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ORIGINAL ARTICLE

Prognostic implications of the intrinsic molecular subtypes in male breast cancer

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Summary

Purpose: *Intrinsic molecular subtyping has been widely used in female breast cancer, and it has proven its significance. In this article, we aimed to study the intrinsic subtypes of male breast cancer (MBC) in correlation with clinicopathological features.*

Methods: *We retrospectively identified 130 MBC cases from 2004 to 2013. Intrinsic molecular subtypes were determined by immunohistochemistry (IHC).*

Results: *From a total of 130 MBC cases, 45.4% of tumors were luminal A subtype, 44.6% were luminal B, 5% were HER2 positive and 5% were triple negative tumors. There were statistically significant differences between different IHC intrinsic subtypes regarding tumor size (p=0.001), estrogen receptor (ER) status (p=0.001), progesterone receptor (PR) status (p=0.001), HER2 status (p=0.001) and Ki67 proliferation index (p=0.001).*

Conclusion*: The distribution of breast cancer intrinsic subtypes in males is different compared to its female counterpart; however, they don't seem to give the same prognostic value.*

Key words: *breast cancer, immunohistochemistry, male, molecular subtyping, outcomes*

Introduction

MBC is rare, accounting for less than 1% of all breast cancers and less than 1% of all malignancies in men. Despite the reported increasing incidence [1,2], MBC is still an understudied disease and the clinical management of male patients with breast cancer is guided by research on female breast cancer. Although male breast cancer does share some similarities with its female counterpart, numerous emerging studies have revealed many differences in hormone receptor [3,4] and human epidermal growth factor receptor2 (HER2) expression levels [5,4] as well as differences in the transcriptional level and the genomic profiling [6,7] and in prognosis and survival [8,9].

Gene expression profiling for female breast carcinoma demonstrated that breast cancer can be classified into 5 main subtypes with distinct molecular features known as luminal A, luminal B, HER2-positive, triple-negative and the classical breast cancer [10-13]. Since gene expression data is often not available, IHC markers have been used as surrogates for DNA microarray in subtyping breast cancer and IHC intrinsic subtypes are routinely used in diagnosis. Although molecular subtyping has been widely used in female breast cancer to identify subsets and provide significant prognostic and therapeutic information, little is known about the prognostic value of these subtypes in MBC. In this study we sought to subclassify MBC using IHC and to evaluate the prognos-

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tic implication of these intrinsic molecular subtypes in MBC patients.

Methods

Patient data

This study was performed at Salah Azaïz Cancer Institute. From 2004 to 2013, data of 130 patients with MBC diagnosed and managed at our institution were collected. Patient details including age at diagnosis, tumor size, lymph node status, tumor grade, TNM stage, distant metastasis and follow-up information were retrieved from medical records. Two experienced pathologists graded independently all tumors following the Nottingham grading system [14]. The study was approved by the local multidisciplinary committee.

Immunohistochemistry

IHC for ER, PR, HER2, proliferation index (Ki67) and cytokeratin 5/6 was conducted on paraffin-embedded tissue slides. Sections of 4 µm were cut, cleared in xylene, rehydrated in ethanol and rinsed in distilled water. The slides were then incubated with specific primary antibodies at room temperature and the reaction was completed through incubation with hydrogen peroxide, chromogen agent diaminobenzidine for 10 min and counterstained with hematoxylin. The slides were then dehydrated and mounted. A positive control with a tissue sample known to express the antigen of interest was included on each histologic slide. Antibodies and dilutions used are shown in Table 1.

Evaluation of IHC staining was done by two experienced pathologists without knowledge of the case outcome. ER and PR stainings were considered positive if at least 1% of the tumor cells showed nuclear staining according to the ASCO/CAP recommendations [15]. According to Hercep Test ™ criteria, tumor scores of 0 or 1+ were considered to be HER2 negative and those scoring 3+ were considered to be HER2 positive.

Chromogenic in situ hybridization (CISH)

CISH was performed for equivocal HER2 cases (score 2+) defined according to the ASCO/CAP 2007 criteria. CISH was performed and interpreted using the ZytoDot SPEC HER2 probe Kit according to the manufacturer's instructions. CISH-amplified cases were considered to be HER2 positive.

IHC intrinsic subtype classification

Immunohistochemical analysis of ER, PR, HER2 and Ki67 proliferation index were used as immunohistochemical surrogate markers for breast cancer intrinsic subtype's definition according to the St. Gallen criteria [16]. All MBC cases were classified into 5 intrinsic subtypes: luminal A (ER+and/or PR+, HER2- and Ki67 low), luminal B (ER+and/or PR+, HER2+ or - and Ki67 high), HER2 positive (ER-, PR- and HER2+) and triple-negative (ER-, PR-, and HER2-).

Statistics

All statistical analyses were performed using SPSS 20.0 (IBM) software package for windows. Differences between breast cancer subtypes regarding clinicopathological characteristics were calculated using Pearson's x^2 test. The results were considered statistically significant if the p value was <0.05. Overall survival (OS) was calculated from diagnosis until death from any cause or last patient follow up. The Kaplan-Meier method was used to generate survival curves and the log rank test was used to compare survival differences. Factors with significant prognostic value in the univariate Cox regression model were evaluated with multivariate Cox regression model to explore the independent effects of survival.

Results

Clinicopathologic features of male breast cancer

The median patient age was 66 years (range 30-96). Invasive ductal carcinoma was the predominant histological type representing 93.8% of the cases (N=126). Tumor size ranged from 0.5 to 10 cm (median 2). Of the cases, 63.8% were grade 2 (N=83) and 59.2% had positive axillary lymph node status (N=77). Of the 130 MBC patients, 90% were ER+ (N=117), 83% PR+ (N=108), 7.7% HER2+ (N=10) and 54.6% (N=71) had a high proliferation index Ki67. Distant metastasis was registered in 46.2% (N=60) of MBC patients.

IHC intrinsic subtypes of male breast cancer

Using IHC surrogates, the majority of our cases were classified as luminal A subtype (45.4%, N=59), followed by luminal B subtype (44.6%,

Table 1. Antibodies used for immunohistochemical characterization of male breast cancer cases

Antibody	*Clone*	*Manufacturer*	*Dilution*	*Antigen retrieval*
ER	6F11	Novocastra	1/75	Citrate buffer
PR	16	Novocastra	1/150	Citrate buffer
HER2	CB11	Novocastra	1/40	Citrate buffer
Ki67	MM1	Novocastra	1/200	EDTA
CK5/6	D5/16B4	Zymed	1/200	Citrate buffer

Table 2. Distribution of clinicopathological features in male breast cancer in relation to IHC studied intrinsic subtypes

		IHC Intrinsic subtypes				
Characteristics	*n (%)*	*Luminal A*	*Luminal B*	*HER2 positive*	*Triple negative*	*p value*
Age, years						0.994
< 60	34 (26.1)	19 (32)	12 (21)	1 (17)	2 (29)	
≥ 60	96 (73.9)	40 (68)	46 (79)	5 (83)	5 (71)	
Pathological type						0.960
IDC	126 (97)	58 (98)	56 (96.5)	6 (100)	6 (86)	
IPC	4 (3)	1 (2)	2 (3.5)	0	1 (14)	
Tumor size, cm						0.001
< 2	41 (31.5)	24 (41)	12 (21)	2 (33)	2 (29)	
≥ 2	89 (68.5)	35 (59)	45 (79)	4 (67)	5 (71)	
SBR grade						0.908
1	21 (16.2)	11 (19)	7 (12)	1 (16.7)	2 (28.6)	
2	84 (64.6)	38 (64)	38 (65.5)	4 (66.7)	4 (57.1)	
3	25 (19.2)	10 (17)	13 (22.5)	1 (16.7)	1 (14.3)	
Axillary lymph node status						0.104
Negative	53 (40.8)	30 (51)	17 (29)	3 (50)	3 (43)	
Positive	77 (59.2)	29 (49)	41 (71)	3 (50)	4 (57)	
Stage						0.457
I-II	58 (44.6)	25 (42)	24 (41)	5 (83)	4 (57)	
III-IV	72 (55.4)	34 (58)	34 (59)	1 (17)	3 (43)	
ER status						0.000
Negative	13 (10)	0	0	6 (100)	7 (100)	
Positive	117 (90)	59 (100)	58 (100)	0	0	
PR status						0.000
Negative	22 (17)	4 (7)	5 (9)	6 (100)	7 (100)	
Positive	108 (83)	55 (93)	53 (91)	0	0	
HER2 status						0.000
Negative	120 (92)	59 (100)	54 (93)	0	7 (100)	
Positive	10 (8)	0	4 (7)	6 (100)	0	
Ki-67 index (%)						0.000
< 20	59 (45.4)	59 (100)	0	0	0	
≥ 20	71 (54.6)	0	58 (100)	6 (100)	7 (100)	
Distant metastasis						0.393
No	70 (54)	36 (61)	28 (49)	2 (33)	4 (57)	
Yes	60 (46)	23 (39)	30 (51)	4 (67)	3 (43)	
Recurrence						0.383
No	123 (94.6)	57 (96.6)	55 (94.8)	5 (83.3)	6 (85.7)	
Yes	7 (5.4)	2 (3.4)	3 (5.2)	1 (16.7)	1 (14.3)	

For abbreviations see text

N=58), HER2 positive (5%, N=6) and triple negative (5%, N=7). Only 7% (N=4) of luminal B cases showed HER2 positivity, the rest were ER positive and had a high Ki67 proliferation index (≥20%).

Association of IHC intrinsic subtypes with clinicopathologic features

The association between IHC intrinsic subtypes and clinicopathologic features in order to determine the significance of this classification in MBC were analyzed. There were statistically significant differences among different IHC intrinsic subtypes regarding tumor size (p=0.001), ER status (p<0.001), PR status (p<0.001), HER2 status (p<0.001) and Ki67 proliferation index (p<0.001). No statistically significant differences were noted among different surrogate intrinsic subtypes regarding age, histological grade, axillary lymph node status, stage, recurrence and distant metastasis. The distribution of clinicopathological features in MBC subtypes are summarized in Table 2.

Prognostic significance of IHC intrinsic subtypes in male breast cancer

With a median follow-up of 12.5 months (range 1-132), 118 deaths were reported. In this series, the 5-year OS rate was 43% (95%CI, 0.04-0.52). Median OS for luminal A subtype was 17

Table 3. Univariate and multivariate Cox regression analyses of different prognostic factors for overall survival in male breast cancer patients

Variables	*Univariate analysis*			*Multivariate analysis*		
	P	*HR*	*95% CI*	*P*	*HR*	*95% CI*
Age						
≤ 60 vs > 60 years	<0.001	0.600	[0.543-0.663]	<0.001	0.600	[0.544-0.661]
Size						
< 2 vs ≥ 2 cm	0.198	0.693	[0.396-1.212]	0.242	0.720	[0.416-1.248]
Grade		0.845	[0.559-1.279]			
1 vs 2-3	0.426			0.293	0.810	[0.548-1.199]
Axillary lymph node status		0.760	[0.438-1.321]			
Negative vs Positive	0.331			0.329	0.767	[0.450-1.306]
Stage		0.939	[0.735-1.199]			
I-II vs III-IV	0.613			0.462	0.914	[0.718- 1.162]
ER status		0.799	[0.237-2.693]			
Negative vs positive	0.717			0.704	0.793	[0.239-2.629]
PR status		0.736	[0.343-1.577]			
Negative vs positive	0.430			0.435	0.741	[0.348-1.574]
HER2 status		1.178	[0.646-2.148]			
Negative vs positive	0.594			0.693	1.125	[0.626-2.0233]
Ki67 index (%)		0.952	[0.350-2.589]			
< 20 vs ≥ 20	0.923			0.884	0.929	[0.344-2.505]
Intrinsic molecular subtypes						
LA vs LB vs HER2 enriched vs TN	0.764	0.893	[0.427-1.867]	0.799	0.911	[0.446-1.864]
Distant metastasis						
No vs Yes	0.292	0.733	[0.412-1.306]	0.727	0.917	[0.563-1.493]
Recurrence						
No vs Yes	0.278	1.859	0.606- 5.701]	0.299	1.798	[0.595-5.437]

HR: hazard ratio, 95% CI: 95% confidence interval, LA: luminal A, LB: luminal B, TN: triple negative

months (range 1-132), 14 months (range 1-118) for luminal B, 5 months (range 1-38) for HER2 positive and 3 months (range 1-18) for triple negative subtype. However, prognosis did not differ regardless this intrinsic classification (p=0.764). Cox proportional hazards regression model was used to examine the association between clinicopathological features including IHC intrinsic subtypes and OS. Univariate analysis showed that only age was significantly associated with OS (p<0.001, HR=0.600, 95%CI 0.543-0.663), whereas IHC intrinsic subtyping failed to define different prognostic groups in MBC (Table 3). Multivariate analysis identified age as a unique independent prognostic factor for OS of MBC patients (p<0.001, HR=24, 95%CI 20-29; Table 3).

Discussion

Molecular subtyping of female breast cancer using the 5 biomarker classification scheme has now become a standard for predicting prognosis and determining appropriate treatment options for females with breast cancer [16,17]. Since MBC is an understudied disease, little is known about the prognostic and therapeutic implications of these intrinsic subtypes, as only few studies on small series have been conducted due to the rarity of this disease. Recently, few studies have attempted to characterize molecular subtypes using IHC markers in MBC and explored their prognostic implications [18-20].

Kornegoor et al. [20] analyzed 134 MBC cases in which luminal A represented the vast majority of the cases (75%), luminal B represented 21% of the cases, basal-like 3% and unclassifiable triple-negative 1% of the cases; no HER2 cases were identified. Nilsson's et al. [19] study on 197 MBC cases, revealed luminal A in 81%, luminal B in 11%, core basal in 1%, but no case of HER2 subtype was identified. A smaller study by Ge et al. [21] was conducted on 42 MBC cases, determining that luminal A was the most common subtype representing 83% of the cases, followed by luminal B (17%). In this study, neither basal-like nor HER2 positive were identified. Another small study by Sánchez-Muñoz et al. [22] looked at 43 MBC patients, and 44% of the cases were luminal A, 51% luminal B and 5% basal-like; no HER2 tumor subtype was identified. Recently, a retrospective study conducted on 111 MBC patients by Abreu et al. also demonstrated that luminal A was the most

common subtype representing 89.2% of the cases, followed by luminal B (7.2%), triple-negative (2.7%) and HER2 positive (1%) [23].

In the present study, we performed for the first time in Tunisia an intrinsic molecular classification of MBC using IHC in order to understand tumor behavior and to confront our results to those reported in the literature. Our analysis demonstrated that MBC is primarily of luminal subtype. Luminal A and luminal B subtypes represented the vast majority of cases (90%). Luminal A was the most common subtype in our series representing 45.4% of the cases using the 1% ER/PR threshold. Same as in female breast cancer, this subtype was associated with a better prognosis.

HER2 subtype is very rare in men; interestingly, we identified 6 cases of males with ER-/PR-/HER2+ breast cancer. A higher incidence of triple-negative subtypes was noted in our series (5%) comparing to previous studies [19-22], and none of the 7 triple-negative cases was classified as basal-like. Taken together, these results demonstrate that the distribution of MBC subtypes is different compared to females [6,21].

In line with the reported results in the literature, our study demonstrated no statistically significant differences between different intrinsic molecular subtypes and age, tumor size, histological grade or distant metastasis. Moreover, in univariate and multivariate analysis, IHC intrinsic subtyping failed to define different prognostic groups in MBC.

In this study, luminal A and B subtypes were found to be by far the most common in MBC and were associated with better prognosis compared to HER2 positive and triple-negative subtypes. HER2 positive and triple-negative breast cancers are rare in males. Intrinsic molecular classification of MBC does not seem to provide similar prognostic information as in female breast cancer.

Conclusions

The results of our study and those of the literature support the notion of considering MBC as a biologically unique entity, different from its female counterpart. More light must be shed on this disease and further research should be conducted in order to improve management and outcome of males with breast cancer.

Conflict of interests

The authors declare no confict of interests.

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