Original paper

Incorporating immunohistochemical markers into screening methods for *BRCA1*-mutated breast cancer

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BRCA1-mutated breast cancer (BC) is responsible for approximately 25% of hereditary breast cancer cases. BRCA1 is a tumor suppressor protein regulating the cell cycle and DNA repair; therefore its dysfunctions play a significant role in carcinogenesis. *BRCA1*-mutated BC is associated with basal-like phenotype, lack of expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) in addition to frequent TP53 mutations and poor prognosis. Currently used criteria for genetic evaluation of BC for the risk of hereditary mutations are based on patients' age and family history, and therefore are prone to be imprecise or incomplete. This review discusses recently developed sets of immunohistochemical markers, promising independent markers (nestin, ALDH1, FOXO3, claudins, topoisomerase 1, EGFR) and their potential to be incorporated into clinical practice as a support tool in oncological counseling. This approach could be applied as a screening method for cost-effective selection of cases requiring genetic testing or adapted in pathology laboratories with limited access to molecular techniques. Although not all of the described predictor models have been validated yet, they could further improve the performance of *BRCA1* screening methods in BC in the near future via increasing the accuracy of criteria for further genetic evaluation.

Key words: BRCA1, breast cancer, immunohistochemistry, genetic evaluation.

Introduction

Breast cancer (BC) is the most common type of cancer among women worldwide, as well as the primary cause of female death. According to widespread estimates, over 2 million BC cases were diagnosed in 2018 [1], and approximately 5-10% of them were hereditary [2]. The risk of BC is increased by various modifiable and non-modifiable factors, including genetic mutations [3]. *BRCA1* belongs to a large group of genes associated with increased risk of BC. Germline mutations occurring in this gene increase the cumulative risk of BC up to 57% for carriers of the mutation at the age of 70 [4]. *BRCA1* is involved in approximately 25% of familial BC cases [2]. As a tumor suppressor protein regulating the cell cycle and DNA repair [5], its dysfunctions play a significant role in carcinogenesis. *BRCA1*-mutated BC is associated with basal-like phenotype and lack of expression of ER (estrogen receptor), PR (progesterone receptor) and HER2 (human epidermal growth factor receptor 2) in addition to frequent *TP53* mutations and poor prognosis [6, 7]. Current criteria for genetic eval-

uation in BC for *BRCA1* (and other) mutations are based mainly on age and personal and family medical history [8], and therefore may be imprecise or incomplete [9, 10]. The insufficient detection of *BRCA1* mutation carriers may hinder provision of targeted therapy and impact the patient's relatives, who – as probable mutation carriers – may require oncological counseling. As there is currently a trend to seek immunohistochemical markers correlating with various clinical data (e.g. overall survival [OS], tumor size, presence of lymph node metastases) [11, 12], this paper summarizes some of the novel approaches to involve immunohistochemistry in the diagnostic workup in order to increase the sensitivity and specificity of the aforementioned criteria at an acceptable cost.

Promising independent markers of *BRCA1* mutation status

Nestin

Nestin, an intermediate filament protein primarily found in the central nervous system as a stem cell/progenitor cell marker, has also been observed in many other undifferentiated non-neuronal tissues, e.g. the myoepithelial layer of the mammary gland and immature blood vessels [13, 14, 15, 16]. Furthermore, its marked expression has been discovered in many processes, such as neural and muscular regeneration or carcinogenesis [17, 18, 19]. Nestin expression has been observed in malignant tumors as a cancer stem cell marker, and its expression intensity tends to correlate with the tumor grade of malignancy [20, 21, 22]. Positive immunohistochemical nestin staining is an independent factor for poor prognosis and is associated with basal-like differentiation of BC cells [23].

The first link between nestin expression and *BRCA1* germline mutation status was based on observation of 8 cases by Li *et al.* in 2007 [16]. The paper by Krüger *et al.* (2017) takes that one step further by analyzing the correlation of nestin expression level with multiple variables, such as overall survival, tumor grade of malignancy, presence of lymph node involvement, *etc*. [24]. When comparing cases with and without a *BRCA1* germline mutation, nestin-positive tumors were more likely to be found in the subgroup with a *BRCA1* mutation (OR = 8.7, sensitivity 62%, specificity 84%). Nestin expression, among many markers included in the study, was able to convincingly predict *BRCA1* germline mutation status in the strongest manner ($p < 0.0005$) in multivariate analysis. It was also the only protein that significantly predicted the presence of a mutation in patients under 40 years old when including the triple negative (TN) profile.

Although no further studies of analytical and clinical value of nestin have been published yet, it could be applied as a predictor in qualification for *BRCA1* germline mutation testing in the future. Statistical data for nestin as well as for other markers described in the present analysis are summarized in Table I.

Nonspecific NAD-dependent aldehyde dehydrogenase 1

Aldehyde dehydrogenase 1 (ALDH1) is a cytosolic enzyme responsible for oxidization of exo- and endogenous aldehydes to carboxylic acids. ALDH1, involved in retinoic acid metabolism, contributes to cell differentiation [25], but also to antitumor drug resistance [26, 27, 28], particularly in BC [29, 30].

ALDH1 expression is observed in around 5% of mammary gland cells representing the stem cell population in physiological conditions [31]. In BC, its expression in cancer stem cells (CSC) was found to be associated with decreased OS, high histological grade, ER negativity, PR negativity and HER2 overexpression [32]. Liu *et al.* (2008) demonstrated that the differentiation of ER(–) stem/progenitor cells to ER(+) luminal cells is conditioned by BRCA1 expression, and its knockdown leads to an increase of ALDH1 expression as well as a decrease of luminal epithelial markers and ER expression in primary breast cells. Since BRCA1 plays a role in DNA repair, it was suggested that *BRCA1* loss may result in an increased percentage of genetically unstable breast stem cells susceptible to carcinogenesis [33]. A study by Madjd *et al.* (2012) revealed a significant inverse correlation between expression of ALDH1 and BRCA1 in BC cells – reduced BRCA1 levels were more often seen in BC cells highly expressing ALDH1 ($p = 0.044$). In their univariate analysis combined ALDH1(+)/BRCA1(low) phenotype was found to be often present in high grade tumors ($p = 0.056$) [34]. These results were in line with previous findings by van Heerma Voss *et al.* (2011), who concluded that ALDH1 expression was significantly higher in both intensity and percentage in *BRCA1*-related BC, implying that these cases had an enlarged CSC component [35]. Similarly to benign tissues, ALDH1 was expressed in stromal and epithelial cells. When comparing tumoral stromal, peritumoral stromal and epithelial ALDH1 levels in groups of hereditary and sporadic BC cases, only peritumoral and epithelial expression levels were independent predictors of *BRCA1* mutation status in multivariate analysis without correlating with each other [36]. In contrast, data provided by Bane *et al.* (2013) show no statistically significant association between *BRCA1* mutation status and ALDH1 expression [37].

To summarize, these conclusions might serve several clinical purposes. Firstly, ALDH1 may be considered as a biomarker for *BRCA1* mutation status as

Table I. Major reports on immunohistochemistry-based predictors identifying BRCA1-associated breast carcinomas

Author	Predictors	R	No. *BRCA1*(+)/(–)	Se (%)	Sp (%)	OR	p
Krüger *et al.* [24]	Nestin	+	45/94	62%	84%	8.7	< 0.0005
Madjd *et al.* [34]	ALDH1	+	43/67	NA	NA	NA	0.044
van Heerma Voss *et al.* [35]	ALDH1	+	41/41	NA	NA	NA	0.001
Bane *et al.* [37]	ALDH1	NS	58/242	NA	NA	NA	0.17
Gong *et al.* [49]	FOXO3 (EZH2 – high)	–	62/150	NA	NA	NA	0.017[a]
	FOXO3 (EZH2 – low)	NS		NA	NA	NA	NS
Danzinger *et al.* [55]	Claudin-3[b]	+	32/17	NA	NA	NA	< 0.001
Van Heerma Voss *et al.* [56]	Membranous claudin-1	+	40/40	NA	NA	7.37	0.02
	Claudin-3	+		NA	NA	3.85	0.04
	Claudin-6	+		NA	NA	4.69	0.03
	Claudin-7	+		NA	NA	9.75	0.04
Arnes *et al.* [70]	CDH3	+	27/226	NA	NA	6.7	< 0.0001
Warmoes *et al.*[c] [72]	CDH3	+	102/102	NA	NA	2.44[d]	0.032
	TOP1	+		NA	NA	3.75[d]	< 0.001
	CDH3 and TOP1	+		NA	NA	5.05[d]	0.003
Danziger *et al.* [55]	EGFR[b]	–	32/17	NA	NA	NA	0.004
van der Groep *et al.* [81]	EGFR	+	22/604	NA	NA	NA	< 0.0001
Arnes *et al.* [82]	EGFR-DA	+	18/157	NA	NA	6.5[e]	0.004
	EGFR-HI	+		NA	NA	4.94[e]	0.015
Collins *et al.* [80]	EGFR[b]	+	20/124	NA	NA	NA	NS

[a] *Versus non-BRCA1-mutated samples*

[b] *Correlation of BRCA1 mutation status in TNBC only*

[c] *Multiple logistic regression data*

[d] *Adjusted odds ratio*

[e] *Hazard ratio defined rather than odds ratio*

NA – not available; No. BRCA1(+)/(–) – total number of BRCA1-mutated cases to total number of non-BRCA1-mutated cases; NS – not significant; OR – odds ratio; p – probability value; R – Relation: (+) factor is found more common in BRCA1-mutated samples; (–) lack of (low) factor is more common in BRCA1-mutated samples; Se – sensitivity; Sp – specificity

well as an additional selection tool for patients requiring genetic testing. Secondly, seeking an established phenotype of *BRCA1*-related tumors could expedite tracking down new or less frequent types of mutation. Finally, ALDH1 might provide promising new treatment strategies in therapy-resistant BC, as it may enable specific targeting of the BC stem cell population.

Forkhead box class O3 and enhancer of zeste homologue 2

Forkhead box class O3 (FOXO3) is a transcription factor protein of the Forkhead box class O family, which in humans consists of four members: FOXO1, FOXO3, FOXO4 and FOXO6. As one of the downstream effectors of the PI3K/PKB signaling pathway, it is involved in various processes, such as cell cycle regulation, DNA damage repair, apoptosis, oxidative stress, drug response, angiogenesis, glucose metabolism and differentiation, as widely discussed by Myatt and Lam (2007) [38]. Recently, FOXO3 has become a target for potential therapies in BC [39, 40, 41, 42]. Its high expression was found to be associated with a low histological grade, low tumor stage, lymph node negativity and better OS rate in luminal-like BC patients [43, 44]. In addition, its overexpression was proven to suppress estrogen-dependent tumorigenesis *in vivo* [45].

Enhancer of zeste homologue 2 (EZH2) is a subunit of the polycomb-repressive complex 2 [46] acting as a methyltransferase in the methylation of H3 histone at Lys 23, therefore linking histone methylation and polycomb group-mediated gene silencing [47]. Its overexpression was found to be associated with distant metastases and poor OS in BC patients [48].

A significant relation between *FOXO3* promoter methylation and *BRCA1* mutation status was shown in a study by Gong *et al.* (2016) [49]. Lack of BRCA1 protein resulted in reduction of FOXO3 expression level through targeting EZH2. On a molecular basis, this research found that *BRCA1*-mutated breast tumors had lower levels of FOXO3 protein than *BRCA2*-mutated or non-mutated cancers, although without statistical significance. Taking into consideration high nuclear EZH2 protein concentration, FOXO3 expression was significantly lower in *BRCA1*-mutated samples in comparison with *BRCA2*-mutated and non-mutated tumors. This discovery confirmed that BRCA1 protein positively regulates FOXO3 expression by suppressing EZH2.

Though this method would be constricted by EZH2 levels, its further research may be worth developing in order to increase the specificity and sensitivity of criteria for genetic testing.

Claudins

Claudins are a group of 24 known proteins involved in the formation of tight junctions between epithelial and endothelial cells. Their ability to determine size of aqueous pores between cells regulates selective paracellular transport of small ions and solutes through tight junctions [50]. Claudin expression and specific physiological functions have been described in a variety of healthy and cancerous tissues, including BC [51], as well as in numerous other pathologies [52]. Aberrant expression levels of certain claudins, frequently observed in neoplasms [52], may result in structural and functional modifications within tight junctions and, as a result, promote tumorigenesis and metastasis through the increase of invasion and survival of tumor cells [53, 54].

The multivariate regression model by Danzinger *et al.* (2018) revealed that claudin-3 was 22 times more likely to be observed in *BRCA1*-mutated triple negative breast cancer (TNBC) in comparison to *BRCA2* and non-mutated TNBC. What is more, expression of claudin-3 was an independent marker of *BRCA1* mutation status in TNBC in a univariate analysis [55]. Van Heerma Voss *et al.* (2014) described overexpression of claudin-3, -6 and -7 in *BRCA1*-mutated tumor tissue in comparison to adjacent healthy tissue. In the samples from the *BRCA1*-mutated group membranous claudin-1 expression was shown to be higher when compared to sporadic BC [56]. The authors proposed claudin-1 and -6 as novel markers of *BRCA1* mutation status. Claudin-1 level was the only significant variable when comparing *BRCA1*-mutated *versus* non-mutated tumors. High expression of claudin-6 in *BRCA1*-mutated tumors in comparison to healthy adjacent tissue was independently associated with *BRCA1* mutation. Claudin-6 expresses the dedifferentiated state of *BRCA1*-mutated cancer cells, as BRCA1 regulates mammary stem cell differentiation.

Claudins may have potential not only for *BRCA1* mutation typing, but also as a diagnostic strategy and treatment target because of their signaling properties, association with multiple signaling pathways and plenitude of regulatory mechanisms [54]. This could be used for further improvement of understanding and managing BC.

Topoisomerase 1 type IB and placental cadherin

Topoisomerase 1 type IB (TOP1) is a nuclear enzyme involved in DNA replication, discovered in 1971 by Wang [57]. It supports the progression of the replication fork by relaxing transcription-related supercoils forming ahead [58]. Its necessity in cell division and development of multicellular organisms was proven by Lee *et al.* in 1993 [59] and later utilized as a treatment target, leading to the FDA's approval of two TOP1 inhibitors: irinotecan and topotecan [60, 61].

Placental cadherin (CDH3), a product of the *CDH3* gene [62], is a calcium-dependent cell-to-cell adhesion protein involved in the formation of adherens junctions [63]. It is primarily expressed in the basal layer of epithelia, including the mammary gland [64, 65]. Upregulation of this protein is correlated with tumor aggressiveness and poor prognosis in BC [66, 67, 68].

CDH3 was proposed to be a serum marker in basal-like BC [69], as well as a poor prognostic marker in BRCA1-deficient BC by Arnes *et al.* (2005) [70], who correlated positive CDH3 status with significantly worse BC-specific survival in univariate analysis, poor prognosis in univariate analysis and presence of a *BRCA1* mutation ($p < 0.0001$). This relation was later confirmed by a preclinical study by Gorski *et al.* (2010), who provided a biological explanation of this phenomenon – BRCA1 protein represses transcription of the *CDH3* gene [71].

Warmoes *et al.* (2016) identified extracellular protein biomarkers of BRCA1-deficient BC murine models in secretomes and exosome-like extracellular vesicles. Identifying well over 2 thousand proteins in BRCA1-deficient secretomes, two abundant proteins – CDH3 and TOP1 – were chosen for validation through immunohistochemical staining of BC specimens. Separately, both proteins presented a significant difference in expression level between *BRCA1*-mutated (higher expression) and sporadic BC (lower expression). For both proteins, their expression was found to be an independent predictor of *BRCA1/2*-related BC, as well as together – TOP1 and CDH3 positivity was significantly correlated with *BRCA1/2* mutation independently of age and TN profile.

These findings suggest a substantial improvement of prediction of *BRCA1/2* mutation if assessment of TOP1 and/or CDH3 was performed routinely, es-

pecially in HER2 or ER positive breast carcinomas in women over 45 years old [72].

Epidermal growth factor receptor

Epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase, one of four members of the epithelial growth factor receptor family [73]. It is involved in a complicated network of signaling pathways, including the Ras/Raf/MEK/ERK pathway mediating cell proliferation, the PI3K/PDK/AKT pathway regulating cell survival and many others, participating in cell adhesion, motility, angiogenesis and organogenesis [74]. Due to its pleiotropic effects on cell metabolism, it became a promising target in anticancer therapy [75].

In BC, EGFR overexpression correlates with reduced disease-free survival and OS rates [76]. Although many analyses have been conducted, there is no consensus about its utility in prediction of the *BRCA1* mutation in BC. Danziger *et al.* (2018) found that the lack of expression of EGFR in tumor tissue was associated with *BRCA1* mutation status when compared to wild-type TNBC despite common expression of this protein in basal-like BC [55], the most common phenotype in *BRCA1*-related breast tumors [77, 78, 79]. In contrast, Collins *et al.* (2009) found no statistically significant difference in EGFR expression between *BRCA1* mutation carriers and negative cases of TNBC [80]. Van der Groep *et al.* (2006) found that EGFR expression was more frequent in *BRCA1*-mutated cases [81]. Arnes *et al.* (2009) reported that EGFR in multiple regression analysis was the strongest predictor of *BRCA1* mutation in models adjusted for age, histological grade and ER status, especially when EGFR expression was measured with the Dako criteria (EGFR-DA) or presented strong staining (EGFR-HI) [82].

Considering the inconsistency in statistical methods throughout the described papers and/or relatively small number of cases, no definite conclusions can be made at this point – additional research should be considered.

Sets of markers useful in selecting probable *BRCA-1* mutation carriers

Apart from searching for independent biomarkers useful in selecting patients for genetic testing, it is also proposed to combine multiple factors and evaluate them simultaneously in a single procedure to assess the probability of *BRCA1* mutation. There are different approaches: from the most basic clinical data, i.e. patient's age and family history [83, 84, 85], adding less or more common immunohistochemical markers [78, 86, 87, 88, 89] to molecular models based on whole-genome sequencing [90], gene expression profiling [91], copy number analysis [92, 93] or array comparative genomic hybridization [94].

One of the most recent suggestions by Vos *et al.* (2018) comprises 14 markers chosen with Lasso logistic regression analysis: age, cyclin D1, ERα, ERβ, FGFR2, FGFR3, FGFR4, GLUT1, IGFR, Ki-67, MAI, MLH1, p120 and TOP2A. This model was able to differentiate *BRCA1*-related tumors with sensitivity never dropping below 80% depending on the chosen probability threshold. The second model proposed in that paper, including age, BCL2, CK5/6, CK8/18, cyclin D1, E-cadherin, ERα, HER2, Ki-67, MAI, MLH1, p16, PMS2, PR and vimentin, was developed with an emphasis on immunochemical markers, clinical and morphological data that are commonly available in pathology laboratories, achieving sensitivity of 78% [87]. Both of these methods have a very good to excellent discriminative performance.

Another attempt to find the best fitting multivariate model was made by Hassanein *et al.* (2013) [88]. Their paper investigated morphological parameters and 21 immunohistochemical markers to arrange the minimum of markers providing the best possible performance. The final model included grade 3, MS110, Lys27H3, vimentin and KI67, achieving specificity of 81% and sensitivity of 83% on a validation set.

Miolo *et al.* (2009) focused on markers used in molecular subtyping of BCs – ER, PR and HER2 [89]. The sensitivity and specificity were 100% and 83.3%, respectively. Adding age to these markers, Spurdle *et al.* (2014) provided very robust insight into likelihood ratios for ER alone, grade alone, combined ER and grade stratified by age and ER/PR/HER2 TN status in a large number of cases: 4477 *BRCA1* mutation carriers, 2565 *BRCA2* mutation carriers and 47 565 assumed *BRCA1* and *BRCA2* mutation-negative BC patients to assess the pathogenicity of *BRCA1* or *BRCA2*-mutated variants [86]. They found that ER positivity predicted lack of a *BRCA1* mutation regardless of tumor grade. Moreover, an ER-negative grade 3 result was better at predicting the presence of a *BRCA1* mutation in women over 50 years old than under that age. Apart from that, TN status had a very high value in predicting *BRCA1* mutation irrespectively of age.

La Cruz *et al.* (2012) also reported that ER negativity was associated with *BRCA1* mutation. They proposed a test comprising ER status and mitotic activity [95]. ER-negative phenotype with a high mitotic rate had specificity and sensitivity of 99% and 43% respectively, in prediction of *BRCA1* mutation. The presence of at least one of those factors decreased specificity to 79%, but increased sensitivity to 67%.

Lakhani *et al.* (2005) focused on basal markers expressed in *BRCA1*- and *BRCA2*-mutated tumors:

Table II. Major reports on immunohistochemistry-based sets of predictors identifying BRCA1-associated breast carcinomas

Author	Included factors	No. *BRCA1*(+)/(–)	AUC	Se (%)	Sp (%)	p
Vos *et al.* [87]	Age, cyclin D1, ERα, ERβ, FGFR2, FGFR3, FGFR4, GLUT1, IGFR, Ki-67, MAI, MLH1, p120 and TOP2A	100/94	0.941	> 80	NA	NA
	Age, BCL2, CK5/6, CK8/18, cyclin D1, E-cadherin, ERα, HER2, Ki-67, MAI, MLH1, p16, PMS2, PR and vimentin		0.856	78	NA	NA
Hassanein *et al.* [88]	Grade 3, MS110, Lys27H3, vimentin and KI67	27/81, 28/28[v]	NA	83	81	NA
Miolo *et al.* [89]	ER, PR, HER2	10/72	NA	100	83	NA
Spurdle *et al.* [86]	Age, ER, PR, HER2	4477/ 47 565[a]	NA	57-67	82-87	NA
La Cruz *et al.* [95]	ER, mitotic activity	37/112	NA	99	43	NA
Lakhani *et al.* [78]	ER, CK5/6	182/109	0.77	56	97	NA
	ER, CK5/6, CK14		0.87	NA	NA	NA
Danziger *et al.* [55]	Claudin-3, EGFR[b]	32/17	0.802	NA	NA	< 0.001
van der Groep *et al.* [81]	Age, Ki-67, EGFR	22/604	Data not provided			
Lee *et al.* [97]	Risk prediction algorithm BOADICEA	537[c]/2785, 211/1934[v]	0.82[d, v]	83[d, v]	64[d, v]	NA[d]

[a] *Presumed noncarriers*

[b] *Correlation of BRCA1 mutation status in TNBC only*

[c] *BRCA1 and/or BRCA2 mutations*

[d] *Data from {99}*

[v] *Validation set*

AUC – area under the curve; NA – not available; No. BRCA1(+)/(–) – total number of BRCA1-mutated cases to total number of non-BRCA1-mutated cases; p – probability value; Se – sensitivity; Sp – specificity

CK14, CK5/6, CK17, EGFR and osteonectin [78]. Despite all markers being more prevalent in mutated tumors, only CK14, CK5/6 and ER remained significant in prediction of *BRCA1* carrier status. Two models were proposed: the first one was based on ER-negative and CK5/6 positive status with specificity and sensitivity of 97% and 56%, respectively, with the area under the receiver operating characteristic (ROC) curve of 0.77. The second test with 3 factors – ER, CK5/6 and CK14 status – resulted in the area under the ROC curve rising to 0.87.

Mavaddat *et al.* (2010) also highlighted a potentially important role of ER, CK5/6 and CK14 in prediction of *BRCA1*-mutated cases [96]. On the other hand, Danzinger *et al.* (2018) found no significant correlation between expression levels of CK5 and CK14 among the *BRCA1*, *BRCA2* and non-mutated group in TNBC. In the performed ROC analysis they found that claudin-3 and EGFR expression levels were able to predict *BRCA1* mutation status in TNBC with *fairly high* sensitivity and specificity (area under curve 0.802, $p < 0.001$) [55].

Quite an interesting approach was proposed by van der Groep *et al.* (2006) – they found that age, Ki-67 and EGFR are the best predictors of *BRCA1* mutation status, and created a decision tree consisting of those factors in order to sort cases into four groups with rising risk of *BRCA1*-mutated BC [81]. This analysis was aimed at those cases where standard screening methods, e.g. based on family history and age, failed to give a definite answer to whether genetic testing should be performed.

BOADICEA (Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm) is a risk prediction model for breast and ovarian cancer. It is used to compute *BRCA1* and *BRCA2* mutation carrier probability based on age, a polygenic component (a large number of genes each contributing in a small part to increase the risk of cancer) and a set of families identified through population-based studies of BC consisting of multiple individuals screened for *BRCA1* and *BRCA2* mutations [97]. In 2010 Mavaddat *et al.* combined BOADICEA with well-known distinctive pathological features of *BRCA1*-related tumors – ER-negative status, TN status and expression of basal markers – in order to achieve improved discrimination of *BRCA1*- and *BRCA2*-related as well as sporadic BC. They

achieved that by subdividing the overall disease into different end points, implementing it in BOADICEA and incorporating the aforementioned markers [96]. In 2012 the model base of families was increased to 2785 via a collaborative data set from the Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA), which distinguished additional differences among *BRCA1*, *BRCA2* and non-mutated tumors. Among many comprehensive analyses, they confirmed that the majority of *BRCA1*-related tumors are ER-negative and TN [98]. In 2008 BOADICEA was validated on 1934 families in the United Kingdom [99] and currently figures as a recommended risk assessment tool in the National Institute for Health and Care Excellence clinical guideline CG164 [100]. BOADICEA is widely accessible to healthcare professionals and members of the public, as its implemented web application is constantly modified to simplify use in the clinical environment [97].

All aforementioned sets of markers are summarized in Table II. Extensive data comparison of selected papers, with clinical and molecular methods taken into account, was also included in a paper by Vos *et al.* (2018) [87].

In conclusion, immunohistochemical staining is a basic method used for assignment of breast cancer to differential histological and molecular subtypes. Therefore, searching for correlations between well-known and widely used tumor division methods and clinically valuable data is a natural consequence. In the case of a hereditary BC, quick and confident qualification for genetic testing is crucial as it results in better therapeutic decisions and facilitates oncological and genetic counselling. The variety of described approaches – validated or not – demonstrates the possibility to further upgrade the accuracy of criteria for further genetic evaluation. There seem to be a few good candidates so far: nestin, ALDH1, claudins, TOP1 with CDH3 as well as sets, especially those which consist of markers used on a regular basis. Though most of them have not been validated yet, they could have advantages over those based on clinical types, as clinical data obtained from a patient could be imprecise or incomplete, particularly in the case of family history. On the other hand, they are burdened with the imperfection of a clinical model and therefore may result in false negatives. Molecular methods, although more precise, are currently more expensive than other methods, and this cannot be expected to change in the near future. Moreover, immunohistochemistry-based methods can be widely used by pathology laboratories with limited access to molecular techniques. Therefore, it is beneficial to utilize immunohistochemistry as a screening test before genetic analyses.

The authors declare no conflict of interest.

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