Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2020 Mar 25; 8(B):133-138.
https://doi.org/10.3889/oamjms.2020.4077
eISSN: 1857-9655
Category: *B - Clinical Sciences*
Section: *Oncology*

The Role of the Molecular Subtypes in the Prognosis of Breast Cancer Patients

Stoyan Nikolov[1], Emil Enchev[1], Georgi Minkov[1], Evgeni Dimitrov[1], Koni Ivanova[2], Maya Gulubova[2], Tatyana Vlaykova[3], Yovcho Yovtchev[1]

[1]Department of Surgery, University Hospital "Prof. Dr Stoyan Kirkovich" Stara Zagora, Bulgaria; [2]Department of Pathology, Faculty of Medicine, Trakia University Stara Zagora, Bulgaria; [3]Department of Biochemistry, Faculty of Medicine, Trakia University Stara Zagora, Bulgaria

Edited by: Ksenija Bogoeva-Kostovska
Citation: Nikolov S, Enchev E, Minkov G, Dimitrov E, Ivanova K, Gulubova M, Vlaykova T, Yovtchev Y. The Role of the Molecular Subtypes in the Prognosis of Breast Cancer Patients. Open-Access Maced J Med Sci. 2020 Mar 25; 8(B):133-138. https://doi.org/10.3889/oamjms.2020.4077
Keywords: Breast cancer; Molecular subtypes; Overall survival
***Correspondence:** Stoyan Nikolov, PhD. Department of Surgery, University Hospital "Prof. Dr Stoyan Kirkovich", 2 General Stoletov Street, 6000, Stara Zagora, Bulgaria. el: +359884922079. E-mail: dr_nikolov@yahoo.com
Received: Nov-18-2019
Revised: Jan-25-2020
Accepted: Mar-06-2020

Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Understanding the biology of the tumor, by dividing it into molecular subtypes, has made it possible to individualize the therapeutic approach in high-risk patients.

AIM: We aimed to determine the importance of established molecular subtypes in the prognosis and the importance of disease-free and overall survival (OS) in patients with non-metastatic breast cancer.

MATERIALS AND METHODS: We analyzed 94 patients with non-metastatic breast cancer for the period 2010–2018. The median follow-up time was 60 months. The mean age in the study group was 60.03 years (SD ± 10.52). According to the characteristics of the studied indicators, we divided the group into Luminal A (n-59 [62.7%]), Luminal B/HER2 (−) (n-2 [2.1%]), Luminal B/HER2 (+) (n-8 [8.5%]), HER2 overexpressing (n-3 [3.2%]), and triple-negative subtype (n-22 [23.5%]). In all patients in the study group, we analyzed the 5-year overall survival (OS) and disease-free survival (DFS) and referred it to molecular subtypes, lymphatic status, HER-2 status, the presence or absence of endocrine therapy for the follow-up period, tumor differentiation, and type of surgery.

RESULTS: We observed the 5-year OS in 92% of patients identified as Luminal A; at 50% of Luminal B/HER2 (−) neg.; in 62.5% with Luminal B/HER2 (+), in 67% with HER2-overexpressing carcinoma; and in 66.7% of patients with triple-negative subtype. The total cancer-associated mortality rate in the analyzed period reached 15.9% (n = 15). Patients with mastectomy (p = 0.019, p = 0.027), positive axilla with more than 4 lymph node (LN) (p = 0.000; p = 0.000), and Luminal B/HER-2 (+) tumors (p = 0.004; p = 0.003) were the independent prognostic factors for worse DFS and OS in our study group. Histological differentiation and HER-2 expression were in unsatisfactory correlation (p = 0.077; p = 0.044 and p = 0.081; p = 0.055, respectively).

CONCLUSION: Molecular subtypes are essential in the prognosis of breast cancer and maybe a criterion for an individualized therapeutic approach.

Introduction

Breast cancer remains a socially significant problem despite the reduced early mortality from the disease in recent years. In a few patients, disease progression after 5 years or more of disease-free survival (DFS) has been observed during follow-up [1], [2]. Established behavioral protocols and principled treatment settings not only determine improvements in treatment outcomes but also have the risk of a lack of individual approach in high-risk groups where the percentage of patients with a 5-year survival rate is unsatisfactory low. An expression of the tendency to understand the biology ("individuality") of a tumor with its specificity of progression and "cellular communication" is its differentiation into molecular subtypes [3], [4], [5]. This has created hope in differentiating and optimizing the therapeutic approach in high-risk groups. The expected treatment outcome improved and allowed for the development of multiple strategies for seemingly "one disease" [6], [7], [8]. Thus, molecular subtypes in breast cancer determine the daily clinical practice [9] proposed in 2011 by the expert panel of St. Gallen [10] and approved in 2013 [11], [12].

The purpose of our study was to determine the importance of established molecular subtypes in the prognosis and the importance of disease-free and overall survival (OS) in patients with non-metastatic breast cancer.

Materials and Methods

The study included 94 patients with non-metastatic breast carcinoma staged and operated at the Clinic for Surgical Diseases at University hospital "Prof. Dr. Stoyan Kirkovich," Stara Zagora for the period 2010–2018. The criteria for inclusion in the group were as follows:

1. Lack of hematogenous metastases in clinical staging
2. T1, T2 tumors
3. Lack of need for neoadjuvant therapy
4. Invasive cancer
5. Immunohistochemically typed carcinoma and defined as Luminal A, Luminal B/HER2 (−), Luminal B/HER2 (+), HER2 overexpressing, and triple-negative
6. 60-month followed up patients with sufficient information on DFS and OS
7. Absence of synchronous tumor of the same side – or contralateral breast
8. Up to N2 in pathohistological staging.

All patients with ultrasound and mammography data for potentially malignant, solid breast tumor underwent clinical staging with:

1. Ultrasound of breast and axilla
2. Mammography
3. CT scan of thorax and abdomen with intravenous contrast
4. Serum CA15-3
5. Pelvic X-ray – until 2008.

All patients after staging were discussed by a multidisciplinary team, and a primary therapeutic approach was defined.

All patients in the study group were treated according to a protocol approved in the clinic, based on the protocols of St. Galen and the National Cancer Center for the country. All analyzed patients underwent primary surgery, followed by pathohistological staging and typing. In our study group, in 67 (71.3%), we performed organ-preserving intervention with lymphatic dissection at the levels I and II and analysis of 14 LNs, tumor bed, and resection lines. In 27 (28.7%), we performed a modified mastectomy with lymphatic dissection at the I and II levels, while the pectoralis minor was preserved and conditions for subsequent subpectoral reconstruction were created. The pathohistological characteristics of the tumor and immunohistological typing are presented in Table 1.

Table 1: Pathohistological characteristics

Tumor characteristics	n (%)
Histology	
Lobular	6 (6.4)
Ductal	82 (87.2)
Other	6 (6.4)
Tumor size (cm)	
≤2	46 (48.9)
>2	48 (51.1)
Grade (G)	
1	13 (13.8)
2	68 (72.4)
3	13 (13.8)
pN Stage	
N0	47 (50)
N1	30 (31.9)
N2	17 (18.1)
ER	
Positive	58 (61.7)
Negative	36 (38.3)
PGR	
Positive	61 (64.9)
Negative	33 (35.1)
HER2	
Positive	11 (11.7)
Negative	83 (88.3)
Molecular subtypes	
Luminal A	59 (62.7)
Luminal B HER 2 (−) neg.	2 (2.1)
Luminal B HER 2 (+) pos.	8 (8.5)
HER 2 overexpressing	3 (3.2)
Triple-negative	22 (23.5)

According to the proposed 2011 St. Gallen classification, we differentiated the study group into the following subtypes (Table 2).

Table 2: Subtypes according to St. Gallen 2011

Subtype	ER and PR status	HER-2 status	Ki-67
Luminal A like	(+) pos.	(−) neg.	<14%
Luminal B/HER2 − like	(+) pos.	(−) neg.	>14%
Luminal B/HER2 + like	(+) pos.	(+) pos.	>14%
HER-2 type	(−) neg.	(+++) overexpressed	NM
Triple-negative	(−) neg.	(−) neg.	NM

All postmenopausal patients in the study group and hormone receptor positives received hormone therapy as follows: 66.7% – tamoxifen, 15.9% – aromatase inhibitors, and 17.4% were without therapy. Polychemotherapy received 64.9% of patients according to the accepted standard for the country – positive axillary LN, high-risk patients (young age, low grade, family, and genetic predisposition). Of the chemotherapy recipients, 14.8% were on CMF (cyclophosphamide, methotrexate, and 5 FU) and 85.2% were treated with anthracycline based therapy. About 66.7% of patients with HER-2 overexpressing carcinoma had positive LN, 50.8% of those with Luminal A, and about half of the patients with Luminal B and triple-negative (50% and 45.5%, respectively).

All patients with organ preserving intervention held adjuvant radiotherapy with a total dosage of 50 ± 2 Gy.

In all patients in the study group, we analyzed the 5-year OS and DFS and referred it to molecular subtypes, lymphatic status, HER-2 status, the presence or absence of endocrine therapy for the follow-up period, tumor differentiation, and type of surgery.

Statistical analysis

The statistical analyzes were performed using SPSS v16.0 (SPSS, Inc.). The Chi-square test and the Fisher exact test were used to compare the categorical data. The comparison between the independent groups was performed with ANOVA, Student's t-test, Mann–Whitney U test, and Kruskal–Wallis test depending on the normality of distribution. Correlations were tested using Spearmen and Person tests. Survival curves were drawn using the Kaplan–Meier method, and the difference in survival was calculated with the log-rank test. Factors with $p < 0.05$ were considered statistically significant.

Results

The first relapse (distant, locoregional, or combined) was the primary starting point for DFS. OS

is calculated from the date of diagnosis to the date of death associated with breast cancer. We observed the 5-year OS in 92% of patients identified as Luminal A; at 50% of Luminal B/HER2 (−) neg.; in 62.5% with Luminal B/HER2 (+), in 67% with HER2-overexpressing carcinoma; and in 66.7% of patients with triple-negative subtype. The total cancer-associated mortality rate in the analyzed period reached 15.9% (n = 15).

As starting points in the prognosis of progression and survival were as follows:

1. Type of intervention
2. Histological differentiation of the tumor (G)
3. HER-2 status
4. LN status
5. The presence or absence of endocrine therapy during the follow-up period
6. Molecular subtypes.

In our study group, in 71.3% of patients, primary surgical treatment included organ-preserving operation with Grades I and II LN dissection, and modified mastectomy was required in 27 patients. In the analysis of the type of surgery for DFS and OS, we found that patients with organ-preserving operation had a significantly longer DFS compared to patients with mastectomy p = 0.019. A similar dependence is also found when assigning the type of operating procedure to OS p = 0.027 (Figure 1).

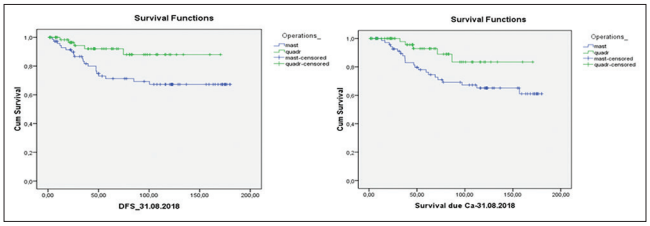

Figure 1: Disease-free survival and overall survival by type of surgery

Of course, the type of surgery is not based entirely not only on the biology of the tumor but also on the concomitant risk factors such as the type of invasive carcinoma, breast volume, family burden, and genetic typing. One way or another, in the patients at risk, we underwent mastectomy, which clearly states that a surgical approach served by “maximum radicalism” is not determinative of the disease.

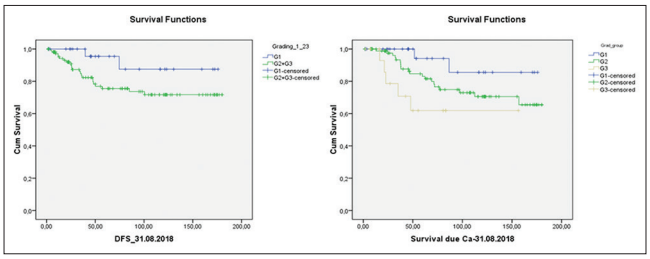

Figure 2: Disease-free survival and overall survival associated with tumor grade

In our study group, the tumor grading factor was associated with longer DFS in patients with well-differentiated carcinomas, although the dependence was not strictly significant p = 0.077. The Kaplan–Meier OS curve, according to tumor differentiation (G), showed an unfavorable prognosis for patients with low differentiation tumors (p = 0.044). Low tumor grade is an independent factor associated with earlier tumor progression and markedly lower OS (Figure 2).

The preformed Kaplan–Meier assays show clearly poorer DFS and OS in HER-2 expressing tumors, but due to the likely small number of patients, a significant dependence was not achieved (p = 0.081; p = 0.055, respectively) (Figure 3).

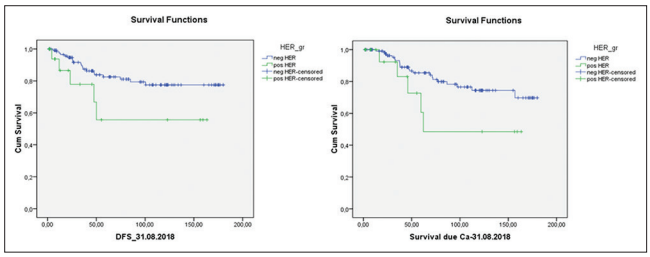

Figure 3: Disease-free survival and overall survival associated HER-2 tumor expression

Patients with pathohistologically negative axilla showed the highest DFS and OS, while patients with metastases in four and more LN had significantly worse prognosis (p = 0.000; p = 0.000, respectively) (Figure 4).

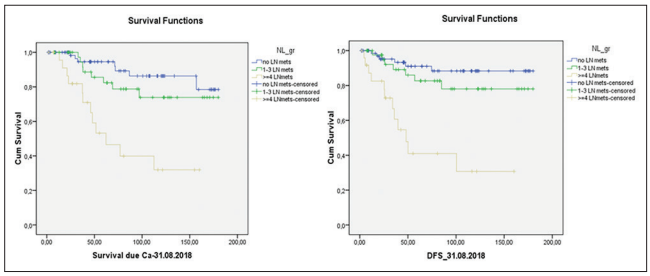

Figure 4: Disease-free survival and overall survival associated with lymphatic status

Cox univariate analysis showed that the evaluation of LN is an independent prognostic factor for survival, with significantly greater than 4 positive LN (pN2) p < 0.001.

Luminal tumors are subject to adjuvant endocrine therapy (ER and/or PR positive). Treatment was performed with selective estrogen modulators or aromatase inhibitors according to menopausal status. Analysis of the association between ET and DFS clearly showed that patients with the Luminal A subtype had significantly better DFS and OS (p = 0.001; p = 0.000, respectively) (Figure 5).

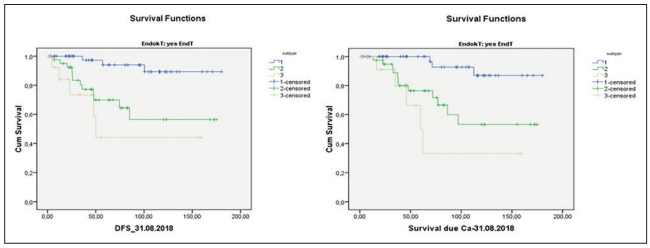

Figure 5: Disease-free survival and overall survival associated with endocrine therapy (1 -Luminal A; 2 - Luminal B/HER-2 [−]; 3 - Luminal B/HER-2 [+])

Although the worse curve was observed in HER-2 positive patients, the significant association was due to the significant difference in prognosis for Luminal A tumors.

Based on immunohistochemical typing, we differentiated tumors into five molecular subtypes:

1. Luminal A
2. Luminal B/HER-2 (−)
3. Luminal B/HER-2 (+)
4. HER-2 overexpressed
5. Triple-negative.

In constructing, the Kaplan–Meier curves for determining the association of molecular type with DFS and OS, we found a significantly better prediction in the Luminal A group (p = 0.003; p = 0.002, respectively) (Figures 6 and 7).

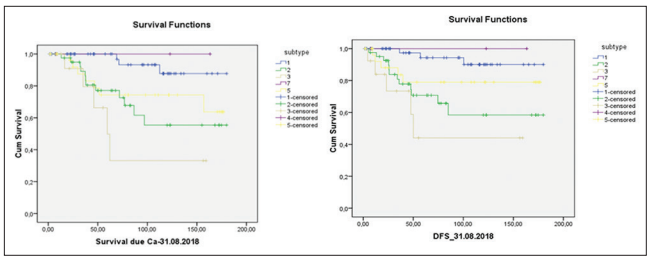

Figure 6: Disease-free survival and overall survival associated with molecular subtypes (1 - Luminal A; 2 - Luminal B/HER-2 (−); 3 - Luminal B/HER-2 (+); 4 - HER-2 overexpressed; 5 – Triple-negative)

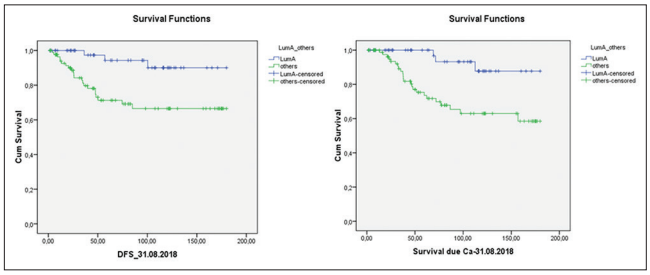

Figure 7: Comparison of disease-free survival and overall survival between the Luminal A group and the other four subtypes

The worst prognosis had patients in the Luminal B/HER-2 (+) group (p = 0.004; p = 0.003, respectively) (Figure 8).

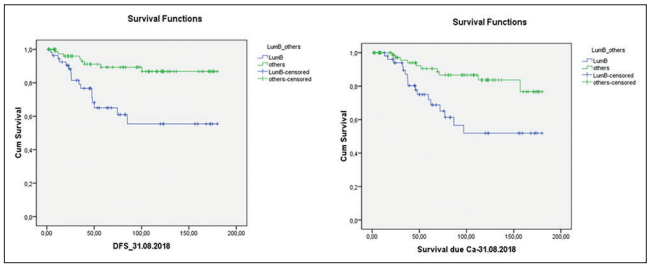

Figure 8: Comparison of disease-free survival and overall survival between Luminal B group and the other four subtypes

Overall, Luminal B, regardless of HER-2 expression, showed a short DFS and OS in direct comparison with the other molecular types (p = 0.002; p = 0.001, respectively) (Figure 8).

Discussion

Breast cancer is the leading cause of death among women worldwide [13], [14]. It is a heterogeneous disease associated with clinical, pathological, and biological factors that are variable from one population to another [15]. They all determine tumor aggression, response to therapeutic approach, and survival. In this aspect, the introduction of a classification aimed at determining the biology of the tumor would improve the healing outcomes. Therefore, molecular classification of breast cancer is an important tool for managing the treatment of these patients. In current practice, the definition of molecular subtypes is routine and mandatory in daily clinical work. However, the real significance of these biological characteristics of the tumor remains underestimated until the past few years [16], [17], [18].

For years, the prognosis of the disease and subsequent “unified” therapy has been conducted solely on the basis of the pathohistological staging of the disease. High-risk groups have clearly emerged over time, showing early progression and short OS beyond early age and poor grade [17], [18]. It was this that determined the need for understanding the biology of the tumor and allowed a better prognosis of the disease. In our study group, 72.4% of patients showed 5-year DFS and nearly 84.1% OS, which defined the low stage as an independent prognostic marker. Of course, the detailed analysis of the group clearly shows that patients with poor differentiation and Luminal B/HER-2 (+), even in the early phase of the disease, showed dramatically lower DFS and OS. On the other hand, positive treatment outcomes were also associated with a high percentage of the Luminal A subtype in the study group. In this study, we focused on a well-defined and homogeneous group of patients. The results obtained can be expected to be the same in other studies, as is evident from the literature to date [19], [20], [8], [21]. The results of this study, the accumulated evidence, support the prognostic role that tumor biology plays in breast cancer.

In our study group, positive axillary LN was a significant, independent prognostic marker for shorter DFS and OS. Although this is a confirmatory finding, we found that patients with up to three positive LN did not have significantly worse DFS and OS than patients with negative axilla. Therefore, we suggest that single, increased LN is likely to be associated with locoregional disease.

The dissociation with the literature regarding HER-2 expressing tumors is interesting, and the lack

of significant association with worse DFS and OS. We attribute this pattern to the small number of patients in the background of a small group who died during the follow-up. One way or another, a statistical trend is observed, which determines the confirmation of results in a larger group.

The surrogate consensus classification of breast cancer in St. Gallen 2011 [10] made it possible to differentiate risk groups into disease-specific indicators – hormonal activity, proliferation, and specific surface expression. This aims at defining not only a therapeutic approach with better end results but also a differentiated prognosis. Defining a high-risk group determines a dynamic change in the overall approach to behavior.

In the group analyzed, we clearly emphasize and confirm the prognostic significance of molecular subtypes in breast cancer patients [20], [8], [21]. We were not surprised by the significantly better prognosis in patients with Luminal A tumors, apparent dependence, and in a number of other studies [22], [18]. In our group, Luminal A is the most common subtype (62.7%), followed by triple-negative (23.5%), Luminal B/HER2 (+) (8.5%), and HER2 overexpressing (3.2%). For the majority of patients who were Luminal A subtype the overall survivall over 5 years is 92%. At the time of more effective systemic treatment, HER2 positive tumors have an uncertain outcome due to the small number of cases. But invariably, in the Luminal B group, we surveyed under the auspices of the high proliferative index had the shortest DFS and OS. Ki-67 evaluation is available in the majority of cases, which allows us to differentiate luminary similar HER2 negative tumors. Nevertheless, the validity and reliability of Ki-67 are still controversial, although it has been widely accepted as a cellular marker of proliferation that is widely available [23]. The 14% limit for Ki-67 recommended on St. Gallen 2011, which was confirmed by Cheang *et al*. [24], is considered critically because of the significant variability of results [25], [26], [27]. This dissonance is very problematic since the recommendation for or against chemotherapy for positive hormonal receptors, negative HER2, and G2 tumors depends mainly on the Ki-67 threshold. Because of this ambiguity in determining the surrogate subtype, it may be difficult to compare the end results with other studies using different surrogate definitions.

Conclusion

Each molecular subtype has its own specific characteristics that can predict DFS and OS, indicating that it is important to consider these characteristics when choosing a treatment strategy. Thus, in the study of this group, patients with Luminal B had a worse prognosis. The decision for adjuvant therapy is appropriate to be refined by taking into account molecular subtypes, which is a prerequisite for individualizing the therapy.

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