

The Association Between Anaplastic Lymphoma Kinase (ALK) with Histological Grading and Molecular Subtypes in Invasive Breast Carcinoma

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ABSTRACT

Background: Breast carcinoma is the second most common cancer in the world and is the most common cancer among women. Research on protein expression is associated with prognosis and opportunities for providing therapeutic agents. Recent research examines ALK with various characteristics in breast cancer. Anaplastic Lymphoma Kinase (ALK) is a tyrosine kinase receptor that plays a role in the signal transduction pathway, including the ERK, JAK / STAT, and PI3K-PKB / Akt pathways, that will cause proliferation and survival of tumour cells.

Objective: To analyzed the immunohistochemical expressions of ALK association with histological grading and molecular subtypes in invasive breast carcinoma patients.

Material dan Methods: Formalin-fixed paraffin-embedded tissue blocks of 44 invasive breast carcinoma patients were immunohistochemically studied for ALK expressions. All clinicopathological characteristics were obtained through medical records and pathology archives.

Results: This study found that positive ALK expression in 40 cases (90.1%), while a negative ALK expression of 4 cases (9.1%). The mean age of patients with invasive breast carcinoma was 47.40 ($\pm$ 10.57) years. Patients with positive ALK expression had an average age of 46.08 $\pm$ 10.64 years, and the highest tumor size was T3 (75%). All cases with negative ALK expression were invasive carcinoma NST. This study also found that there was no relationship of ALK expression with grading and molecular subtypes in invasive breast carcinoma.

Conclusions: These findings can be considered in using ALK as a determinant of prognosis and therapeutic agents. The use of other biological markers and validation by using larger samples and more even distribution is needed for the determination of a more precise prognosis in this study.

Keywords: invasive breast carcinoma, ALK, ALK inhibitor

1. Introduction

Anaplastic Lymphoma Kinase (ALK) is a tyrosine kinase receptor that belongs to the superfamily insulin receptor homologous to leucocyte tyrosine kinase (LTK). The ALK gene is located in the chromosome segment 2p23.[1] The ALK gene is known to play a genetic and biological role in many tumors including anaplastic large cell lymphoma, neuroblastoma, inflammatory myofibroblastic tumors, diffuse large B cell lymphoma, renal carcinoma, serous carcinoma of the ovary, esophageal squamous cell carcinoma, colon carcinoma and recently non-small cell lung carcinoma. Only a few studies investigated the role of ALK in breast carcinoma. Recent studies showed ALK in inflammatory breast cancer and triple negative breast cancers, but the results were still controversial.[2]

The association between ALK mutation or expression with breast carcinoma is not fully understood, and various studies have shown different results, even with different methodologies.[3] Perez-Pinera et al. showed an association between high-grade nuclear pleomorphism and cytoplasmic ALK expression in various histological types of breast carcinoma.[4] Kim et al. found that an increase in

the number of copies of the ALK gene was associated with inflammatory breast cancer, but no significant correlation with positive immunohistochemical staining which was found.[5]

Nowadays, curative therapy for patients with ALK gene mutation has been done in anaplastic large cell lymphoma (ALCL), non-small cell lung carcinoma (NSCLC), neuroblastoma and others, while in patients with breast carcinoma is still debated. ALK inhibitors therapeutic agent such as crizotinib, a first-line oral drug from tyrosine kinase inhibitors has been given to cancer patients with the ALK gene mutation. Assessing histological types and molecular subtypes in invasive breast carcinoma can be expected to assist in establishing more precise clinical diagnosis and determining patient survival. To know the aggressiveness of invasive breast carcinoma based on the ALK expression might help the clinician to determine the optimal treatment and prognosis.

2. Material and Methods

We examined 44 cases of invasive breast carcinoma patients in the Anatomic Pathology Unit of the Haji Adam Malik General Hospital, in Medan with a cross sectional approach. We collected clinical data, including age, tumor size, and molecular subtypes from medical records. The histological grade and type were evaluated by three researchers through microscopic examination of hematoxylin and eosin staining slides.

Immunohistochemical staining was carried out using ALK (FNab00310, Fine Test, China) with the Diagnostic BioSystems method (Diagnostic BioSystems, Pleasanton, CA, USA). Positive control is human lymphoma [6,7].

ALK expression was evaluated semi-quantitatively by three researchers by assessing the number of expressed cell (score 0-3) and the intensity of staining (score 0-3), with a total score was 6. The intensity of staining was 0 (not expressed), 1 (weak), 2 (medium), 3 (strong). The percentage of cells expressed was 0 (<10%), 1 (11–40%), 2 (41–70%), and 3 ($\geq 71\%$). The two scores are then summed. The cut-off value is 3, where ≤ 3 was negative and > 3 was positive. ALK staining was expressed in cytoplasmic.[8]

3. Results

The expression of ALK immunohistochemical staining in patients with invasive breast carcinoma in this study was 40 cases (90.9%) showed positive expressions, while 4 cases (9.1%) showed negative expressions (Table 4.1)

Table 4.1. Distribution of invasive breast carcinoma patient based on ALK expression

ALK expression	total (n)	Percentage (%)
Positive	40	90,9
Negative	4	9,1
Total	44	100

3.1 Distribution of ALK expression based on invasive breast carcinoma characteristics

Based on clinical data obtained from medical records, the samples obtained in this study had an average age of 47.40 ± 10.57 years, with the youngest was 23 years and the oldest was 72 years. The majority of 19 cases (43.2%) was 35 to 49 years, followed by 17 cases (38.6%) aged 50 to 64 years, under 35 years and over 65 years each as many as 4 cases (9.1%) (Table 4.1). Most tumor size status was T4 in 20 cases (45.5%), followed by T3 in 17 cases (38.6%), T2 in 6 cases (13.6%), and T1 in 1 case (2.3%). Based on the results of ER, PR, HER2, and Ki-67 immunohistochemistry staining examination, the most molecular subtypes in this study were 18 cases of luminal B (40.9%) followed by HER2-enriched 14 cases (31.8%), Luminal A 8 cases (18.1%), and triple-negative 4 cases (9.0%) (Table 4.1).

Microscopic examination results of HE preparations showed the majority of samples had non-specific breast carcinoma histopathological subtypes in 39 cases (88.6%), while 5 other cases had histopathological subtypes of specific breast carcinoma consisting of 3 cases (6.8%) lobular invasive carcinoma, 1 case carcinoma with medullary feature (2.3%), and 1 case (2.3%) mucinous carcinoma. The

majority of samples showed grade 1 and grade 2 each of 19 cases (43.2%), followed by grade 3 of 6 cases (13.6%) (Table 4.1).

Table 4.2 ALK immunohistochemical expression based on the characteristics of invasive breast carcinoma patient

Variable	Total (n)	Percentage (%)	ALK expression			
			Positive N=40		Negative N=4	
Age, mean $\pm$ SD	47,40 $\pm$ 10,57		46,08 $\pm$ 10,64		48,75 $\pm$ 9,78	
<35	4	9,1	4	10	0	0
35-49	19	43,2	17	42,5	2	50
50-64	17	38,6	15	37,5	2	50
≥ 65	4	9,1	4	10	0	0
Tumor Size						
T1	1	2,3	0	0	1	25
T2	6	13,6	6	15	0	0
T3	17	38,6	14	35	3	75
T4	20	45,5	20	50	0	0
Molecular Subtype						
• Luminal A	8	18,1	7	17,5	1	25
• Luminal B	18	40,9	17	42,5	1	25
• HER2-enriched	14	31,8	13	32,5	1	25
• Triple-negative	4	9,0	3	7,5	1	25
Histological type						
Non-specific						
• Invasive carcinoma NST	39	88,6	35	87,5	4	100
Specific						
• Invasive Lobular Carcinoma	3	6,8	3	7,5	0	0
• Mucinous Carcinoma	1	2,3	1	2,5	0	0
• Carcinoma with medullary feature	1	2,3	1	2,5	0	0
Grading						
• Grade I	19	43,2	17	42,5	2	50
• Grade II	19	43,2	17	42,5	2	50
• Grade III	6	13,6	6	15	0	0
Total	44	100	40	100	4	100

The mean age of patients with invasive breast carcinoma with positive ALK expression was 46.08 ± 10.64 years. Meanwhile, the mean age of patients with invasive breast carcinoma with negative ALK expression was 48.75 ± 9.78 years, slightly older than the average age with positive ALK expression (Table 4.2).

Based on tumor size, the majority cases showed positive ALK expression in T4 in 20 cases (50%), T3 in 14 cases (35%), and T2 in 6 cases (15%). While negative ALK expression was found in T3 in 3 cases (75%), and T1 in 1 case (25%) (Table 4.2).

In this study, we found 35 cases (90%) of invasive breast carcinoma with non-specific histological types, include invasive carcinoma non special type (NST) and 5 cases (100%) of invasive breast carcinoma with specific histological types showed positive ALK expression. Specific histological types include 3 cases of invasive lobular carcinoma, 1 case of carcinoma with medullary features, and 1 case of mucinous carcinoma. Whereas 4 cases of invasive breast carcinoma (100%) with invasive carcinoma non special type (NST) showed negative ALK expression. (Table 4.2).

3.2 Distribution of ALK expression based on histopathological grading and molecular subtypes in invasive breast carcinoma

From 19 cases of each grade 1 and grade 2, about 17 cases (42%) from each grade were found positive ALK expression. Whereas, all cases of grade 3 group showed positive ALK expression (Table

4.9). Negative expressions were found in 2 cases (50%) respectively in grade 1 and 2 invasive breast carcinomas (Table 4.3).

Table 4.3 Relationship between ALK expression and grading of invasive breast carcinoma

No	ALK expression	Grade						p-value*
		Grade I		Grade II		Grade III		
		n	%	n	%	n	%	
1.	Negative	2	50,0	2	50,0	0	0	0,592
2.	Positive	17	42,5	17	42,5	6	15,0	

* Mann-Whitney U Test

The majority cases showed positive ALK expression in 17 cases of luminal B (42.5%), followed by HER2-enriched in 13 cases (32.5%), luminal A in 7 cases (17.5%), and triple-negative in 3 cases (7.5%). Negative ALK expression was spread evenly in each molecular subtype, about 1 case (25%) for each subtype (Table 4.4).

Table 4.4 Relationship between ALK expression and molecular subtype of Invasive Breast Carcinoma

No	ALK expression	Subtype molekuler								p-value*
		Luminal A		Luminal B		HER2-enriched		Triple negative		
		n	%	n	%	n	%	n	%	
1.	Negative	1	25,0	1	25,0	1	25,0	1	25,0	0,73
2.	Positive	7	17,5	17	42,5	13	32,5	3	7,5	

* Mann-Whitney U Test

Based on the Mann-Whitney U statistical test, there was no significant relationship between ALK expression with histopathological grading (p-value 0,592) and molecular subtypes (p-value 0,73) in invasive breast carcinoma.

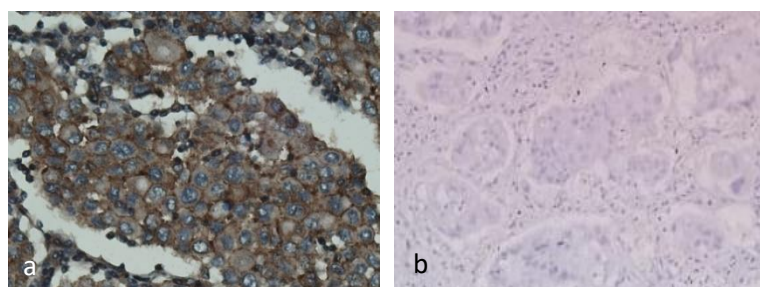


Figure 1. Immunohistochemical expression. (a) Positive ALK expression (b) Loss of ALK expression in the cytoplasm.

4. Discussion

In this study, the majority of cases with positive ALK expression were 40 cases (90.9%) and only 4 cases (9.1) with negative ALK expression. Supported by Ali et al. that researched women in Pakistan found 57.9% more positive ALK tests than negative ALK tests.[9] Research To et al. positive ALKs were obtained in all cases of pulmonary adenocarcinoma.[7] But contrary to Mehrjardi et al. in Iran, which found 47% fewer cases of breast carcinoma with positive ALK expression than negative ALK expression. The relationship of ALK expression with breast carcinoma is not fully understood, and various studies have shown different results, albeit with different methodologies.[10] Perez-Pinera et al. found an association between high-grade nuclear pleomorphism and cytoplasmic ALK expression in various histological types of breast carcinoma.[4]

The mean age of patients with invasive breast carcinoma with positive ALK expression was 46.08 ± 10.64 years, slightly younger than the age of invasive breast carcinoma patients with negative ALK expression of 48.75 ± 9.78 years. Supported by Mehrjardi et al. who found the average age of patients with positive ALK was 48.6 years younger than that with negative ALK which was 50.3 years.[10] There are now many invasive breast cancer sufferers younger. This might be due to the frequent behavior patterns of eating fast food and lack of sports activities.

Tumor size in this study was obtained only from medical record, so that it cannot be seen in detail. However, it can be estimated that positive ALK expression is often found in cases with tumor sizes T3 and T4, which range above 5 cm. This shows the low rate of early detection and the lack of public awareness of breast carcinoma. Mehrjardi et al. found a mean tumor size with positive ALK expression lower than in this study which was 3.1 ± 1.8 cm and negative ALK expression was 4.3 ± 2.4 cm.[10]

In this study, all cases with negative ALK expression were found in the subtype of invasive carcinoma Non Special Type (NST). Ali et al. and Mehrjardi et al. found the histological subtype is not significantly related to positive ALK expression. [9,10] Siraj et al. found overexpression from ALK in 75% infiltrating ductal carcinoma in 22 of the patients, and 17.5% of lobular subtypes in their study.[11] Previous study, Perez-Pinera et al. shows ALK overexpression in 50% of histological subtypes.[4] Mehrjardi et al. did not even find ALK overexpression in any of the lobular subtype cases in their study.[10] This might be due to the wide range of histological types, while the sample distribution in this study was not evenly distributed.

Molecular subtypes of invasive breast carcinoma are determined based on ER, PR, HER2, and Ki-67 tests. The literature suggests that molecular subtypes are used to determine chemotherapeutic agents and prognosis for patients with invasive breast carcinoma.[12,13] Luminal subtypes tend to be associated with low grade and are less aggressive, whereas basal-like tumors and positive HER-2 are more sensitive to chemotherapy.[14] In this study it was found that the most luminal B subtypes showed positive ALK expression and there was no significant relationship between ALK expression and molecular subtypes after statistical tests. Supported by Mehrjardi et al. that also found no significant relationship between ALK expression and molecular subtypes.[10] Contrary to Siraj et al. found overexpression of ALK in aggressive breast carcinoma such as triple negative breast cancer.[11]

Many studies show histological grade is significantly related to the molecular subtype of breast carcinoma. Grade I is associated with luminal A, while grade III is associated with HER2-enriched and TNBC.[14-20] Researchers found the same number of positive ALK expressions in grades 2 and 3 in 17 cases (42.5%), whereas all grade 3 tumor groups had positive ALK expression. After conducting statistical tests, there was no significant relationship between ALK expression and invasive breast carcinoma grade levels. It was similar to Mehrjardi et al., but contrary to Siraj et al. that found a significant relationship between ALK expression and grade 3 invasive breast carcinoma.[10,11]

No significant relationship was found between ALK expression with histological grading and molecular subtypes in this study, perhaps because the distribution of breast carcinoma samples for each grading and molecular subtypes not evenly distributed, while there is so many histopathological grading and molecular subtypes.

Many study compared the three immunohistochemical methods, real-time polymerase chain reaction (RT-PCR), and fluorescence in situ hybridization (FISH) to detect ALK mutations. they showed RT-PCR has high sensitivity and specificity in detecting ALK mutations.[21,22] However, RT-PCR is still an inadequate method as an initial check to detect positive ALK, because it has a high false negative. The immunohistochemical method is a more effective and more cost-effective method. Whereas FISH examination is difficult to obtain adequate samples.[22,23] Ding et al. compared ALK expression based on immunohistochemical, FISH, and RT-PCR examination techniques in cases of non-small cell lung carcinoma and found that the immunohistochemical examination method is one of the effective methods for detecting ALK. However, the immunohistochemical examination method is only a screening method that has a high false positive, so another method of examination is needed to further verify.[24]

5. Conclusion

There was no significant relationship between histological grading and molecular subtypes with ALK expression in invasive breast carcinoma. But it does not rule out the possibility of using anti-ALK drugs as a new therapeutic drug for invasive breast carcinoma patients. Larger studies is needed to know the survival rates of invasive breast carcinoma patients based on ALK expression.

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