

Table 1: Baseline clinicopathological characteristics of all patients and those in the training and validation cohorts.

Variables	All cohort ($n = 19617$)	Training cohort ($n = 13732$)	Validation cohort ($n = 5885$)	P
Age				0.026
<60	4203(21.4)	4958 (36.1)	1302 (22.1)	
60–69	7054(36.0)		2096 (35.6)	
70–79	6588(33.6)	4619 (33.6)	1969 (33.5)	
>80	1772(9.0)	1254 (9.1)	518 (8.8)	
Race				0.019
White	16312(83.2)	11445 (83.3)	4867 (82.7)	
Black	1814(9.2)	1262 (9.2)	552 (9.4)	
Others	1491(7.6)	1025 (7.5)	466 (7.9)	
Sex				0.013
Male	9807(50.0)	6839 (49.8)	2968 (50.4)	
Female	9810(50.0)	6893 (50.2)	2917 (49.6)	
Stage				0.017
I	11543(58.8)	8047 (58.6)	3496 (59.4)	
II	4572(23.3)	3226 (23.5)	1346 (22.9)	
III	3502(17.9)	2459 (17.9)	1043 (17.7)	
Histology				0.009
Adenocarcinoma	12278(62.6)	8579 (62.5)	3702 (62.9)	
Squamous	7336(37.4)	5153 (37.5)	2183 (37.1)	
Surgery				0.014
Complete resection	1092(5.6)	778 (5.7)	314 (5.3)	
Partial resection	18525(94.4)	12954 (94.3)	5571 (94.7)	
Positive nodes				0.005
Yes	4812(24.5)	3360 (24.5)	1452 (24.7)	
No	14805(75.5)	10372 (75.5)	4433 (75.3)	

Table 2: Univariate and multivariate analyses of each factor's ability in predicting OS.

	Univariate analyses				Multivariate analyses		
Variable	HR	95% CI	P	C-index	HR	95% CI	P
Age				0.563			
<60	Reference				Reference		
60–69	1.110	1.010–1.220	0.038		1.174	1.065–1.294	0.001
70–79	1.430	1.300–1.570	<0.001		1.604	1.455–1.768	<0.001
>80	2.00	1.780–2.260	<0.001		2.367	2.095–2.674	<0.001
Race				0.516			
White	Reference				Reference		
Black	0.913	0.813–1.025	0.120		1.022	0.909–1.148	0.717
Others	0.748	0.649–0.863	<0.001		0.777	0.673–0.897	<0.001
Sex				0.562			
Male	Reference				Reference		
Female	0.649	0.607–0.694	0.030	<0.001	0.714	0.667–0.764	<0.001
Stage				0.615			
I	Reference				Reference		
II	2.100	1.940–2.270	<0.001		1.832	1.672–2.006	<0.001
III	2.610	2.410–2.830	<0.001		2.287	2.047–2.554	<0.001
Histology				0.566			
Adenocarcinoma	Reference				Reference		
Squamous	1.570	1.470–1.6770	<0.001		1.325	1.237–1.420	<0.001
Surgery				0.528			
Complete resection	Reference				Reference		
Partial resection	1.990	1.780–2.230	<0.001		1.297	1.150–1.462	<0.001
Positive nodes			<0.001	0.574			
Yes	Reference				Reference		
No	2.030	1.900–2.170			1.183	1.077–1.299	<0.001

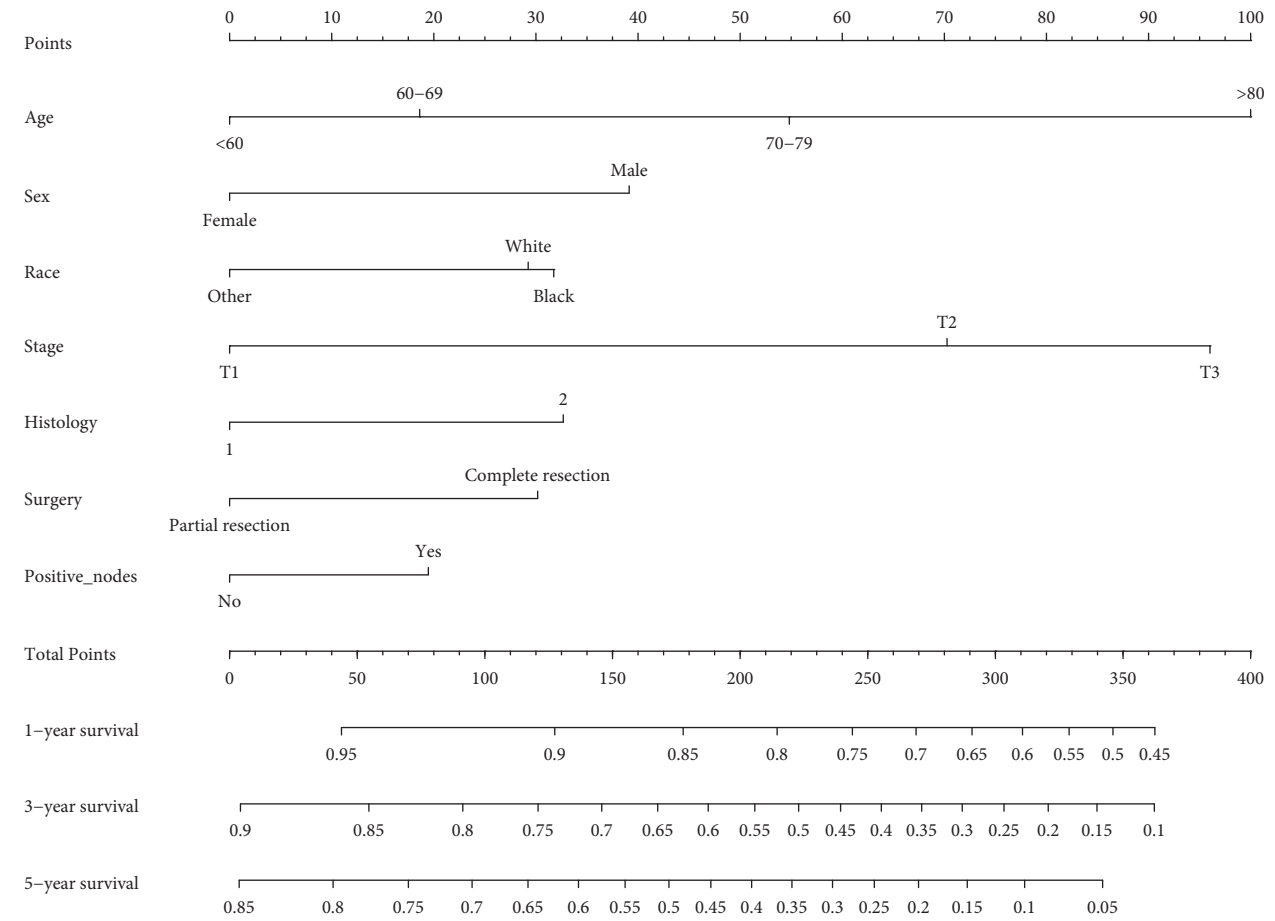

Figure 1: A nomogram for prediction of 1-, 3-, and 5-year OS rates of stages I–III NSCLC patients after surgery.

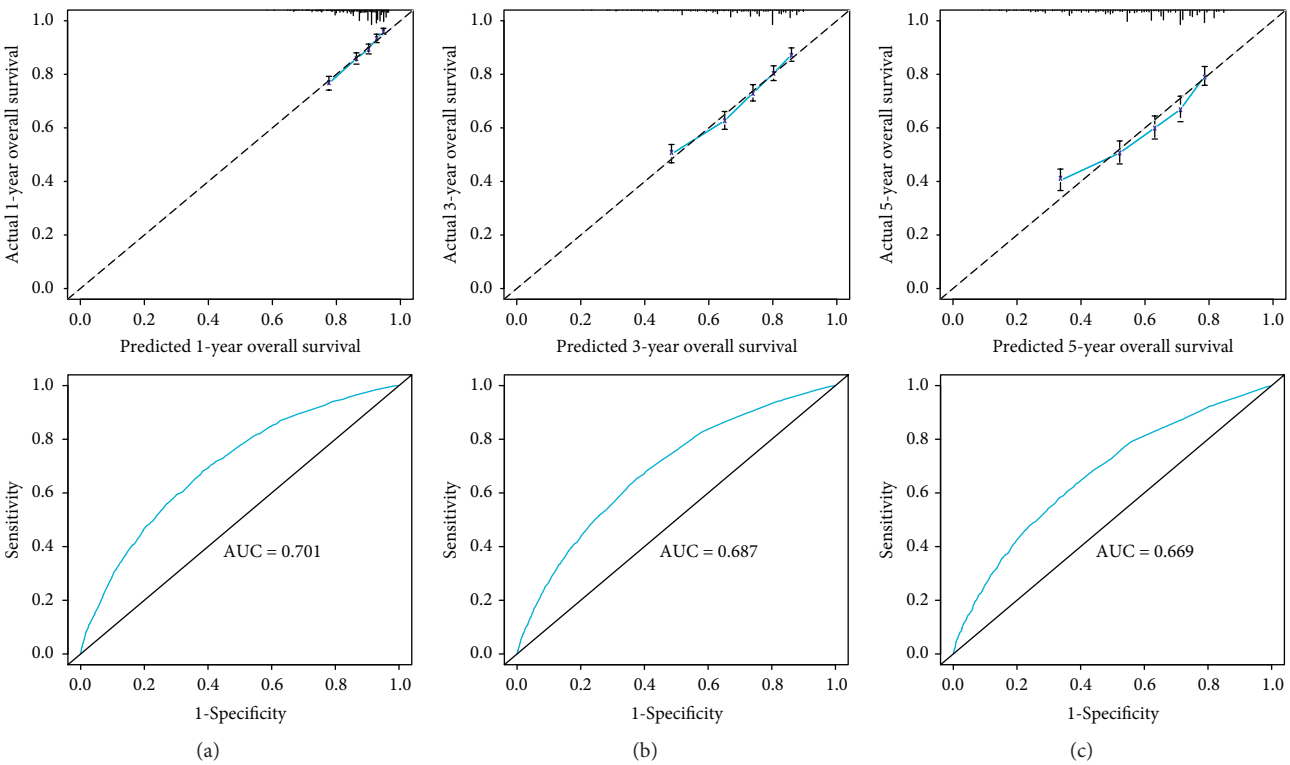

Figure 2: Calibration curves of the nomogram predicting 1-year, 3-year, and 5-year OS rates of stages I–III NSCLC patients after surgery. On the calibration plot, the *x*-axis is nomogram-predicted probability of over survival. The *y*-axis is the actual over survival.

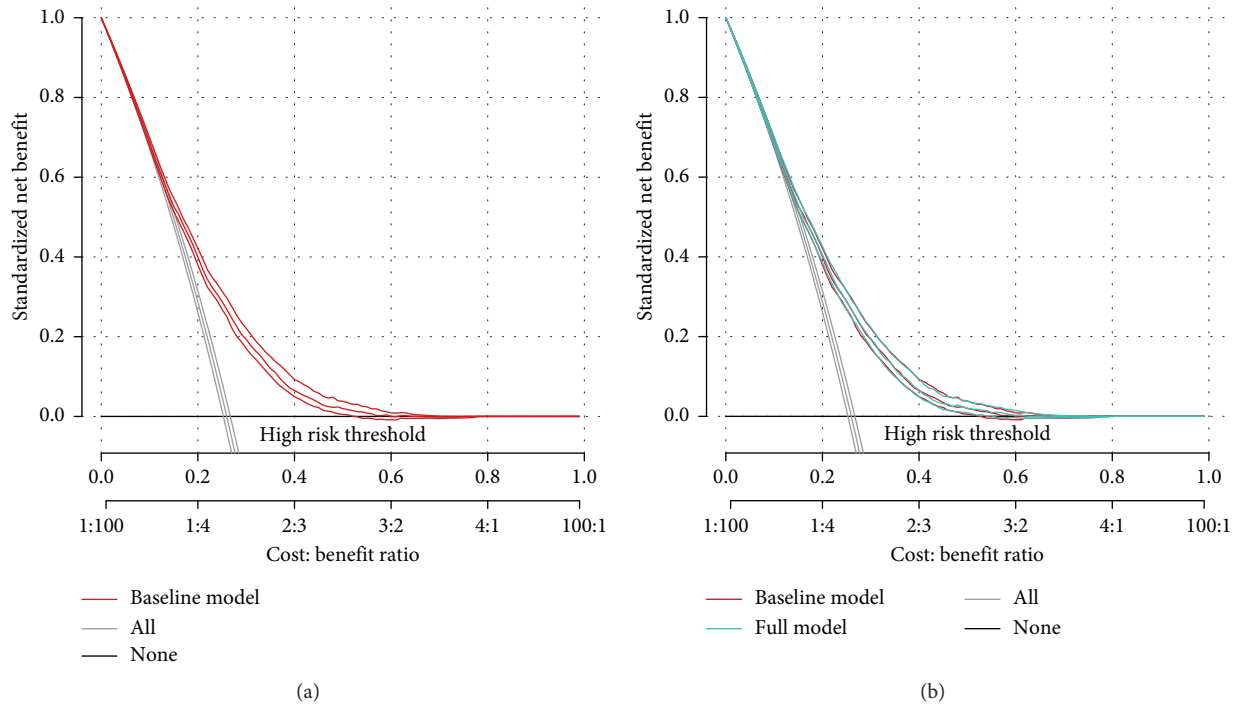

Figure 3: Decision curves of the nomogram predicting OS. The x-axis represents the threshold probabilities, and the y-axis measures the net benefit calculated by adding the true positives and subtracting the false positives.

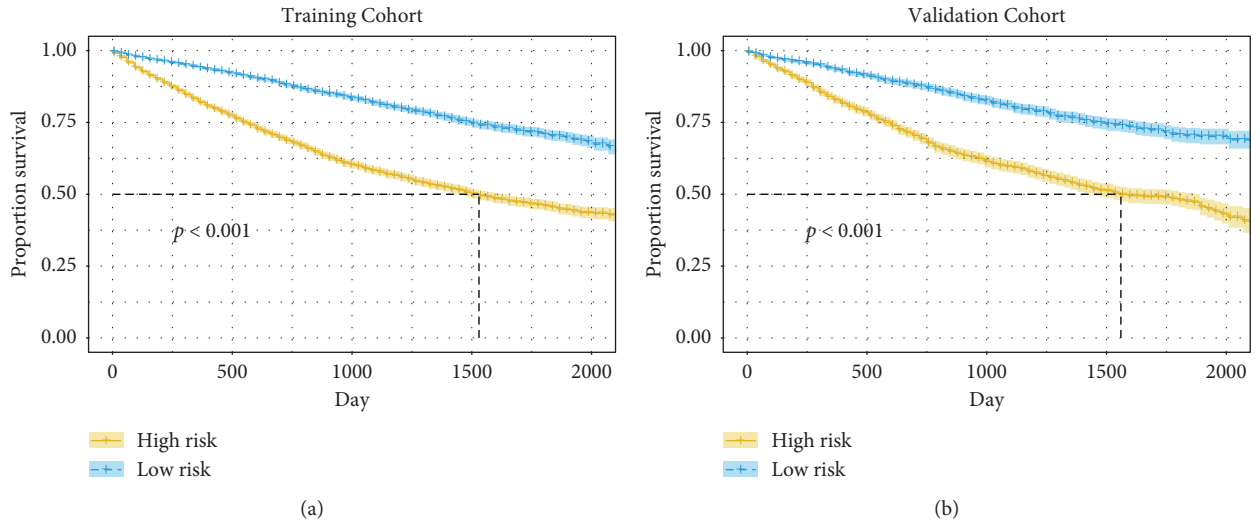

Figure 4: Kaplan–Meier curves of OS for patients in the low- and high-risk groups. (a) Kaplan–Meier curves of OS for patients in the low- and high-risk groups in the training cohort. (b) Kaplan–Meier curves of OS for patients in the low- and high-risk groups in the validation cohort.

These findings are consistent with previous reports on risk factors for non-small-cell lung cancer [7,8,20]. It is necessary to validate the nomogram and avoid excessive fitting of the model and determine the extensibility [11]. Notably, according to our nomogram, stage is the most powerful predictor of OS, and C-index (C-index = 0.615) was the highest among all predictors. One of the possible reasons is that TNM staging is the current important tool to make decision about the stage-specific therapeutic strategy and assess the prognostic survival [21]. However, in the present study, we did not divide these stages into specific T and N category, which were reported as the significant and independent factors in other research studies. We need future studies to assess each factor of stage which may impact on survival for patients with resected NSCLC.

In addition, positive lymph node was another important predictor for OS and the C-index was 0.574. Several research studies [22,23] reported the relationship between

positive lymph nodes and survival. The reason may be that with more positive lymph nodes being cleared out, potential metastatic lymph nodes will be removed. For patients with resected NSCLC, the number of positive lymph nodes was also demonstrated as an important prognostic factor [24,25]. And in many other cancers, positive lymph node is an important factor affecting survival [26–28]. Moreover, complete sampling of lymph nodes results in precise staging and, therefore, appropriate adjuvant treatments for patients.

In this study, we defined 1-, 3-, and 5-year survival rates as our endpoints. Calibration curves showed good agreement between nomogram prediction and actual observation. The nomogram performed well by AUC at every measured time point, which revealed that the nomogram had good performance to predict 1-, 3-, and 5-year OS rates for patients with resected NSCLC. Kaplan–Meier curves showed that OS in the different groups was accurately differentiated by the risk classification system in the training cohort and validation cohort, both of $P < 0.05$.

Although surgery is the first choice treatment for patients with stages I, II, and partial III NSCLC [29, 30], postoperative adjuvant treatment could decrease the risk of disease recurrence and improve outcome [30–32]. It should be noted that postoperative adjuvant therapies including chemotherapy, radiotherapy, target treatment, and any other adjuvant therapies were not selected as candidate factors because they were only recommended for a proportion of patients with potentially high risk of locoregional recurrence.

In addition, patients with N2 disease were a heterogeneous group [33]. Operation may have some limitations for these patients, and the treatment should be individualized [34]. Mao et al. [35] showed that the C-index of the nomogram was 0.673 in the training cohort and 0.664 in the validation cohort. In our study, we did not specify the proportion of these patients with N2 disease who were treated with surgery from SEER database. The future studies are necessary to validate this result.

However, there are several limitations in our study. First, this was a retrospective study from the SEER database which could not represent the global population. Second, some other factors affecting survival, including smoking history, tumor location, and resection type, were not included in the present study. These data also may have an impact on clinical prognosis. Third, due to the limitations of the SEER database, the details of specific adjuvant therapy, such as chemotherapy and radiochemotherapy which may have some effect on survival for these patients, could not be obtained. Finally, although we use a large cohort to establish the nomogram and risk classification and validated in validation cohort, further validation of the predictive model is still essential.

5. Conclusion

We established a nomogram and a corresponding risk classification system predicting survival for NSCLC patients who underwent surgery. The results proved that the model had better performance to predict survival for NSCLC patients who underwent surgery than AJCC stage. Although future validation is necessary, these tools may be helpful for clinicians to evaluate prognostic indicators of patients undergoing operation.

Abbreviations

NSCLC: Non-small-cell lung cancer
SEER: Surveillance epidemiology and end results
OS: Overall survival
CT: Computed tomography
HR: Hazard ratio
CI: Confidence interval
AJCC: American Joint Committee on Cancer
C-index: Concordance index
ROC: Receiver operating characteristic
AUC: Area under the curve.

Data Availability

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

Conflicts of Interest

All the authors have no conflicts of interest to declare.

Authors' Contributions

X-L S was in charge of analysis and wrote the article.H-N Y, Z-X L, and J-M L helped with acquisition and analysis of the data. C-L Z helped in editing language. J S and H-Y W, the corresponding authors, were in charge of guidance of the design and analysis the whole research. H-Y W and X-L S were major contributors to the manuscript. All authors read and approved the final manuscript.

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Research Article

Mucinous Histology, *BRCA1/2* Mutations, and Elevated Tumor Mutational Burden in Colorectal Cancer

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Mucinous colorectal carcinomas (MC) constitute 10% of colorectal malignancies. Recently, an increased risk of colorectal cancer has been demonstrated in germline *BRCA1/2* mutation carriers. Furthermore, *BRCA1/2* germline mutation carriers have exhibited a higher-than-expected frequency of MC tumors. Here, we investigate the relationship between *BRCA* mutations and mucinous histology in colorectal carcinoma patients, using both an existing cohort of sequenced colorectal tumors and a prospective case-control study comparing MC and conventional adenocarcinoma (AC) patients tested for *BRCA* mutations. We discovered that MC tumors exhibit a statistically significantly higher incidence of *BRCA* mutations in addition to a higher average mutation count when compared to AC tumors in the existing cohort. The strongest predictor of the mutation count was mucinous histology, independently of other variables including microsatellite instability. Contrary to our hypothesis, the first association did not recur in the prospective case-control study, likely due to our pathological definition of MC tumors and small sample size. Finally, we observed a higher tumor mutational burden (TMB) in MC tumors compared with AC tumors. We suggest that the association between MC histology, *BRCA* mutations, and increased TMB may open the door to the utilization of simple tests (such as histopathologic characterization) to detect patients who may benefit from immunotherapy in colorectal cancer.

1. Introduction

Colorectal cancer (CRC) is the third most common malignancy worldwide, currently accounting for 700,000 deaths worldwide per year. The global burden of CRC, according to recent estimations, is anticipated to rise by 60% by 2030 [1].

While colorectal tumors were previously considered to be a single homogenous entity, it is now known that they are in fact a heterogeneous collection of tumors, each with its own distinct histological and molecular features that vary in their treatment and prognosis. The heterogeneous population of CRC is mainly comprised of two histological subtypes: 10–15% mucinous carcinomas (MC) and 85–90% adenocarcinomas (AC) [2].

MC tumors have a tendency to develop in young patients and are associated with late diagnosis at advanced stages, possibly because their typical location in the proximal colon is associated with less symptomatic presentation and a faster disease progression [3]. Clinically, MC prognosis has proven to be slightly worse than AC, with 2–8% increased hazard of death even when corrected for stage at presentation [4]. A limited response to systemic therapy in metastatic disease has also been reported [5]. MC histology has therefore been considered as an unfavorable prognostic indicator of CRC. This consensus has been recently challenged due to the identification of the importance of the sidedness (right vs. left colon) in the prognosis. This has led to an understanding that for colonic MC tumors there is no difference in overall

survival after correction for stage and sidedness [6]. Yet, for rectal MC tumors, there is a reduced rate of complete response and tumor downstaging following neoadjuvant chemoradiotherapy [7].

The carcinogenesis of MC is not clearly understood, though the higher prevalence of MC in hereditary and acquired conditions such as inflammatory bowel diseases, hereditary nonpolyposis colorectal cancer (HNPCC), and past radiotherapy treatment suggests that MC may derive from an alternative oncogenic pathway [8]. Regarding the genetic and molecular patterns, MC tumors tend to overexpress the *MUC2* and *MUC5AC* genes which are responsible for the formation of excess mucous. Other common molecular aberrations in MC include higher incidence of *PI3K*, *SMAD4*, and *BRAF* mutations[5, 9–12]. Importantly, MC tumors are associated with microsatellite instability (MSI), which is known to be involved in most cases of HNPCC and in 15% of sporadic CRCs. MSI is caused by inactivation of DNA mismatch repair genes (e.g., *MLH1* and *MSH2*), triggering an uncontrolled tumor growth [5, 13–15].

Classically, *BRCA1/2* genes encode important proteins responsible for maintenance of genome integrity and response to DNA damage [15, 16]. Hereditary mutated *BRCA1/2* tumor suppressor genes are key factors for pathogenesis and development of breast and ovarian cancers. *BRCA1/2* role in the carcinogenesis of CRC is currently unknown. Recent retrospective study of *BRCA1/2* carriers who developed CRC detected a higher-than-expected incidence of left-sided MC tumors [17]. Ending long-lasting debate, a new meta-analysis has clearly shown a statistically significant increased risk of colorectal cancer development in carriers of *BRCA1* mutations [18].

In this study, we aim to further investigate the relationship between BRCA mutations and mucinous histology in colorectal cancer patients.

2. Methods

2.1. Patients. Patients were eligible if they were 18 years of age or older and had a colorectal malignancy with valid histology of adenocarcinoma or mucinous features. Patients were considered as MC if the tumor pathology was described as having one of the following features: mucin-producing cells, signet ring cells, a focal mucinous component, or a mucin predominant feature. All patients provided written informed consent for any genetic research. The study was approved by the Institutional Review Board.

Excluded patients were those who did not have available pathology slides or a sufficient quality of material for BRCA analysis.

2.2. Database Analysis. A cohort of targeted sequencing of 1134 metastatic colorectal cancer (MSKCC [19]) was accessed via cBioPortal (https://www.cbioportal.org) for analysis. Patients were considered MC if their tumor exhibited one of the following features: mucinous carcinoma, signet ring cells, and a mucinous component. Patients were considered AC if their diagnosis was a conventional adenocarcinoma.

2.3. Study Design

2.3.1. Prospective Study Measurements. A prospective case-control study was conducted based on a large academic hospital's cancer center between January 2017 and August 2019 (Hadassah Medical Center). CRC patients with mucinous histology were recruited, along with conventional adenocarcinoma histology controls. Clinical and pathological data were extracted from digital records. Genetic data was analyzed and validated by the pathology department in Hadassah Medical Center or Foundation Medicine tests. Mismatch repair (MMR) status was evaluated by immunostaining for the mismatch repair proteins hMLH1, hPMS2, hMSH6, and hMSH2. Next-generation sequencing tests were conducted to identify alternations in hotspot regions in a few key factor functioning genes by Ion Torrent system. For library construction of *KRAS*, *BRAF*, and *PI3K* genes, Oncomine™ Solid Tumour DNA Kit was used; for *BRCA1/2* genes, Ion AmpliSeq™ Oncomine BRCA primers were used.

Tumor mutation burden (TMB) results were based on either (1) commercial kits (such as 324-gene panel assay FoundationOne® CDx test, validated comparing to whole-exome sequencing (WES) [20]) or (2) local analysis by Pathology Department with Ion Torrent system sequencing and assessed by the Oncomine Tumor Mutation Load Assay (Thermo Fisher Cat. No. A37910), also validated comparing to WES [21].

2.4. Database Analysis of TCGA Measurements. It is important to mention that TMB assessed by WES is usually reported as the total number of mutations per tumor, while TMB outputs from gene panel assays are usually normalized to mutations per megabase (mut/Mb) because they differ in the number of genes and target region size [20].

In our paper, we utilize a measure called "mutation count," defined as somatic nonsynonymous variants in encoding genes by exome sequencing as determined by TCGA [19, 20].

An additional measure we utilize is the MSI score. This measure was also derived from the TCGA database and is evaluated by MSIsensor, a software tool that quantifies MSI in paired tumor-normal genome sequencing data and reports the somatic status of corresponding microsatellite sites in the human genome [22].

2.5. Statistical Analysis. In order to compare different variables between the two groups, we used the chi-squared test and Fisher's exact test for categorical variables and the Student t-test and Mann–Whitney U test for quantitative variables. Analysis of more than two groups was conducted by the Kruskal–Wallis test for quantitative variables and by the chi-squared test for categorical variables. Spearman's rank-order correlation was used to compare two quantitative

Table 1: Investigation of mutation count characteristics in metastatic colorectal cancer database (MSKCC, Cancer Cell 2018). Significant **p** values are marked in bold.

Parameters	Test for mutation	p value for mutation count	Corrected p value for mutation count
Age at diagnosis	Spearman	**p** value <0.05	**p** value <0.05
Sex	Mann–Whitney	p value >0.05	p value >0.1
First site of metastasis	Kruskal–Wallis	*p value >0.05*	*p value >0.1*
Fraction of genome altered	Spearman	**p** value <0.05	**p** value <0.1
Tumor sample histology	Mann–Whitney	**p** value <0.05	**p** value <0.05
Stage at diagnosis	Kruskal–Wallis	**p** value <0.05	**p** value <0.05
Primary tumor location	Mann–Whitney	**p** value <0.05	**p** value <0.05
Primary tumor site	Kruskal–Wallis	**p** value <0.05	**p** value <0.05
MSI score	Spearman	**p** value <0.05	**p** value <0.05
BRCA mutations	Mann–Whitney	**p** value <0.05	**p** value <0.05
Additional analyses			
BRCA mutations and tumor sample histology	Chi-square	**p** value <0.05	**p** value <0.05
Stratified mutation count analysis			
BRCA mutations among MC	Mann–Whitney	**p** value <0.05	**p** value <0.05
BRCA mutations among AC	Mann–Whitney	**p** value <0.05	**p** value <0.05
Tumor sample histology and *BRCA* mutations	Mann–Whitney	**p** value <0.05	**p** value <0.05
Tumor sample histology and *BRCA* WT	Mann–Whitney	**p** value <0.05	**p** value <0.05

*Threshold for significance after correction for multiple hypotheses was 0.1.

variables. In the MSKCC cohort, a linear regression model was constructed for all variables that were statistically significantly linked to the mutation count. All p values are corrected for multiple hypotheses by the Bonferroni method [23].

3. Results

3.1. BRCA Mutations Are Linked to MC Histology and a Higher Mutation Count in an Existing Database. To assess whether there is a higher incidence of *BRCA1/2* mutations in MC tumors than in AC tumors, we performed an analysis of a cohort of targeted sequencing of 1134 metastatic colorectal cancer samples [19] (hereby the MSKCC database). The database included 128 MC patients and 725 AC patients (conventional adenocarcinoma), while other histological subtypes were excluded. Our analysis showed a significantly higher incidence of *BRCA* mutations in the MC tumors compared to AC (19/128 MC 14.8%, 30/725 AC 4.1%, p value <0.001, by chi-squared). The MSKCC database also includes the mutation count for each sample, defined as somatic nonsynonymous variants in encoding genes by exome sequencing as determined by TCGA; this feature is known to be prominent among MC tumors and is often linked to MSI [24]. Interestingly, several other variables in the MSKCC database presented a similar behavior, several of which were known features of MC tumors (Table 1): age at diagnosis, fraction of genome altered, and primary tumor location (average mutation count for right colon tumor was 20.1 versus 9.5 for left colon tumors). For the latter variable, this relation remained even when examining the exact tumor site (cecum—22, ascending colon—19, hepatic flexure—18.6, and no specific location in right colon—19.5 average mutation count). As expected, the MSI score (see Methods) was also statistically significantly correlated with mutation count (p value <0.001).

The average mutation count in tumors with MC histology was 24.8, indeed much larger than the average mutation count of 8.9 for tumors with AC conventional histology (p value <0.001). We noticed that *BRCA* mutations were linked to a higher mutation count in a statistically significant manner. We found a much larger amount of mutations in patients with mutated *BRCA* somatic genotypes versus patients with the wild-type (WT) somatic *BRCA* genotype (average of 59.4 versus 9.4, respectively, p value <0.001).

3.2. BRCA-Mutated Tumors Can Be Divided into a High Mutation Count Group with Mucinous Histology and a Low Mutation Count Group with Adenocarcinoma Histology. While tumors with *BRCA* mutations indeed tended to have higher mutation counts (Figure 1(a)), the analysis revealed two distinct groups of *BRCA*-mutated tumors that differ significantly in their mutation count: a group with high mutation count and group with low mutation count. While some of the variability between these two groups could be explained by MSI score, some of the *BRCA*-mutated tumors did not have a high MSI score despite a high mutation count (Figure 1(b)). We decided to employ two parallel strategies in order to further explore this phenomenon. (A) We compared the different variables in the MSK database between the two groups. (B) We studied the relationship of the different variables with the mutation count directly among *BRCA*-mutated tumors. We suspected that some features would discriminate between the two groups, and, indeed, fraction of genome altered, tumor sample histology, stage at diagnosis, primary tumor locations, and MSI score were significantly different between the two groups (Table 2), a result that was in complete agreement between the two strategies we employed. Finally, we constructed a linear regression model for the mutation count among *BRCA*-mutated tumors, utilizing the features found to be

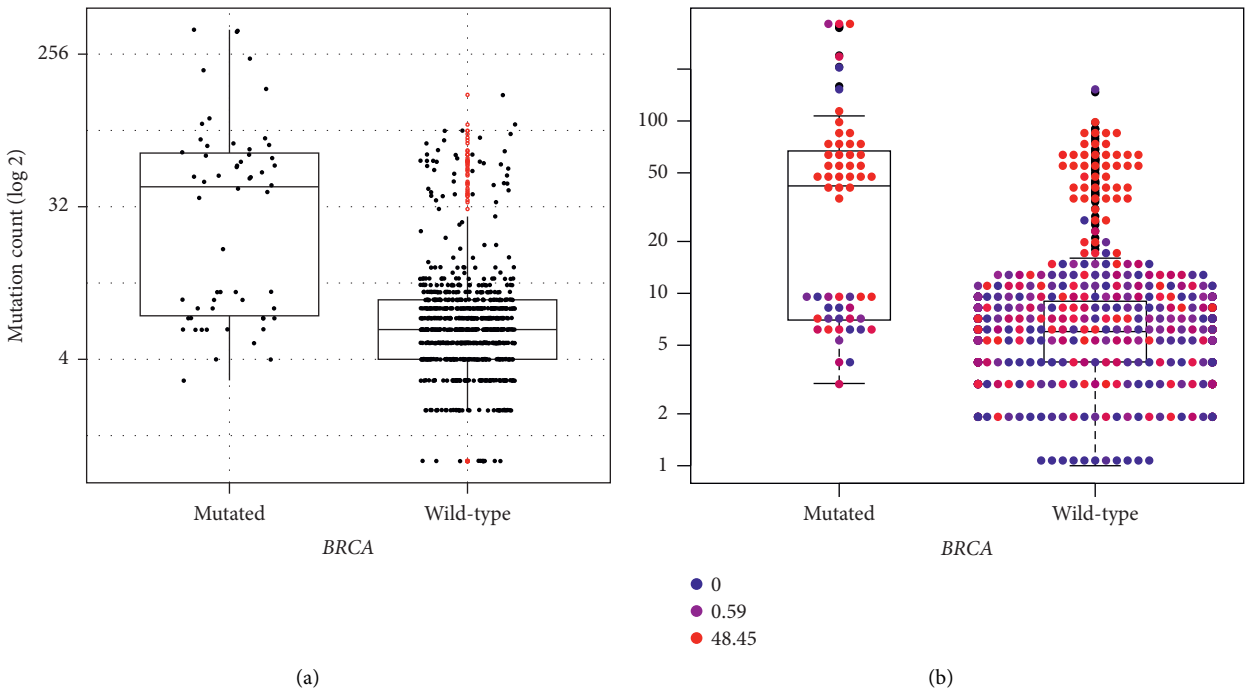


Figure 1: Relationship between *BRCA*-mutated vs. WT tumors and overall mutation count (a). Boxplot-swarmplot with the individual swarmplot points colored by MSI (b). MSI level score is calculated by MSIsensor (Niu et al. [25]) from blue (0) to red (48.45). Both plots are log-scaled.

Table 2: Association of different variables and mutation count involved in discrimination between the *BRCA*-mutated/high-mutation-count group and the *BRCA*-mutated/low-mutation-count group. Significant *p* values are marked in bold.

Parameters	Test for mutation	*p* value for mutation count	Corrected *p* value for mutation count	Test for *BRCA*_mut_group	*p* value for *BRCA*_mut_group	Corrected *p* value for *BRCA*_mut_group
Age at diagnosis	Spearman	*p* value >0.05	*p* value >0.1	Mann–Whitney	*p* value >0.05	*p* value >0.1
Sex	Mann–Whitney	*p* value >0.05	*p* value >0.1	Chi-square	*p* value >0.05	*p* value >0.1
First site of metastasis	Kruskal–Wallis	*p* value >0.05	*p* value >0.1	Fisher's exact test	*p* value >0.05	*p* value >0.1
Fraction of genome altered	Spearman	**p** *value <0.05*	**p** *value <0.05*	Wilcoxon	**p** *value <0.05*	**p** *value <0.05*
Tumor sample histology	Mann–Whitney	**p** *value <0.05*	**p** *value <0.05*	Chi-square	**p** *value <0.05*	**p** *value <0.1*
Stage at diagnosis	Kruskal–Wallis	**p** *value <0.05*	**p** *value <0.05*	Fisher's exact test	**p** *value <0.05*	**p** *value <0.05*
Primary tumor location	Mann–Whitney	**p** *value <0.05*	*p* value >0.1	Chi-square	**p** *value <0.05*	*p* value >0.1
MSI score	Spearman	**p** *value <0.05*	**p** *value <0.05*	Mann–Whitney	**p** *value <0.05*	**p** *value <0.05*

*Threshold for significance after correction for multiple hypotheses was 0.1.

statistically significant in the previous analysis (Table 3). Nearly 0.40 of the variances in the mutation count between the different patients with *BRCA* mutations could be explained using these variables alone. The strongest predictor of the mutation count was mucinous histology, independently of other variables.

3.3. A Prospective Cohort Questions the Relationship between BRCA Mutations and Histological Features. At our cancer center, we prospectively enrolled 93 CRC patients, 53 cases of patients with MC tumors and 40 with AC tumors. Of 53 MC patients, 30 were included (Figure 2). None of the background features differed significantly between the mucinous histology and adenocarcinoma histology groups, indicating that the two groups were not biased by their background properties (Table 4). Since *KRAS*, *BRAF*, and *PI3K* mutations are known to have a higher frequency in MC patients [5, 9–12], we performed sequencing tests for those mutations. However, we found no statistically significant differences in the frequencies of these mutations between the two groups, though there was a positive trend in the *KRAS*

Table 3: Linear regression model for the mutation count in the MSKCC database.

Parameters	Estimate	Std error	t value	Pr($>\|t\|$)
Intercept	38.9395	31.4677	1.237	0.22332
Fraction of genome altered	−134.5028	50.6571	−2.655	0.01142
Tumor sample, mucinous	51.2031	18.705	2.737	0.00928
MSI score	−0.5486	0.6408	−0.856	0.39719
Primary tumor location, right	35.694	17.7	2.017	0.05066
Stage at diagnosis II	−6.9323	32.7837	−0.211	0.83363
Stage at diagnosis III	9.7213	30.1277	0.323	0.74867
Stage at diagnosis IV	−5.253	28.6696	−0.183	0.85557
Multiple R-squared	0.3829			
Adjusted R-squared	0.2721			
F-statistic	3.457 on 7 and 39 DF			
p value	0.005653			

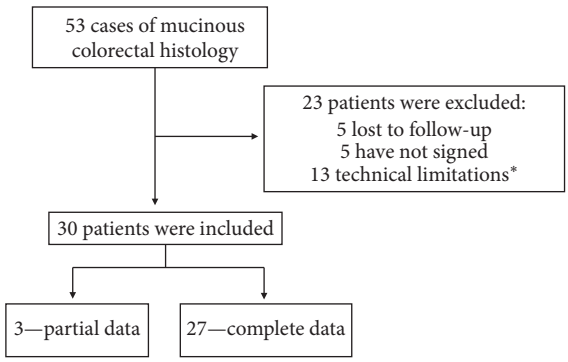


Figure 2: Prospective trial enrollment of patients with mucinous colorectal cancer.

mutations towards MC group ($p = 0.08$). In addition, no association was found between MSI and the MC group.

All patients were tested for somatic *BRCA1/2* mutations (Figure 3); among 70 CRC patients, 23 revealed a nonsynonymous *BRCA* mutation (i.e., 32%). Our cohort presents a trend towards a higher frequency of nonsynonymous mutations in either *BRCA1* or *BRCA2* in MC tumors compared to AC tumors, but it is not statistically significant (12/30, 40% of MC group, 11/40, 27% of AC group, p value = 0.2705, by chi-squared test). However, when analyzing *BRCA2* mutations separately, we did observe a trend towards a higher frequency of mutations in the MC group (9/29, 31% of MC group, 6/40, 15% of AC group). Additionally, two pathogenic mutations of *BRCA2* were present only in the MC group (c.7480 C > T and c.1670 T > C). Notably, one common mutation (c.8850 G > T) comprised half of the *BRCA2* mutations detected in the AC group. On the *BRCA1* gene, the same pathogenic mutation c.68_69delAG was present in both MC and AC groups. The distribution of mutations along the genes by the cBioPortal mutation mapper tool (https://www.cbioportal.org/mutation_mapper) does not indicate a bias for specific or hotspot locations or domains along the proteins between the two groups (Figure 4).

Lastly, since we observed a higher mutation count in MSKCC data for both MC tumors and *BRCA*-mutated tumors, we have decided to perform Tumor Mutation Burden (TMB) analysis in our patients. Fourteen patients were assembled in an attempt to provide a further outlook towards the role of *BRCA* mutations as a marker of high TMB and the relation to MC (MC: 4 *BRCA*-mutated, 5 *BRCA* wild-type (WT); AC: 2 *BRCA*-mutated, 3 *BRCA* WT). Only a single case of MC had MSI. Taking Foundation Medicine cutoff for TMB (low <6, intermediate 6–19, and high >20) [24], we observed that MC tumors are enriched for intermediate-high TMB tumors (Figure 5, Table 5, $p = 0.07$). In addition, *BRCA*-mutated tumors had numerically elevated TMB, in comparison to *BRCA* WT cancers ($p = 0.14$).

4. Discussion

In the current work, we have suggested a novel correlation between CRC histology, mutational burden, and *BRCA* mutations.

Our analysis of the MSKCC database detected a statistically significant higher incidence of *BRCA* mutations in the MC group as listed above (19/128 MC 14.8%, 30/725 AC 4.1%, p value <0.001, by chi-squared test). Additionally, average mutation counts in tumors with MC histology were higher compared with the AC group (24.8 and 8.9, respectively, p value <0.001). Our analysis might shed a light into the relationship between *BRCA* mutations and high mutation counts, since the mutated *BRCA* group has shown higher mutation counts compared with the *BRCA* WT group (average of 59.4 versus 9.4, respectively, p value <0.001).

Furthermore, we demonstrated two distinct groups of tumors with *BRCA* mutations: a high-mutation-count group with both mucinous histology and high MSI and a low-mutation-count group with both adenocarcinoma histology and low MSI score.

This finding can be explained by the well-known association between mucinous histology and MSI, suggesting MSI as a reasonable explanation for the high mutation counts in the MC group. Nevertheless, our analysis further revealed a small group of *BRCA*-mutated tumors with high mutation counts and a low MSI score (Figure 1(b)), possibly implicating *BRCA* as an independent predictor of high mutation count.

It is interesting to ponder what characterizes these different subgroups and what causes the high mutation

Table 4: Prospective cohort patients' characteristics.

	Adenocarcinoma ($n = 40$)	Mucinous ($n = 30$)
Sex		
Female	23 (57.5%)	12 (40.0%)
Male	17 (42.5%)	18 (60.0%)
Age at diagnosis		
Mean (SD)	59.1 (13.8)	60.2 (14.7)
Mean (min, max)	60.0 (20.0, 86.0)	63.5 (22.0, 78.0)
Ethnic origin		
Arab	9 (22.5%)	6 (20.0%)
Jewish-Ashkenazi	16 (40.0%)	15 (50.0%)
Jewish-non-Ashkenazi	12 (30.0%)	7 (23.3%)
Missing	3 (7.5%)	2 (6.7%)
Family breast history		
No	33 (82.5%)	23 (76.7%)
Yes	7 (17.5%)	7.(23%)
Previous malignancy		
No	36 (90.0%)	26 (86.7%)
Yes	4 (10.0%)	4 (13.3%)
Stage at diagnosis		
I	3 (7.5%)	0 (0%)
II	4 (10.0%)	5 (16 7%)
III	10 (25.0%)	13 (43 3%)
IV	23 (57.5%)	11 (36 7%)
Missing	0.(0%)	1 (3.3%)
Primary tumor site		
Left	25 (65.5%)	18 (60.0%)
Right	10.(25.0%)	11 (36.7%)
Missing	5 (12.5%)	1 (3.3%)
Metastases primary site		
Abdomen	4 (10.0%)	6 (20.0%)
Distant	4 (10.0%)	3 (10.0%)
Liver	22 (55.0%)	6 (20.0%)
Pelvis	3 (7.5%)	4 (13.3%)
Missing	7 (17.5%)	11 (36.7%)
Surgery		
No	4 (10.0%)	6 (20.0%)
Yes	34 (85.0%)	23 (76.7%)
Missing	2 (5.0%)	1 (3.3%)
Adjuvant treatment		
FOLFOX	6 (15.0%)	7 (23.3%)
None	22 (55.0%)	12 (40.0%)
Oxaliplatin, Fluorouracil	0 (0%)	1 (3.3%)
XELODA	1 (2.5%)	(2.6.7%)
XELOX	3 (7.5%)	7 (23.3%)
Missing	8 (20.0%)	1 (3.3%)

count in each case. To further study what variables determine the mutation count, we constructed a linear regression model demonstrating that nearly 0.4 of the variance in the mutation count between the different patients with *BRCA* mutations could be explained using a small number of variables (Table 2, Table 3). Some of the variables were not statistically significantly linked to the mutation count within the regression model, indicating additional correlations between variables within the model which explain the same variance in the mutation count. The strongest predictor of mutation counts was mucinous histology, independently of other variables, possibly suggesting that this feature determines the mutation count in patients with *BRCA* mutations.

Since the linear regression model indicated that mucinous histology, and not MSI, is the best predictor of mutation counts, it is possible that the *BRCA*-mutated low-MSI, high-mutation-count group is associated with mucinous histology. Our data also correlates with a previous report by Ciriello et al. [26], who characterized a subset of ultramutated CRC with an altered double-strand break repair mechanism. Notably, >50% of these tumors had somatic mutations in *BRCA1/2* genes. However, a further study should be done to validate and establish the existence of this specific subgroup.

With the intention to robustly establish the link between MC histology and *BRCA* mutations, we tested a cohort of AC and MC patients with similar background features in our medical center (Figure 3). Unfortunately, we could not reestablish the statistically significant link between *BRCA* mutations and the MC group (12/30, 40% of MC group and 11/40, 27% of AC group were *BRCA*-mutated).

Notably, even mutations that are known to be found in significantly higher incidence in MC tumors such as *KRAS*, *BRAF*, and *PI3K* were not seen in our cohort, prompting a suspicion that the lack of association is related to limitations of this specific cohort itself, and may explain our failure to reestablish the link between *BRCA* mutations and MC histology. Indeed, this analysis was performed on a limited sample size and with a broad definition of MC histology. This broad definition was linked to the variance between observers and to the MC WHO criteria, which are based on the evaluated amount of mucin, a component that is difficult to define accurately. However, we observed a trend towards a higher frequency of *BRCA2* mutations in the MC group (9/29, 31% of MC group, 6/40, 15% of AC group).

Lastly, since we observed a higher mutation count in MSKCC data, we have decided to further investigate the implications of this finding and to reestablish it in our local cohort. To link mutation count and TMB, we relied on a previous method described by Chalmers et al. [24], where mutation count was divided by the estimated exome sample size of 38 Mb to calculate mutation count per MB. Mutation count per MB was found equivalent to TMB per MB as both represent the total number of mutations counted divided by the size of the coding region of the targeted territory.

Later, TMB analysis was performed in a prospective cohort (Figure 5, Table 5). We observed that MC tumors are enriched for intermediate-high TMB tumors (Figure 5, $p = 0.07$). A study by Naseem et al. [27] may hint at the importance of this finding; this impressive study presented 6396 CRC tumor samples tested with next-generation sequencing for pathogenic mutations, MSI and TMB. *BRCA* pathogenic mutations were detected in 1.1% ($n = 72$) of tumors, while *BRCA2* in 2.8% ($n = 179$). *BRCA1/2*mutations were associated with higher TMB in all CRCs, including MSI-H and MSS cases ($p < 0.001$). Among MSS cases with *POLE* wild-type status, *BRCA1* ($p = 0.0269$) and *BRCA2* ($p = 0.0151$) mutations were associated with high

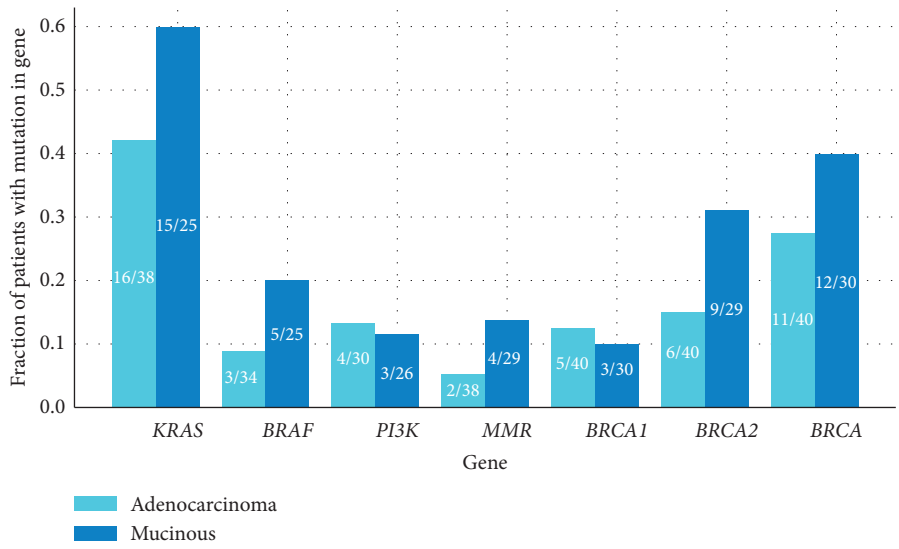


Figure 3: Common mutations in colorectal cancers from patients enrolled to the prospective cohort. The frequency of all genes does not differ in a statistically significant manner between the two groups.

Figure 4: Lollipop plot of identified somatic mutations in *BRCA1* and *BRCA2* in mucinous (MC) and adenocarcinoma (AC) colorectal cancers.

TMB and combining both *BRCA1/2* mutations led to an even higher TMB (3.6%; $p = 0.001$). *BRCA1/2* mutations are more frequent in MSI-H and independently associated with higher TMB, pathogenic *POLE* mutations, and right-sided tumors in MSI-H CRCs [27]. Potentially, the findings may indicate that the lack of a functioning DNA repair

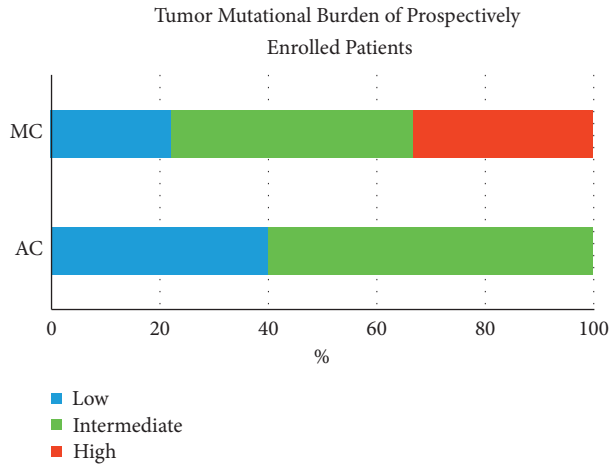
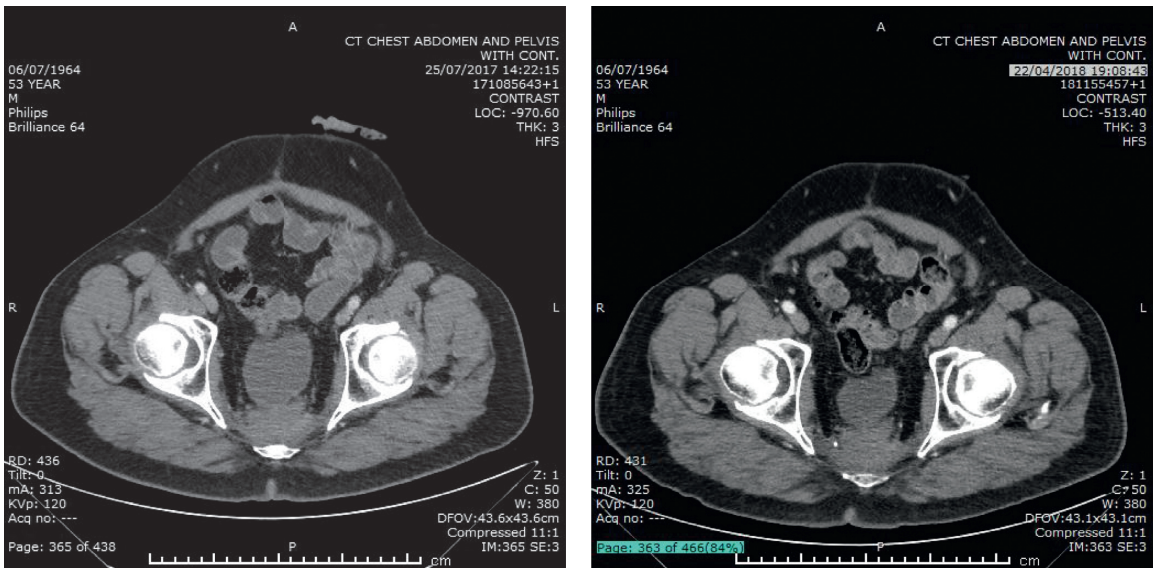


Figure 5: Tumor mutation burden (TMB) of the prospective cohort. Mucinous (MC, $n = 9$) tumors have higher TMB versus adenocarcinoma cancers (AC, $n = 5$), $p = 0.07$.

Table 5: Tumor mutation burden (TMB) of the prospective cohort. Mucinous (MC, $n = 9$) tumors have higher TMB versus adenocarcinoma cancers (AC, $n = 5$), $p = 0.07$.

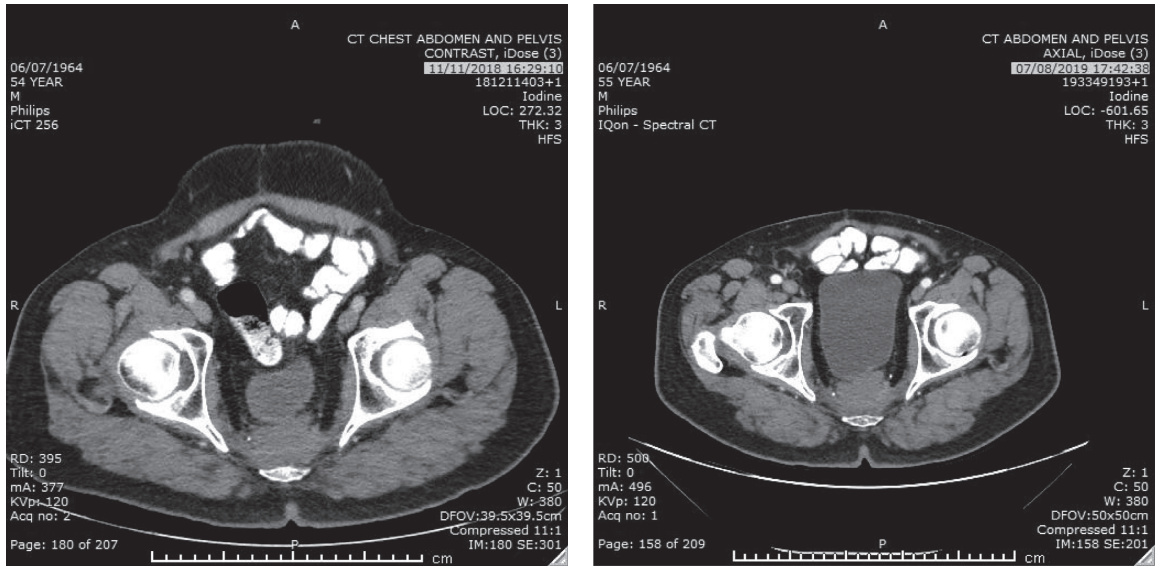
	Number of patients	Average TMB (mut/megabase)	High TMB*	Low TMB*
Mucinous	9	43.07	3, 33%	6, 66%
Mucinous *BRCA* mut	4	84.03	2, 50%	2, 50%
Mucinous *BRCA* WT	5	10.3	1, 20%	4, 80%
Adenocarcinoma	5	5.9	0, 0%	5, 100%
Adenocarcinoma *BRCA* mut	2	4.91	0, 0%	2, 100%
Adenocarcinoma *BRCA* WT	3	6.56	0, 0%	3, 100%

*Number of patients, % of patients with high/intermediate/low TMB. **High TMB using standard cutoff of >20.



(a) (b)

Figure 6: Continued.



(c) (d)

Figure 6: Stability of presacral CRC metastatic lesion on PARP inhibitor therapy. Axial computed tomography scans during treatment are provided: (a) July 2017, (b) April 2018, (c) November 2018, and (d) August 2019.

mechanism might be the driver for a higher-mutation load or alternatively that the mutations in the *BRCA* genes themselves are passenger mutations due to the overall increased mutations load.

An intriguing question might be "what is the further impact of our findings on the evaluation of CRC patients of Jewish-Ashkenazi ancestry, for whom the incidence of germline *BRCA* and Lynch syndrome mutations are higher [28]?" It is important to emphasize that genetic testing for germline mutations involves important ramifications regarding the genetic counseling needed for descendants and the potential cascade testing. Thus, testing for germline mutations warrants patients' consent and understanding. Moreover, we tried to utilize PARP inhibition approach in one of the patients in our cohort, as PARP inhibition is synthetically lethal in *BRCA*-deficient tumors (FDA approved for ovarian, pancreatic, and breast tumors with *BRCA1/2* mutation [5, 25, 29, 30]). The patient was a 53-year-old male with rectal adenocarcinoma (mismatch repair proficient, KRAS and BRAF wild-type) with pelvic and lung metastases. He underwent somatic tumor analysis that showed pathogenic *BRCA1* mutation (c.68_69delAG), later proved to be germline. Following achievement of maximal response to first-line chemotherapy with FOLFOX and anti-EGFR antibody (Panitumumab), the patient started Veliparib (PARP inhibitor, kindly provided by AbbVie) on July 2017. The treatment was well tolerated on 300 mg BID and the patient remained with stable disease (Figure 6) for almost 23 months (June 2019), when new mediastinal and pulmonary lesions appeared. As represented here, PARP inhibitors might serve as a potential future therapeutic approach in *BRCA*-mutated CRC, especially for challenging MC patients.

In addition, emerging evidence suggests that *BRCA1* mutation may even influence the survival outcomes among metastatic CRC patients treated with Oxaliplatin or Irinotecan-based regimens [31].

Taken together, this data imply that *BRCA1/2* and MC histology may serve as a potential surrogate marker for tumors with higher TMB. This "low-tech" biomarker can increase the number of patients who may benefit from novel treatment strategies based on immunotherapy for TMB-high tumors [28]. Further studies are required to elucidate the real-world value of TMB analysis in MC colorectal cancer with or without *BRCA1/2* mutation.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Acknowledgments

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Supplementary Materials

Anonymized individual patient data used to support the findings of this study are included within Supplementary Materials. (*Supplementary Materials*)

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Research Article

Effects of *BRCA* Germline Mutations on Triple-Negative Breast Cancer Prognosis

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Germline *BRCA1* and *BRCA2* mutations confer an increased lifetime risk for breast cancer and ovarian cancer. Several studies have investigated prognosis among *BRCA1/2* mutation carriers and noncarriers, but the prognostic impact on outcomes of breast cancer patients has not been determined. The aim of this study was to determine the prognosis of TNBC patients with and without *BRCA1/2* germline mutation. Among 502 patients diagnosed with TNBC between 2005 and 2008, 124 patients with a strong family history of breast cancer or ovarian cancer as well as TNBC patients diagnosed under 45 years were referred to the Genetic Counseling Unit for genetic counselling and genetic tests. In 30 (24%) of them, the *BRCA1/2* mutation was detected (the most common 5382insC in 18 (60%) patients). The median follow-up of the entire group was 60 months. *BRCA1/2* mutation carriers were statistically significantly younger at TNBC diagnosis compared with nonmutation patients (41 vs 47 years, respectively). Patients with the *BRCA1/2* mutation had smaller tumors (stage I: 47% vs 24.5% in noncarriers), but there was no significant difference in the regional nodal status (58.5–63% with cN0). Contralateral breast cancer developed in 26.5% of *BRCA1/2* mutation carriers and in 14% of noncarriers. Other primary cancers were also slightly more common in *BRCA1/2* mutation carriers (16.5% vs 9.5%). The performed analysis did not show any significant differences between the groups in recurrence-free survival ($p = 0.312$). There was no significant difference between patients with or without *BRCA1/2* mutation as regards overall survival ($p = 0.649$) and the risk of TNBC death ($p = 0.333$). The survival from detection of metastases was similar in two groups ($p = 0.865$). Our study demonstrated that the *BRCA1* mutation does not affect TNBC patients' outcomes.

1. Introduction

BRCA1 and *BRCA2* are tumor suppressor genes involved in DNA damage repair, cell cycle control, gene transcription regulation, and apoptosis. The common germline mutations of the *BRCA1* gene are 5382insC, 185delAG, 3819del5, and 4153delA and of *BRCA2* are 4075delGT and 580del4 [1]. In the western population, about 5% of the breast cancer patients may carry heritable cancer susceptibility gene mutations, with *BRCA1* being the most common mutation [2]. The mutation rate can be higher in Ashkenazi Jews [3, 4]. Interestingly, *BRCA1/2* mutation rates in Asians are lower than those in whites [5].

1.1. Prevalence of Breast/Ovarian Cancer. Germline *BRCA1* and *BRCA2* mutations confer an increased lifetime risk for breast cancer and ovarian cancer. Women with *BRCA1/2* germline mutations have a higher incidence of breast cancer than those without these genetic abnormalities. The cumulative incidence of breast cancer by age 70–80 years in female mutation carriers is 71.4–87% for the *BRCA1* mutation and 77–88% for the *BRCA2* mutation [6–8]. The ovarian cancer risk is 59–65% for the *BRCA1* mutation and 34.5–37% for the *BRCA2* mutation [6, 8]. The high lifetime risk of breast and ovarian cancers in *BRCA1/2* carriers is crucial for counselling, intensive breast and ovarian screening (annual MRI commenced from the age of 25 with

the additional annual mammography from the age of 30, 6-monthly ovarian cancer screening with transvaginal ultrasound, and Ca125 serum measure started at the age of 30), and risk-reducing surgery (bilateral salpingo-oophorectomy and bilateral risk-reducing mastectomy including skin-sparing and nipple-sparing mastectomy) [9, 10].

Compared to *BRCA2* carriers and noncarriers, *BRCA1*-associated breast cancers are often high-grade and poorly differentiated infiltrating ductal carcinoma and are more often triple-negative with higher expressions of cytokeratin 5/6, cyclin E, and p53. Patients with *BRCA1*-associated breast cancers are younger than those with the *BRCA2* mutation and those without mutation [11, 12].

1.2. Prognosis. Several studies have investigated prognosis among *BRCA1/2* mutation carriers and noncarriers, but the prognostic impact on outcomes of breast cancer patients has not been definitely determined. It is controversial whether *BRCA1/2* mutations in breast cancer are associated with poor prognosis. Some studies revealed that *BRCA1/2* mutation carriers with breast cancer had worse overall survival (OS) than noncarriers [13–15], others showed no difference [16–20], and some studies indicated that *BRCA1/2* mutation carriers had better survival than noncarriers [21–23]. Differences could be partly the result of the analysis of different ethnic populations (Ashkenazi Jewish population [24], central-eastern population [15], western population [19], or Asian population [20, 25]), small study group with mutations, variations in mutation assay techniques, mutation types, cancer treatment modalities, or length of follow-up.

Among all biological subtypes of breast cancer, triple-negative breast cancer (TNBC) is more likely to harbor a germline *BRCA1/2* mutation, with reported prevalence rates varying from about 10% to 20% [20, 22, 26, 27]. The effect of the *BRCA1/2* mutation on the prognosis in TNBC patients has not been well examined, with divergent findings reported in the previous studies [18, 20, 22, 28–30].

2. Aim

The aim of this study was to determine the prognosis of TNBC patients with and without *BRCA1/2* germline mutation.

3. Materials and Methods

Five hundred two consecutive TNBC patients treated at the Department of Breast Cancer and Reconstructive Surgery, Maria Skłodowska-Curie Institute–Cancer Center (MSCI), Warsaw, Poland, between 2005 and 2008, were selected and analyzed to assess risk factors of recurrence, recurrence-free survival (RFS), and OS. Among them, 124 patients with a strong family history of breast cancer or ovarian cancer as well as TNBC patients diagnosed under 45 years were referred to the Genetic Counseling Unit of Cancer Prevention Department in MSCI, Warsaw, for genetic counselling and genetic tests. The patients were tested for the following *BRCA1/2* mutations: *BRCA1* gene: c.5266dupC (5382insC), c.181T>G (C61G, 300T>G), c.3700_3704delGTAAA (3819del5), c.68_69delAG (185delAG), c.676delT (p.Cys226Valfs), c.1687C>T (p.Gln563Ter), c.3756_3759delGTCT (3875del4), c.4035delA (4153delA), c.5251C>T (5370C>T), and c.5345G>A (p.Trp1782X) and *BRCA2* gene: c.658_659del GT (p.Val220fs), c.5946delT (6174delT), c.9371A>T (p.Asn3124Ile), and c.5744C>T (C5972T). Characteristics of the whole group of 502 TNBC patients and 124 patients in whom genetic tests were performed are presented in Tables 1 and 2. The Ki-67 expression and vimentin expression were conducted additionally due to the fact that, in the analyzed period of time, these markers were not assessed as standard practice (vimentin still remains as an experimental biomarker, expressed more often in mesenchymal tumors). The decisions on therapy were made regardless of the *BRCA1/2* mutation status.

3.1. Statistical Analysis. Univariate analysis was performed in order to compare patient and tumor characteristics (age at diagnosis, clinical stage, HER2 expression, histological grade G, Ki-67 expression, and vimentin expression) as well as therapy (type of surgery, radiotherapy, and (neo)adjuvant chemotherapy) depending on the *BRCA1/2* mutation status. R Development Core Team (R 3.1.3., 2009) software was used for these analyses.

The following definitions of events were used:

(i) RFS—time from TNBC diagnosis to recurrence

(ii) OS—time from TNBC diagnosis to death from any cause

(iii) Breast cancer-specific survival (BCSS)—time from TNBC diagnosis to death from breast cancer

(iv) Survival from dissemination—time from recurrence to death from any cause

Then, RFS, OS, and survival from dissemination of the disease in both groups were assessed. Additionally, risk of breast cancer death using the competing risk method was evaluated. Finally, the *BRCA1/2* mutation was assessed as one of the seven prognostic factors for recurrence and survival in multivariate analysis using the multistep Cox model. The other prognostic factors in the Cox model were age at diagnosis, TNM stage (I, II, or III), Ki-67 expression, vimentin expression, histological grade G (G1, G2, or G3), and histological type (no special type—NST or others).

4. Results

Finally, 124 (25%) out of 502 TNBC patients had undergone genetic counselling with *BRCA1/2* mutation tests and were included for further analysis. In 30 (24%) of them, the *BRCA1/2* mutation was detected. Only in one case, the mutation of the *BRCA2* gene was found, and for the *BRCA1* gene, 29 mutated cases were detected. The following *BRCA1* mutations were found: c.5266dupC (5382insC) in 18 patients, c.181T>G (C61G, 300T>G) in 5 patients, c.3700_3704delGTAAA (3819del5) in 2 patients, and c.5251C>T (5370C>T), c.5345G>A (p.Trp1782X), c.3756_3759delGTCT (3875del4), and c.68_69delAG (185delAG) in 1 patient each, respectively. One patient harbored *BRCA2* gene mutation c.5744C>T (C5972T). The

Table 1: Characteristics of 502 TNBC patients.

Factor		Rate (%)
Number of patients	502	100
Age at diagnosis (years)		
Median	55	
Mean	**56**	
Range	24–98	
Clinical staging (cTNM)		
I	97	19.5
II	246	49
III	132	26
IV	27	5.5
Initial clinical tumor staging		
cT0	7	1
cT1	111	22
cT2	248	49.5
cT3	58	12
cT4	76	15
No available data	2	0.5
Initial clinical node staging		
cN0	243	48
cN1	180	36
cN2	58	11.5
cN3	19	4
No available data	2	0.5
HER2 expression		
0 or 1+	431	86
2+, FISH negative	71	14
Histological type		
NST	416	83
Lobular	25	5
Medullar	11	2
Apocrine	11	2
Metaplastic	20	4
Others	20	4
G		
1	21	4
2	165	33
3	310	62
No available data	6	1
Ki-67 expression		
<14%	140	28
14–30%	183	36.5
>30%	133	26.5
No available data	46	9
Vimentin expression assessed		
Yes	443	88
No	59	12
Vimentin		
Positive	71/443	16
Negative	372/443	84
Contralateral breast cancer	41	8
Other primary cancer (other than contralateral breast cancer)	45	9

FISH: fluorescence in situ hybridization.

comparison between *BRCA1/2* mutation carriers and noncarriers is presented in Table 2. The median follow-up of the entire group was 60 months. *BRCA1/2* mutation carriers were statistically significantly younger at TNBC diagnosis compared with nonmutation patients (41 vs 47 years, respectively). Patients with the *BRCA1/2* mutation had smaller tumors (stage I: 47% vs 24.5% in noncarriers), but there was no significant difference in the regional nodal status (58.5–63% with cN0). The most common histological type was NST in both groups with a similar rate of medullar

Table 2: Characteristics of 124 TNBC patients assessed for *BRCA1/2* mutations.

Factor	Patients tested for *BRCA* mutations				p value (*BRCA*-positive vs *BRCA*-negative)
	BRCA noncarriers	Rate (%)	*BRCA* carriers	Rate (%)	
Number of patients	94	100	30	100	
Age at diagnosis (years)					
Median	49		40		
Mean	47.5		41.4		0.0115
Range	25–67		24–76		
Clinical staging (cTNM)					
I	23	24.5	14	47	
II	51	54	13	43	0.0006
III	19	20	2	7	
IV	1	<0.5	1	3	
Initial clinical tumor staging					
cT0	0	0	0	0	
cT1	28	30	16	53	
cT2	56	59.5	9	30	0.0004
cT3	4	4	2	7	
cT4	6	6.5	3	10	
No available data	0	0	0	0	
Initial clinical node staging					
cN0	55	58.5	19	63	
cN1	27	28.5	10	33	
cN2	9	9.5	1	4	0.1063
cN3	3	3.5	0	0	
No available data	0	0	0	0	
HER2 expression					
0 or 1+	79	84	29	97	0.0091
2+, FISH negative	15	16	1	3	
Histological type					
NST	80		21	70	
Lobular	5	85	1	3.5	
Medullar	5	5.5	1	3.5	0.0023
Apocrine	2	5.5	1	3.5	
Metaplastic	2	2	2	6	
Others	0	2	4	13.5	
G					
1	0	0	2	6.5	
2	29	30	12	40	0.0065
3	64	68	16	53.5	
No available data	1	2	0	0	
Ki-67 expression					
<14%	26	27.5	5	16.5	
14–30%	29	31	10	33.5	0.0761
>30%	28	30	13	43.5	
No available data	11	11.5	2	6.5	
Vimentin expression assessed					
Yes	82	87	26	86.5	0.8361
No	12	13	4	13.5	
Vimentin					
Positive	14	15	8	26.5	0.0372
Negative	68	85	18	73.5	
Contralateral breast cancer	13	14	8	26.5	0.0228
Other primary cancer (other than contralateral breast cancer)	9	9.5	5	16.5	0.1475

FISH: fluorescence in situ hybridization.

cancer (3.5–5.5%). Noncarriers had more often G3 tumors. Contralateral breast cancer developed in 26.5% of *BRCA1/2* mutation carriers and in 14% of noncarriers. In both groups, almost half contralateral breast cancers developed before TNBC diagnosis. Other primary cancers were also slightly more common in *BRCA1/2* mutation carriers (16.5% vs 9.5%). Almost all cases occurred after TNBC diagnosis in both groups (only 2 cases of lymphoma and one ovarian cancer developed before TNBC). The summary of these results is presented in Table 2.

In 72 patients (58% of all TNBC), the primary operation was performed. In other 47 (38%) patients, surgery was carried out after neoadjuvant chemotherapy. Breast-conserving surgery was more common in *BRCA1/2* mutation carriers (41.5% vs 33.5%). Adjuvant chemotherapy was performed in 87 patients (90% after primary surgery). Overall, (neo)adjuvant chemotherapy was performed in a similar percentage of patients with or without *BRCA1/2* mutation. The summary of patient therapy is presented in Table 3.

We compared RFS, OS, risk of breast cancer death, and survival from distant metastases in *BRCA1/2* carriers and noncarriers. The performed analysis did not show any significant differences between the groups in RFS ($p = 0.312$), also after taking into account the clinical stage of TNBC (in patients in the following stages: I: $p = 1.0$, II: $p = 0.454$, and III: $p = 0.197$) or (neo)adjuvant chemotherapy ($p > 0.05$). The risk of the recurrence depending on the *BRCA1/2* mutation status is shown in Figure 1. There was no significant difference between patients with or without *BRCA1/2* mutation regarding overall survival ($p = 0.649$). The *BRCA1/2* mutation was not a prognostic factor of patient survival. The results are presented in Figure 2. The risk of TNBC death did not differ significantly in both groups (Figure 3).

In 13% (4/30) of *BRCA1/2* mutation patients and in 21% (20/94) of noncarriers, the recurrence of the disease was detected. In both groups, there was one patient with primary metastatic TNBC. There was no significant difference in survival from detection of metastases between these two groups ($p = 0.865$). The results are presented in Figure 4.

Among seven variables taken in multivariate analysis, TNM stage was the only factor significantly influencing recurrence and death. There was no correlation between RFS or OS and other analyzed risk factors, including the *BRCA1/2* germline mutation. The results are shown in Tables 4 and 5.

5. Discussion

Our study showed that the outcome of TNBC patients did not differ depending on the *BRCA* mutation status. We aimed to clarify the prognostic value of *BRCA1/2* mutations on breast cancer-specific outcomes after conventional treatment. In our study, RFS, OS, and risk of death from TNBC were similar between patients with breast cancer and *BRCA1* germline mutation and noncarriers. Because of the fact that among our patients with *BRCA1/2* mutations only one had *BRCA2* mutation, the results and discussion concern about patients with breast cancer and *BRCA1* mutation.

5.1. All Biological Types of Breast Cancer. The meta-analysis of 11 studies performed by Lee et al. revealed that patients with breast cancer and *BRCA1* mutation had worse OS compared to noncarriers (HR = 1.92). The *BRCA2* mutation did not affect survival in patients with breast cancer (HR = 1.30) [31].

In meta-analysis by Zhong et al. [32], based on 13 studies with 10 016 women with breast cancer, concerning breast cancer survival, the *BRCA1* mutation carriers had worse OS than noncarriers (HR = 1.5, $p = 0.009$) but were not significantly different from noncarriers in terms of progression-free survival (HR = 1.35, $p = 0.09$).

In other meta-analysis performed by Zhu et al. [3], based on 34 studies, event-free survival (EFS), OS, and BCSS were compared in three groups of breast cancer patients: *BRCA1* carriers, *BRCA2* carriers, and *BRCA1/2* noncarriers. In patients with *BRCA1* and *BRCA2* mutations, OS was worse than that in patients without mutation ($p < 0.001$ and $p = 0.034$, respectively) but did not translate into poor BCSS ($p = 0.448$ and $p = 0.401$, respectively) or EFS ($p = 0.438$ and $p = 0.558$, respectively) [3]. The *BRCA1* mutation was significantly associated with worse OS in studies conducted in Europe ($p < 0.001$) and studies assessing patients diagnosed before 1995 ($p < 0.007$).

The POSH prospective cohort study analyzed patients with young-onset breast cancer (≤40 years) regarding the *BRCA1/2* mutation status [33]. Recently published results indicated no significant difference in OS or distant disease-free survival between patients carrying *BRCA1/2* mutations and patients without those mutations after a diagnosis of breast cancer.

A study by Wang et al. performed on the Chinese cohort revealed that patients with *BRCA1/2* mutations had worse survival outcomes than noncarriers [25]. *BRCA1/2* mutation carriers were more likely to have lymph node involvement at initial diagnosis than noncarriers [25]. In our study, we did not observe these kinds of relations.

5.2. Triple-Negative Breast Cancer. Studies that have evaluated the prognostic role of the *BRCA1/2* mutation in patients with TNBC have shown inconclusive results, but the newest and larger ones are in line with our study.

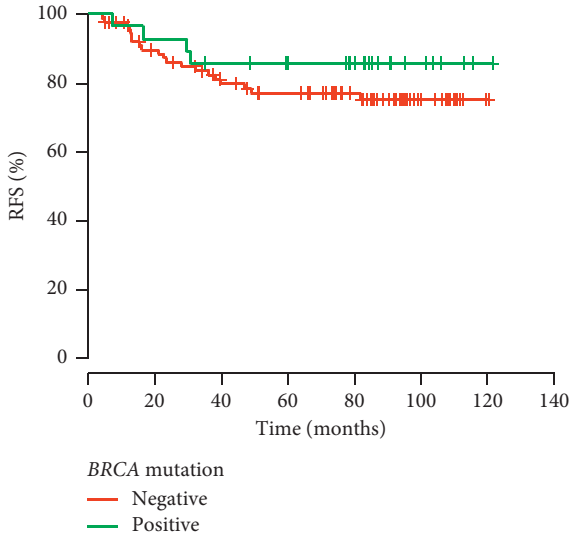
In the study performed by Yadav et al. [34], 266 TNBC patients had undergone *BRCA1/2 mutation* tests. In 27% of them, *BRCA1/2* mutations were detected. No statistically significant difference was found in locoregional recurrence, distant recurrence, RFS, and OS between the breast cancer patients with and without *BRCA1/2* mutations. 5-year OS for *BRCA1/2*-positive and *BRCA1/2*-negative breast cancer patients was 83% and 90% and 5-year RFS was 83% and 80%, respectively. The differences were not statistically significant [34].

In the study by Gonzales-Angulo et al. [22], based on 77 TNBC patients, RFS was better for patients with the *BRCA1/2* mutation and OS was similar between carriers and noncarriers.

In another study, Maksimenko et al. [30] compared the outcomes of 78 TNBC patients without *BRCA1* mutation

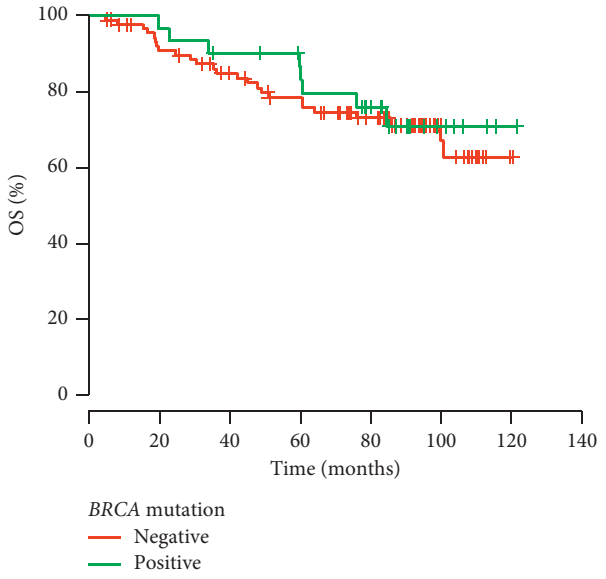
Table 3: Therapy of 124 TNBC patients assessed for *BRCA1/2* mutations.

Type of therapy	Patients tested for *BRCA* mutations				*p* value (*BRCA*-positive vs *BRCA*-negative)
	BRCA noncarriers	Rate (%)	*BRCA* carriers	Rate (%)	
Number of patients	94	100	30	100	
Surgery					
Yes	90	96	29	97	0.7004
No	4	4	1	3	
Type of surgery					
Mastectomy	60/90	66.5	17/29	58.5	0.2438
Breast-conserving surgery	30/90	33.5	12/29	41.5	
Radiotherapy					
Yes	55	58.5	17	56.5	0.7751
No	39	41.5	13	43.5	
Radiotherapy					
After mastectomy	27/55	49	5/17	29.5	0.0044
After breast-conserving surgery	28/55	51	12/17	70.5	
Neoadjuvant chemotherapy					
Yes	20	21.5	4	13.5	0.0940
No	74	78.5	26	86.5	
Regimens in neoadjuvant chemotherapy					
AT⟶CMF	5/20	25	1/4	25	<0.0001
Anthracycline + taxane	9/20	45	2/4	50	
Anthracycline	5/20	5	1/4	25	
Others	1/20	25	0	0	
Adjuvant chemotherapy					
Yes	64	68	23	76.5	0.1541
No	30	32	7	23.5	
Regimens in adjuvant chemotherapy					
Anthracycline (AC)	41/64	64	12/23	52	0.0574
FEC/FAC	11/64	17.5	4/23	17.5	
Anthracycline + taxane	8/64	12.5	5/23	21.5	
CMF	2/64	3	0	0	
Taxane	2/64	3	2/23	9	



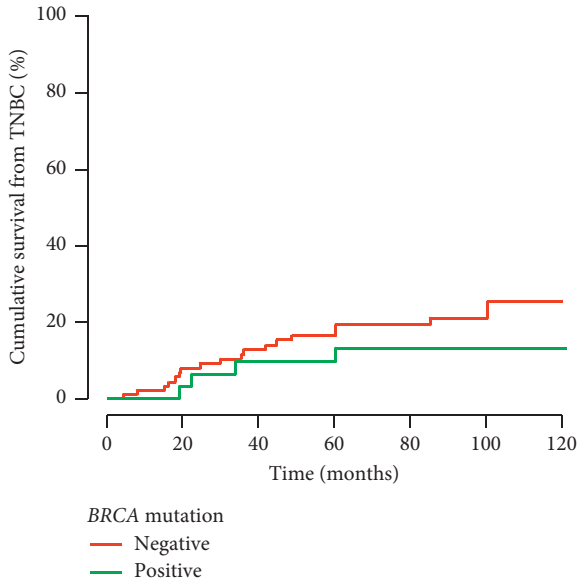
BRCA mutation	*n*	RFS					Median RFS (months)	*p*
		12 months	24 months	36 months	60 months	120 months		
Negative	93	96.6%	86.0%	83.6%	77.2%	75.3%	Not reached	$p = 0.312$
Positive	29	96.4%	92.9%	85.7%	85.7%	85.7%	Not reached	

Figure 1: Risk of recurrence in TNBC patients depending on the *BRCA* mutation status.



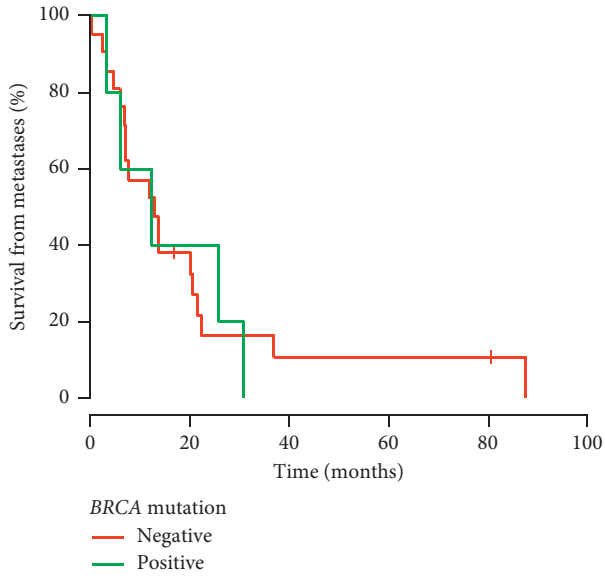
BRCA mutation	*n*	OS					Median OS (months)	*p*
		12 months	24 months	36 months	60 months	120 months		
Negative	93	97.8%	90.8%	86.1%	78.5%	62.8%	Not reached	$p = 0.649$
Positive	29	100%	93.5%	90.3%	86.7%	70.8%	Not reached	

Figure 2: Risk of death in TNBC patients depending on the *BRCA* mutation status.



BRCA mutation	*n*	Cumulative probability of death from TNBC					Median OS from TNBC (months)	*p*
		12 months	24 months	36 months	60 months	120 months		
Negative	93	2.2%	8.0%	11.6%	16.6%	25.3%	Not reached	$p = 0.333$
Positive	29	0.0%	6.4%	9.7%	9.7%	13.3%	Not reached	

Figure 3: Relationship between the presence of the *BRCA1/2* mutation and the risk of death due to TNBC.



BRCA mutation	*n*	Survival from metastasis development				Median survival from metastasis development (months)	*p*
		12 months	24 months	36 months	60 months		
Negative	21	52.4%	16.3%	16.3%	10.9%	12.85	$p = 0.865$
Positive	5	60.0%	40.0%	0.0%	0.0%	12.23	

Figure 4: Survival time counted from relapse depending on the *BRCA1/2* mutation status.

Table 4: Multivariate analysis: final model for RFS.

Factor	HR	95% CI		*p*
Clinical stage: I or II		Reference		
Clinical stage: III	43.26	2.13	880.64	0.014

Table 5: Multivariate analysis: final model for OS.

Factor	HR	95% CI		*p*
Clinical stage: I		Reference		
Clinical stage: II	2.359	1.385	4.016	0.002
Clinical stage: III	8.353	4.918	14.188	<0.001

with those of 38 TNBC patients with the *BRCA1* mutation. The BCSS and distant recurrence were significantly lower in the *BRCA1*-positive patients. In 4 other larger studies, there was no difference found in recurrence and survival between TNBC carriers and noncarriers of *BRCA1/2* mutations [18, 20, 28, 29]. A meta-analysis of 11 papers performed by Xie et al. also revealed that RFS and OS in TNBC patients with and without *BRCA1/2* mutations did not differ [20].

Baretta et al. [24] performed a meta-analysis concerning the relation between *BRCA1/2* mutation and prognosis of breast cancer based on 105 220 breast cancer patients including 3588 (3.4%) *BRCA1/2* mutation carriers. OS, BCSS, RFS, and distant metastasis-free survival (DMFS) were estimated. The authors found that *BRCA1* mutation carriers had a 30% higher risk of dying than *BRCA1*-negative/sporadic cases (OS), but they did not find association between *BRCA1* and the risk of death from breast cancer (BCSS). Contrary to patients with all subtypes of breast cancer, 1748 patients with TNBC and *BRCA1/2* mutations had better OS than *BRCA1/2*-negative ones (HR = 0.49) [24]. The risk of recurrence in TNBC was not statistically different between *BRCA1/2* carriers and *BRCA1/2* noncarriers ($p = 0.82$). BCSS and DMFS of *BRCA1* mutation carriers did not differ from those of *BRCA1*-negative TNBC patients ($p = 0.76$ and $p = 0.65$, respectively) [24].

In the present study, all investigated TNBC cases were diagnosed and treated in one breast cancer department. The used methods did not differ depending on the *BRCA1/2* mutation status, and patients had a long time of follow-up (up to 10 years). Nowadays, new drugs such as poly(-adenosine diphosphate-ribose) polymerase (PARP) inhibitors (olaparib and talazoparib) are dedicated to metastatic *BRCA1/2*-positive TNBC as well as immunotherapy for PDL-1-positive metastatic TNBC [35–37]. These drugs can influence the survival of *BRCA1/2* carriers with TNBC in the future. In the analyzed cohort with metastatic disease, the survival did not depend on the *BRCA1/2* mutation status. In contrast, Larson et al. showed that *BRCA* carriers with metastatic TNBC had clinically significant improved OS at 3 years compared to patients without *BRCA* mutations (3-year

OS of 63% vs 28%). In that study also, no patients received treatment with the PARP inhibitor [38].

6. Limitations of the Study

The retrospective nature of the study and a small number of recurrences or deaths in patients who had undergone genetic tests are two main limitations of this study.

Out of 502 consecutive TNBC patients referred to MSCI between the years 2005 and 2008, only 124 (25%) patients underwent genetic tests for the *BRCA1/2* mutation. From them, the *BRCA1/2* mutation was found only in 30 cases, which gives 6% (30/502) *BRCA1/2* carriers among 502 TNBC patients. According to the current NCCN guideline and ESMO recommendations, 65% of all TNBC patients from our analysis met the genetic test criteria solely by their age at diagnosis of TNBC (up to 60 years); therefore, the tests should be performed [10, 39]. This number might be even higher considering other criteria such as a strong family history of breast/ovarian cancer. In the years 2005–2008, genetic tests were offered at our institution only for patients with a strong family history of breast/ovarian cancer and for those under 45 years at the initial diagnosis of breast cancer.

7. Conclusion

Our study demonstrated that the *BRCA1* mutation does not affect RFS and OS in patients diagnosed with TNBC. The outcome of breast cancer in *BRCA1* carriers and noncarriers was comparable. The *BRCA1* germline mutation did not influence the prognosis of the TNBC patients.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Acknowledgments

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