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The Molecular Classification of Women's Breast Cancer by Immunohistochemical, Experience of Sidi Bel Abbes, Algeria.

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ABSTRACT

Breast cancer is the first cancer of women in Algeria but it remains a poorly known disease. Breast cancer is a heterogeneous disease from the clinical, histopathological and biological point of view with different prognoses and responses to different therapies. Traditional classifications including histological assessment and clinical staging are used to guide patient management. However, these prognostic and predictive factors are relatively crude measures and many patients are over treated or undertreated as a result. Therefore, we propose, similarly to many series in the literature with 237 cases of invasive breast carcinoma (IBC) diagnosed at the Department of Pathology, Hospital-University-Center, Sidi Bel Abbes, Algeria, to address the new molecular classification of breast cancer by a conventional immune histochemical (IHC) approach, validated as a replacement technique for microarray analysis, which allows an individualisation of treatment. Our study showed 63.4% luminal tumors A, 10.5% luminal tumors B, 13.6% HER2 phenotype tumors, 12.5% of triple negative tumors whose 84.3% of normal-breast-like, 15.7% of non-basal-like tumors.

Key words: Breast cancers, molecular classification, luminal, immunohistochemical.

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INTRODUCTION

In spite of the progress of treatments and the discovery of targeted therapies, breast cancer is the most common cancer in women worldwide, with nearly 1.7 million new cases diagnosed in 2012 (second most common cancer overall) acquired from the global cancer project (GLOBOCAN 2012) [1], with 7500 cases of breast cancer are registered with approximately 3500 registered deaths each year, in Algeria [2]. It is also the first cancer to get benefits from target therapies against hormone receptors and now HER2 [3, 4, 5].

Clinically, breast cancer is a remarkably heterogeneous disease. Traditionally, pathologic determinations of tumor size, lymph node status, endocrine receptor status, and human epidermal growth factor receptor 2 (HER2) statuses have driven prognostic predictions and, ultimately, adjuvant therapy recommendations for patients with early stage breast cancer [6, 7]. However, these factors are insufficient to account for the evolutionary heterogeneity of the disease and to adapt the treatment and many patients are overtreated or undertreated as a result [8].

The clinical and morphological heterogeneity of breast cancers was associated with molecular heterogeneity. The pioneering works on molecular classification (MC) for invasive breast carcinoma (IBC) by Perou [9] and Sorlie et al [10,11] with global gene expression profiling (GEP) for IBC identified 5 intrinsic subtypes of IBC: luminal A, luminal B, normal breast-like, HER2-enriched, and basal-like, with differing clinical outcomes and responses to neoadjuvant chemotherapy,[12] a bimodal age distribution,[13] and different risk factors.[14] Luminal A: tumors expressing at least one hormone receptor and not expressing HER2neu (RE + and / or RP +, HER2-).Luminal B: tumors expressing at least one hormone receptor and expressing HER2neu (RE + and / or RP +, HER2 +). HER2: tumors overexpressing HER2neu without hormone receptor expression (RE-, RP-, HER2 +).Negative Triple: tumors that express neither hormone receptor nor HER2neu (RE-, RP-, HER2-). Tumors classified in the basal group had the following phenotype based on the Nielsen definition: RE-, RP-, HER2-, CK5-6 + and / or p53 +, EGFR, vimentine. [15, 16, 17]

Therefore, we propose, similarly to many series in the literature to address this new molecular classification of breast cancer.

MATERIALS AND METHOD

An experimental study, from 2010 to 2016, included 237 cases of invasive breast carcinoma diagnosed at the Department of Pathology, Hospital-University-Center, Sidi Bel Abbes, Algeria.

Fragments from paraffin-embedded specimens were made necessary to confirm the histological type.

Many investigators have used IHC-based molecular classification to study IBC and have shown predictive/ prognostic values comparable with that of GEP. In 2013, IHC-based MC was recommended in the St Gallen guidelines for clinical decision-making. [18]

Accurate IHC analyses for estrogen receptor (ER), progesterone receptor (PR), and HER2 are critical for IHC based MC. [19] Semiquantitative evaluation of RH is helpful for further typing of the luminal subtype, [20–22] and evaluation of cytokeratin 5/6 (CK5/6) and P53 help to identify the basal like. [23, 24]

Interpretation was made according to the guidelines immunohistochemistry procedures and scoring methods for each biomarker.

RESULTS AND DISCUSSION

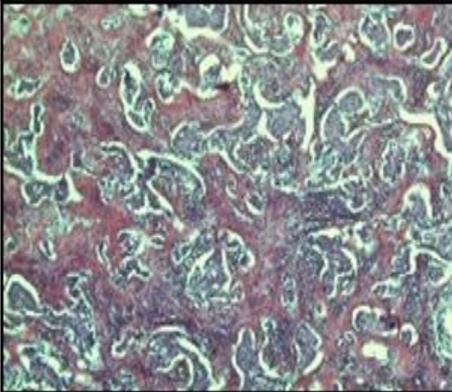
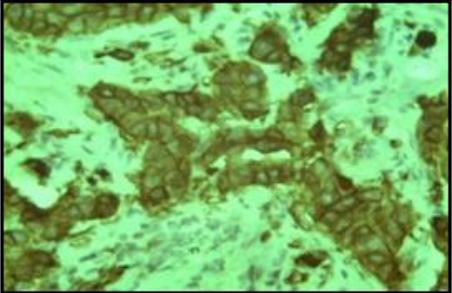
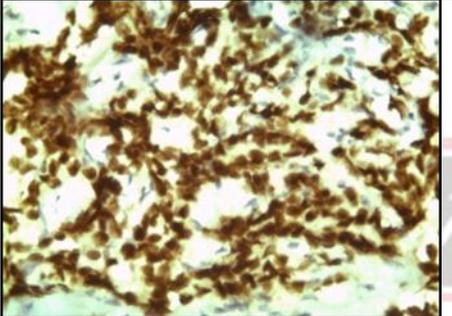
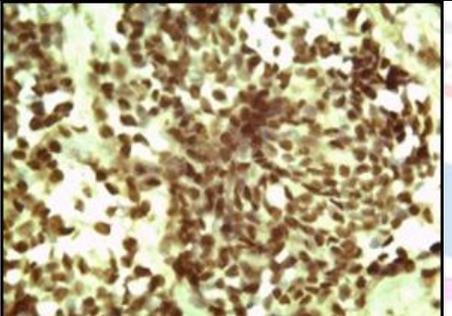
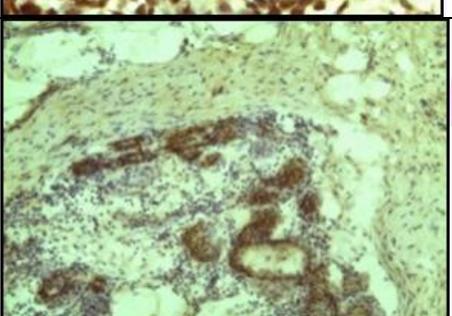
The mean age of patients was 43.7 years (30–63 years). Interpretation results of IHC procedures was made according to the guidelines and scoring methods for each biomarker are briefly described here.

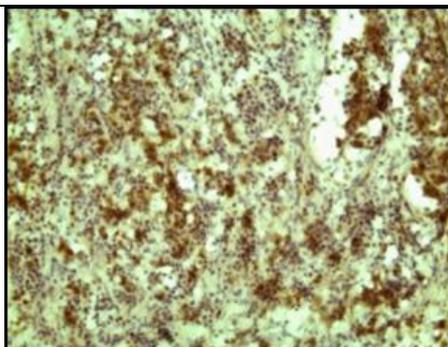
ER and PR : The American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guideline recommends that ER, PR be considered positive if 1% or more of tumor cells have nuclear staining of any intensity. [25]

The ASCO/CAP guideline recommends that HER2 be defined as positive if 10% or more of tumor cells exhibit strong uniform membrane staining [26, 27] and we used the Fluorescence In Situ Hybridization (FISH) for score HER2 (+2) with average copy number of HER-2 / neu inferior <4: Negative: No amplification, between 4 to 6: Low amplification and superior > 6: Positive: Amplification. [28]

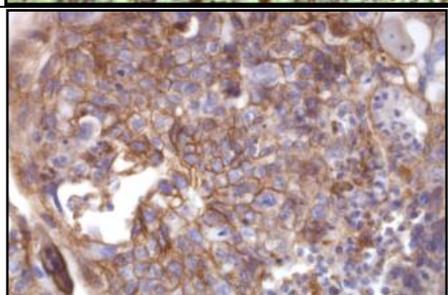
CK5/6 is a high–molecular weight cytokeratin and is expressed in normal myoepithelial cells. Its expression in breast cancer is associated with p53, and increased cytogenetic abnormalities [29]; CK5/6 is often expressed in BRCA1-related breast cancers. [30] Nielsen et al [23] report that a panel of 4 markers (ER, HER2, CK5/6, and EGFR) accurately identifies basal-like breast cancer (BLBC).[31] The cut-off for its positivity in the literature range from any positive cytoplasmic staining to 20% of tumor cells.[27]

Positivity for p53 is associated with a worse prognosis and diminished response to therapy. [32,33] Status of p53 has been proposed to divide triple-negative breast cancer (TNBC) into 2 biologically distinct subgroups: a p53-negative normal breast-like TN subgroup, and a p53-positive basal-like subgroup with worse overall and event-free survival.[32,34,35] Currently, most investigators use 10% of nuclear staining in tumor cells as the cut-off for p53 positivity. (Markers of MC is illustrated in table with Figures).

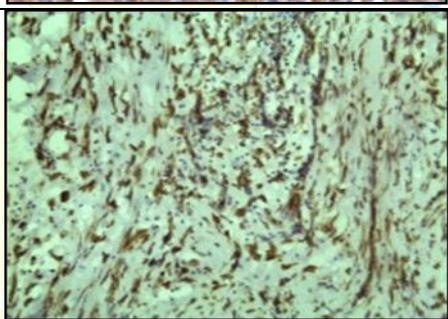
Markers	Interpretation
	<p>Hematoxylin-Eosin sample of Invasive breast carcinoma (IBC) GX 10</p>
	<p>Immuno-histochemical patterns of Invasive breast carcinoma Complete membrane staining of more than 10% of tumor cells with strong intensity showing Her-2/neu expression (IHC) 3+ GX 40</p>
	<p>Immuno-histochemical patterns of Luminal Invasive breast carcinoma ER (estrogen receptor) nuclear staining of any intensity of more than 1% of tumor cells GX 40 (ASCO)</p>
	<p>Immuno-histochemical patterns of Luminal Invasive breast carcinoma PR (Progesterone receptor) nuclear staining of any intensity of more than 1% of tumor cells GX 40 (ASCO)</p>
	<p>Immuno-histochemical patterns of Basal-like subtype carcinoma of CK 5/6 positive cytoplasmic staining to 20% of tumor cells GX 20</p>



Immuno-histochemical patterns of Basal-like subtype carcinoma of P53 positive nuclear staining to 10% of tumor cells GX 20



Immuno-histochemical patterns of Basal-like subtype carcinoma of EGFR positive membrane staining to 10% of tumor cells GX 20



Immuno-histochemical patterns of Basal-like subtype carcinoma of Vimentine positive membrane staining to 10% of tumor cells GX20

Molecular classification by IHC showed: 63.4% luminal tumors A, 10.5% luminal tumors B, 13.6% HER2 phenotype tumors, 12.5% of triple negative tumors.

The study of the expression of cytokeratins 5/6 for the triple negative phenotype identified: 84.3% of normal-breast-like, 15.7% of non-basal-like tumors.

For many years, breast cancer has been considering as a single entity with identical therapeutic modalities however, it is a heterogeneous disease, comprising multiple entities associated with distinctive histological and biological features, clinical presentations and behaviours and responses to therapy, whose current histological and clinical classifications do not fully predict evolution. [6,7,8, 36]

Traditionally, morphologic classification, histologic grade, status of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2), along with tumor stage, are used to guide clinical management, currently only factors are considering for therapeutic choice. [6, 7]

Although many genes and proteins have been studying in breast cancer, and proved that breast cancer is a molecularly heterogeneous disease [37]. Evidence from gene expression microarrays suggests the presence of multiple molecular subtypes of breast cancer. [38, 39, 40] The recent commercial availability of gene expression profiling techniques that predict

risk of disease recurrence as well as potential chemotherapy benefit have shown promise in refining clinical decision making. The pioneering works on molecular classification (MC) for invasive breast carcinoma (IBC) by Perou [9] and Sorlie et al [10,11], using complementary DNA (cDNA) microarrays representing 8102 human genes to characterize gene expression patterns in a set of 65 surgical specimens of human breast tumors from 42 different individuals, they demonstrated that the phenotypic diversity of breast tumors was associated with corresponding gene expression diversity which means molecular heterogeneity. They identified with global gene expression profiling (GEP) for IBC 5 intrinsic subtypes of IBC: luminal A, luminal B, normal breast-like, HER2-enriched, and basal-like, with differing clinical outcomes and responses to neoadjuvant chemotherapy, [12] a bimodal age distribution, [13] and different risk factors. [14]

A conventional immunohistochemical approach was used in the study, validated as a replacement technique for microarray analysis, which allows an individualisation of treatment [41, 42]. Because the application of gene expression profiling in daily practice is not economical or practical now, many investigators have studied the use of immunohistochemical surrogates as a substitute for determining the MC of IBC [43]. In 2013, IHC-based MC was recommended in the St Gallen guidelines for clinical decision-making. [18]

The subtype of invasive breast cancer

Luminal A Subtype. Luminal A breast cancers account for about 30% to 40% of all IBC. They do not overexpress HER2, and about 13% of luminal A tumors have a p53 mutation, it is associated with better prognosis.[44] Morphologically, most luminal A tumors are well differentiated carcinomas of no special type, tubular carcinomas, classical lobular carcinomas, mucinous carcinomas, and neuroendocrine carcinomas.[45]

Luminal A tumors often have an IHC profile of high ER and PR expression, negative HER2, and low Ki-67. The original IHC surrogates were ER and/or PR positive, and HER2 negative. [46] Subsequent studies showed that a Ki-67 of 14% was the cut-off point to separate luminal A from luminal B subtypes. [20] More recently, a 20% cut-off point of PR to separate luminal A and B subtypes has been proposed, [21] and this was substantiated in a separate study.[47] Based on currently available data, the most commonly used IHC surrogates for luminal A subtype are ER, PR of 20% or greater, HER2, and Ki-67 of less than 14%.

Luminal B Subtype. Luminal B subtype accounts for about 20% to 30% of all IBC. Compared with luminal A subtype, it shows a higher frequency of p53 mutations (32%). Morphologically, this group of tumors is less well differentiated and consists mostly of invasive ductal carcinomas of no special type, and also some invasive micropapillary

carcinomas.[45] By IHC analysis, these tumors show a lower level of ER expression, a lower level of or negative PR expression, and a higher level of Ki-67 labelling. However, molecular studies have shown that luminal B is not simply a more proliferative variant of luminal A, because both luminal A and B cancers have their own specific oncogenic drivers.[48] but later most investigators used ER and HER2 to describe this subtype if the score of Ki-67 was 14% or higher,[24, 49] or higher than 20%.30

HER2-Positive.This subgroup consists of 12% to 20% of all IBC. HER2positive tumors are very heterogeneous at the molecular level. [50, 51] A recent study from Fountzilias et al [52] clearly demonstrated that luminal HER2 (ER, PR, HER2) and HER2-enriched subtypes (ER, PR, HER2) are clinically distinct, with different survival curves and metastatic patterns. Thus, HER2-positive tumors should be divided into two subtypes: luminal HER2 subtype (ER and/or PR positive/HER2 positive) and HER2-enriched subtype (ER and/or PR negative/HER2 positive).[52–54]

Triple-Negative Breast Cancer. Triple negative" breast cancers represent about 17% of breast cancers and constitute a heterogeneous group characterized by the absence of estrogen and progesterone hormone receptors and the absence of overexpression of HER-2 growth factor in immunohistochemistry, is associated with an adverse clinical profile with a high risk of early metastatic relapse due to the aggressive nature of the tumors, their partial response to chemotherapy and the use of targeted therapies used in clinical practice [55].

TNBC patients are usually younger,[56] with higher-grade tumors111 and a higher risk of distant recurrence and death within the first 3 to 5 years after diagnosis.[57] Most importantly, there is no targeted therapy available for TNBC at the present time.

Morphologically, most TNBCs are ductal carcinoma of no special type; others include special types of ER-negative tumors, such as adenoid cystic carcinoma, secretory carcinoma, metaplastic carcinoma, and carcinoma with medullary features, each with a distinctive morphology and clinical behavior.

Basal-like breast cancer can also be defined using IHC surrogates, including ER, PR, HER2, CK5, and EGFR, according to Nielsen definition: RE-, RP-, HER2-, CK5-6 + and / or p53 + [15, 16, 17, 58].

There have been p53 mutations reported in 82% of these BLBCs, and most are positive for keratins 5/6. Morphologically, BLBCs are frequently high grade and large size, with pushing borders, with a poor Nottingham Prognostic Index, and a high rate for local recurrence and distant visceral organ metastasis, especially within the first 5 years. There is a small group of TNBCs that are also negative for CK5 and EGFR. [59] Rakha et al [60] showed that this

subgroup of tumors is less likely to be associated with a BRCA1 mutation, and they have better breast cancer-specific survival and disease-free survival compared with BLBC.

Our results were similar to the literature except with higher rate luminal A in our study was a much larger with 63% versus 30%.

CONCLUSION

Clearly, MC of IBC has enhanced our understanding of breast carcinogenesis significantly in recent years. The capacity to define subtypes of breast cancer provides a framework for understanding the mechanism of breast carcinogenesis, and opportunities for improving therapeutic intervention. The IHC surrogates have been shown to be useful for advancing the understanding of the prognostic and predictive values of MC, unlike the methods of express gene, difficulty of realization in current practice, the need for frozen tumor material for the majority of these tests, the impossibility of guaranteeing good quality RNAs of all operated breast tumors. Much more work needs to be done, especially in the standardization of IHC analysis and scoring for each biomarker, standardization of the definition for each classification, and the continued addition of newly discovered biomarkers, before MC can be used routinely in the clinical setting. In order to apply the most appropriate treatment to each patient, depending on the type and severity of her disease, it is necessary to address breast cancer in its complexity.

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