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The Effect of Blood Group Phenotypes on the Ki-67 Proliferation Index in Breast Cancer

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ABSTRACT
The Ki-67 proliferation index is an established biomarker reflecting tumor aggressiveness and treatment response in breast cancer. ABO blood group phenotypes have been associated with cancer susceptibility and inflammatory–immunological pathways, yet their potential influence on tumor proliferative behavior remains unclear. This study aimed to investigate the relationship between ABO/Rh blood groups and Ki-67 expression levels in breast cancer.

This retrospective study included 189 patients diagnosed with breast malignancy between 2020 and 2024. Ki-67 values were recorded as the percentage of positively stained tumor nuclei. Differences among ABO phenotypes were analyzed using the Kruskal–Wallis test, followed by Dunn post-hoc analysis with Bonferroni correction. Rh groups were compared using the Mann–Whitney U test. All p-values were standardized to three decimal places. A post-hoc power analysis indicated that the sample size (n=189) was adequate to detect a moderate effect size (Cohen's f=0.25) with 80% power at α=0.05.
The overall mean Ki-67 index was 25.9 ± 18.7% (median 22%). Ki-67 levels differed across ABO groups with borderline statistical significance (p=0.060). After Bonferroni correction, the largest differences were observed between group O and groups B/AB (adjusted p=0.078 and p=0.083), suggesting a trend toward higher proliferation in non-O phenotypes. No significant association was detected between Rh factor and Ki-67 levels (p=0.810).
Although statistical significance was not reached, patients with blood group O demonstrated lower Ki-67 values, whereas B and AB groups tended to exhibit higher proliferation levels. This pattern may indicate a possible biological influence of ABO antigen expression on tumor proliferation. Larger and multicenter studies are warranted to clarify this potential association.

Keywords: Ki-67; Proliferation Index; ABO Blood Group; Rh Factor; Breast Cancer; Tumor Biology; Prognostic Biomarker

Introduction

Cellular proliferation is a central biological process driving tumor development, progression, and treatment resistance (1). Among proliferation markers, Ki-67 has become a widely accepted and clinically relevant indicator due to its expression in all active phases of the cell cycle (G_1 , S, G_2 , and M) and absence in quiescent cells, providing a reliable measure of tumor cell proliferation (2). High Ki-67 levels in breast cancer are strongly associated with higher histological grade, increased recurrence risk, and poorer survival, making this marker a key component of prognostic classification and therapeutic decision-making (3).

Beyond its classical role in transfusion medicine, the ABO blood group system has gained considerable attention for its potential associations with cancer susceptibility and tumor biology (4). ABO antigens, expressed not only on erythrocytes but also on epithelial and endothelial cells, are involved in intercellular adhesion, immune recognition, and inflammatory regulation (5). These mechanisms raise the possibility that antigenic differences may influence tumor behavior through pathways related to cell–cell and cell–matrix interactions, cytokine signaling, and microenvironmental modulation (6).

Epidemiological studies have reported varying cancer risks among different ABO phenotypes, with non-O blood groups often showing higher incidence and more aggressive tumor characteristics in several malignancies, including gastric, pancreatic, and hepatic cancers (7,8). Such findings suggest that ABO phenotypes may act as immunogenetic modifiers of tumor development through alterations in inflammation, coagulation profiles, and adhesion molecule pathways (9).

However, despite the growing evidence supporting a link between ABO antigens and cancer biology, the relationship between ABO phenotypes and specific proliferative markers such as Ki-67 remains poorly defined. Only limited clinical data have evaluated whether genetically determined blood group antigens

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may influence tumor cell proliferation, and existing studies present inconsistent results. Moreover, most available evidence is derived from small or heterogeneous cohorts, and few investigations have specifically focused on breast cancer.

In this context, the present study aimed to evaluate the association between ABO and Rh blood group phenotypes and Ki-67 proliferation index in patients with breast malignancy. Given that both Ki-67 expression and ABO antigen profiles are routinely available in clinical practice, identifying a potential link between these parameters could contribute to a better understanding of breast tumor biology and may provide a simple, cost-effective adjunct to existing prognostic tools.

Materials and Methods

Study Design and Population: This retrospective, cross-sectional study was conducted at the Department of General Surgery, Malatya Turgut Özal University. A total of 487 patients diagnosed with malignant breast disease between January 2020 and June 2024 were initially screened. Patients with missing or incomplete data regarding ABO blood group, Rh factor, or Ki-67 proliferation index were excluded. After applying these exclusion criteria, a final cohort of 189 patients with complete datasets was included in the analysis.

Data Collection and Variables: Demographic and clinical data—including age, sex, ABO blood group, and Rh factor—were obtained from electronic hospital records. Blood group typing was performed using standard hemagglutination methods, and Rh factor was determined through direct agglutination testing. The Ki-67 proliferation index was defined as the percentage of positively stained tumor nuclei among at least 1,000 counted tumor cells. Molecular subtype data (Luminal A/B, HER2-enriched, triple-negative) were inconsistently available and therefore excluded from the analysis to avoid bias arising from missing information.

Histopathological and Immunohistochemical Assessment: All tissue specimens were fixed in 10% neutral-buffered formalin and paraffin-embedded. Sections of 4 μm thickness were stained with monoclonal anti–Ki-67 antibody (clone MIB-1, Dako, Denmark) using an automated immunostaining system in accordance with standard protocols. Two independent pathologists, blinded to the patients' blood group information, evaluated all Ki-67 slides. In cases where inter-observer variation exceeded 5%, the slides were re-evaluated jointly to reach consensus.

Statistical Analysis: Statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). The distribution of continuous variables was assessed using the Shapiro–Wilk test. As Ki-67 values were not normally distributed, non-parametric tests were employed.

Differences in Ki-67 levels among the ABO blood groups were evaluated using the Kruskal–Wallis test, followed by Dunn's post-hoc test with Bonferroni correction to control for type I error in multiple comparisons. Comparisons between Rh-positive and Rh-negative patients were performed using the Mann–Whitney U test. Continuous variables were expressed as mean ± standard deviation (SD), median (interquartile range, IQR), and range (minimum–maximum), while categorical variables were presented as counts and percentages.

Sample Size and Power Analysis: Although an a prior sample size calculation was not feasible due to the retrospective design, a post-hoc power analysis was conducted to assess the adequacy of the final sample. Based on the observed variation in Ki-67 values and assuming a moderate effect size (Cohen's f = 0.25), a minimum of 180 patients was required to achieve 80% power at a 0.05 alpha level for detecting differences among ABO groups. With 189 patients, the sample size was deemed sufficient for detecting an effect of this magnitude. Nonetheless, borderline significance in group comparisons may be partly attributable to the smaller sizes of specific ABO subgroups, particularly B and AB.

A two-tailed p-value <0.05 was considered statistically significant, while p-values between 0.05 and 0.10 were interpreted as indicating a biological trend warranting further investigation.

Ethical Considerations: The study protocol was approved by the Ethics Committee of Malatya Turgut Özal University (Approval No: E-30785963-020-322365, Date: 11 August 2025). All procedures were conducted in accordance with the ethical standards of the Declaration of Helsinki. Owing to the retrospective design, the requirement for informed consent was waived.

Results

A total of 189 female patients with histopathologically confirmed malignant breast disease and complete datasets were included in the final analysis. The mean age was 57.7 ± 13.7 years (range: 28–94 years), and approximately half of the cohort (50%) fell within the 47–67-year range, which represents the most common age interval

Table 1: Demographic and Clinical Characteristics of The Study Population

Variable	Mean ± SD / n (%)
Age (years)	57.7 ± 13.7
Age range	28–94
Sex (female)	189 (100%)
Blood group A	79 (41.8%)
Blood group O	65 (34.4%)
Blood group B	29 (15.3%)
Blood group AB	16 (8.5%)
Rh (+)	161 (85.2%)
Rh (–)	28 (14.8%)

SD: Standard deviation; n: Number of patients

Table 2: Distribution of Ki-67 Proliferation Categories According to ABO Blood Groups

Ki-67 Category	O (n=65)	A (n=79)	B (n=29)	AB (n=16)
≤10%	25 (38.46%)	21 (26.58%)	6 (20.69%)	4 (25.00%)
11–30%	27 (41.54%)	35 (44.30%)	13 (44.83%)	6 (37.50%)
>30%	13 (20.00%)	23 (29.11%)	10 (34.48%)	6 (37.50%)

Ki-67: Nuclear proliferation index expressed as the percentage of positively stained tumor nuclei

for breast cancer diagnosis in the Turkish population.

The distribution of ABO blood groups and Rh factor is summarized in Table 1. Blood group A was the most prevalent (41.8%), followed by O (34.4%), B (15.3%), and AB (8.5%). Rh-positive phenotype predominated (85.2%), while Rh-negative status accounted for 14.8%. This distribution was consistent with the general Turkish population.

Distribution of the Ki-67 Proliferation Index: The overall mean Ki-67 proliferation index was 25.9 ± 18.7%, with a median value of 22% (range: 1–95%). The distribution curve showed right skewness, indicating that most tumors exhibited moderate proliferative activity, with a notable subset demonstrating markedly high Ki-67 expression.

When stratified into three categories, 56 patients (29.6%) had low Ki-67 values (≤10%), 81 patients (42.9%) exhibited moderate expression (11–30%), and 52 patients (27.5%) had high proliferation rates (>30%). The distribution of these categories among ABO phenotypes is shown in Table 2.

Group O patients were more frequently represented in the low-proliferation (≤10%) category (38.46%), whereas groups B and AB demonstrated a higher proportion of tumors with Ki-67 >30% (34.48% and 37.50%, respectively). This distribution pattern suggests a potential biological tendency toward increased proliferative activity in non-O blood groups (Table 2).

Comparison of Mean Ki-67 Values Across Blood Groups: Evaluation of Ki-67 as a continuous variable revealed clear differences among the ABO phenotypes. Mean Ki-67 levels were 19.7% in group O, 27.7% in group A, 31.9% in group B, and 31.2% in group AB, demonstrating a progressive increase from O toward non-O blood groups.

The Kruskal–Wallis test indicated a borderline significant difference across the groups ($p = 0.060$). Post-hoc pairwise comparisons using Dunn's test with Bonferroni correction showed that the largest discrepancies were observed between group O and group B (adjusted $p = 0.078$) and between group O and group AB (adjusted $p = 0.083$). Although these comparisons did not reach statistical significance, group O consistently exhibited lower Ki-67 values, whereas groups B and AB displayed higher proliferative activity. Group A demonstrated intermediate levels, suggesting a gradual increase that parallels the antigenic complexity of A and B glycosylation patterns.

Overall, the pattern formed by these results—despite the lack of formal statistical significance—illustrates a biologically coherent trend in which non-O phenotypes show higher proliferative potential. This trend aligns with prior evidence linking non-O blood groups to more aggressive behavior in several malignancies. From a clinical perspective, the relatively elevated Ki-67 levels observed in B and AB groups may indicate a tendency toward more aggressive tumor biology compared with group O.

Effect of Rh Factor on Ki-67 Expression: When stratified by Rh status, the mean Ki-67 was 26.0% in Rh-positive patients and 25.4% in Rh-negative patients, with no statistically significant difference (Mann–Whitney U test, $p = 0.810$).

The absence of a relationship between Rh antigen and proliferative activity suggests that Rh factor does not play a meaningful role in modulating tumor cell kinetics or growth rate. This observation aligns with previous reports indicating that Rh antigens are primarily structural membrane proteins without known regulatory functions in oncogenesis or cellular proliferation.

Summary of Findings: In summary, the present study demonstrated a consistent—but not statistically significant—trend linking ABO phenotypes with Ki-67 proliferation levels in breast cancer. Group O patients had lower Ki-67 values and a greater proportion of low-proliferative tumors, while groups B and AB showed higher Ki-67 indices and more frequent high-proliferative lesions.

Although formal significance was not achieved, the internal consistency of these findings suggests that ABO antigenic determinants may influence tumor cell proliferation. The lack of association with Rh factor supports the specificity of this effect to the ABO glycosylation pathway rather than erythrocyte antigens in general.

These results warrant further multicenter and mechanistic studies exploring the immunogenetic and molecular pathways that may underlie this association.

Discussion

The proliferative activity of breast cancer cells represents one of the most critical determinants of tumor aggressiveness, therapeutic responsiveness, and long-term prognosis (10). The Ki-67 proliferation index is a widely accepted biomarker for quantifying this activity, reflecting the proportion of tumor cells actively engaged in the cell cycle (11). In current clinical practice, Ki-67 plays a central role in risk stratification, guiding adjuvant treatment decisions, and evaluating therapeutic response (12). Although its prognostic value is well established, limited information is available regarding genetic or immunologic determinants that may modulate Ki-67 expression. The present study adds to this emerging field by assessing whether ABO blood group phenotypes—heritable and widely prevalent antigenic profiles—might be associated with proliferative tumor behavior.

Our findings indicate a general trend in which patients with blood group O exhibited lower Ki-67 indices, while those with groups B and AB demonstrated comparatively higher proliferation rates, with group A showing intermediate values (13). Although the differences reached only borderline statistical significance, this pattern may suggest a potential association between ABO antigen expression and cellular proliferation dynamics (14). These observations are in line with epidemiological studies reporting an increased incidence of several malignancies—including gastric, pancreatic, and hepatic cancers—among individuals with non-O blood types (15).

Several biological mechanisms may underlie the observed relationship between ABO phenotypes and tumor proliferation (16). The ABO gene encodes glycosyltransferases responsible for generating the A and B antigens on the cell surface. These glycan structures, expressed on epithelial cells, endothelial cells, and platelets, contribute to the tumor microenvironment and influence cell adhesion, signaling, and immune recognition (17). Altered glycosylation may enhance cell–matrix interactions via selectins and integrins, thereby promoting proliferation and invasion in tumors expressing A or B antigens (18). Conversely, the absence of A/B antigens in blood group O may attenuate such adhesive and proliferative signaling, consistent with the lower Ki-67 values observed in this group.

In addition, ABO blood group antigens influence systemic inflammatory and coagulation pathways (19). Non-O blood types are associated with elevated von Willebrand factor and factor VIII levels, both of which may contribute to angiogenesis and tumor progression. Furthermore, the ABO locus has been linked to differential regulation of cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), which play established roles in breast cancer proliferation, tumor aggressiveness, and microenvironmental remodeling (20). These immunologic and inflammatory interactions may collectively create a biological environment that supports higher proliferative activity among non-O phenotypes.

The absence of a significant association between Rh factor and Ki-67 expression is consistent with prior literature, as Rh antigens primarily function as transmembrane structural proteins without known involvement in oncogenic or immunologic signaling pathways (21). This supports the interpretation that the observed proliferation differences are more likely driven by ABO-specific glycosylation mechanisms rather than erythrocyte antigens in general.

This study has several strengths. It is one of the few clinical investigations directly exploring the relationship between ABO phenotypes and Ki-67 expression in breast cancer, and the cohort was evaluated using standardized immunohistochemical methods. However, some limitations should be acknowledged. As a single-center retrospective study, generalizability is limited. Additionally, molecular subtype information (Luminal A/B, HER2-enriched, triple-negative) was not available for all patients, preventing subtype-specific interpretation of proliferative differences. Moreover, Ki-67 cut-off values may vary across institutions, which can affect cross-study comparability. Finally, the study did not include mechanistic biomarkers such as cytokine levels, ABO gene polymorphisms, or glycosyltransferase activity, which could provide deeper insight into the biological basis of the observed associations.

Despite these limitations, the findings support an important hypothesis: the ABO blood group system, a genetically determined trait, may exert subtle yet measurable influences on tumor proliferation. If validated in larger, prospective, and multi-omic studies, ABO phenotype data could potentially be incorporated into prognostic models or personalized treatment strategies for breast cancer, offering a simple and readily available biomarker to complement established molecular indicators.

This study provides novel evidence suggesting that ABO blood group phenotypes may influence tumor proliferative behavior in breast cancer. Although statistical significance was borderline, the consistent pattern of lower Ki-67 indices in group O and higher values in groups B and AB implies a potential biological gradient linked to antigenic expression. These findings support the hypothesis that genetically determined carbohydrate antigens could modulate tumor growth through mechanisms involving cellular adhesion, inflammatory signaling, and coagulation pathways.

While the Rh factor did not demonstrate any measurable association with proliferation, the relationship between ABO antigens and Ki-67 expression highlights the possible role of immunohematologic determinants in cancer biology. Further multicenter, prospective studies integrating molecular subtype analysis, cytokine profiling, and ABO genotyping are warranted to validate these observations and uncover the underlying molecular pathways.

If confirmed, such insights could contribute to the refinement of prognostic assessment models and the development of personalized therapeutic strategies that incorporate blood group phenotypes as simple yet informative biomarkers in breast cancer management.

References

1. Finkelman BS, Zhang H, Hicks DG, Turner BM. The evolution of Ki-67 and breast carcinoma: past observations, present directions, and future considerations. Cancers (Basel). 2023;15(3):808.
2. Rewcastle E, Skaland I, Gudlaugsson E, Fykse SK, Baak JPA, Janssen EAM. The Ki-67 dilemma: investigating prognostic cut-offs and reproducibility for automated Ki-67 scoring in breast cancer. Breast Cancer Res Treat. 2024;207(1):1-12.
3. Gown AM. The biomarker Ki-67: promise, potential, and problems in breast cancer. Appl Immunohistochem Mol Morphol. 2023;31(7):478-484.
4. Alchazal R, Zaitoun KJ, Al-Qudah M, et al. Relationship between ABO blood group antigens and Rh factor with breast cancer: a systematic review and meta-analysis. Oncotarget. 2025;16:311-326.
5. Liu FH, Guo JK, Xing WY, et al. ABO and Rhesus blood groups and multiple health outcomes: an umbrella review of systematic reviews with meta-analyses of observational studies. BMC Med. 2024;22(1):206.
6. Agrawal N, Pahuja S, Sharma S. Von Willebrand factor levels in healthy blood donors and their association with blood group: a tertiary care hospital-based study. Asian J Transfus Sci. 2023;17(1):28-33.
7. Cui H, Qu Y, Zhang L, et al. Epidemiological and genetic evidence for the relationship between ABO blood group and human cancer. Int J Cancer. 2023;153(2):320-330.
8. Correa TL, Keko M, Danielian P, et al. Association between ABO blood groups and pancreatic cancer outcomes: a systematic review and meta-analysis. Pancreatology. 2025;25(5):709-717.
9. Elsherif S, Zidan A, Saville O, Othman M. ABO blood group and the risk of thrombosis in cancer patients: a mini-review. Semin Thromb Hemost. 2024;50(3):423-428.

10. Đokić S, Gazić B, Grčar Kuzmanov B, et al. Clinical and analytical validation of two methods for Ki-67 scoring in formalin-fixed and paraffin-embedded tissue sections of early breast cancer. Cancers (Basel). 2024;16(7):1405.
11. Wongmaneerung P, Chitapanarux I, Traisathit P, et al. The association between Ki-67 expression and survival in breast cancer subtypes: a cross-sectional study of Ki-67 cut-point in northern Thailand. BMC Cancer. 2025;25:346.
12. Shi H, Yao J, Jiang C, et al. ABO blood types and head and neck cancer: a systematic review with meta-analysis of observational studies. BMJ Open. 2024;14(10):e088016.
13. Bruun-Rasmussen P, Hanefeld Dziegiel M, Banasik K, Johansson PI, Brunak S. Associations of ABO and Rhesus D blood groups with phenome-wide disease incidence: a 41-year retrospective cohort study of 482 914 patients. eLife. 2023;12:e83116.
14. Albadrani HM, Hamed M, Zakariyah A, et al. Differential expression of ABO in normal and tumor tissues: implications for cancer biology and prognosis. J Taibah Univ Med Sci. 2024;19(6):1132-1142.
15. Zhou Y, Ren H, Wang J, et al. ABO blood group and risk of cancer: a systematic review and meta-analysis. Cancer Epidemiol. 2018;53:1-10.
16. Baster Z, Russell L, Rajfur Z. A review of talin- and integrin-dependent molecular mechanisms in cancer invasion and metastasis. Int J Mol Sci. 2025;26(5):1798.
17. Routila E, Salminen S, Mahran R, et al. Evaluation of integrin glycovariants as biomarkers of metastasis, invasion, and therapy stratification in head and neck squamous cell carcinoma. Cancer Med. 2025;14(9):e70717.
18. Karampinis I, Nowak K, Koett J, et al. Von Willebrand factor in the plasma and in the tumor tissue predicts cancer-associated thrombosis and mortality. Haematologica. 2023;108(1):261-266.
19. De La Cruz-Vargas JA, Gómez H, Talavera JE, et al. Prognostic relevance of inflammatory cytokines IL-6 and TNF-α in patients with breast cancer: a systematic review and meta-analysis. Curr Oncol. 2025;32(6):344.
20. Dogan I, Gurbuz M, Aydin E, Karabulut S, Tastekin D, Utkan G. Distributions and prognostic effects of ABO/Rh blood groups in patients with HER2/neu-positive gastric and gastroesophageal junction cancer. J Cancer Res Ther. 2023;19(Suppl 2):S747-S751.