Section: Pathology

Original Research Article

THE PROGNOSTIC VALUE OF AGNOR PARAMETER IN VARIOUS GRADES OF BREAST CARCINOMAS

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ABSTRACT

Background: Breast carcinoma is a heterogeneous malignancy with variable biological behavior, necessitating reliable prognostic markers to guide clinical management. Argyrophilic Nucleolar Organizer Regions (AgNORs) reflect cellular proliferative activity and may serve as an adjunct prognostic tool. **Objectives:** This study aimed to histopathologically grade infiltrating ductal carcinoma (IDC) cases, calculate the mean AgNOR counts, and compare these counts across tumor grades and lymph node status to evaluate their prognostic significance. **Materials and Methods:** A prospective study was conducted over six months at the Department of Pathology, Victoria Hospital and Bowring and Lady Curzon Hospital, Bangalore, including 30 cases of histologically confirmed IDC. Tumors were graded using the Nottingham grading system. AgNOR staining was performed on paraffin-embedded tissue sections, and counts were assessed in 100 tumor cell nuclei per case under oil immersion microscopy. Mean AgNOR counts were expressed as mean ± standard deviation. Descriptive statistics and appropriate comparative tests were used to analyze the data. **Result:** Mean AgNOR counts significantly increased with tumor grade: Grade I (2.75 ± 0.43), Grade II (4.03 ± 0.60), and Grade III (6.25 ± 0.81) ($p < 0.001$). Tumors with positive lymph node status showed higher mean AgNOR counts (5.89 ± 0.75) compared to node-negative cases (3.38 ± 0.58) ($p < 0.001$). Moreover, higher-grade tumors were significantly associated with lymph node metastasis ($p = 0.045$). **Conclusion:** AgNOR quantification correlates positively with tumor grade and lymph node involvement, reflecting increased proliferative activity in aggressive breast carcinomas. This simple, cost-effective technique may serve as a valuable adjunct prognostic marker to augment the histopathological evaluation of breast cancer, particularly in resource-limited settings.

INTRODUCTION

Breast cancer remains the most prevalent malignancy and the leading cause of cancer-related mortality among women worldwide. The heterogeneity in its biological behavior underscores the importance of identifying robust prognostic markers to guide clinical management and predict outcomes. Classical prognostic factors such as tumor size, lymph node status, histological grade, and hormone receptor status are routinely evaluated but may not fully capture the proliferative potential or aggressiveness of individual tumors.[1]

Nucleolar organizer regions (NORs) are chromosomal segments comprising ribosomal DNA, playing a critical role in ribosome biogenesis and cell proliferation. These regions are transcriptionally active and can be selectively visualized by silver staining, resulting in argyrophilic nucleolar organizer regions (AgNORs). The quantification of AgNORs in histological sections has emerged as a marker of cellular proliferation, as the number of AgNORs per nucleus often increases with the malignant transformation of cells and correlates with proliferative activity.[2]

Since the introduction of AgNOR staining in tumor pathology by Ploton et al., there has been considerable interest in the prognostic role of AgNOR analysis across a wide range of human cancers, including breast carcinoma. Numerous studies have reported a strong association between higher AgNOR counts and increased tumor grade, advanced stage, lymph node positivity, and poorer clinical outcomes. The proliferative rate, reflected by AgNOR count, is intrinsically linked to tumor aggressiveness; a higher AgNOR count may indicate a faster cell cycle and higher ribosomal synthesis, both hallmarks of malignancy.[3]

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In breast carcinoma, AgNOR assessment through morphometric analysis has been correlated not only with well-established prognostic indices but also with survival outcomes. For example, studies have shown that patients with tumors exhibiting high AgNORvalues tend to have significantly shorter disease-free and overall survival compared to those with lower AgNOR counts. Additionally, AgNOR quantification is a relatively simple, rapid, and cost-effective technique, enhancing its appeal as a potential adjunct to standard histopathological grading and proliferative markers such as Ki-67.[4]

However, the prognostic value of AgNOR is not absolute and is influenced by the molecular characteristics of the tumor, particularly the status of tumor suppressor proteins such as p53 and retinoblastoma protein (pRb). Evidence suggests that AgNOR counts are most informative when interpreted alongside these molecular markers, as alterations in p53 and pRb modulate both ribosome biogenesis and cell cycle progression.[5]

Overall, the assessment of AgNOR parameters represents a valuable addition to the histopathological evaluation of breast carcinomas. It holds promise for stratifying patients by risk and tailoring adjuvant therapies, especially in resource-limited settings where advanced molecular assays may not be readily available. Continuous research exploring its integration with other established and emerging biomarkers will further clarify its role in the nuanced management of breast cancer.

The objective of this study is to comprehensively assess the prognostic significance of AgNOR (Argyrophilic Nucleolar Organizer Region) parameters in breast carcinomas. Specifically, the study aims to grade breast carcinoma lesions histopathologically according to established criteria, to quantitatively determine the mean AgNOR counts within these tumors, and to systematically compare these counts across various histological grades and lymph node involvement statuses.

MATERIALS AND METHODS

The study was designed as a prospective observational analysis conducted over a six-month period from January 2017 to June 2017 in the Department of Pathology at Victoria Hospital and Bowring and Lady Curzon Hospital in Bangalore. A minimum of 30 cases of histologically confirmed infiltrating ductal carcinoma of the breast, received during this period, were included in the study. Cases involving other types of breast carcinomas or benign breast diseases were excluded to maintain homogeneity in the study population. Each tumor was graded histopathologically using the Nottingham grading system, which evaluates parameters such as tubule formation, nuclear pleomorphism, and mitotic count to assign a grade reflecting tumor differentiation.

For assessing proliferative activity, all cases underwent AgNOR (Argyrophilic Nucleolar Organizer Region) staining. The staining involved preparation of two working solutions: the first by mixing 25 ml of formic acid solution with gelatin, and the second by combining 0.1 ml of the first solution with 0.2 ml of silver nitrate immediately before application. Formalin-fixed, paraffin-embedded tissue sections were deparaffinized, rehydrated, and stained with the silver solution, resulting in black dots representing nucleolar organizer regions within tumor cell nuclei.

AgNOR quantification was performed by counting the number of distinct black dots in the nuclei of 100 randomly selected tumor cells per case under a 100x oil immersion objective lens. The counts were expressed as mean ± standard deviation for each tumor. Descriptive statistics were used to analyze the data, enabling comparison of mean AgNOR counts across different histological grades and correlation with lymph node status and other clinicopathological parameters. This methodological approach provided an objective measure of cellular proliferation, facilitating assessment of AgNOR's prognostic value in infiltrating ductal carcinoma of the breast.

RESULTS

The study population consisted entirely of female patients, reflecting the predominance of breast cancer in women. The majority of patients (50%) were between 41 and 60 years of age, with a smaller proportion younger than 40 years (26.7%) and older than 60 years (23.3%). Tumor sizes were most frequently in the range of 2.1 to 5 cm (50%), with fewer cases having small tumors (≤2 cm) or large tumors (>5 cm). This demographic distribution provides a typical representation of breast cancer cases in terms of age and tumor size (Table 1).

There was a statistically significant increase in mean AgNOR counts with advancing tumor grade ($p < 0.001$). Grade I tumors showed the lowest mean AgNOR count (2.75 ± 0.43), indicating lower proliferative activity. Grade II tumors had a moderate mean count (4.03 ± 0.60), while Grade III tumors exhibited the highest proliferative activity with a mean count of 6.25 ± 0.81. These findings demonstrate a strong correlation between tumor histological grade and cellular proliferation as measured by AgNOR counts, supporting AgNOR as a marker of tumor aggressiveness (Table 2).

Patients with positive lymph node status had significantly higher mean AgNOR counts (5.89 ± 0.75) compared to those without lymph node involvement (3.38 ± 0.58), with $p < 0.001$. This indicates that tumors with regional lymphatic spread show higher proliferative activity. The association between elevated AgNOR count and nodal metastasis suggests the potential role of AgNOR as

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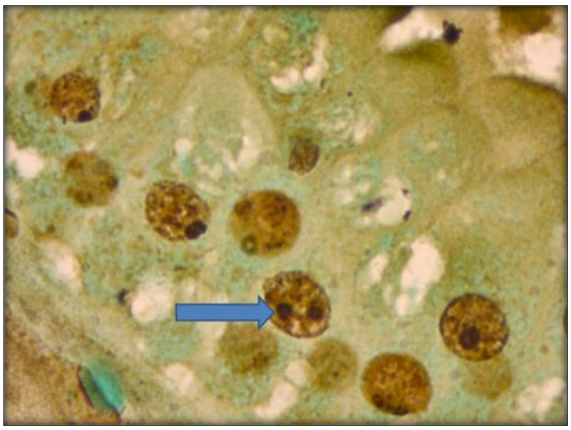
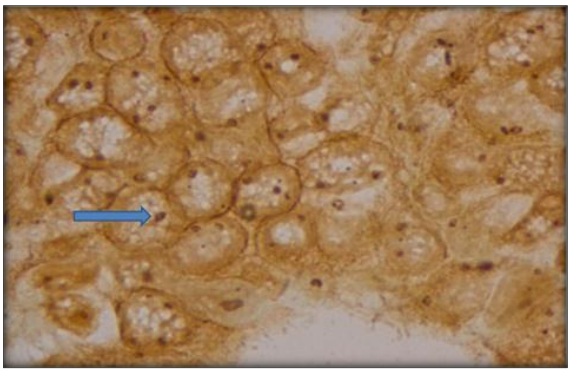
a prognostic marker linked with tumor dissemination (Table 3).

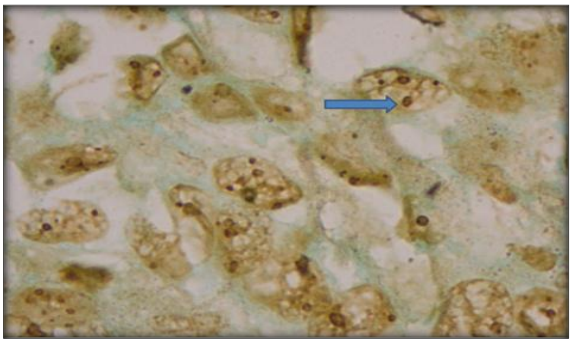
The distribution of tumor grades according to lymph node involvement showed a statistically significant association (p = 0.045). Grade I tumors were predominantly lymph node negative (38.9%) with very few lymph node positive cases (8.3%). In contrast, Grade III tumors were more frequent in lymph node positive patients (50%) compared to node-negative patients (22.2%). Similarly, Grade II tumors were nearly equally distributed but had a higher percentage among lymph node positive cases. This demonstrates that higher histological grade is correlated with increased likelihood of lymph node metastasis, reinforcing the aggressive biological behavior of poorly differentiated tumors (Table 4).



CONTROL SHOWING DUCTAL EPTHELIAL CELLS:AgNOR seen as black dots



Histological grade II:Mean AgNOR count 3



Histological grade III:Mean AgNOR count 5

Table 1: Demographic Characteristics of the Study Population (N = 30)

Characteristic	Frequency (%)
Age (years)	
≤ 40	8 (26.7)
41–60	15 (50.0)
> 60	7 (23.3)
Gender	
Female	30 (100)
Tumor Size (cm)	
≤ 2	10 (33.3)
2.1–5	15 (50.0)
> 5	5 (16.7)

Table 2: Comparison of Mean AgNOR Counts acrossTumor Grades

Tumor Grade	Number of Cases	Mean AgNOR Count ± SD	p-value*
Grade I	8	2.75 ± 0.43	
Grade II	12	4.03 ± 0.60	< 0.001
Grade III	10	6.25 ± 0.81	< 0.001

***ANOVA test comparing means among grades**

Table 3: Comparison of Mean AgNOR Counts by Lymph Node Status

Lymph Node Status	Number of Cases	Mean AgNOR Count ± SD	p-value*
Negative	18	3.38 ± 0.58	< 0.001
Positive	12	5.89 ± 0.75	

**Independent t-test comparing means between groups*

Table 4: Distribution of Tumor Grade by Lymph Node Status

Tumor Grade	Lymph Node Negative N (%)	Lymph Node Positive N (%)	p-value*
Grade I	7 (38.9)	1 (8.3)	0.045
Grade II	7 (38.9)	5 (41.7)	
Grade III	4 (22.2)	6 (50.0)	

**Chi-square test for association*

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DISCUSSION

This study examined the prognostic significance of the Argyrophilic Nucleolar Organizer Region (AgNOR) parameter in infiltrating ductal carcinoma (IDC) of the breast, examining its correlation with histopathological grade and lymph node status. The results demonstrated a clear and statistically significant association between increasing tumor grade and elevated mean AgNOR counts, as well as higher AgNOR counts in tumors with lymph node metastasis. These findings affirm the utility of AgNOR quantification as a reliable, cost-effective marker reflective of tumor proliferative activity and aggressiveness in breast carcinoma.

The demographic profile of the study cohort, consisting entirely of female patients predominantly aged 41–60 years, aligns with epidemiological patterns documented in breast cancer prevalence worldwide. This middle age group remains a peak incidence window for breast cancer development, underscoring the clinical relevance of the studied population.[1]

A fundamental observation from this study was the progressive increase in mean AgNOR counts correlating with the Nottingham histological grade of breast carcinoma. Grade I tumors exhibited the lowest mean AgNOR scores, indicative of relatively slower proliferative capacity and well-differentiated tumor cells, whereas Grade III tumors demonstrated markedly higher AgNOR counts, consistent with aggressive, poorly differentiated phenotypes. Grade II tumors fell between these extremes, which is in concordance with prior investigations demonstrating similar trends.[2,3,5]

This gradation of AgNOR counts reflects the biological behavior of breast tumors, since AgNORs are markers of ribosomal DNA transcription activity associated with protein synthesis and cell proliferation. Increased numbers of AgNOR dots per nucleus signify heightened nucleolar activity, which serves as a surrogate for the rapid cell cycling and growth characteristic of malignant transformation. Studies by Derenzini et al. and others have reported analogous findings; for instance, they observed that higher AgNOR counts were significantly associated with increased tumor grade in breast carcinomas, reiterating AgNOR's role in reflecting the proliferative potential of neoplastic cells.[2,4,6]

Beyond tumor grade, the current study found significantly elevated mean AgNOR counts in cases with positive lymph node status. Patients exhibiting lymph node metastasis had higher proliferative indices as evidenced by mean AgNOR values when compared to those without nodal involvement. This observation is clinically meaningful, as lymphatic spread remains one of the most critical prognostic factors in breast cancer, indicating systemic dissemination risk and poorer survival outcomes. The positive correlation of AgNOR counts with lymph node status supports its applicability in predicting tumor aggressiveness and metastatic potential.[3,5]

Several previous studies have substantiated the association between AgNOR quantification and lymph node metastasis. For example, a study conducted by Aleskandarany et al. demonstrated that high AgNOR counts correlated with the presence of lymphatic metastasis and shorter disease-free survival. Similarly, qualitative and quantitative assessment of AgNORs in metastatic lymph nodes has been linked with adverse prognosis, further affirming the aggressiveness of tumors with higher proliferative indices.[7-9]

Moreover, the relationship observed between histological grade and lymph node involvement reiterates the established paradigm that poorly differentiated tumors (Grade III) exhibit increased likelihood of metastasis compared to well-differentiated tumors (Grade I). This study's finding that Grade III tumors predominate in lymph node positive cases reflects the tumor's biological propensity for local invasion and systemic spread. The statistically significant association ($p = 0.045$) between tumor grade and nodal status strengthens the rationale for integrating proliferative indices such as AgNOR counts in comprehensive pathological evaluation.[3,10]

AgNOR counting has several advantages when applied to breast cancer prognostication. The technique is relatively inexpensive, reproducible, and can be performed on routinely processed formalin-fixed paraffin-embedded sections, making it accessible in resource-limited settings where advanced molecular assays may not be feasible. Compared to other proliferative markers such as Ki-67, AgNOR staining provides a direct visualization of nucleolar proteins involved in ribosomal synthesis, addressing a fundamental aspect of cell proliferation.[5,11]

Despite these advantages, the prognostic significance of AgNORs must be interpreted in the context of other molecular and clinicopathological factors. The literature suggests that combining AgNOR parameters with biomarkers such as p53, pRb, and hormone receptor status yields a more robust predictive model. Alterations in tumor suppressor genes influence ribosomal biogenesis and cell cycle regulation, which in turn impact AgNOR expression and tumorbehavior. Therefore, AgNOR should complement rather than replace established prognostic markers.[12,13]

Notably, some studies have raised concerns regarding the specificity and standardization of AgNOR quantification. Variability in staining protocols, counting techniques, and interpretation criteria has led to conflicting results in certain cohorts. Nonetheless, with methodical standardization and appropriate training, these limitations can be surmounted, enabling reliable integration of AgNOR evaluation into routine pathology.[4]

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CONCLUSION

In conclusion, this study demonstrates that AgNOR quantification is a valuable prognostic marker in infiltrating ductal carcinoma of the breast, exhibiting a strong positive correlation with tumor histological grade and lymph node involvement. Elevated mean AgNOR counts reflect increased cellular proliferative activity, which is characteristic of more aggressive and poorly differentiated tumors with a higher propensity for metastasis. Given its simplicity, cost-effectiveness, and reproducibility, AgNOR assessment can serve as a useful adjunct to conventional histopathological evaluation, aiding in risk stratification and guiding treatment decisions, especially in resource-constrained settings where advanced molecular tests may not be readily available.

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