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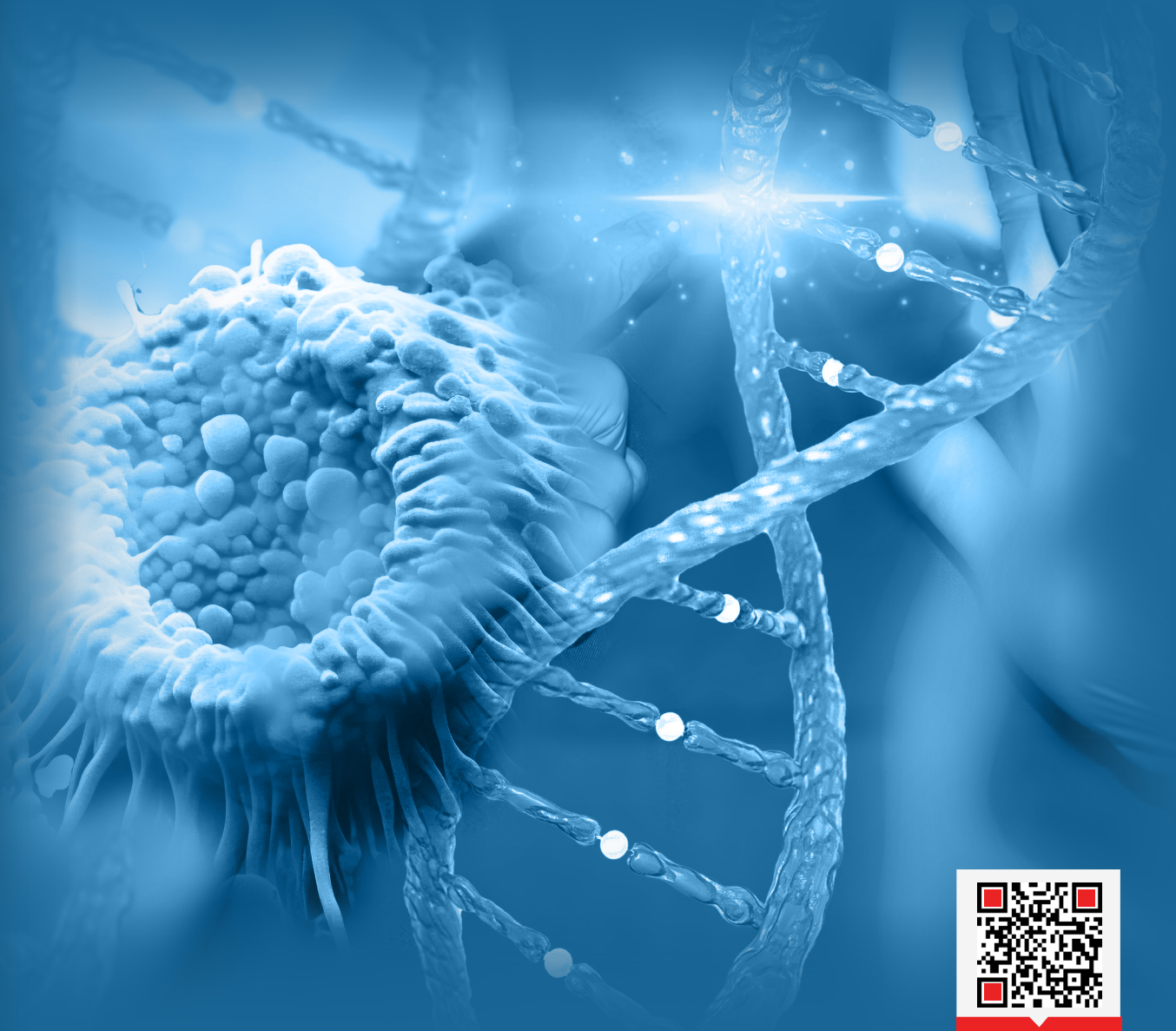
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Review

Antibody-drug Conjugates (ADCs) in Breast Cancer

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ABSTRACT

Breast cancer is the most common cancer in women worldwide and comprises numerous molecular subtypes. Antibody-drug conjugates (ADCs) are a new class of drugs that combine specific monoclonal antibodies with a cytotoxic drug and are also used in breast cancer treatment. This review discusses the ADCs ado-trastuzumab emtansine (T-DM1), trastuzumab deruxtecan (T-DXd), sacituzumab govitecan (SG), and datopotamab deruxtecan (Dato-DXd). These drugs have demonstrated significant benefits in overall survival in patients with human epidermal growth factor receptor 2 (HER2)-positive, HER2-low, hormone receptor-positive, and triple-negative breast cancer. T-DXd has extended the reach of HER2-targeted therapy to patients with HER2-low and HER2-ultralow disease; SG and Dato-DXd have emerged as effective options for patients with extensive prior treatment. In addition to therapeutic effectiveness, the individual adverse effect profiles of these medications must be carefully evaluated and managed. Important safety issues with these medications include thrombocytopenia and liver toxicity with T-DM1, interstitial lung disease/pneumonia with T-DXd, neutropenia and diarrhea with SG, and stomatitis and ocular damage with Dato-DXd. Ongoing clinical trials include investigations of early-stage therapies, new combination medicines, and next-generation research targeting resistance mechanisms associated with ADCs. As a result, ADCs have established a new standard in breast cancer treatment and play a vital role in personalized oncology.

Keywords: Antibody-drug conjugates, breast cancer, drug-related side effects and adverse reactions, molecular targeted therapy

Introduction

Accounting for 32% of all female cancer diagnoses, breast cancer is the most common cancer in the United States (US). Among women less than 50 years old, it is the top cancer killer; yet, when all age groups are taken into account collectively, it ranks second, after lung cancer [1]. Thanks to advances in the genomic era, breast cancer may now be classified into intrinsic molecular subgroups, which have improved our understanding of the disease's biological heterogeneity: luminal A, luminal B, human epidermal growth factor receptor 2 (HER2)-positive, and basal-like/triple-negative. This classification has provided greater accuracy in predicting clinical course and treatment response [2,3]. Thanks to this molecular classification, endocrine therapy (ET) developed for hormone receptor (HR) positive disease [4] or anti-HER2 drugs used when HER2 amplification is available have significantly improved individual clinical survival [5]. However, in individuals with aggressive types of breast cancer, such as triple-negative breast cancer (TNBC), the effect of the medications used on survival has been significantly limited [6]. There is growing interest in

developing more selective and effective therapeutic strategies for cases in which certain breast cancer subtypes are resistant to conventional treatments. In this framework, the paradigm of breast cancer treatment has changed substantially with the development of novel targeted agents in recent years [7].

The structure of antibody-drug conjugates (ADCs) comprises a monoclonal antibody targeting a tumor-associated antigen, a cytotoxic payload, and a chemical linker that connects them. The adverse effects of ADCs are caused by a number of factors, including the distribution of the target antigen, the payload, the linker, the conjugation process, the structure of the antibody, and its Fc receptor expression [8]. The ADCs ado-trastuzumab emtansine (T-DM1), trastuzumab deruxtecan (T-DXd), sacituzumab govitecan (SG), and datopotamab deruxtecan (Dato-DXd) have been approved by the US Food and Drug Administration (FDA) for the treatment of breast cancer and have entered practice (Table 1) [9]. These agents combine target specificity with strong cytotoxic activity and show promising results, especially in patient populations with drug resistance or limited treatment options.

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This review examines the growing importance of ADCs in breast cancer treatment and the research performed on them. It summarizes the most recent clinical data for all molecular subtypes and emphasizes their significance, especially for HER2-positive and TNBC subtypes. It also includes toxicity profiles and the management of side effects.

T-DM1

T-DM1 is an ADC created by conjugating trastuzumab, an HER2-targeted antibody, with DM1, a microtubule-inhibiting cytotoxic agent derived from maytansine [10]. The anticancer effects of trastuzumab are seen when T-DM1 binds to the HER2 receptor [11,12]. Following binding of the HER2-T-DM1 complex, it is taken up into the cell via endocytosis and lysosome fusion, and the breakdown of this intracellular complex releases the cytotoxic load known as DM1 [13]. In this way, T-DM1 causes cells to undergo apoptosis and mitotic breakdown [14].

In the first human study published in 2010, an intravenous (IV) dose regimen of 3.6 mg/kg every three weeks was determined for T-DM1 administration [15]. Trastuzumab in combination with docetaxel has long been recognized as an effective treatment for HER2-positive metastatic breast cancer (MBC). In the initial randomized phase II study, T-DM1 was compared with this treatment. In this context, T-DM1 has emerged as a highly effective treatment option for improving progression-free survival (PFS). The median PFS in the T-DM1 group was statistically significantly longer than in the trastuzumab plus docetaxel group [14.2 months vs. 9.2 months, hazard ratio (HR): 0.59, $p=0.035$]. Objective response rates (ORR) were similar (64.2% vs. 58.0). The tolerance profile was enhanced, and the number of adverse events graded 3 or higher decreased with T-DM1 [16].

The results of the phase II study served as the foundation for the EMILIA study. This phase III trial, which was randomized and open-label, investigated the safety and efficacy of T-DM1 in individuals with HER2-positive, locally advanced disease or MBC who had previously received trastuzumab and taxane therapy. In this study involving 991 patients, one group received oral lapatinib and capecitabine, while the other received T-DM1. T-DM1 demonstrated improvements in both PFS and overall survival (OS) compared with the other treatment. Increases of approximately 6 months in median OS and 3 months in median PFS were observed (OS: 25.1 vs. 30.9 months; PFS: 9.6 vs. 6.4 months). The T-DM1 group had a higher ORR (43.6% vs. 30.8%), and responses lasted

longer (12.6 months). In terms of safety analyses, the T-DM1 group experienced fewer grade ≥ 3 adverse events (57% vs. 41%). The most prevalent adverse effects of T-DM1 were increased transaminases and thrombocytopenia, whereas the control group experienced diarrhea, palmar-plantar erythrodysesthesia, vomiting, and thrombocytopenia more frequently [10]. In 2013, the European Medicines Agency and the FDA approved the use of T-DM1, which was the first ADC to be used in the treatment of breast cancer [17]. In the most recent study, the median OS was 29.9 months in the T-DM1 group and 25.9 months in the control group. Additionally, the risk of mortality was reduced by 25% (HR: 0.75) [18]. Overall, T-DM1 has been shown to be a safe standard of care option for previously treated HER2-positive MBC. It not only increases the chances of survival, but it is also safe.

The TH3RESA trial demonstrated that T-DM1 was effective in treating HER2-positive MBC in patients who had previously received other treatments without success. In patients treated with trastuzumab, lapatinib, and a taxane, T-DM1 added 2.9 months to PFS compared to physician-choice chemotherapeutic regimens (HR: 0.52, $p<0.0001$). A considerable improvement in OS (HR: 0.55) was observed as well. The T-DM1 group had a higher incidence of grade 3 or higher adverse events (43% vs. 32%). In the T-DM1 group, thrombocytopenia was the most common serious adverse event [19]. Patients who have previously received trastuzumab and lapatinib for HER2-positive MBC may find T-DM1 a more acceptable alternative because of its lower toxicity profile.

The MARIANNE trial investigated the efficacy of T-DM1 as a first-line treatment for HER2-positive MBC. Patients were divided into three groups and received trastuzumab plus a taxane, T-DM1, or T-DM1 plus pertuzumab. T-DM1 was as effective for PFS and OS as the standard trastuzumab-taxane combination. The frequency of adverse events of grade 3 and above was lower in the T-DM1 group. In the control group, alopecia, peripheral neuropathy, and gastroenteritis occurred more frequently. Conversely, T-DM1-containing regimens were more frequently associated with thrombocytopenia and increased hepatic toxicity [20]. These data suggest that although T-DM1 does not show superiority to standard therapy in the first-line setting, it may be a better-tolerated alternative.

The KAMILLA study evaluated the clinical benefit of T-DM1 in breast cancer with HER2-positive brain metastases. Furthermore, an ORR of 21.4% in patients with brain metastases and a 42.9% response rate (defined as $>30\%$ reduction in intracranial lesions) demonstrated that T-DM1

Table 1. Structural and functional characteristics of ADCs used in breast cancer

ADC	Target antigen	Cytotoxic payload	Linker type	DAR	Bystander effect
T-DM1	HER2	DM1 (microtubule inhibitor)	Non-cleavable	~3.5	No
T-DXd	HER2	DXd (topoisomerase I inhibitor)	Cleavable	~8	Yes
SG	Trop-2	SN-38 (topoisomerase I inhibitor)	CL2A (cleavable)	~8	Yes
Dato-DXd	Trop-2	DXd (topoisomerase I inhibitor)	Cleavable	~4	Yes

ADC: Antibody-drug conjugate, DAR: Drug-to-antibody ratio, Dato-DXd: Datopotamab deruxtecan, HER2: Human epidermal growth factor receptor 2, HR: Hormone receptor, T-DM1: Trastuzumab emtansine, T-DXd: Trastuzumab deruxtecan, SG: Sacituzumab govitecan, TNBC: Triple-negative breast cancer

can also exhibit clinical activity in central nervous system (CNS) metastases. The preponderance of the adverse events was hematologic and hepatic in nature, and the most common events were thrombocytopenia and elevated liver function tests. The low treatment discontinuation rate (5.7%) supports the manageability of the toxicity profile [21]. The incorporation of atezolizumab into T-DM1 therapy did not improve PFS in the overall HER2-positive MBC population in the KATE2 study. However, a signal of enhanced clinical activity was observed in the programmed death-ligand 1 (PD-L1)-positive subgroup [22].

T-DM1 has been studied in early-stage HER2-positive breast cancer and in disseminated disease. The pathological complete response (pCR) rate for T-DM1 plus pertuzumab in the neoadjuvant phase of the KRISTINE study was 55.7%, which was higher than the 44.4% observed with the standard chemotherapy strategy of trastuzumab plus carboplatin plus pertuzumab. The patients' quality of life was preserved because there were a lot fewer grade 3 or higher adverse events in the T-DM1+P group (31.8% vs. 67.7%) [23].

The KATHERINE study is an adjuvant TDM-1 study of early-stage HER2-positive breast cancer patients who underwent surgery after trastuzumab and taxane-based chemotherapy and who experienced persistent invasive disease. Adjuvant T-DM1 demonstrated a significant improvement in invasive disease-free survival (iDFS) compared with adjuvant trastuzumab. T-DM1 significantly increased the 3-year iDFS rate (88.3% vs. 77.0%). It also reduced the rate of distant metastasis (10.5% vs. 15.9%). The rate of discontinuation due to grade 3 or higher adverse events was higher in the T-DM1 group (15.4% and 2.1% vs. 25.7% and 18.0%) [24]. Based on the results of the final long-term analysis of a 7-year follow-up, T-DM1 increased the iDFS rate to 80.8%, representing an absolute gain of 13.7 percentage points, compared with trastuzumab; it also increased the OS rate to 89.1%, representing an absolute improvement of 4.7 percentage points [25]. Therefore, 14 cycles of T-DM1 are now considered the established standard of care for patients with persistent HER2-positive disease after neoadjuvant treatment.

The T-DM1+P combination did not exhibit superiority over the conventional regimen of taxane + trastuzumab + P (THP) in terms of iDFS in the KAITLIN trial, which evaluated adjuvant therapy in patients with high-risk HER2-positive early-stage breast cancer [26]. Nevertheless, the ATEMPT study, which investigated the efficacy of adjuvant T-DM1 in patients with stage I HER2-positive breast cancer, compared it with the conventional regimen of paclitaxel and trastuzumab. A three-year iDFS rate of 97.8% was achieved after one year of adjuvant T-DM1 therapy [27].

T-DM1: Toxicity and Management

The safety profile of T-DM1 treatment has been comprehensively evaluated for hematologic, hepatic, neurological, pulmonary, and cardiac toxicities. With a frequency of 12.9% in the EMILIA study, 5% in the TH3RESA trial, and 5.7% in the adjuvant KATHERINE trial, thrombocytopenia was the most commonly reported grade ≥ 3 adverse event in clinical

studies [10,19,24]. Thrombocytopenia is generally observed during the initial two treatment cycles and is believed to be the result of the suppression of megakaryocyte differentiation [22]. The KAMILLA study, however, demonstrated that severe thrombocytopenia is rarely associated with clinically significant bleeding events [21]. In the context of clinical management, it is advisable to maintain the current dose of treatment for patients with grade 1-2 thrombocytopenia. In the event of grade 3 thrombocytopenia, treatment should be discontinued until the platelet count reaches grade 1 or lower, at which point it should be resumed at the same dose. For patients developing thrombocytopenia grade 4, treatment should be interrupted and resumed at a reduced dose level after hematological recovery [28].

Hepatotoxicity is a characteristic toxicity of T-DM1, most often presenting as asymptomatic transaminase elevations. In the EMILIA trial, grade 3 or higher increases in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were reported in 4.3% and 2.9% of patients, respectively. In the KATHERINE study, the rates of AST and ALT elevations at all grades were 28.4% and 23.1%, respectively [24]. Nodular regenerative hyperplasia, an uncommon yet serious complication, has been reported, especially in the KATHERINE and KAITLIN studies [24,26]. If hepatotoxicity is grade ≥ 3 , temporary suspension of treatment is recommended. If there is significant elevation of bilirubin: permanent discontinuation of treatment is recommended [10].

Compared with conventional taxane-based therapies, which are associated with neurological toxicity, T-DM1 has been linked to a lower incidence of peripheral neuropathy. In the KATHERINE study, the rate of peripheral neuropathy of all grades was 18.6%, while grade ≥ 3 neuropathy was reported in only 1.4% of patients [24]. In the KRISTINE study, the rate of grade 3 or higher peripheral neuropathy was found to be 3.1% [23]. In patients who develop severe neuropathy, it is recommended to interrupt treatment until symptoms resolve and to reduce the dose as necessary.

Pulmonary toxicities, particularly pneumonitis and interstitial lung disease (ILD), are uncommon but potentially life-threatening complications. While the incidence of pneumonitis was reported as 2.6% in the KATHERINE study [24], the incidence of radiation-associated pneumonia was reported as 2.3% in the KAITLIN study [26]. Therefore, permanent discontinuation of T-DM1 therapy is recommended when pneumonitis or ILD is suspected [20,22].

Clinically substantial reductions in left ventricular ejection fraction with T-DM1 are uncommon when cardiac safety is assessed. In the EMILIA trial, 1.7% of patients experienced a reduction to less than 50%, and the left ventricular ejection fraction decreased by over 15% from baseline [10]. In the KATHERINE study, the rate of cardiac events was found to be quite low (0.1%) [24]. However, in line with the general safety approach for HER2-targeted therapies, regular cardiac monitoring is recommended.

The cornerstones of toxicity management in T-DM1 therapy are dose delay and dose reduction. The standard regimen is

an IV dose of 3.6 mg/kg every 21 days; if toxicity develops, the dose can be reduced to 3.0 mg/kg and then to 2.4 mg/kg, respectively [10,22]. Treatment may be delayed for up to 42 days until toxicity resolves; however, discontinuation of treatment is recommended if tolerance cannot be achieved even at lower doses [22]. In adjuvant studies, allowing patients who could not continue T-DM1 due to toxicity to complete their treatment with trastuzumab has been considered an important approach for maintaining treatment continuity [26]. Additionally, in a study evaluating the real-world analysis of adjuvant T-DM1, it demonstrated a manageable toxicity profile [29].

T-DXd

T-DXd, the next-generation ADC, is composed of a humanized anti-HER2 immunoglobulin G1 monoclonal antibody, a cleavable tetrapeptide binder, and a topoisomerase I inhibitor payload (DXd) [30]. T-DXd has a superior drug-to-antibody ratio compared to T-DM1 (approximately 8 vs. approximately 3.5) [30-32]. The bystander effect is a mechanism by which the released lethal payload can affect nearby cells to eradicate cancer by more easily passing through their membranes [33]. T-DXd employs a tumor-agnostic approach targeting HER2 expression, with HER2 expression determined by central/local evaluation as immunohistochemistry (IHC) 3+ or 2+ using the DAKO HER2 HercepTest [34].

Participants with HER2-positive MBC who had already received extensive therapy, including T-DM1, were evaluated for T-DXd in the Phase II DESTINY-Breast01 trial. The OS was 62% after an average of 26.5 months of follow-up, with the shortest PFS and OS at 19.4 and 29.1 months, respectively. Patients were most severely affected by ILD/pneumonitis, which occurred in 15.8% of cases and was associated with a 2.7% mortality rate [35].

DESTINY-Breast03 was a study designed to evaluate the efficacy of T-DXd versus T-DM1 in HER2-positive MBC patients who had previously received trastuzumab and taxane [36]. T-DXd provided a 20-month advantage in PFS and a 36% reduction in overall mortality risk (HR: 0.64). In the T-DXd arm, ILD/pneumonitis cases were low-grade, with an incidence of 15.2%; no fatal cases occurred. This demonstrates that toxicity can be managed with appropriate monitoring. These results strengthen the status of T-DXd as a standard second-line treatment for HER2-positive MBC [37].

Just as some breast cancers lack HER2 amplification, overexpression, or both, a large proportion exhibit low but treatable levels of HER2 expression. In these patients with “HER2-low” cancer, current HER2-targeted therapies have been ineffective. HER2-low expression is indicated by IHC findings of 1+ or 2+ and by negative *in situ* hybridization (ISH) results. In DESTINY-Breast04, patients with HER2-low MBC who had already received one or two rounds of chemotherapy were compared in two groups: those who received T-DXd and those who received chemotherapy given at the discretion of the clinician. Whether evaluated in the overall patient group or in the HR-positive arm, T-DXd demonstrated a substantial

clinical advantage. The ORR was substantially higher with T-DXd than with chemotherapy (52.3% vs. 16.3%), and the risk of mortality with T-DXd was 36% lower. In safety analyses, although grade ≥ 3 adverse events were observed at a lower rate compared to chemotherapy, ILD/pneumonia (0.8% fatal) developing in 12.1% of patients emerged as the primary toxicity requiring attention. Subgroup analyses showing similar efficacy in HER2 IHC 1+ and HER2 IHC 2+/ISH-negative patients support that T-DXd provides broad clinical benefit in the HER2-low disease group [38].

Patients with HER2-ultralow expression were included in the clinical use of T-DXd in the DESTINY-Breast06 study, which followed the DESTINY-Breast04 study. We included patients with HR-positive MBC who had not undergone chemotherapy for metastatic disease and whose disease had progressed after at least one ET session. Patients were considered to have HER2-low expression if their IHC scores were 1+ or 2+/ISH-negative, and HER2-ultra-low expression if their IHC scores were 0 with mild membrane staining. T-DXd was compared with investigator-selected single-agent chemotherapy. T-DXd improved PFS in both the HER2-low and HER2-ultralow groups (HRs: 0.62 and 0.78, respectively). This demonstrates that T-DXd is effective across a broader spectrum of HER2 expression [39]. Based on these results, T-DXd appears to be a viable alternative to other ET for patients with HR-positive, HER2-low, or ultra-low MBC [40].

The DAISY study is another trial evaluating the efficacy of T-DXd in patients with HER2-overexpressing, HER2-low, and HER2-negative MBC. The ORR was 70.6%, 37.5%, and 29.7%, respectively. These results indicate that clinical responses observed in HER2-negative patients, the bystander effect, and alternative biological mechanisms may play a role in treatment response [41].

The DESTINY-Breast02 study compared T-DXd to the physician’s recommended treatment for individuals with HER2-positive MBC whose disease had progressed on T-DM1. T-DXd demonstrated a significant advantage in terms of PFS, OS, and ORR (70% vs. 29%). ILD/pneumonia, observed in 10% of patients, emerged as the primary toxicity of concern [42]. These results provided the first randomized evidence suggesting that resistance developed against one ADC can be overcome by another ADC.

The DESTINY-Breast12 trial evaluated the effectiveness of T-DXd in patients with brain metastases. Participants were patients with HER2-positive MBC who had previously received anti-HER2 treatment; the trial was a phase 3b/4 study. Among patients with brain metastases, T-DXd demonstrated a 71.7% CNS response rate and a 61.6% PFS rate at 12 months. The ORR was 62.7% in patients who did not have brain metastases [43]. Even in cases where brain metastases are present, our data show that T-DXd is an effective therapeutic option.

Patients with HER2-positive advanced-stage disease or MBC were enrolled in the DESTINY-Breast09 trial, a first-line study that assessed the effectiveness of T-DXd + P. T-DXd+P outperformed the gold-standard regimen of THP, achieving a median increase in PFS of 44% (40.7 months vs. 26.9 months).

The T-DXd+P group had a higher ORR (87% vs. 81%) [44]. Even at the outset of therapy, these results support the proposition that T-DXd may be an appropriate therapeutic option. Thanks to NCT04784715, we can still obtain OS data and conduct long-term follow-up. In the DESTINY-Breast05 study, researchers evaluated T-DXd and T-DM1 in women with high-risk HER2-positive breast cancer who had residual disease after neoadjuvant therapy. T-DXd showed superior 3-year iDFS compared with T-DM1 (92.4% vs. 83.7%; HR: 0.47). The decreased risk of distant recurrence in the T-DXd arm provides additional evidence that the medicine has the ability to be an effective post-neoadjuvant therapy for early-stage HER2-positive cancer (NCT04622319) [45].

T-DXd: Toxicity and Management

Although the safety profile of T-DXd therapy is generally manageable, it is characterized by gastrointestinal and hematologic toxicities, as well as adverse events such as ILD and pneumonia, which require close monitoring [35].

Nausea, vomiting, fatigue, alopecia, and constipation were the most frequently reported adverse effects in clinical trials [42]. Nausea, in particular, is observed in the majority of patients and is more pronounced during the early stages of treatment [35]. Given that the drug is classified as having a high emetogenic risk, appropriate prophylactic antiemetic measures is important. For primary prophylaxis before treatment, a triple antiemetic regimen of dexamethasone, a 5-hydroxytryptamine receptor antagonist, and a neurokinin-1 receptor antagonist is suggested [46].

Neutropenia, anemia, and leukopenia are the major hematologic toxicities, and regular monitoring of complete blood count and dose adjustment as required is recommended [37].

ILD/pneumonitis is the most serious and potentially fatal toxicity associated with T-DXd, with an incidence of approximately 10-15.8%. Therefore, patients should be carefully evaluated for symptoms such as cough, dyspnea, and fever [35]. In the case of suspected ILD/pneumonitis, T-DXd treatment should be held, and the patient should be clinically, radiologically, and, if needed, pulmonologically assessed.

In grade 1 (asymptomatic) cases, treatment should be stopped until the radiological findings have completely resolved and regressed to grade 0, with close monitoring of symptoms and oxygen saturation for 2-7 days; a follow-up chest computed tomography scan should be performed within 1-2 weeks. During this period, systemic corticosteroid therapy with prednisone or equivalent at a dose of at least 0.5 mg/kg/day should be considered. After clinical recovery, the dose should be gradually reduced over at least 4 weeks. T-DXd can be resumed at the same dose if ILD/pneumonitis has fully resolved within 28 days of onset; if resolution occurs later than 28 days after onset, treatment should be resumed at a reduced dose level. Conversely, if the condition has not completely resolved within 49 days, T-DXd treatment should be permanently discontinued.

Symptomatic grade 2 ILD/pneumonitis represents a clinically significant toxicity requiring definitive discontinuation of

T-DXd and prompt administration of systemic corticosteroids at therapeutic doses. Steroid medication must be maintained for a minimum of 14 days or until clinical and radiological abnormalities have fully resolved, followed by a progressive dosage reduction over at least four weeks. If clinical improvement is not evident within the initial five days, it is advisable to either escalate the steroid dosage to 2 mg/kg/day or to transition to IV corticosteroid therapy.

Grade 3 or grade 4 ILD/pneumonitis is a serious clinical condition requiring urgent and aggressive treatment. In these patients, T-DXd therapy should be permanently discontinued, and the patients should be hospitalized. Treatment should be initiated immediately with high-dose IV methylprednisolone (typically 500-1000 mg/day for three days), followed by at least 1 mg/kg/day of oral prednisone or an equivalent. After 14 days of corticosteroid treatment, or earlier if radiological and clinical improvements are seen, the dosage should be gradually reduced over four weeks. In cases where a clinical response is not achieved within 3-5 days despite steroid therapy, additional immunosuppressive agents, such as infliximab or mycophenolate mofetil, should be considered.

Patients at all stages should be closely monitored until clinical symptoms and radiological findings have completely resolved, even if T-DXd treatment has been discontinued. Additionally, the increased risk of opportunistic infections in patients using prednisone or equivalent corticosteroids at doses higher than 20 mg daily should be considered, and appropriate prophylactic treatments, particularly for *Pneumocystis jirovecii* pneumonia, should be evaluated [47].

Furthermore, it is necessary to conduct routine assessments of cardiac function both prior to and during treatment, as there is a low incidence of decreased left ventricular ejection fraction with other HER2-targeted agents [35].

In general, the majority of adverse events can be effectively managed through treatment interruptions, dose reductions, and supportive care approaches [44].

SG

Targeting trophoblast cell-surface antigen-2 (Trop-2), SG is an ADC composed of an anti-Trop-2 monoclonal antibody conjugated to an SN-38 payload via a hydrolyzable CL2A linker. Owing to its high drug-to-antibody ratio (~8:1) and efficient cellular internalization, SG exhibits selective cytotoxic effects on tumor cells expressing Trop-2. The released SN-38 inhibits cell proliferation by causing deoxyribonucleic acid (DNA) damage and inducing apoptosis. Additionally, free SN-38, which can diffuse into the tumor microenvironment, exerts a cytotoxic effect on neighboring cells, contributing to the "bystander effect" mechanism [48].

SG has become an important part of the Phase 1/2 IMMUGEN-132-01 project to demonstrate the efficacy of the ADC method in treating metastatic TNBC. It demonstrated a 33.3% ORR in individuals with advanced TNBC who had previously undergone extensive treatment. Neutropenia and anemia occurred frequently as grade 3 side effects; 3% of people stopped their medication. Overall, SG demonstrated a manageable

safety profile [49]. SG and physician-selected chemotherapy were evaluated in the phase 3 ASCENT trial in patients with metastatic TNBC who had previously undergone two or more lines of treatment or who had relapsed. The OS was 12.1 months, the median PFS was 5.6 months, and the ORR was 35% in patients without brain metastases, compared with 5% in those with brain metastases. Diarrhea and myelosuppression were more common side effects of SG therapy [50]. These findings further establish SG as the treatment of choice for patients with metastatic TNBC who have undergone two or more prior treatments. Furthermore, since SG demonstrated a PFS advantage over chemotherapy across all Trop-2 expression levels, its efficacy was shown to persist across a broad biological spectrum, suggesting that routine pre-treatment Trop-2 assessment may not be mandatory [51].

Particularly in HR⁺/HER2-MBC, SG showed encouraging effectiveness in the Phase I/II IMMU-132-01 basket trial [49]. In endocrine-resistant, previously treated patients with HR⁺/HER2-negative MBC, the TROPiCS-02 trial compared SG with physician-selected chemotherapy. SG enhanced ORR (21% vs. 14%) and increased median PFS (from 4 months to 5.5 months; HR: 0.66) [52]. Initial results showed a numerical advantage in OS; however, after adjusting for confounding factors, the SG group had a higher median OS (14.4 vs. 11.2 months; HR: 0.79). A survival advantage was observed regardless of the level of Trop-2 expression. The occurrence of diarrhea was 10% greater with SG treatment compared to chemotherapy (1% lower), and grade ≥ 3 neutropenia was 51% higher (compared to 39%). No instances of treatment-associated pneumonitis were seen in the SG group [53]. The results support SG's status as the first approved ADC for HR⁺/HER2-negative MBC in patients with a history of ET, taxanes, and cyclin-dependent kinase 4/6 (CDK4/6) inhibitors who have completed 2-4 cycles of chemotherapy for metastatic disease.

Patients with HER2-negative breast cancer who are at high risk of recurrence and have residual disease after neoadjuvant chemotherapy are being studied in the SASCIA phase 3 trial to determine the safety and effectiveness of SG. According to an interim safety analysis, The adverse events, such as nausea, vomiting, diarrhea, alopecia, and hematologic toxicities, were more prevalent in the SG group. The SG group also had a higher rate of grade ≥ 3 adverse events than the physician's choice group (66.7% vs. 20.9%). No new safety signals were found. Although dose delays were more common with SG therapy, the rates of dose decrease were equal in both arms. The data support the notion that ADCs might be useful in treating early-stage breast cancer and demonstrate that SG has an acceptable safety profile despite its increased toxicity burden [54]. The study has reported only interim safety analyses, and primary efficacy results are not yet mature (NCT04595565). The NeoSTAR study is a phase 2 trial evaluating neoadjuvant SG in early-stage TNBC in combination with pembrolizumab. In this study, a pCR was achieved in 34% of patients. The most frequently reported events in patients were hair loss, fatigue, and diarrhea. While these results indicate that the combination of SG and pembrolizumab demonstrates promising antitumor

activity in early-stage TNBC, they also indicate a need for further studies to determine the optimal treatment duration and sequence [55].

SG: Toxicity and Management

Hematologic and gastrointestinal adverse events are the most prevalent, and the toxicity profile of SG treatment is essentially consistent with the known effects of SN-38.

The most frequent side effect of grade 3 or higher was neutropenia. The ASCENT and TROPiCS-02 studies reported an incidence of approximately 51% for grade ≥ 3 neutropenia [50,52]. The approach for neutropenia includes dose skipping, dose reduction as needed, and granulocyte-colony stimulating factor (G-CSF) supplementation [49,50]. Clinical studies have shown that approximately 49-54% of patients require G-CSF support, and dose modifications can be applied in 25-50% of cases involving recurrent severe hematologic toxicity [49,50,52].

Gastrointestinal toxicities are also a notable component of SG therapy [49,50]. In particular, diarrhea is observed to some degree in approximately 59-62% of patients, while the rate of grade ≥ 3 diarrhea is approximately 10%. Therefore, the early initiation of anti-motility agents, such as loperamide, and the implementation of supportive care measures are important. Common side effects include nausea in 59% of cases and vomiting in 29% [50]; therefore, prophylactic antiemetic therapy is recommended as an important part of clinical practice [49]. Alopecia is observed in approximately 46-48% of patients and is considered an expected side effect related to the cytotoxic burden of SG [50].

Peripheral neuropathy should be considered, and, in particular, ILD/pneumonia is significantly lower with SG than with other ADCs [50]. Furthermore, the uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) *28/*28 genotype, which affects SN-38 metabolism, has been linked to an elevated risk of hematologic toxicity. Although neutropenia and anemia may be observed more frequently in these patients, current data do not support routine UGT1A1 screening prior to SG treatment; instead, close clinical monitoring is recommended. It is evident that SG toxicities may be effectively treated with proactive supportive techniques and appropriate dosage changes, since the treatment discontinuation rates owing to adverse events remain low (5-6%) [52].

Dato-DXd

One ADC that stands out is Dato-DXd. Combines a conjugated humanized anti-Trop-2 antibody with the powerful topoisomerase I inhibitor DXd [56]. The half-life of Dato-DXd is greater than that of SG [57]. Patients with solid tumors who had previously received therapy were assessed for safety and effectiveness of Dato-DXd in the TROPION-PanTumor01 phase 1 study. Remarkably, the TNBC subgroup achieved an ORR of 31.8% and a median PFS of 4.4 months [58]. Participants in the TROPION-Breast01 trial's third phase were patients with HR⁺/HER2-negative inoperable disease or MBC who were receiving investigational treatment, had previously undergone

one or two cycles of chemotherapy in a metastatic setting, and were resistant to or not eligible for ET; they were compared with investigator-selected single-agent chemotherapy for safety and efficacy. Compared with chemotherapy, Dato-DXd demonstrated a considerable improvement in PFS, with a 37% lower likelihood of death or progression. Final OS analysis did not reveal any statistically significant differences between Dato-DXd and chemotherapy after controlling for variables such as median follow-up time (22.8 months vs. 18.3 months; HR: 1.01). On the other hand, Dato-DXd outperformed the other treatments secondary outcomes such as time to second advancement, duration of reaction, and ORR. Mild nausea and stomatitis were the most often reported toxicities, and treatment-related adverse events grade ≥ 3 occurred far less frequently in the Dato-DXd group than in the chemotherapy group (45.6% vs. 22.2%) [59]. Adults who have already received endocrine-based treatment or chemotherapy and have HR⁺ but HER2-negative breast cancer (IHC: 0, IHC: 1⁺, or IHC 2⁺/ISH⁺) are eligible for treatment with Dato-DXd according to the TROPION-Breast01 investigation.

The BEGONIA study examined the efficacy and tolerability of Dato-DXd with durvalumab in previously untreated individuals with advanced or metastatic TNBC. A full recovery was noted in 10% of patients, contributing to an ORR of 79%. The treatment response was shown to be independent of PD-L1 expression levels, and the median PFS was reported to be 13.8 months. According to the safety studies, 65% of patients had nausea and stomatitis as adverse events, while 57% of patients reported adverse events of grade ≥ 3 . ILD/pneumonitis occurred in 5% of patients, and no fatalities were reported as a result of therapy. These results provide credence to the idea that Dato-DXd and durvalumab should be the subject of additional clinical trials [60].

The effectiveness of neoadjuvant Dato-DXd in treating early-stage breast cancer was assessed in the I-SPY2.2 study. Although 30% of patients in the other group achieved pCR using Dato-DXd, only 6% of patients with HR⁺/HER2⁻ and TNBC did so. The pCR incidence was particularly high (41% in this category) among patients who tested negative for HR, HER2, immune status, and DNA repair deficiency. Based on these results, it seems that selecting patients based on their molecular traits is crucial for maximizing Dato-DXd therapy efficacy [61].

Dato-DXd: Toxicity and Management

The Dato-DXd group had a better tolerability profile, as evidenced by a reduced frequency of grade ≥ 3 treatment-related side events (45% vs. 21-22%) in clinical studies compared to the investigator-selected chemotherapy regimen [59].

The most commonly observed adverse events include stomatitis, nausea, vomiting, fatigue, skin rash, decreased appetite, and alopecia. Stomatitis is a characteristic toxicity of

Dato-DXd therapy; it was reported in 72-83% of patients in the studies and was predominantly mild to moderate (Grade 1-2). Dexamethasone-containing mouthwashes are recommended to reduce the risk of stomatitis [58,61]. Additionally, ocular toxicities such as dry eyes, keratitis, and blurred vision have been observed in approximately 38-44% of patients [61]. The use of lubricating eye drops is emphasized for the management of ocular toxicities.

As with other ADCs containing T-DXd, ILD/pneumonitis is a clinically significant adverse event with an incidence ranging from approximately 2% to 4%; most cases are mild [53,54]. Dose delays or dose reductions are implemented as needed, and close clinical monitoring of patients is recommended, particularly for those with ILD.

Infusion-related reactions have been reported in approximately 18-20% of cases. Dato-DXd's lower rates of neutropenia and diarrhea compared to other Trop-2-targeted ADCs are attributed to its linker structure, which is more stable in plasma and thus contributes significantly to the drug's safety profile.

Table 2 provides a comprehensive comparative overview of clinical indications, efficacy outcomes from landmark trials, characteristic toxicity profiles, and clinical management strategies for the evaluated ADCs.

Future Perspectives

As part of research into ADCs, current agents are used in the early stages of treatment, and next-generation ADCs and novel combination strategies are being developed. In this context, studies are being conducted to assess the appropriate use of T-DXd in metastatic and early-stage HER2-positive breast cancer and to redefine its role in current treatment algorithms. Additionally, the Phase III DESTINY-Breast11 trial showed higher pCR rates with neoadjuvant T-DXd followed by THP than with dose-dense anthracycline-based chemotherapy followed by THP in patients with high-risk HER2-positive early breast cancer. Although longer follow-up is needed to clarify event-free and OS outcomes, these results may support the incorporation of T-DXd-based, anthracycline-free strategies into future treatment algorithms [62]. Furthermore, the potential synergistic antitumor benefits and the ability to overcome resistance mechanisms achieved by combining ADCs with immune checkpoint inhibitors, tyrosine kinase inhibitors, CDK4/6 inhibitors, and other targeted therapies are under intensive investigation.

Furthermore, supportive therapies such as bispecific ADCs, dual-loaded ADCs, probody-drug conjugates, and immune-activated ADC platforms aim to overcome current limitations such as tumor heterogeneity, treatment resistance, and off-target toxicities [9]. The results of these ongoing trials are expected to significantly improve the therapeutic index of ADCs and set new standards in breast cancer treatment.

Table 2. Comparative clinical summary of ADCs in breast cancer: current indications, landmark efficacy outcomes, and toxicity profiles

Ado-trastuzumab emtansine (T-DM1) HER2	• Adjuvant eBC: residual invasive HER2-positive eBC after neoadjuvant taxane- and trastuzumab-based therapy. • mBC: previously treated unresectable locally advanced or metastatic HER2-positive breast cancer.	• EMILIA • TH3RESA • KATHERINE • KAMILLA	• Residual eBC (KATHERINE): at 8.4 yrs follow-up, 7-yr iDFS was 80.8% vs. 67.1% with trastuzumab (HR: 0.54); 7-yr OS was 89.1% vs 84.4% (HR: 0.66). • Prior mBC (EMILIA): mPFS 9.6 vs. 6.4 months; mOS 30.9 vs. 25.1 months compared with lapatinib + capecitabine. • Brain metastases (KAMILLA): intracranial ORR ~21% in patients with measurable brain metastases.	• Thrombocytopenia (inc. grade ≥3) • Hepatotoxicity/transaminase elevation • Periphereal neuropathy • Fatigue & nausea • Left ventricular dysfunction • Rare ILD/pneumonitis • Nodular regenerative hyperplasia	• Established standard of care for residual HER2-positive disease after neoadjuvant therapy; future treatment algorithms may evolve with emerging comparative evidence. • Monitor CBC and LFTs prior to each dose. • Withhold or reduce dose for clinically significant thrombocytopenia or transaminitis. • Discontinue permanently for nodular regenerative hyperplasia, severe DILI, or clinically significant ILD/pneumonitis. • Monitor LVEF at baseline and periodically.
Trastuzumab deruxtecan (T-DXd) HER2	• Neoadjuvant eBC: T-DXd → THP (Stage II-III HER2+). • Adjuvant eBC: residual invasive HER2+ eBC post-neoadjuvant trastuzumab/taxane therapy. • 1L mBC: T-DXd + pertuzumab for unresectable/metastatic HER2+. • Previously treated mBC: unresectable/metastatic HER2+. • HER2-low mBC: unresectable/metastatic after prior chemo in metastatic setting or early relapse. • HR+/HER2-low or ultralow: unresectable/metastatic post-≥1 ET.	• DESTINY-Breast04 • DESTINY-Breast05 • DESTINY-Breast06 • DESTINY-Breast09 • DESTINY-Breast11 • DESTINY-Breast12	• Neoadjuvant eBC (DB-11): pCR 67.3% (T-DXd→THP) vs. 56.3% (ddAC→THP). • Residual eBC (DB-05): 3-yr iDFS 92.4% vs. 83.7% with T-DM1 (HR: 0.47). • 1L mBC (DB-09): T-DXd + pertuzumab mPFS 40.7 vs. 26.9 months (HR: 0.56); ORR 87% vs. 81%. • Prior mBC (DB-03): Sustained PFS & OS superiority over T-DM1. • HER2-low mBC (DB-04): mPFS 9.9 vs. 5.1 months (HR: 0.50); mOS 23.4 vs. 16.8 months; ORR 52.3% vs. 16.3%. • HR+/HER2-low/ultralow (DB-06): mPFS 13.2 vs. 8.1 months (HR: 0.64). • Brain metastases (DB-12): CNS ORR 71.7% in patients with active or stable brain metastases; 12-month PFS 61.6%	• ILD/pneumonitis (potentially fatal) • Nausea & vomiting • Neutropenia, anemia, thrombocytopenia • Fatigue & alopecia • Left ventricular dysfunction • Infusion-related reactions	• Educate patients to report new respiratory symptoms immediately. • Obtain prompt chest CT and specialist evaluation on ILD suspicion. • Grade 1 ILD: hold T-DXd until resolution; consider systemic corticosteroids (prednisone ≥0.5 mg/kg/day). • Grade ≥2 ILD: discontinue permanently; promptly initiate systemic corticosteroids (prednisone ≥1 mg/kg/day with prolonged taper). • Require 3-drug antiemetic prophylaxis. • Monitor CBC and LVEF baseline and periodically.

Table 2. Continued

Sacituzumab govitecan (SG) Trop-2	• 1L mTNBC: monotherapy for patients not candidates for PD-1/PD-L1 inhibitors. • 1L PD-L1+ mTNBC: SG + pembrolizumab (CPS ≥10). • Prior mTNBC: unresectable/metastatic after ≥2 prior therapies (≥1 for mBC). • HR+/HER2- mBC: post-ET and ≥2 chemo regimens in mBC setting.	• IMMU-132-01 • ASCENT • TROPICS-02 • ASCENT-03 • ASCENT-04 / KEYNOTE-D19 • SASCI	• 1L TNBC (ASCENT-03): mPFS 9.7 vs. 6.9 months (HR: 0.62); ORR 50% vs 47%. • 1L PD-L1+ TNBC (ASCENT-04): SG + pembro mPFS 11.2 vs. 7.8 months (HR: 0.65); ORR 61% vs 55%. • Prior mTNBC (ASCENT): mPFS 5.6 vs 1.7 months; mOS 12.1 vs 6.7 months; ORR 35% vs. 5%. • HR+/HER2- mBC (TROPICS-02): mPFS 5.5 vs 4.0 months; mOS 14.4 vs 11.2 months. • Efficacy observed regardless of Trop-2 expression; routine testing not required.	• Neutropenia (inc. grade ≥3 & febrile neutropenia) • Diarrhea (inc. severe) • Nausea & vomiting • Alopecia & fatigue • Anemia • Infusion/hypersensitivity reactions • Increased toxicity risk in UGT1A1-deficient patients • Severe or life-threatening neutropenia and diarrhea. • Monitor CBC before dosing and during treatment. • Consider primary G-CSF prophylaxis in high-risk patients; use secondary prophylaxis post-neutropenic events. • Initiate loperamide promptly at onset of uncomplicated diarrhea. • Close monitoring for patients homozygous for UGT1A1*28.
Datopotamab deruxtecan (Dato-DXd) Trop-2	• HR+/HER2- mBC: Post-ET and chemotherapy in metastatic setting. • 1L mTNBC: Unresectable/metastatic in patients not candidates for PD-1/PD-L1 inhibitors. • Additional early-stage and combination strategies remain under clinical investigation	• TROPION-PanTumor01 • TROPION-Breast01 • TROPION-Breast02 • BEGONIA • I-SPY2.2	• HR+/HER2- mBC (TROPION-Breast01): mPFS 6.9 vs. 4.9 months (HR: 0.63); ORR 36% vs. 23%; mOS 18.6 vs. 18.3 months (HR: 1.01). • 1L mTNBC (TROPION-Breast02): mPFS 10.8 vs. 5.6 months (HR: 0.57); mOS 23.7 vs. 18.7 months (HR: 0.79); ORR 64% vs. 30%. • BEGONIA: ORR 79%; mPFS 13.8 months with Dato-DXd + durvalumab in 1L TNBC (exploratory).	• Stomatitis/oral mucositis (predominantly Gr 1-2) • Ocular toxicities (dry eye, keratitis, blepharitis, blurred vision) • Nausea & vomiting • Fatigue • ILD/pneumonitis • Anemia & neutropenia (generally lower vs. SG) • High incidence of stomatitis; prophylactic dexamethasone oral rinse (swish-and-spit) is strongly recommended from cycle 1 to prevent oral mucositis. • Recommend preservative-free eye drops; avoid contact lenses. • Perform baseline and periodic ophthalmologic examinations. • Hold for suspected ILD; permanently discontinue for Grade ≥2 ILD.

Current indications are summarized based on the United States Food and Drug Administration approvals as of July 2026. Regulatory approvals, treatment sequencing, and availability may differ across countries and regions. The DESTINY-Breast05, DESTINY-Breast11, ASCENT-03/04, and TROPION-Breast02 trials have recently reported primary Phase III results. Although these findings have led to regulatory approvals in selected settings, incorporation into regional clinical practice guidelines (including ESMO and NCCN) may vary according to the timing of guideline updates.

ADC: Antibody-drug conjugate, BC: Breast cancer, CBC: Complete blood count, CPS: Combined positive score, ddAC: Dose-dense doxorubicin and cyclophosphamide, DLI: Drug-induced liver injury, eBC: Early breast cancer, ESMO: European Society for Medical Oncology, ET: Endocrine therapy, G-CSF: Granulocyte-colony stimulating factor, HER2: Human epidermal growth factor receptor 2, HR+: Hormone receptor, HR: Hazard ratio, iDFS: Invasive disease-free survival, ILD: Interstitial lung disease, LFT: Liver function test, LVEF: Left ventricular ejection fraction, mBC: Metastatic breast cancer, mOS: Median overall survival, mPFS: Median progression-free survival, mTNBC: Metastatic triple-negative breast cancer, NCCN: National Comprehensive Cancer Network, ORR: Objective response rate, OS: Overall survival, pCR: Pathological complete response, PD-1: Programmed cell death protein 1, PD-L1: Programmed death-ligand 1, THP: Taxane, trastuzumab, and pertuzumab, TNBC: Triple-negative breast cancer, Trop-2: Tropheblast cell-surface antigen 2, UGT1A1: Uridine diphosphate glucuronosyltransferase 1A1

Conclusion

The efficacy and safety profiles of ADCs make them novel therapeutic modalities in cancer therapy. T-DM1, T-DXd, SG, and Dato-DXd are approved for breast cancer, have shown efficacy in relevant clinical studies, and are currently in use. Moreover, the existence of many agents in late-phase clinical development is a positive development for breast cancer treatment. A better understanding of ADCs through clinical practice will foster rational decision-making as well as planning for effective research. Analyses of toxicity and management issues arising during ADC use in the real world will further advance our understanding. This will facilitate the development of superior next-generation ADCs with more reliable and better-characterized toxicity profiles.

Footnotes

Authorship Contributions

Concept: M.N.B., B.D., Design: M.N.B., Analysis or Interpretation: M.N.B., B.D., Literature Search: M.N.B., Writing: M.N.B., B.D.

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Toxicities of High-dose Chemotherapy Followed by Autologous Hematopoietic Stem Cell Transplantation: Relationship with Survival and Transplantation Outcomes in a Single-center Experience

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ABSTRACT

Aim: This study evaluated the toxicities associated with mobilization and conditioning regimens and examined the relationship of these toxicities to survival and transplant outcomes in patients with multiple myeloma (MM) and lymphoma undergoing high-dose chemotherapy followed by autologous hematopoietic stem cell transplantation.

Methods: We conducted a retrospective analysis of 58 patients treated at a single tertiary referral center in Türkiye. The median follow-up was 24 months. Toxicities were categorized by organ system and graded according to the Common Terminology Criteria for Adverse Events version 5.0.

Results: For the entire cohort, overall survival (OS) was 89.5% at one month, 75.7% at twelve months, and 70.2% at the last follow-up. In MM, the three month treatment response was strongly associated with OS; OS was 94.1 percent for patients in remission and 35.7 percent for those with refractory disease ($p<0.001$). Across toxicity categories, no statistically significant associations were observed with OS or disease-free survival (DFS). In MM, mucositis and anemia did not show a statistically significant association with shorter DFS, yet the observed trend may be clinically relevant.

Conclusion: In this single-center experience, regimen-related toxicities did not independently predict transplant outcomes or OS. Nevertheless, the direction and consistency of effects on mucositis and anemia in MM justify prospective confirmation. Multicenter studies with homogeneous patient populations are warranted to clarify the prognostic role of treatment-related toxicities and to refine supportive care strategies.

Keywords: Lymphoma, multiple myeloma, transplant, toxicity, mobilization, conditioning, chemotherapy

Introduction

For nearly 30 years, high-dose chemotherapy (HDT) followed by autologous hematopoietic stem cell transplantation (AHST) has been one of the most critical treatments for multiple myeloma (MM) and has been used for a similar duration to treat aggressive non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL) [1]. This approach allows the administration of intensive cytotoxic therapy while enabling rapid hematopoietic recovery through reinfusion of autologous stem cells. Initial HDT/AHST has demonstrated efficacy and become the standard of care, especially for eligible patients with newly

diagnosed MM. In recent years, the therapeutic landscape of MM has evolved considerably with the introduction of novel agents, including proteasome inhibitors, signaling lymphocytic activation molecule family member 7 inhibitors, histone deacetylase inhibitors, immunomodulatory drugs, and anti-cluster of differentiation 38 monoclonal antibodies. These agents have significantly improved response rates and survival outcomes in patients with MM. Despite these advances, HDT/AHST continues to play an important role in the management of eligible patients. Previous studies have demonstrated that HDT/AHST can provide clinical benefit for MM patients with both high risk and standard risk genetic profiles [2].

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Numerous complications can occur during HDT/AHSCT. These complications may involve multiple organ systems and may adversely affect quality of life, prolong hospitalization, contribute to long-term complications, and potentially influence transplantation outcomes. The type and severity of complications observed after hematopoietic stem cell transplantation may vary according to several patient and treatment-related factors. These include previous treatments, the disease status at the time of transplantation, existing comorbid conditions, and the intensity of the conditioning regimen. In addition, the duration of cytopenias and immunosuppression, as well as underlying or treatment-related organ dysfunction, may influence the occurrence and severity of post-transplant complications [3]. Although Many studies have examined the toxicities associated with chemotherapy protocols used in HDT/AHSCT, the relationship between these toxicities and transplant-related outcomes remains incompletely understood. In this study, we aimed to evaluate the toxicity profiles of HDT/AHSCT protocols and to investigate their potential association with survival and transplant outcomes in patients with lymphoma and MM. By jointly analyzing these factors, this study aims to improve understanding of how treatment-related toxicities may influence clinical outcomes.

Methods

Patients

We retrospectively analyzed the clinical and laboratory records of patients with NHL, HL, and MM who underwent HDT/AHSCT between March 2018 and November 2020 (n=58). All patients provided informed consent prior to HDT/AHSCT.

Study Design

This retrospective, single-center, non-interventional study was designed to evaluate the toxicity of mobilization and conditioning regimens, and their effects on transplantation outcomes and survival in patients with MM and lymphoma who underwent HDT/AHSCT at our centre. The median follow-up duration was 24 months. The study was approved by the Clinical Research Ethics Committee of İzmir Bozyaka Training and Research Hospital, University of Health Sciences Türkiye (approval no: 10, date: 21.10.2020). Patients with incomplete clinical records or insufficient follow-up data were excluded from the analysis. Cases with missing information regarding treatment-related toxicities were also excluded. In addition, patients who did not undergo autologous stem cell transplantation and those younger than 18 years were not included in the study.

Chemotherapy Regimens, Protocols, and Supportive Care

Chemotherapy regimens and protocols

Tables 1 and 2 summarize the mobilization and conditioning regimens used for patients with MM and lymphoma, respectively.

Supportive care

Patients were admitted to the bone marrow transplant unit in designated rooms equipped with high-efficiency particulate air filters to maintain a sterile environment. Blood transfusions were performed when hemoglobin levels dropped below 7 g/dL or platelet counts fell below $10 \times 10^3/\mu\text{L}$. All patients received fluconazole and acyclovir prophylaxis, beginning with the conditioning regimen.

Assessment of Toxicity

Mucositis was monitored daily and recorded in the patients' charts. Toxicities were assessed according to the Common Terminology Criteria for Adverse Events v5.0 criteria. Toxicities

Table 1. Multiple myeloma mobilization and conditioning regimens

	Regimen	n (%) =40
Mobilization	G-CSF alone	2 (5%)
	Cyclophosphamide + G-CSF	33 (82.5%)
	Cyclophosphamide + G-CSF + plerixafor	5 (12.5%)
Conditioning	Melphalan 200 mg	30 (75%)
	Melphalan 140 mg	9 (22.5%)
	No conditioning given	1 (2.5%)

G-CSF: Granulocyte colony-stimulating factor

Table 2. Lymphoma mobilization and conditioning regimens

	Regimen	n (%) =18
Mobilization	IVAC + G-CSF	1 (5.5%)
	Cyclophosphamide + G-CSF	2 (11.1%)
	R-ICE + G-CSF	1 (5.5%)
	R-ESHAP + G-CSF	7 (38.8%)
	Gemcitabine + G-CSF	1 (5.5%)
	R-IDARAM + G-CSF	2 (11.1%)
	Etoposide + G-CSF after R-IDARAM	1 (5.5%)
	Etoposide + G-CSF after cyclophosphamide + plerixafor	1 (5.5%)
	Etoposide + G-CSF	2 (11.1%)
Conditioning	BEAM	4 (22%)
	CBV	3 (16%)
	BuCYE	2 (11%)
	Mitoxantrone	1 (5%)
	Mitoxantrone + melphalan	4 (22%)
	TBC	4 (22%)

G-CSF: Granulocyte colony-stimulating factor, IVAC: Ifosfamide, etoposide, cytarabine, R-ICE: Rituximab, ifosfamide, carboplatin and etoposide, R-ESHAP: Rituximab, etoposide, methylprednisolone, cytarabine and cisplatin, R-IDARAM: Rituximab, cytarabine, idarubicin, methotrexate and dexamethasone, BEAM: Carmustine, etoposide, cytarabine and melphalan, CBV: Cyclophosphamide, carmustine and etoposide, BuCYE: Busulfan, cyclophosphamide and etoposide, TBC: Thiotepa, busulfan and cyclophosphamide

were graded on a five-level scale based on their severity and clinical impact. Grade 1 corresponds to mild or asymptomatic events that do not require specific medical intervention. Grade 2 represents moderate adverse events that may require minimal, local, or non-invasive interventions and limit age-appropriate instrumental activities of daily living. Grade 3 indicates severe or medically significant events that are not immediately life-threatening but may require hospitalization or prolong hospitalization, and may limit self-care activities of daily living. Grade 4 refers to life-threatening adverse events requiring urgent medical intervention. Grade 5 corresponds to death related to the adverse event [4].

Nine toxicity categories were evaluated: gastrointestinal, cardiac, pain, neurological, pulmonary, dermatological, hematological, renal, and neutropenic fever (infectious toxicity).

Neutropenic fever was defined as a single oral temperature of ≥ 38.3 °C or ≥ 38 °C lasting for at least one hour, accompanied by an absolute neutrophil count of <500 cells/ μ L [5].

Gastrointestinal toxicities were subcategorized into seven groups: nausea, vomiting, anorexia, mucositis, dyspepsia, diarrhea, and liver toxicity.

Hematological toxicities were classified into three groups: anemia, thrombocytopenia, and neutropenia.

Cardiac toxicities were classified into five categories: sinus tachycardia, new-onset atrial fibrillation, sinus bradycardia, acute coronary syndrome, and reduced ejection fraction.

Neurological toxicities were classified into three categories: epileptic seizures, cerebral ischemic injury, and posterior reversible encephalopathy syndrome.

Pulmonary toxicities were classified into three categories: respiratory distress, pulmonary edema, and pulmonary hemorrhage.

Renal toxicities were assessed based on the progression of creatinine levels.

Dermatological toxicities included rash, dry skin, and hair loss. These skin-related adverse events were assessed through clinical examination during follow-up visits and documented according to their severity and clinical significance.

Pain toxicities were evaluated based on patient-reported symptoms and their effect on daily functioning.

Overall survival (OS) and disease-free survival (DFS) analyses were performed for toxicities observed in a sufficient number of patients. The toxicities and number of patients in each group are listed in Table 3. When patients within the same toxicity group experienced multiple subgroup toxicities, the highest-grade toxicity was recorded.

Evaluation and Description of Clinical Response

Treatment response was evaluated in accordance with the 2016 International Myeloma Working Group uniform response criteria for MM and the International Working Group consensus response evaluation criteria for lymphoma [6,7]. Assessments included physical examinations, laboratory tests, and radiologic evaluations. Patients were evaluated for response at three months post-HDT/AHSCT and for final response.

Outcomes

The primary endpoints were DFS and OS. DFS was defined as the time from HDT/AHSCT to the detection of disease recurrence or progression. OS was defined as the time from HDT/AHSCT to death from any cause [8].

Statistical Analysis

For descriptive analysis, a database was created using SPSS 21.0 (IBM Corp., Armonk, NY, USA), with data presented as frequencies, percentages, means, standard deviations, and median values.

Toxicities induced by mobilization and conditioning regimens were categorized as Grades 1 and 2, Grades 3-5, or as present/absent. OS and DFS were analyzed using the Kaplan-Meier method. A p value of <0.05 was considered statistically significant.

Results

Patient Characteristics

A total of 58 patients diagnosed with lymphoma (n=18) or MM (n=40) were included in this study. The median age was 56.5

Table 3. Toxicity group, subgroups and number of patients

Toxicity	Mobilization number of events n		Conditioning number of events n	
	Grade 1 and 2	Grade 3-5	Grade 1 and 2	Grade 3-5
Cardiac	3	1	6	4
Hematological	4	51	0	57
Gastrointestinal	16	1	26	16
Neurological	2	1	0	1
Pulmonary	1	1	0	2
Renal	0	0	2	0
Neutropenic fever	0	1	0	17
Dermatological	0	0	4	0
Pain	8	2	1	0

years (range, 25-72 years). Patient clinical characteristics are summarized in Table 4.

Outcomes

OS was evaluated in all patients (n=58). The OS rates were 84.1% at 4 months, 75.7% at 12 months, 66.0% at 18 months,

Table 4 . Patient characteristics	
Characteristic	n (%)
Sex	
Male	36 (62.1%)
Female	22 (37.9%)
Age	
<60 years	35 (60.3%)
≥60 years	23 (39.7%)
Diagnosis	
Hodgkin lymphoma	7 (12.1%)
Non-Hodgkin lymphoma	11 (19%)
Multiple myeloma	40 (68.9%)
Comorbidities	
Cardiac disease	10 (17.2%)
Diabetes mellitus	7 (12%)
Pulmonary disease	8 (13.7%)
Neurological disease	2 (3.4%)
Renal disease	4 (6.8%)
Acquired immunodeficiency syndrome	2 (3.4%)
No comorbidities	38 (65.5%)
Multiple Myeloma Subgroup (n=40)	
ISS Stage I	6 (15%)
ISS Stage II	18 (45%)
ISS Stage III	16 (40%)
IgA lambda	9 (22.5%)
IgA kappa	6 (15%)
IgG lambda	5 (12.5%)
IgG kappa	16 (40%)
Kappa light chain	2 (5%)
Lambda light chain	2 (5%)
High cytogenetic risk	4 (10%)
Standard cytogenetic risk	32 (80%)
Not evaluated	4 (10%)
Lymphoma Subgroup (n=18)	
Mixed cellularity Hodgkin lymphoma	4 (22.4%)
Lymphocyte depleted Hodgkin lymphoma	1 (5.6%)
Nodular sclerosis Hodgkin lymphoma	2 (11.2%)
Diffuse large B-cell lymphoma	8 (44.2%)
Follicular lymphoma	1 (5.6%)
Burkitt lymphoma	1 (5.6%)
Mantle cell lymphoma	1 (5.6%)
ISS: International staging system, IgA: Immunoglobulin A, IgG: Immunoglobulin G	

and 70.2% at the end of the follow-up period (Figure 1). The presence of comorbidities was not significantly associated with OS (70.4% vs. 70.0%, p=0.911).

DFS was analyzed in 53 evaluable patients (Figure 2). The DFS rates were 61.5%, 52.4%, and 46.2% at 1, 12, and 24 months, respectively. Five patients lacking response evaluations were excluded from the analysis.

Among patients with MM (n=40), 38.8% remained in remission, 7.5% were refractory, and 17.5% relapsed at 24 months. Survival analysis by sex revealed higher OS in males than in females (86.4% vs. 58.8%), reaching borderline statistical significance (p=0.053). In patients with MM, DFS was 65.7% at 1 month, 56.1% at 12 months, and 45.7% at the end of the study. No significant associations were observed between age and either DFS or OS in patients with MM. For patients with lymphoma, OS was 77.8% at 2 months, 53.5% at 14 months, and 61.1% at 24 months.

Among the 18 patients with lymphoma, seven (38.8%) died before the final response evaluation: three deaths were transplant-related; three were due to coronavirus disease-2019 (COVID-19) (one in remission and two refractory); and two resulted from sepsis. In the MM group, seven patients died:

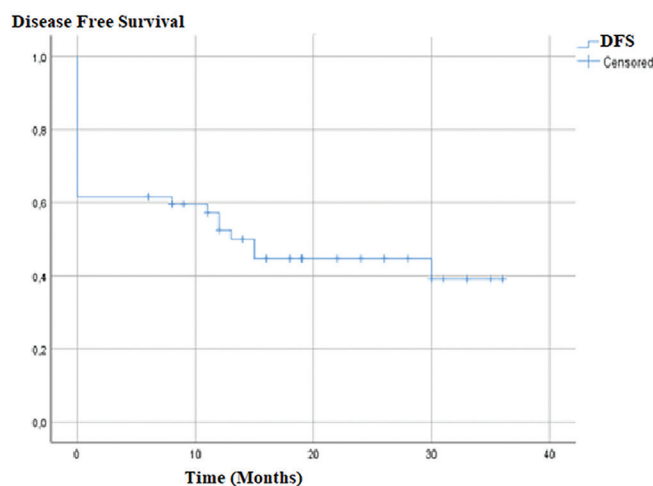


Figure 1. DFS was assessed in the all patient
DFS: Disease-free survival

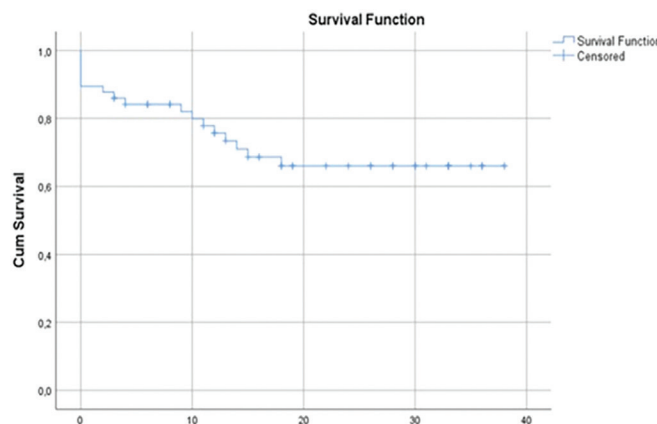


Figure 2. Evaluation of all patients for overall survival

six had treatment-refractory disease, and one experienced a relapse after a nine-month remission. The mean age at death was 58.1 years. Causes of death included sepsis ($n=4$), intracranial abscess ($n=1$), and rapidly progressive viral pneumonia ($n=1$); the cause of one death remained uncertain because the patient was followed at another center. At the last follow-up, seven patients with lymphoma (38.8%) were in remission, two (11.1%) were refractory, one (5.5%) had relapsed, and one (5.5%) was lost to follow-up.

A significant association was observed between treatment response at 3 months and OS ($p=0.001$). Patients who achieved remission had a markedly higher OS rate (94.1%) than those with refractory disease (35.7%).

Assessment of treatment-associated toxicities revealed no statistically significant associations with OS or DFS. Patients experiencing low-grade and high-grade gastrointestinal toxicity had similar survival outcomes: OS: 74.1% vs. 62.5% ($p=0.260$) and DFS: 36.8% vs. 57.1% in patients with MM ($p=0.456$). Likewise, cardiac toxicity was not significantly associated with OS (61.5% vs. 72.7%, $p=0.101$) or DFS (38.5% vs. 48.7%, $p=0.177$). Neutropenic fever had no significant effect on DFS (43.8% vs. 47.2; $p=0.830$).

Pain and dermatological toxicities were documented during follow-up; however, the available data were insufficient to permit statistical evaluation of their relationships with survival and transplantation outcomes. Therefore, these variables were not included in the final analysis.

Although these results were not statistically significant, several findings may have clinical relevance. Notably, among patients with MM, those with mucositis had a DFS nearly twofold lower than that of patients without mucositis (28.6% vs. 57.1%), a difference that approached statistical significance ($p=0.056$). This finding suggests that mucositis may adversely affect post-transplant management and that it warrants additional investigation in larger cohorts. A similar pattern was observed in MM patients with higher-grade anemia, who had lower DFS than those with low-grade anemia (35.7% vs. 52.6%, $p=0.263$), indicating a potentially unfavorable effect. Although the sample size was limited, necessitating cautious interpretation, these findings collectively underscore the toxicities that may substantially affect patient outcomes and warrant further investigation.

Detailed subgroup analyses of treatment-related toxicities are provided in Supplementary Table 1.

Discussion

Toxicities associated with mobilization and conditioning regimens in HDT/AHSCT have been extensively investigated [9]. Unlike several previous studies that evaluated mobilization or conditioning regimens separately, the present single-center analysis examined survival outcomes and treatment-related toxicities in patients with MM and lymphoma undergoing HDT/AHSCT, considering both treatment phases together. Our objective was to determine whether transplantation outcomes and survival were associated with treatment-related toxicities

and to contextualize our real-world results within the current literature.

Among all patients, OS was 84.1% at 4 months, 75.7% at 12 months, and 66.0% at 18 months, with 70.2% alive at last follow-up (Figure 1). DFS was 61.5% at 1 month, 52.4% at 12 months, and 46.2% at 24 months (Figure 2). Early treatment response emerged as the strongest predictor: patients in remission at 3 months had markedly higher OS than those with refractory disease (94.1% vs 35.7%). This finding aligns with prior studies in MM demonstrating that depth of response after induction and early remission status are critical predictors of long-term survival [10].

HDT/AHSCT is a well established treatment modality in MM and is the standard of care in relapsed or refractory HL [11-13]. In NHL, the use of HDT/AHSCT remains histology-dependent, with heterogeneous outcomes reflecting differences in salvage regimens [13].

Although the heterogeneity of regimens in our lymphoma subgroup and limited sample size precluded direct comparisons, hematologic toxicities emerged as the most frequent adverse events, consistent with other real world series [14].

The COVID-19 pandemic markedly affected outcomes. Several patients failed to attend follow-up evaluations, and infections were directly responsible for mortality in both the remission and refractory groups. Similar findings have been documented by transplant programs worldwide, indicating that COVID-19 was associated with considerable disruptions and resulted in increased non-relapse mortality among hematopoietic cell transplant patients [15,16]. This may account for our slightly lower day-100 survival rate (89.5%) compared with the ~95-99% reported in previous MM and lymphoma transplantation cohorts [17-19].

No significant associations were observed between treatment-related toxicities (gastrointestinal toxicity, cardiac toxicity, and neutropenic fever) and survival outcomes. However, two observations in our cohort merit attention. MM patients with mucositis had a nearly twofold lower DFS than those without mucositis (28.6% vs. 57.1%), with the difference approaching statistical significance ($p=0.056$). Likewise, higher-grade anemia was associated with a numerically worse DFS (35.7% vs. 52.6%). These trends are biologically plausible and consistent with literature describing mucositis and cytopenia as notable complications of transplantation that warrant careful supportive care [20,21].

Fanning et al. [21] reported that severe mucositis in patients with lymphoma undergoing HDT/AHSCT was linked to poorer OS, highlighting its potential prognostic relevance. In our study, mucositis appeared to affect DFS in patients with MM, with DFS rates of 28.6% among patients who developed mucositis versus 57.1% among patients who did not develop mucositis. Taken together, these findings suggest that while mucositis is consistently a clinically relevant toxicity, its prognostic significance may vary depending on disease type and on whether OS or DFS is considered. Although our study is underpowered to confirm these associations, they may nevertheless have prognostic value and warrant validation in larger, multicenter cohorts.

Study Limitations

This study has some limitations that must be addressed. The relatively small and heterogeneous patient population reduced the statistical power of subgroup analyses and precluded regimen-level comparisons. The retrospective design and pandemic-related loss to follow-up further limited the evaluation of complete response. Nevertheless, this study contributes to the literature by systematically assessing mobilization and conditioning toxicities, emphasizing early remission status as a crucial prognostic factor, and identifies mucositis and anemia as toxicities that may affect long-term outcomes in patients with MM.

Conclusion

We evaluated HDT/AHSCT-related toxicities in patients with MM and lymphoma in relation to demographic characteristics and survival outcomes. Early response at three months was a strong predictor of OS, underscoring its value as a prognostic marker. Although no significant correlation was observed between specific toxicities and OS, toxicities such as anemia and mucositis were associated with reduced DFS in patients with MM. These findings highlight the importance of careful, supportive care and provide clinicians with practical guidance for early outcome assessment and management of treatment-related complications. Future multicenter studies are needed to confirm these associations and inform the development of more effective supportive care strategies.

Ethics

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of İzmir Bozyaka Training and Research Hospital, University of Health Sciences Türkiye (approval no: 10, date: 21.10.2020).

Informed Consent: This is a retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.S., F.G., Concept: M.S., F.G., Design: F.G., Data Collection or Processing: M.S., F.G., Analysis or Interpretation: M.S., Literature Search: M.S., Writing: M.S.

Conflict of Interest: No conflict of interest was declared by the authors.

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Supplementary Table 1: <https://d2v96fxpocvxx.cloudfront.net/e6921826-40a4-4ec2-8c4c-3b8dc63111d7/content-images/8e25d99b-af37-4152-847c-5b09636d79de.pdf>

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Original Article

Surgical Management and Clinical Outcomes of Mediastinal Mass Resections: A Single-center Retrospective Analysis

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ABSTRACT

Aim: The surgical management of mediastinal masses is complex due to anatomical constraints and diverse pathologies. This study aims to provide a descriptive analysis of surgical outcomes, risk factors for complications, and survival trends in patients undergoing mediastinal mass resection.

Methods: We retrospectively reviewed the records of 36 patients who underwent surgical resection of mediastinal masses between 2019 and 2026. Postoperative complications were graded using the Clavien-Dindo classification. Demographic data, tumor characteristics, and pathological subtypes were analyzed. Long-term survival was estimated using the Kaplan-Meier method, and risk factors for major complications (Grade $\geq$ III) were evaluated.

Results: The study cohort consisted of 26 females and 10 males with a mean age of 53.69 ± 14.19 years. Major complications occurred in 13.9% (n=5) of patients. A significant association was found between major complications and smaller tumor diameters (31.14 ± 14.0 mm vs. 53.55 ± 23.8 mm, $p=0.022$) and a history of malignancy ($p=0.028$). The 30-day mortality rate was 2.8% (n=1). While major complications did not adversely affect 5-year overall survival ($p=0.486$), a strong trend toward prognostic divergence was observed across different pathological subtypes (log-rank $p=0.056$), with metastatic lesions showing poorer outcomes.

Conclusion: Mediastinal mass resection remains a clinical necessity and is inherently challenging. Given the vast pathological variety and technical demands of the mediastinum, continuous documentation of institutional experiences is essential for refining preoperative planning and complication management strategies. Successful outcomes depend on the integration of surgical precision with robust perioperative care.

Keywords: Mediastinal neoplasms, thoracic surgery, video-assisted thoracoscopic surgery, postoperative complications, survival analysis, tumor size

Introduction

The mediastinum is the central compartment of the thoracic cavity, bordered laterally by the pleural cavities, anteriorly by the sternum, and posteriorly by the vertebral column. This complex space is anatomically divided into superior, anterior, middle, and posterior compartments, each harboring distinct vital structures, including the heart, great vessels, trachea, esophagus, and major autonomic nerves [1-3]. Given this dense concentration of critical organs, any pathological mass arising within these boundaries poses a significant clinical challenge [4].

Mediastinal tumors are relatively uncommon, accounting for approximately 3% of all primary tumors in the thoracic cavity [5,6].

Despite their low incidence, they present a significant diagnostic and therapeutic challenge due to their proximity to vital thoracic structures [5,6]. The anatomical position and internal characteristics of a lesion are pivotal in refining the differential diagnosis [6].

Mediastinal masses are highly variable in size, location, and biological nature; therefore, surgical strategies must be carefully planned and adapted when dealing with these lesions [7,8]. The evolution of surgical techniques, from open sternotomy and thoracotomy to minimally invasive approaches, has allowed for more tailored interventions based on the specific characteristics of the mass and the patient's clinical status [8,9].

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The objective of this study was to analyze our institutional experience with mediastinal masses and evaluate our clinical findings in the context of existing literature. By documenting our surgical outcomes, complication profiles, and survival data, we aim to contribute to the collective understanding of these diverse clinical entities.

Methods

Study Design and Patient Selection

This retrospective study was conducted in the department of thoracic surgery at a tertiary-care oncology hospital. Medical records of 36 patients who underwent surgical resection for mediastinal masses between 2019 and 2026 were retrospectively reviewed. The study protocol was approved by the Institutional Ethics Committee of University of Health Sciences Türkiye, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital (approval no: 2026-02/16, date: 05.02.2026). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Inclusion and Exclusion Criteria

Patients who underwent surgical resection for a primary mediastinal mass in our thoracic surgery department between 2019 and 2026 were analyzed retrospectively. The inclusion criteria for this study were defined as follows: (1) the presence of a primary mediastinal tumor confirmed by preoperative imaging, including computed tomography (CT) or magnetic resonance imaging (MRI); (2) the availability of complete medical, surgical, and pathological records; and (3) the performance of surgical resection with curative intent (achieving either R0 or R1 resection). Exclusion criteria were: patients with incomplete follow-up data, and patients treated solely with biopsy without definitive resection.

Follow-up Protocol

The standardized follow-up protocol involved clinical examination and radiological assessment. Following discharge, patients were evaluated at the outpatient clinic within the first postoperative month for early complications. Long-term follow-up consisted of physical examinations and thoracic CT scans every 6 months for the first 2 years and annually thereafter. Survival status and recurrence data were obtained from hospital records and national health databases.

Preoperative Evaluation and Surgical Procedure

All patients underwent comprehensive preoperative evaluation, including physical examination, routine laboratory tests, and pulmonary function tests. Contrast-enhanced CT was performed in all cases to assess tumor size, location, and relationship with adjacent vital structures. MRI was used in selected cases to better visualize neurovascular involvement.

Surgical approaches were tailored to the tumor's anatomical location and size. The primary goal in all cases was complete (R0) resection.

Due to the limited number of major complications (n=5), univariate analysis was primarily used to identify potential risk factors. A multivariate model was not constructed to avoid overfitting and maintain the validity of the statistical conclusions in this small-scale cohort.

Classification of Complications

Postoperative complications were recorded and graded according to the Clavien-Dindo classification [10].

Survival

Overall survival (OS) was defined as the time from the date of surgery to the date of death from any cause or to the date of last follow-up. The 30-day mortality rate was also documented.

Statistical Analysis

Statistical analysis was performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean $\pm$ standard deviation, and categorical variables were presented as frequencies and percentages. The Independent Samples t-test was used to compare continuous variables between groups. Categorical data were analyzed using Pearson's chi-square test or Fisher's exact test, as appropriate. Survival rates were estimated using the Kaplan-Meier method, and differences between groups (pathological subtypes and complication status) were compared using the log-rank (Mantel-Cox) test. A p value <0.05 was considered statistically significant.

Results

Patient Demographics and Clinical Characteristics

A total of 36 patients who underwent surgical resection for mediastinal masses were included in this study. The study population consisted of 26 (72.2%) females and 10 (27.8%) males, with a mean age of 53.69 ± 14.19 years. The anatomical distribution of the masses revealed that the anterior mediastinum 25 (69.4%) was the most common site of involvement. The mean tumor diameter, as measured on preoperative CT, was 49.19 ± 24.05 mm.

Surgical Outcomes and Pathological Diagnosis

Surgical interventions were performed via various approaches; sternotomy and thoracotomy were the most frequently used, with each accounting for 12 cases (33.3%). Other techniques included video-assisted thoracoscopic surgery, mediastinotomy, and collar incisions. The mean operative duration was 202.08 ± 81.31 minutes, summarized in Table 1.

The most frequent diagnoses were schwannoma, thymic squamous cell carcinoma (SCC), Breast cancer metastasis, and thymoma, each identified in 4 patients (11.1%). Other pathological entities encountered in the study included ectopic thyroid tissue, reactive lymph node, synovial sarcoma, thymic cyst, ganglioneuroma, metastasis of teratoma, neuroendocrine carcinoma, mesothelial cyst, solitary fibrous

tumour, cervical SCC metastasis, and bronchogenic cyst, which are listed in Table 2. The presence of metastatic lesions and malignant primary tumors highlights the clinical complexity of the cases managed in our institution.

Analysis of Postoperative Complications

Postoperative complications were observed in 10 (27.7%) patients. According to the Clavien-Dindo classification:

- Minor complications (Grade I-II): Occurred in 5 (13.9%) patients.
- Major complications (Grade $\geq$ III): Occurred in 5 (13.9%) patients.

Comparative analysis between patients with major complications (n=5) and those without (n=31) revealed several significant findings. A statistically significant correlation was found between smaller tumor size and a higher incidence of major complications (31.14 $\pm$ 14.0 mm vs. 53.55 $\pm$ 23.8

mm, p=0.022). Furthermore, a history of malignancy was significantly associated with increased major morbidity (p=0.028).

The overall 30-day postoperative mortality rate was 2.8% (n=1). One death occurred in the major complication group (1/5, 20%), whereas no deaths were observed among patients without major complications (0/31, 0%). The difference did not reach statistical significance due to the limited number of events (Fisher's exact test, p=0.139).

Patients in the major complication group tended to be younger (44.57 $\pm$ 15.2 vs. 55.89 $\pm$ 13.0 years) and had longer operative durations (248.5 $\pm$ 95.6 vs. 190.8 $\pm$ 75.0 minutes); however, these differences did not reach statistical significance as seen in Table 3 (p=0.056 and p=0.094, respectively). Other factors, including gender, surgical approach, and anatomical compartment, showed no significant correlation with major complications (p>0.05).

Table 1. Comparative analysis of surgical approaches

Surgical approach	n (%)	Mean operation time (minute)	Complication rate (%)	Mean hospital stay (days)
Sternotomy	12 (33.3%)	215 $\pm$ 45	16.6%	7.2 $\pm$ 2.1
Thoracotomy	12 (33.3%)	190 $\pm$ 60	16.6%	6.8 $\pm$ 1.8
VATS (minimally invasive)	8 (22.2%)	160 $\pm$ 35	12.5%	3.5 $\pm$ 1.2
Other (Mediastinotomy, etc.)	4 (11.1%)	145 $\pm$ 25	0%	2.1 $\pm$ 0.9

VATS: Video-assisted thoracoscopic surgery, min: Minimum

Table 2. Distribution of pathological diagnoses and survival outcomes

Pathological groups	n (%)	5 year survival rate (%)	Clinical behavior
Neurogenic tumors (schwannoma, ganglioneuroma)	5 (13.9%)	100%	Benign/indolent
Thymic epithelial tumors (thymoma, thymic SCC/CA)	9 (25.0%)	77.8%	Low-intermediate malignancy
Metastatic lesions (breast, cervix, teratoma)	6 (16.7%)	33.3%	Aggressive/poor prognosis
Cystic lesions (bronchogenic, thymic, mesothelial)	4 (11.1%)	100%	Benign
Miscellaneous (thyroid, SFT, synovial sarcoma, etc.)	12 (33.3%)	83.3%	Variable
Total	36 (100%)	83.3% (overall)	--

SCC: Squamous cell carcinoma, CA: Carcinoma, SFT: Solitary fibrous tumor

Table 3. Association between demographic/clinical characteristics and major complications (Clavien-Dindo Grade $\geq$ III)

Variable	Total (n=36)	Major comp. (+) (n=5)	Major comp. (-) (n=31)	p value
Age (years), mean $\pm$ SD	53.69 $\pm$ 14.19	44.57 $\pm$ 15.2	55.89 $\pm$ 13.0	0.056
Tumor size (mm), mean $\pm$ SD	49.19 $\pm$ 24.05	31.14 $\pm$ 14.0	53.55 $\pm$ 23.8	0.022*
Operative duration (min), mean $\pm$ SD	202.08 $\pm$ 81.31	248.5 $\pm$ 95.6	190.8 $\pm$ 75.0	0.094
Gender, n (%)				0.061
- Male	10 (27.8%)	3 (30.0%)	7 (70.0%)	
- Female	26 (72.2%)	2 (7.7%)	24 (92.3%)	
History of malignancy, n (%)				0.028*
- Present	7 (19.4%)	3 (42.8%)	4 (57.2%)	
- Absent	29 (80.6%)	2 (6.9%)	27 (93.1%)	

*: p<0.05 indicates statistical significance based on Fisher's exact test

SD: Standard deviation

Survival Analysis

The impact of postoperative morbidity on long-term prognosis was evaluated using the Kaplan-Meier method. The median follow-up period was 25.8 (0-108) months. There were 6 (16.7%) events (deaths) recorded during the follow-up period. There was no statistically significant difference in 5-year OS between patients with major complications and those without ($p=0.486$, log-rank test; see Figure 1). The lack of a statistically significant association between major complications and long-term survival may be attributed to the relatively small sample size of our cohort, which limits the statistical power to detect such a difference.

The relationship between pathological subtypes and OS was analyzed using the Kaplan-Meier method. A notable trend toward statistical significance was observed among the different pathological groups (log-rank $p=0.056$). Patients with metastatic breast cancer demonstrated a poorer survival profile compared with patients with benign pathologies, such as Schwannomas, who maintained a 100% survival rate during the follow-up period (see Figure 2). Although the difference did not reach the conventional threshold of $p<0.05$, the observed trend ($p=0.056$) suggests a prognostic difference that may achieve statistical significance in a larger patient cohort with greater statistical power.

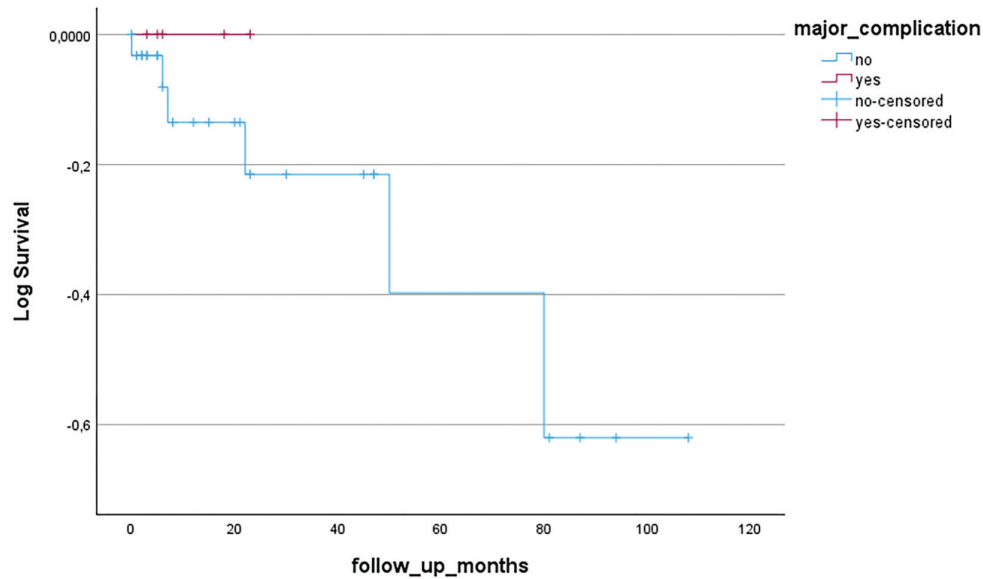


Figure 1. Kaplan-Meier analysis of overall survival stratified by major complications

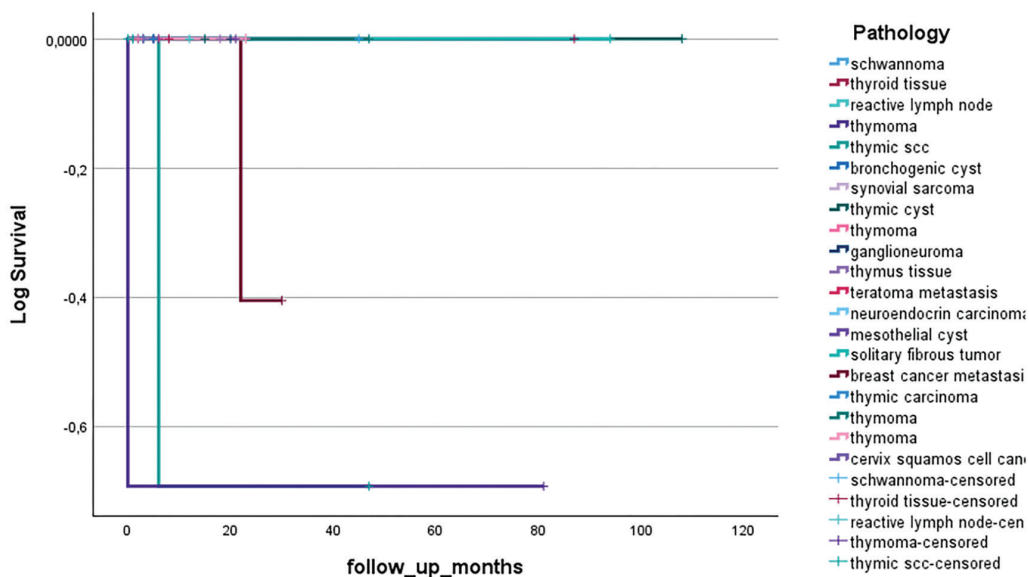


Figure 2. Long-term survival outcomes based on histopathological diagnoses

Discussion

The surgical management of mediastinal masses remains a challenge due to the complex anatomical landscape and the proximity of vital neurovascular structures. In this study, we evaluated the perioperative outcomes and long-term survival of 36 patients, focusing on the impact of postoperative complications.

We also identified a significant correlation between a history of malignancy and major postoperative complications ($p=0.028$). Patients with previous oncological treatments, such as chemotherapy or radiotherapy, often present with tissue fibrosis and increased vascular fragility. These factors significantly complicate surgical planes and increase the risk of perioperative bleeding or organ injury. Our results underscore the need for heightened vigilance and potentially more conservative surgical planning in patients with a history of malignancy.

The primary objective in the management of mediastinal masses is achieving a definitive pathological diagnosis and, where possible, a complete surgical resection. Our study highlights the intrinsic complexity of mediastinal surgery, dictated by the narrow confines of the thoracic compartments. Regardless of the pathological subtype—ranging from benign schwannomas to aggressive thymic SCC—surgical intervention remains the cornerstone of treatment to prevent local invasion and provide long-term symptom relief. However, the surgeon must navigate an “anatomical minefield” in which even minor deviations can lead to injury to great vessels or the tracheobronchial tree. The diverse pathological etiologies observed in our cohort (spanning 11 different diagnoses) underscore that mediastinal surgery is not a monolithic practice but requires a tailored approach for each histopathological entity.

In our series, major complications occurred in 13.9% of patients, which serves as a reminder of the inherent risks of these procedures. The management of these complications is as crucial as the surgery itself. In this descriptive study, our data reflect that a high level of perioperative vigilance is mandatory, particularly for patients with the risk factors we identified, such as prior malignancy and smaller, deep-seated lesions.

In our analysis, smaller tumor size was significantly associated with major complications ($p=0.022$). Although this finding appears paradoxical, it is most likely attributable to the limited sample size of our cohort. In small-scale clinical studies, the presence of a few high-risk cases with smaller tumor diameters can skew univariate results, leading to potentially misleading statistical significance. Therefore, this correlation should be interpreted with caution and viewed as an exploratory finding rather than a definitive clinical rule. Future research involving larger patient populations is essential to determine whether tumor size is a genuine independent predictor of surgical morbidity in mediastinal resections.

Our surgical outcomes and clinical findings are consistent with several significant studies. Kilic et al. [10] reported in their 118-case series that anterior mediastinal location was predominant

and that postoperative complications significantly prolonged hospital stay ($p=0.018$), a finding that mirrors our own clinical observations. Furthermore, the 20-year long-term experience presented by Harrison et al. [11] confirms that achieving complete (R0) resection remains the most critical prognostic factor for OS. Consistently, in the landmark study by Caronia et al. [12], the importance of surgical radicality was emphasized, with morbidity rates (around 15-20%) comparable to our cohort. Our high R0 resection rate and favorable survival trends are consistent with established national and international benchmarks, confirming the reliability of our single-center surgical approach.

The successful management of the diverse pathologies encountered in our series—ranging from benign nerve sheath tumors to metastatic lesions—reflects the evolution of surgical strategies in a tertiary care cancer center. Our data suggest that the diversity of mediastinal etiologies necessitates a versatile surgical repertoire, from minimally invasive approaches to complex open resections. As surgical teams navigate the learning curve associated with these high-stakes procedures, the continuous documentation and sharing of institutional outcomes become paramount. This descriptive process is not merely a statistical exercise but a vital tool for the transfer of experience, helping to refine preoperative risk stratification and intraoperative decision-making, thereby further minimizing perioperative morbidity.

Study Limitations

This study has several limitations that should be considered when interpreting the results. The primary limitations are its retrospective design and the relatively small sample size, which precluded performing a multivariate analysis. Although our univariate analyses identified small tumor size and a history of malignancy as significant risk factors for major morbidity, we were unable to adjust for potential confounding variables. Furthermore, the heterogeneity of pathological entities within the cohort and the limited number of primary events may reduce statistical power to detect subtle differences in survival or to draw definitive conclusions for specific tumor types. Despite these constraints, our findings provide valuable clinical insights into the risk factors for major morbidity in mediastinal surgery and serve as a foundation for future larger-scale studies.

Conclusion

This study provides a descriptive analysis of the surgical management of mediastinal masses, highlighting that while resection is a clinical necessity, it remains inherently challenging. Given the anatomical complexity and the potential for major morbidity, continuous updates of institutional experiences in the literature are essential.

Ethics

Ethics Committee Approval: The study protocol was approved by the Institutional Ethics Committee of University of Health Sciences Türkiye, Dr. Abdurrahman Yurtaslan Ankara Oncology

Training and Research Hospital (approval no: 2026-02/16, date: 05.02.2026).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: H.Ç., M.K., H.N., Concept: H.Ç., M.K., M.A.B., Design: H.Ç., M.K., M.A.B., Data Collection or Processing: H.Ç., M.K., H.N., G.F., Analysis or Interpretation: H.Ç., M.K., G.F., Literature Search: H.Ç., M.K., H.N., Writing: H.Ç., M.K., M.A.B., G.F.

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Original Article

Serum Vitamin D as a Predictor of Immunotherapy Response in Advanced Non-small Cell Lung Cancer

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ABSTRACT

Aim: Vitamin D is known to exert immunomodulatory effects and may influence the therapeutic efficacy of immune checkpoint inhibitors in patients with non-small cell lung cancer (NSCLC). This study aimed to evaluate the relationship between baseline serum vitamin D levels and survival outcomes in NSCLC patients treated with nivolumab.

Methods: In this retrospective study, we analyzed patients with stage IV NSCLC who received nivolumab as second-line therapy at a single tertiary center between October 2022 and September 2024. Patients were stratified based on baseline serum 25-hydroxyvitamin D levels into sufficient (≥ 20 ng/mL) and insufficient (< 20 ng/mL) groups. Progression-free survival (PFS) and overall survival (OS) were assessed using the Kaplan-Meier method and compared using the log-rank test.

Results: A total of 39 patients were included in the final analysis. Of these, 16 (41.0%) had sufficient vitamin D levels, whereas 23 (59.0%) were classified as insufficient. Median PFS was longer in the sufficient group than in the insufficient group (10.1 vs. 4.6 months), although this difference was not statistically significant ($p=0.292$). Similarly, median OS favored the sufficient group (17.3 vs. 11.7 months), although this difference did not reach statistical significance ($p=0.823$).

Conclusion: Baseline vitamin D status may be associated with survival outcomes among patients with NSCLC receiving nivolumab. Although statistical significance was not achieved, the observed trends suggest a potential prognostic role of vitamin D. Further prospective studies are warranted to confirm these findings.

Keywords: Non-small cell lung cancer, immunotherapy, nivolumab, vitamin D, prognosis

Introduction

Non-small cell lung cancer (NSCLC) represents the most frequently diagnosed subtype of lung cancer and continues to be a major contributor to cancer-related mortality on a global scale [1]. Despite ongoing improvements in systemic treatment approaches, outcomes in advanced-stage disease remain limited [2].

The development of immune checkpoint inhibitors (ICIs), particularly those targeting the PD-1/PD-L1 pathway, has improved treatment options for patients with advanced NSCLC by enhancing anti-tumor immune activity [3]. However, not all patients benefit from these therapies, and considerable variability in treatment response persists. Although biomarkers such as PD-L1 expression and tumor mutational burden are commonly used in clinical practice, their predictive capacity remains incomplete [4].

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Beyond tumor-related characteristics, patient-related factors may also influence treatment outcomes. Vitamin D has been increasingly recognized for its role in immune regulation in addition to its classical functions in bone metabolism [5]. Its active metabolite, calcitriol, interacts with the vitamin D receptor, which is expressed in various immune cells, including T lymphocytes, dendritic cells, and macrophages [6]. Through these interactions, vitamin D may affect both innate and adaptive immune responses, potentially influencing anti-tumor immunity [7,8].

Low vitamin D levels are frequently observed in oncology patients and have been linked to less favorable clinical outcomes across different malignancies [9,10]. However, the relationship between vitamin D status and survival remains inconsistent across studies. While some reports have not identified a clear association [11], others have demonstrated improved outcomes in patients with higher circulating levels of 25-hydroxyvitamin D [25(OH)D] [12].

More recently, attention has focused on the potential interaction between vitamin D and immunotherapy outcomes. Some studies suggest that sufficient vitamin D levels may be associated with improved survival in patients receiving ICIs [13]. In addition, evidence from melanoma indicates that vitamin D supplementation may enhance response to PD-1 inhibition [14].

Given the limited data specifically addressing patients with NSCLC treated with ICIs, the present study aimed to evaluate the association between baseline serum vitamin D levels and survival outcomes, including progression-free survival (PFS) and overall survival (OS) in patients with advanced NSCLC receiving nivolumab.

Methods

This retrospective, single-center study, conducted at a tertiary oncology center, included patients with stage IV NSCLC who received nivolumab as second-line treatment between October 2022 and September 2024. Clinical data were retrospectively collected from electronic medical records.

Patient eligibility was determined based on the availability of key clinical information. Patients were eligible if they were aged ≥ 18 years, had histologically confirmed metastatic NSCLC, received at least one cycle of nivolumab, and had available baseline serum 25(OH)D measurements obtained before treatment initiation. Cases with missing essential data or insufficient follow-up were excluded from the analysis.

The study was approved by the University of Health Sciences Türkiye, Ankara Etlik City Hospital Scientific Research Evaluation and Ethics Committee (approval no: AEŞH-BADEK-2024-931, date: 27.11.2024). The research was conducted in line with accepted ethical principles, and patient identities were not disclosed at any stage of data handling.

Relevant clinical details were retrieved from hospital records. These included demographic characteristics, disease-related variables, and treatment timelines. Information regarding disease status and follow-up was also reviewed.

Patients were followed during routine clinical visits and underwent radiological evaluations performed according to institutional practice standards.

Disease progression was determined based on radiological and clinical evaluation according to institutional practice.

Vitamin D status was assessed using baseline serum measurements. For analytical purposes, patients were grouped according to a predefined threshold into those with sufficient vitamin D levels and those with insufficient levels.

PFS was defined as the interval between nivolumab initiation and documented disease progression or death from any cause.

OS was defined as the interval from nivolumab initiation to death from any cause or to the last follow-up.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics software (version 26.0; IBM Corp., Armonk, NY, USA). Survival analyses were conducted using the Kaplan-Meier method and compared using the log-rank test. Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. A two-sided p value < 0.05 was considered statistically significant.

Results

Patient Characteristics

The analysis included 39 patients who met the study criteria. According to baseline serum vitamin D levels, 16 patients (41.0%) were classified as vitamin D sufficient (≥ 20 ng/mL), whereas 23 patients (59.0%) were categorized as vitamin D deficient (< 20 ng/mL). The median baseline vitamin D level was 17 ng/mL (range: 4-45 ng/mL).

The clinical and demographic characteristics of the cohort are summarized in Table 1. The proportion of male patients was similar in the two groups (81.3% in the sufficient group vs. 78.3% in the deficient group; $p=0.820$). Patients in the sufficient vitamin D group were more frequently aged ≥ 65 years compared to those in the deficient group (81.3% vs. 47.8%, $p=0.035$).

The groups were comparable in terms of ECOG performance status ($p=0.514$), histological subtype ($p=0.440$), and extent of metastatic disease ($p=0.357$).

Survival Outcomes

Patients with sufficient baseline vitamin D levels had a longer median PFS compared with those with deficient levels [10.1 months [95% confidence interval (CI): 0.1-20.8] vs. 4.6 months (95% CI: 2.9-6.2)]. However, this difference was not statistically significant ($p=0.292$; Figure 1).

A similar pattern was observed for OS. The median OS was 17.3 months (95% CI: 3.8-30.9) in the vitamin D sufficient group and 11.7 months (95% CI: 0.1-23.5) in the vitamin D deficient group, and this difference was not statistically significant ($p=0.823$; Figure 2).

Table 1. Baseline clinical and demographic characteristics of patients with advanced NSCLC receiving nivolumab, according to serum vitamin D status			
Characteristic	Sufficient vitamin D (≥20 ng/mL) n (%)	Insufficient vitamin D (<20 ng/mL) n (%)	p value
Sex			0.820
- Female	3 (19%)	5 (22%)	
- Male	13 (81%)	18 (78%)	
Age			0.035
- < 65 years	3 (19%)	12 (52%)	
- ≥ 65 years	13 (81%)	11 (48%)	
ECOG performance status			0.514
- 0	3 (19%)	5 (22%)	
- 1	11 (69%)	12 (52%)	
- 2	2 (12%)	6 (26%)	
Histological subtype			0.440
- Squamous cell carcinoma	5 (31%)	10 (44%)	
- Adenocarcinoma	11 (69%)	13 (56%)	

ECOG: Eastern Cooperative Oncology Group, NSCLC: Non-small cell lung cancer

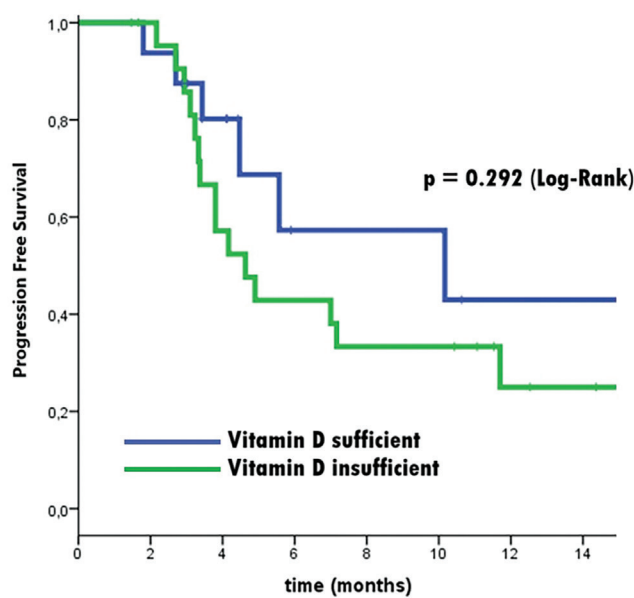


Figure 1. Kaplan-Meier curve for progression-free survival according to baseline serum vitamin D status (p=0.292, log-rank test)

Given the limited sample size, multivariable Cox regression analysis was not performed.

Discussion

The findings of this study indicate a trend toward improved survival in patients receiving nivolumab who had higher baseline vitamin D levels. Although these differences did not reach statistical significance, the direction of the findings suggests that vitamin D status may still have clinical relevance in patients receiving ICIs.

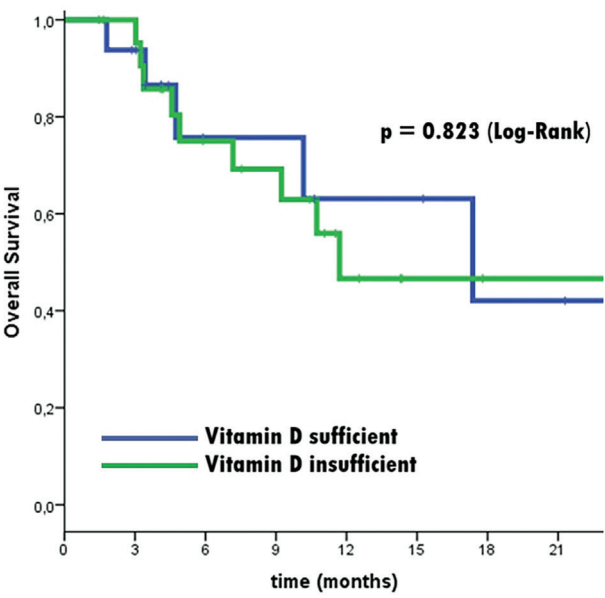


Figure 2. Kaplan-Meier curve for overall survival according to baseline serum vitamin D status (p=0.823, log-rank test)

The lack of statistical significance is likely attributable to the limited sample size and the inherent variability of retrospective cohort studies. Small patient numbers reduce the ability to detect modest differences, even when a consistent pattern is present. Despite this limitation, our observations are in line with previous reports suggesting that adequate vitamin D levels may be associated with improved outcomes and reduced treatment-related toxicity in patients receiving immunotherapy [13-17].

Rather than acting through a single pathway, vitamin D appears to contribute to overall immune balance. It may

support a more controlled immune response by influencing inflammatory signaling and maintaining equilibrium between immune activation and regulation. In the setting of cancer, such an effect could help sustain anti-tumor activity while preventing excessive immune reactions. This broader perspective may partially explain the differences observed between patient groups.

An additional finding in our cohort was that patients with sufficient vitamin D levels tended to be older, yet had numerically better survival outcomes. This observation suggests that vitamin D status may have prognostic value beyond age alone. Considering that aging is associated with gradual decline in immune function, adequate vitamin D levels may help preserve immune competence in older individuals [18-20].

Vitamin D status is also influenced by individual biological variability. Differences in metabolism, distribution, and binding proteins may affect circulating 25(OH)D levels and their functional impact. These factors indicate that vitamin D biology is complex and may not be fully reflected by a single baseline measurement [21].

Although vitamin D deficiency has been associated with poorer outcomes in various malignancies, data specific to NSCLC patients receiving ICIs remain limited. Our findings contribute to this field by suggesting that vitamin D status may represent a relevant host-related factor influencing treatment outcomes [9,22,23].

From a clinical perspective, vitamin D is a potentially modifiable parameter. Given its accessibility, low cost, and favorable safety profile, assessing and correcting deficiencies may represent a simple supportive strategy in oncology practice. However, whether supplementation directly improves immunotherapy efficacy requires further investigation.

Study Limitations

Several limitations should be considered when interpreting these results. The retrospective single-center design introduces potential selection bias, information bias, and unmeasured confounding, which should be considered when interpreting the findings. The relatively small sample size limits statistical power. In addition, factors such as nutritional status, comorbidities, and vitamin D supplementation were not fully evaluated. The absence of longitudinal vitamin D measurements also prevents assessment of dynamic changes during treatment. The limited sample size may have reduced the ability to detect statistically significant differences in survival, despite the observed numerical trends favoring patients with sufficient vitamin D levels.

Future Directions

Larger prospective studies are needed to confirm these findings. Future research integrating clinical, molecular, and immunological parameters may provide a more comprehensive understanding of the relationship between vitamin D and immunotherapy. In addition, interventional studies evaluating vitamin D supplementation could help clarify its potential therapeutic role.

Conclusion

Baseline vitamin D levels may be associated with clinical outcomes in patients with advanced NSCLC receiving nivolumab. Although statistical significance was not observed, the consistent trend toward improved outcomes supports further investigation in larger prospective studies.

Ethics

Ethics Committee Approval: The study was approved by the University of Health Sciences Türkiye, Ankara Etlik City Hospital Scientific Research Evaluation and Ethics Committee (approval no: AEŞH-BADEK-2024-931, date: 27.11.2024).

Informed Consent: This is a retrospective study, therefore patient consent was not required.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.Ç., Ö.A.İ., B.Ö., D.M.K., Concept: S.Ç., O.S., K.B., S.D.Ş., B.Ö., D.M.K., Design: S.Ç., O.S., Ö.A.İ., A.T.A., B.Ö., D.M.K., Data Collection or Processing: S.Ç., K.B., S.D.Ş., Ş.E., D.M.K., Analysis or Interpretation: S.Ç., A.T.A., D.M.K., Literature Search: S.Ç., S.K., E.Z., Ş.E., D.M.K., Writing: S.Ç., S.K., E.Z., D.M.K.

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Original Article

Concordance and Discordance Between PET/CT and CEA/CA19-9 Dynamics and Their Predictive Value for Clinical Prognosis in Colorectal Cancer

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ABSTRACT

Aim: The study aimed to evaluate the concordance between treatment-related changes in fluorodeoxyglucose (FDG) positron emission tomography/computed tomography (PET/CT) -derived metabolic parameters and serum tumor markers [carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9)] and to investigate the clinical significance of concordant and discordant response patterns in colorectal cancer.

Methods: This retrospective study included 81 patients with histologically confirmed colorectal cancer who underwent FDG PET/CT before and after systemic chemotherapy. Metabolic response was defined as a $\geq 30\%$ maximum standardized uptake value reduction, and $\geq 20\%$ decrease in CEA or CA19-9 levels defined as tumor marker response. Patients were categorized into concordant-good-response, concordant-poor-response, and discordant-response groups based on PET and CEA responses. Survival was analyzed using Kaplan-Meier and Cox regression methods.

Results: Following systemic chemotherapy, treatment responses were evaluated using changes in FDG PET/CT metabolic parameters and serum tumor markers. Significant reductions were observed in all PET-derived metabolic parameters ($p < 0.001$) and in CEA levels ($p = 0.008$) after treatment, whereas CA19-9 did not show a significant change ($p = 0.751$). No significant association was found between PET response and CEA response ($p = 0.492$) or between PET response and CA19-9 response ($p = 0.807$); however, a significant association was observed between CEA and CA19-9 responses ($p < 0.001$). Concordance groups were significantly associated with mortality ($p = 0.036$), and overall survival (OS) differed among groups (log-rank $p = 0.043$). The concordant good-response group had the longest survival, whereas the concordant poor-response group had the shortest. The discordant response group exhibited intermediate survival outcomes.

Conclusion: The combined assessment of PET-derived metabolic response and CEA response is associated with OS in colorectal cancer. Concordance patterns may provide clinically meaningful information, while discordant responses likely reflect heterogeneous tumor biology.

Keywords: Colorectal cancer, FDG PET/CT, CEA blood test, treatment, prognosis

Introduction

Colorectal cancer continues to rank as the third most frequently diagnosed malignancy worldwide, with rising incidence in developing regions despite a decline in high-income countries [1]. Current global estimates indicate substantial regional and population-based variation in the

burden of colorectal cancer, which is anticipated to increase in the years ahead [2]. Current treatment strategies for colorectal cancer include surgical resection for localized disease and multimodal approaches for advanced stages, primarily involving chemotherapy, with radiotherapy and immunotherapy used in selected cases. Biomarkers such as

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carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) are primarily used in clinical practice for monitoring disease progression [3].

In contrast, functional imaging modalities, particularly fluorine-18-fluorodeoxyglucose (^{18}F -FDG) positron emission tomography/computed tomography (PET/CT), enable the evaluation of tumor metabolism and may provide a more comprehensive assessment of treatment response. In the setting of serial CEA elevation, ^{18}F -FDG PET/CT is recommended as a problem-solving tool in accordance with National Comprehensive Cancer Network guidelines [4].

However, the relationship between metabolic imaging findings and serum tumor markers is not always consistent, and discordant results are frequently encountered in clinical practice. The clinical implications of such discordance remain unclear.

Therefore, this study aimed to evaluate the concordance between treatment-related changes in ^{18}F -FDG PET/CT findings and in serum tumor markers and to investigate the clinical significance of discordant results in relation to patient outcomes.

Methods

This retrospective analysis comprised 81 patients with histologically confirmed colorectal cancer who underwent FDG PET/CT examinations both before and after systemic chemotherapy. Clinical, imaging, and laboratory data were obtained from institutional records. Ethical approval has been obtained from the University of Health Sciences Türkiye, Ankara Etlik City Hospital No. 1 Scientific Research Ethics Committee (approval no: AEŞH-BADEKI-2025-735, date: 24.12.2025). The requirement for informed consent has been removed.

All patients received systemic chemotherapy between pre-treatment and post-treatment PET/CT examinations, and treatment response was evaluated based on changes in PET-derived parameters and serum tumor markers.

Patients who underwent surgical resection between the two PET/CT examinations were excluded.

Demographic data (age, sex), tumor location (colon or rectum), serum tumor markers (CEA and CA19-9), and primary tumor PET parameters were recorded from both the pre- and post-treatment PET/CT scans. PET parameters included the maximum standardized uptake value (SUV_{max}) derived from the primary tumor, as well as metabolic tumor volume (MTV) and total lesion glycolysis (TLG), which were both calculated using the same volume of interest. Serum tumor markers were measured within one month of the PET/CT to ensure temporal consistency.

Metabolic response was defined based on changes in SUV_{max} . A reduction of $\geq 30\%$ was considered a metabolic response, based on thresholds inspired by PET response criteria in solid tumors (PERCIST) criteria, although SUV_{max} rather than peak SUV_{max} normalized to lean body mass was used [4]. Tumor marker response was defined as a $\geq 20\%$ decrease in CEA or CA19-9; additionally, a $\geq 50\%$ reduction in CEA was explored

for sensitivity analysis [5]. Patients with complete metabolic response (CMR), defined as the absence of FDG-avid lesions, were classified as responders.

Based on PET (SUV_{max}) and CEA responses, patients were categorized into three groups: concordant good response (both responses present), concordant poor response (no response in either parameter), and discordant response (mismatch between PET and CEA responses).

Percentage changes were calculated as $(\text{post-pre})/\text{pre} \times 100$. However, analyses were primarily based on directional changes (increases vs. decreases). For volumetric parameters (MTV and TLG), only patients with measurable values at both time points were included.

Statistical Analysis

Statistical evaluation was performed using IBM SPSS Statistics for Mac (version 27.0; IBM Corp., Armonk, NY, USA).

Shapiro-Wilk test was used to evaluate the distribution of continuous variables and results were expressed accordingly as either mean $\pm$ standard deviation or median with interquartile range. Changes between pre- and post-treatment measurements were examined with the Wilcoxon signed-rank test, whereas categorical data were compared using the chi-square or Fisher's exact tests, where applicable.

The association between PET-derived metrics and tumor markers was explored using Spearman correlation analysis. Overall survival (OS) was calculated from the date of baseline PET/CT to death or the last known follow-up. Survival probabilities were estimated using Kaplan-Meier curves and differences between groups were assessed with the log-rank test. To further investigate the effects of the variables on OS, Cox proportional hazards models were applied. Statistical significance was defined as a two-sided p value below 0.05.

AI-assisted language tools were used for English editing of the manuscript.

Results

A total of 81 patients who underwent systemic chemotherapy were included, and their treatment responses were evaluated by changes in FDG PET/CT metabolic parameters and serum tumor markers. The mean age was 64.1 ± 11.3 years (range: 25-90), and 47 (58.0%) were male and 34 (42.0%) were female. 50 patients (61.7%) had colon cancer and 31 (38.3%) had rectal cancer. The mean follow-up duration was 20.6 ± 10.1 months (range: 6-70). According to the PET-CEA concordance classification, 22.2% of patients had a concordant good response, 18.5% had a concordant poor response, and 59.3% had a discordant response. No significant association was observed between PET-CEA concordance groups and metastatic disease status ($\chi^2=0.457$, $p=0.796$). The baseline demographic and clinical characteristics of the study population are summarized in Table 1.

Comparison of pre- and post-treatment values demonstrated a significant reduction in CEA levels (median, 7.30 vs. 4.80; $p=0.008$), whereas the decrease in CA19-9 levels was not statistically significant (median, 22.80 vs. 18.20; $p=0.751$).

Table 1. Baseline demographic and clinical characteristics of the study population (n=81)

Variable	Value
Age (years), mean $\pm$ SD	64.1 $\pm$ 11.3
Age (range)	25-90
Sex, n (%)	
Male	47 (58.0%)
Female	34 (42.0%)
Diagnosis, n (%)	
Colon cancer	50 (61.7%)
Rectal cancer	31 (38.3%)
Follow-up duration, mean $\pm$ SD	20.6 $\pm$ 10.1
Follow-up duration, range	6-70
PET-CEA concordance groups, n (%)	
Concordant good	18 (22.2%)
Concordant poor	15 (18.5%)
Discordant	48 (59.3%)

SD: Standard deviation

All PET-derived metabolic parameters, including SUV_{max}, MTV, TLG, and mean SUV (SUV_{mean}), showed significant reductions after treatment (all $p < 0.001$) (Table 2).

No significant association was observed between PET response and CEA response ($\chi^2=0.472$, $p=0.492$) or between PET response and CA19-9 response ($\chi^2=0.060$, $p=0.807$). In contrast, a significant association was found between CEA and CA19-9 responses ($\chi^2=19.147$, $p < 0.001$).

A significant association was observed between concordance groups and mortality ($\chi^2=6.65$, $p=0.036$). Mortality rates were 33.3% in the concordant good response group, 40.0% in the concordant poor response group, and 12.5% in the discordant response group (Table 3).

Among discordant cases, a significant difference in mortality was observed between the PET-discordant group (PET-responsive/CEA-non-responsive) and the CEA-discordant group (CEA-responsive / PET-non-responsive) (2/38 vs. 4/10, $p=0.013$, Fisher's exact test).

Correlation analysis based on directional changes (increase vs. decrease) demonstrated a moderate positive association of CEA with both SUV_{max} ($r=0.405$, $p=0.002$) and SUV_{mean} ($r=0.463$, $p < 0.001$), and a weaker but significant correlation with TLG ($r=0.336$, $p=0.013$). No significant correlation was observed between CEA and MTV ($p=0.421$) or between CA19-9 and PET parameters ($p > 0.05$).

Kaplan-Meier analysis demonstrated a statistically significant difference in OS among concordance groups (log-rank $p=0.043$). Patients in the concordant good response group had the longest survival (mean: 49.1 months), while those in the concordant poor response group had the shortest survival (mean: 26.9 months). The discordant group showed survival outcomes comparable to those of the concordant good response group (Figure 1).

In univariate Cox regression analysis, both age (hazard ratio: 1.059, $p=0.019$) and concordance status ($p=0.045$) were significantly associated with OS. However, in multivariable analysis that included both variables, concordance status was no longer statistically significant ($p=0.221$), and age also lost statistical significance ($p=0.101$).

CMR was observed in 16 patients. There was no significant difference in OS between patients with and without CMR (log-rank $p=0.409$).

No significant association was observed between tumor location (colon vs. rectum) and PET-CEA concordance groups ($\chi^2=0.679$, $p=0.878$), indicating similar distributions of concordance patterns across tumor sites.

Table 2. Changes in tumor markers and PET-derived metabolic parameters before and after treatment

Variable	Pre-treatment median (IQR)	Post-treatment median (IQR)	p value*
CEA	7.30 (3.27-44.70)	4.80 (2.17-19.00)	0.008
CA19-9	22.80 (8.50-75.70)	18.20 (10.60-55.70)	0.751
SUV _{max}	15.15 (8.48-20.28)	7.25 (4.53-14.30)	<0.001
MTV	13.40 (7.43-30.25)	7.50 (4.78-14.10)	<0.001
TLG	102.00 (53.23-230.05)	37.55 (17.08-91.28)	<0.001
SUV _{mean}	8.20 (5.20-10.65)	4.60 (3.05-8.00)	<0.001

*: Wilcoxon signed-rank test

IQR: Interquartile range, PET: Positron emission tomography, CEA: Carcinoembryonic antigen, CA19-9: Carbohydrate antigen 19-9, SUV_{max}: Maximum standardized uptake value, MTV: Metabolic tumor volume, TLG: Total lesion glycolysis, SUV_{mean}: Mean standardized uptake value**Table 3. Mortality according to response groups**

Response group	Total (n)	Deaths (n)	Mortality (%)
Concordant good	18	6	33.3%
Concordant poor	15	6	40.0%
Discordant	48	6	12.5%
Total	81	18	22.2%

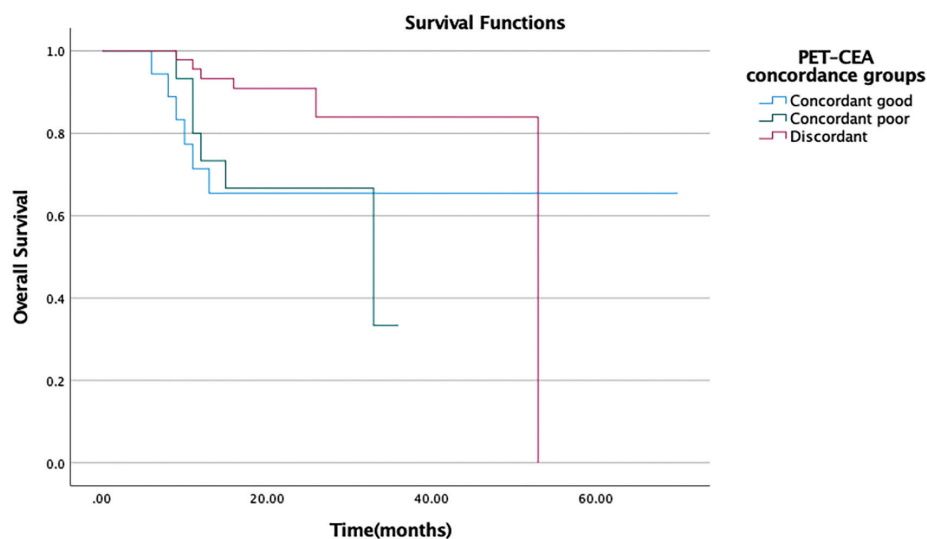


Figure 1. Kaplan-Meier survival curves according to PET-CEA concordance groups (concordant good response, concordant poor response, and discordant response). Patients in the concordant good response group demonstrated the longest overall survival, whereas those in the concordant poor response group had the shortest overall survival. The discordant response group showed survival outcomes comparable to those of the concordant good-response group. Differences between groups were statistically significant (log-rank test, $p=0.043$). Censoring marks are omitted for clarity

PET: Positron emission tomography, CEA: Carcinoembryonic antigen

Discussion

Colorectal cancer prognosis is influenced by multiple clinicopathological and molecular factors, reflecting the multifactorial nature of disease outcomes [5]. Within this framework, reliance on a single biomarker is unlikely to fully represent disease dynamics. Consequently, discordance between different assessment modalities may occur in clinical practice. Accordingly, in the present study, treatment response was evaluated using both FDG PET- derived parameters and serum tumor markers.

In the present study, a significant association was observed between PET-CEA concordance groups and mortality. Within the discordant response group, mortality differed significantly by discordance type, with higher mortality in the CEA-discordant subgroup (CEA responders with no PET response). Previous studies have reported only a moderate correlation between FDG PET-derived metabolic parameters and serum tumor markers, suggesting that these modalities reflect different aspects of tumor biology rather than interchangeable measures of disease burden [6]. Taken together, these findings support the concept that PET-derived parameters and serum tumor markers provide complementary information in the assessment of treatment response.

FDG PET/CT has an established role in evaluating treatment response in colorectal cancer, particularly because it can detect metabolic changes earlier than conventional morphological imaging. Previous studies have demonstrated that therapy-induced alterations in tumor glucose metabolism are significantly associated with both OS and progression-free survival [7]. In addition, metabolic response assessment using simplified parameters, such as SUV, has been shown to

be clinically reliable, supporting the use of semiquantitative approaches in routine practice. These findings are consistent with our results, in which metabolic response, defined by changes in SUV_{max} , was associated with survival outcomes.

In the present study, no significant association was observed between concordance groups and metastatic disease status. This finding suggests that the observed discordance between PET and serum tumor markers cannot be solely attributed to differences in tumor burden. Although PET-derived parameters were obtained from the primary tumor rather than whole-body disease, both PET metabolic parameters and serum tumor markers demonstrated significant reductions following treatment. Therefore, the discordance observed in this study may reflect differences in the biological information captured by these modalities rather than merely differences in the extent of disease.

No significant association was observed between tumor location (colon vs. rectum) and PET-CEA concordance groups, suggesting that concordance patterns are not influenced by primary tumor site. This finding further supports the notion that concordance reflects underlying tumor biology rather than anatomical or site-related factors.

Despite improvements in treatment and early detection, colorectal cancer remains a major cause of cancer-related mortality worldwide, although survival rates have improved over time [8]. Prognosis in colorectal cancer is influenced by multiple clinical and biological factors, including performance status, tumor burden, and systemic inflammatory markers [9]. Previous studies have demonstrated that elevated preoperative CEA and CA19-9 levels are associated with poorer survival outcomes, with the worst prognosis observed when both markers are increased, suggesting their potential

prognostic value [10]. Treatment response in colorectal cancer is commonly assessed using standardized criteria such as Response Evaluation Criteria in Solid Tumors, which incorporate both radiologic tumor burden and normalization of tumor marker levels; in this context, serum markers such as CEA are frequently used as adjuncts in response evaluation rather than standalone indicators [11].

In our study, the combined assessment of tumor marker response (CEA) and metabolic response, defined as a $\geq 30\%$ reduction in SUVmax inspired by PERCIST-based criteria, was significantly associated with OS. Patients with a concordant good response (i.e., a decrease in both PET and CEA) demonstrated more favorable survival outcomes, whereas those with a concordant poor response exhibited the worst survival outcomes. Discordant patterns were associated with intermediate outcomes. Although discordant patterns were associated with intermediate survival outcomes, this group exhibited the lowest mortality rate. This apparent discrepancy may be explained by the heterogeneous biological behavior underlying discordant responses. Previous studies have demonstrated that prognosis in colorectal cancer is influenced by multiple clinical and biological factors, including performance status, tumor burden, and laboratory parameters. However, in our study, no significant difference was observed between metastatic and non-metastatic patients. This finding may be explained by the characteristics of our study population: all patients received systemic chemotherapy and did not undergo upfront surgical resection. Therefore, even patients classified as non-metastatic may belong to a more advanced, biologically aggressive subgroup that is not suitable for surgery, and this may have attenuated the expected prognostic differences between metastatic and non-metastatic disease.

The prognostic role of age in colorectal cancer has been widely investigated; however, its independent impact on survival remains controversial. Some studies suggest that survival differences become more evident in patients older than 60 years, whereas younger age groups show comparable outcomes [12]. Consistent with previous reports, chronological age alone does not appear to be an independent determinant of survival outcomes in colorectal cancer, suggesting that other factors such as tumor biology and treatment-related variables may play a more prominent role [13]. Previous studies have indicated that, although older age is associated with increased morbidity and mortality, its independent effect on survival becomes less clear when patients with similar disease stages and treatments are compared, which further supports this concept. In our study, age was significantly associated with OS in univariate analysis; however, this association was not maintained in multivariate analysis. The apparent prognostic impact of age in colorectal cancer may be explained by its association with comorbidities and differences in treatment patterns, as older patients are more likely to receive less aggressive therapy and have reduced tolerance to treatment [13]. These findings support the notion that age may act as a surrogate marker rather than an independent determinant of prognosis.

In our study, the combined assessment of PET and CEA response was significantly associated with OS in univariate analysis; however, this association did not remain significant in multivariate models. Nevertheless, the observed survival patterns across concordance groups suggest that the integration of metabolic and tumor marker responses may still provide clinically meaningful information. This may be explained by the fact that PET-derived parameters and serum tumor markers reflect complementary aspects of tumor biology, including metabolic activity and systemic tumor burden. While the prognostic impact of age may largely be influenced by comorbidity burden and treatment selection, response-based parameters may offer a more direct reflection of tumor behavior and treatment-related disease dynamics.

CA19-9 is a well-established tumor marker primarily used in pancreatic cancer, where it has demonstrated prognostic value and utility in monitoring treatment response [14]. However, its role in colorectal cancer is less consistent compared to CEA [15]. In our study, CA19-9 showed an association with CEA but did not demonstrate a significant relationship with treatment response. This finding may reflect its more limited applicability to colorectal cancer and its lower sensitivity in capturing treatment-related disease dynamics compared to CEA.

MTV is defined as the volume of tumor tissue with increased FDG uptake and represents the sum of voxels with SUV values above a predefined threshold. This volume can be measured semiquantitatively using contouring methods on attenuation-corrected PET/CT images. TLG is a parameter that combines tumor volume and metabolic activity, and is calculated as the sum of the products of SUV_{mean} and MTV [16]. In our study, all PET-derived metabolic parameters and CEA levels showed significant reductions following treatment, whereas CA19-9 did not demonstrate a significant change. In addition, only a weak correlation was observed between TLG and CEA, suggesting that these parameters may reflect different aspects of tumor biology. While TLG integrates metabolically active tumor burden by combining lesion volume and uptake, CEA may better represent systemic disease dynamics. This is consistent with our finding of no significant association between PET and CEA responses, which further supports the conclusion that PET-derived metrics and serum tumor markers provide complementary rather than overlapping information in the assessment of treatment response.

Study Limitations

This study has several limitations. First, its retrospective design and relatively small sample size may limit the generalizability of the findings. Second, PET-derived parameters were obtained from the primary tumor rather than from whole-body tumor burden, which may have affected the assessment of the extent of metabolic disease. Finally, the heterogeneity of treatment regimens may have influenced survival outcomes and response patterns.

Overall, these findings highlight that integrating PET-derived metabolic parameters with serum tumor markers may offer a more comprehensive and clinically meaningful approach for evaluating treatment response in colorectal cancer.

Conclusion

The combined assessment of FDG PET/CT- derived metabolic response and serum tumor marker (CEA) response was associated with OS in patients with colorectal cancer. Concordance patterns, particularly concordant good and concordant poor responses, demonstrated distinct survival outcomes, while discordant patterns reflected heterogeneous disease behavior. Although these associations were not maintained in multivariate analysis, the observed trends suggest that integrating metabolic and tumor marker responses may provide clinically meaningful insights into treatment response. Further studies with larger cohorts and assessment of whole-body tumor burden are warranted to validate these findings.

Ethics

Ethics Committee Approval: Ethical approval has been obtained from the University of Health Sciences Türkiye, Ankara Etlik City Hospital No. 1 Scientific Research Ethics Committee (approval no: AEŞH-BADEKI-2025-735, date: 24.12.2025).

Informed Consent: Informed consent was obtained from all participants.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.D.Ş., A.E.K., E.Y., Ö.A.İ., D.M.K., Ö.Ö., Concept: S.D.Ş., D.M.K., Design: S.D.Ş., D.M.K., Data Collection or Processing: S.D.Ş., A.E.K., E.Y., Analysis or Interpretation: S.D.Ş., Literature Search: S.D.Ş., A.E.K., Writing: S.D.Ş., Ö.Ö.

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Original Article

Quantifying the Survival Benefit of Trimodality Therapy in Esophageal Squamous Cell Carcinoma: An IPTW-adjusted Retrospective Analysis

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Aim: The survival advantage of surgery following chemoradiotherapy in esophageal squamous cell carcinoma (ESCC) remains debated due to potential selection bias in non-randomized studies. We aimed to quantify the magnitude of survival benefit associated with trimodality therapy after rigorous adjustment for baseline imbalances.

Methods: We retrospectively analyzed 54 patients with stage II-III ESCC treated between 2022 and 2025. Patients received either neoadjuvant chemoradiotherapy followed by surgery (trimodality group, n=24) or definitive chemoradiotherapy alone (dCRT group, n=30). To address treatment selection bias, inverse probability of treatment weighting (IPTW) based on propensity scores was applied. Progression-free survival (PFS) and overall survival (OS) were evaluated using weighted Kaplan-Meier estimates and Cox proportional hazards models.

Results: At a median follow-up of 23.5 months, trimodality therapy was associated with a substantially prolonged PFS compared with dCRT (median 67.6 vs. 9.2 months, p=0.007). Similarly, OS was significantly improved (median 72.1 vs. 16.6 months, p=0.009). In multivariable analysis, surgery remained independently associated with improved OS [hazard ratio (HR): 0.41, 95% confidence interval (CI): 0.22-0.78, p=0.006] and PFS (HR: 0.44, 95% CI: 0.24-0.81, p=0.008). These findings were consistent after IPTW adjustment (OS HR: 0.46; PFS HR: 0.49, both p<0.01), indicating a robust and clinically meaningful survival benefit.

Conclusion: After rigorous adjustment for baseline differences, trimodality therapy was associated with a substantial survival advantage over dCRT in patients with locally advanced ESCC. These findings suggest that the magnitude of benefit with surgery may be greater than previously appreciated in real-world populations, supporting its use in appropriately selected patients.

Keywords: Cancer research, cell therapy, chemotherapy, clinical hematology, clinical oncology, clinical trials

ABSTRACT

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Introduction

Esophageal cancer is considered to be one of the most aggressive malignancies on a global scale [1]. esophageal squamous cell carcinoma (ESCC) is the predominant histologic subtype in Eastern and Central Asia, and it also constitutes a major proportion of esophageal cancers in some regions of Türkiye [2]. Despite advances in multimodal treatment strategies, long-term survival remains poor, with 5-year survival rates rarely exceeding 25%, largely due to the high prevalence of locally advanced disease at diagnosis [3].

For patients with stage II-III ESCC, multimodality treatment has become the cornerstone of care [4]. Neoadjuvant chemoradiotherapy followed by surgery (trimodality therapy) is widely considered the standard approach for resectable disease, largely based on randomized trials such as the CROSS study, which demonstrated improved overall survival (OS) compared with surgery alone [5]. However, the role of surgery following chemoradiotherapy remains controversial, particularly when compared with definitive chemoradiotherapy (dCRT), which is often used in patients deemed medically inoperable or with tumors of borderline resectability.

Several studies have attempted to compare trimodality therapy with dCRT, but the interpretation of these findings is inherently limited by treatment selection bias [6]. Patients undergoing surgery are typically younger, have better performance status, and present with less advanced disease, making it difficult to determine whether observed survival differences reflect a true treatment effect or simply differences in baseline characteristics [7-9]. Even in studies employing multivariable adjustment, residual confounding remains a major concern.

Therefore, a critical unresolved question is not only whether surgery improves survival, but also to what extent the observed benefit reflects a true therapeutic effect rather than patient selection. Quantifying this distinction is essential for optimizing treatment decisions in real-world clinical practice.

In this context, we conducted a retrospective analysis of patients with locally advanced ESCC who were treated at a high-volume tertiary center. Using inverse probability of treatment weighting (IPTW) to minimize baseline imbalances, we aimed to quantify the magnitude of the survival benefit associated with trimodality therapy compared with dCRT.

Methods

Study Design and Patient Selection

This retrospective, single-center cohort study was conducted in the Department of Medical Oncology at University of Health Sciences Türkiye, Ankara Etlik City Hospital and included patients diagnosed with locally advanced ESCC between January 2022 and March 2025. The study was approved by the University of Health Sciences Türkiye, Ankara Etlik City Hospital Scientific Research Evaluation and Ethics Committee No. 1 (approval no: AEŞH-BADEK1-2025-694, date: 17.12.2025). It was conducted in accordance with the Helsinki Declaration.

Patients were identified through the institutional oncology database. Eligibility criteria included: histopathologically confirmed ESCC, clinical stage II-III disease according to the American Joint Committee on Cancer 8th edition staging system [10], completion of definitive or neoadjuvant CRT and availability of follow-up and survival data. Exclusion criteria were the presence of distant metastasis (M1disease), histological subtypes other than squamous cell carcinoma, prior thoracic radiotherapy or systemic chemotherapy for another malignancy, and incomplete clinical or survival data.

Clinical staging was based on endoscopic ultrasound [11], contrast-enhanced computed tomography (CT), and/or positron emission tomography-CT (PET-CT). Patients with incomplete staging data were categorized as having “unknown” clinical- stage variables.

To address missing stage information, the clinical T (cT) and clinical N (cN) variables were incorporated into multivariate Cox regression models using dummy coding, with “unknown” included as a separate category. Sensitivity analyses excluding patients with unknown cT values were also performed to assess the robustness of the results.

Treatment Groups

Patients were categorized into two groups based on treatment strategy and operability:

1. Trimodality (Surgery) Group: Patients who received neoadjuvant CRT followed by curative-intent esophagectomy.
2. Definitive CRT Group: Patients treated with definitive CRT without surgery due to medical inoperability, advanced local invasion, or patient refusal.

Decisions regarding surgical eligibility were made by a multidisciplinary tumor board consisting of medical oncologists, radiation oncologists, thoracic surgeons, and radiologists.

Chemoradiotherapy Protocol

All patients received concurrent CRT with curative intent over a 5-6- week period.

Chemotherapy: Weekly carboplatin (area under the curve: 2, day 1) combined with paclitaxel (50 mg/m², day 1) administered concurrently radiotherapy.

Radiotherapy: External-beam radiotherapy was delivered to a total dose of 50.4-60 Gy in daily fractions of 1.8-2.0 Gy over 5-6 weeks, using three-dimensional conformal or intensity-modulated radiotherapy techniques. This dose range is consistent with commonly accepted standard-dose dCRT schedules, which have been shown to provide an optimal balance between efficacy and treatment-related toxicity [12].

In the neoadjuvant setting, surgical resection was planned 6-8 weeks after completion of CRT, provided an adequate clinical response and an acceptable performance status [Eastern Cooperative Oncology Group (ECOG) 0-2].

Surgical Approach and Pathologic Assessment

Patients in the trimodality group underwent transthoracic or transhiatal esophagectomy with regional lymph node dissection. The surgical approach (Ivor-Lewis or McKeown procedures) was determined based on tumor location and the surgeon's discretion. Pathological evaluation included assessment of resection margin status (R0/R1), pathological tumor and pathological nodal stage, and treatment response. Pathological complete response was defined as the absence of residual viable tumor cells in the resected specimen.

Data Collection and Variables

Demographic, clinical, treatment-related, and pathological data were retrospectively collected from electronic medical records. Collected variables included age, sex, smoking status, comorbidities, primary tumor localization, cT and cN stage, treatment modality, radiation dose, number of chemotherapy cycles, progression-free survival (PFS), and OS.

Outcome Definitions and Follow-up

OS was defined as the time from the initiation of chemoradiotherapy (day 1 of treatment) to death from any cause.

PFS was defined as the time from the same starting point to the first documented disease progression or death, whichever occurred first.

For patients undergoing surgery, postoperative recurrence or death was considered a PFS event.

Patients who were alive and progression-free at the last follow-up were censored on that date.

Sensitivity analyses were repeated using the date of diagnosis as time zero, yielding consistent results.

Patients were followed every 3 months during the first 2 years and every 6 months thereafter. Disease recurrence was confirmed by imaging (CT or PET-CT) and/or by endoscopic biopsy.

Statistical Analysis

Survival outcomes were estimated using the Kaplan-Meier method and compared with the log-rank test. Multivariable Cox proportional hazards regression models were used to assess the independent association between surgery and survival outcomes, adjusting for age, sex, cT stage, cN stage, tumor location, ECOG performance status, and treatment modality. The proportional hazards assumption was verified using Schoenfeld residuals. To further control for treatment selection bias, IPTW based on propensity scores was performed. Propensity scores were estimated using logistic regression incorporating the same covariates. Covariate balance before and after weighting was assessed using standardized mean differences (SMDs), with values <0.10 indicating adequate balance (Supplementary Table 1). Weighted Kaplan-Meier curves and weighted Cox regression models were generated to estimate adjusted hazard ratios (HRs). A two-sided p

value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 30.0 and BluSky Statistics (version 10.3.2).

Results

A total of 54 patients with locally advanced ESCC were included in the analysis. Of these, 24 patients (44.4%) underwent chemoradiotherapy followed by surgery (trimodality group), while 30 patients (55.6%) underwent dCRT group.

Patient Characteristics

Baseline demographic and clinical characteristics by treatment modality are summarized in Table 1. The median age of the entire cohort was 62.0 years (range, 36.0-86.0). Female patients constituted 52% of the study population; no statistically significant difference in sex distribution was observed between the treatment groups ($p=0.161$). Most patients (88.9%) were active smokers, and smoking status did not differ significantly between groups ($p=0.561$).

The primary tumor's location was found to be most commonly in the upper thoracic region in both cohorts (41.7% in the trimodality group vs 60.0% in the dCRT group), with no statistically significant difference ($p=0.381$).

Incomplete clinical staging data were present for 39% of patients. The absence of cT stage data was observed more frequently in the dCRT group than in the trimodality group (83.3% vs. 58.3%). Baseline differences between patients with known and unknown cT stage are summarized in Supplementary Table 2. In the sensitivity analysis restricted to patients with known cT stage ($n=25$), surgery remained significantly associated with improved OS (median 70.4 vs. 17.1 months, $p=0.018$) and PFS ($p=0.021$). Thus, the impact of surgery on survival was consistent even after excluding patients with incomplete staging.

cT stage differed significantly between groups ($p=0.004$). Early T1-T2 stages were observed only in the surgical cohort, whereas T4 disease was present exclusively in patients who did not undergo surgery. cN stage showed a near-significant difference ($p=0.054$), with a higher proportion of N3 disease in the surgical group (43.5% vs. 10.0%). The proportion receiving concurrent chemoradiotherapy was comparable between the surgical and non-surgical groups (70.8% vs. 80.0%, $p=0.434$).

Postoperative Outcomes and Recurrence Patterns

Among the 24 patients who underwent surgery, no postoperative mortality occurred within 90 days.

During follow-up, 12 patients (50.0%) in the surgery group and 25 patients (83.3%) in the definitive CRT group experienced disease recurrence or progression. In the surgical group, recurrence was predominantly distant metastasis (8 patients, 66.7%), whereas locoregional recurrence occurred in 4 patients (33.3%).

Conversely, patients treated with definitive CRT had a higher proportion of locoregional failures (16 patients, 64.0%) and a lower proportion of distant relapses (9 patients, 36.0%).

Table 1. Baseline demographic and clinical characteristics of patients according to surgical status						
Variable	Category	Surgery (n=24) n	%	No surgery (n=30) n	%	p value
Gender	Female	15	62.5	13	43.3	0.161
	Male	9	37.5	17	56.7	
Smoking status	Smoker	22	91.7	26	86.7	0.561
	Never	2	8.3	4	13.3	
	Former (quitted)	0	0.0	0	0.0	
Primary tumor location	Upper thoracic	10	41.7	18	60.0	0.381
	Middle thoracic	8	33.3	6	20.0	
	Esophagogastric junction	6	25.0	6	20.0	
Clinical T stage (cT)	Unknown	14	58.3	25	83.3	0.004
	T1	3	12.5	0	0.0	
	T2	6	25.0	0	0.0	
	T3	1	4.2	1	3.3	
	T4	0	0.0	4	13.3	
Clinical N stage (cN)	Unknown	1	4.3	3	10.0	0.054
	N0	6	26.1	9	30.0	
	N1	5	21.7	9	30.0	
	N2	1	4.3	6	20.0	
	N3	10	43.5	3	10.0	
Concurrent chemoradiotherapy	Yes	17	70.8	24	80.0	0.434
	No	7	29.2	6	20.0	

The median interval to disease recurrence was 15.2 months after surgery, compared with 8.4 months after definitive CRT. These findings indicate that surgery improves locoregional control, whereas systemic relapse remains the primary mode of failure in both groups (Table 2).

Progression-free and OS

When chemoradiotherapy initiation was used as the common time-zero point, the median PFS and OS remained significantly longer in the surgery group. A sensitivity analysis using the date of diagnosis as time zero yielded similar findings, confirming that differences in survival were not explained by time-definition bias.

At a median follow-up of 23.5 months, median PFS for the cohort was 19.9 months (95% CI: 0.7-39.0). Patients who

underwent surgery had significantly longer PFS than those treated with dCRT (67.6 months, 95% CI: 17.8-117.4 vs. 9.2 months, 95% CI: 4.9-13.4; $p=0.007$, log-rank test) (Figure 1). Survival comparisons were performed using the log-rank test. Median OS and PFS values are shown with 95% confidence intervals.

Patients who underwent neoadjuvant chemoradiotherapy followed by surgery had significantly longer PFS than those treated with dCRT (median PFS: 67.6 vs. 9.2 months, $p=0.007$, log-rank test).

Similarly, OS was significantly longer in the surgical cohort (72.1 months, 95% CI: 24.9-119.2) than in the dCRT group (16.6 months, 95% CI: 7.2-25.9; $p=0.009$, log-rank test) (Figure 2).

Table 2. Postoperative mortality and recurrence patterns			
Outcome	Surgery group (n=24)	Definitive CRT (n=30)	p value
90-day mortality	0 (0.0%)	3 (10.0%)	0.091
Any recurrence/progression	12 (50.0%)	25 (83.3%)	0.007
Locoregional recurrence	4 (33.3%)	16 (64.0%)	0.028
Distant metastasis	8 (66.7%)	9 (36.0%)	0.042
Median time to recurrence (months)	15.2 (95% CI: 9.8-20.7)	8.4 (95% CI: 5.6-11.2)	0.01
Recurrence patterns were categorized as locoregional (tumor bed, anastomotic site, or regional lymph nodes) and distant (lung, liver, bone, or non-regional lymph nodes) CRT: Chemoradiotherapy, CI: Confidence interval			

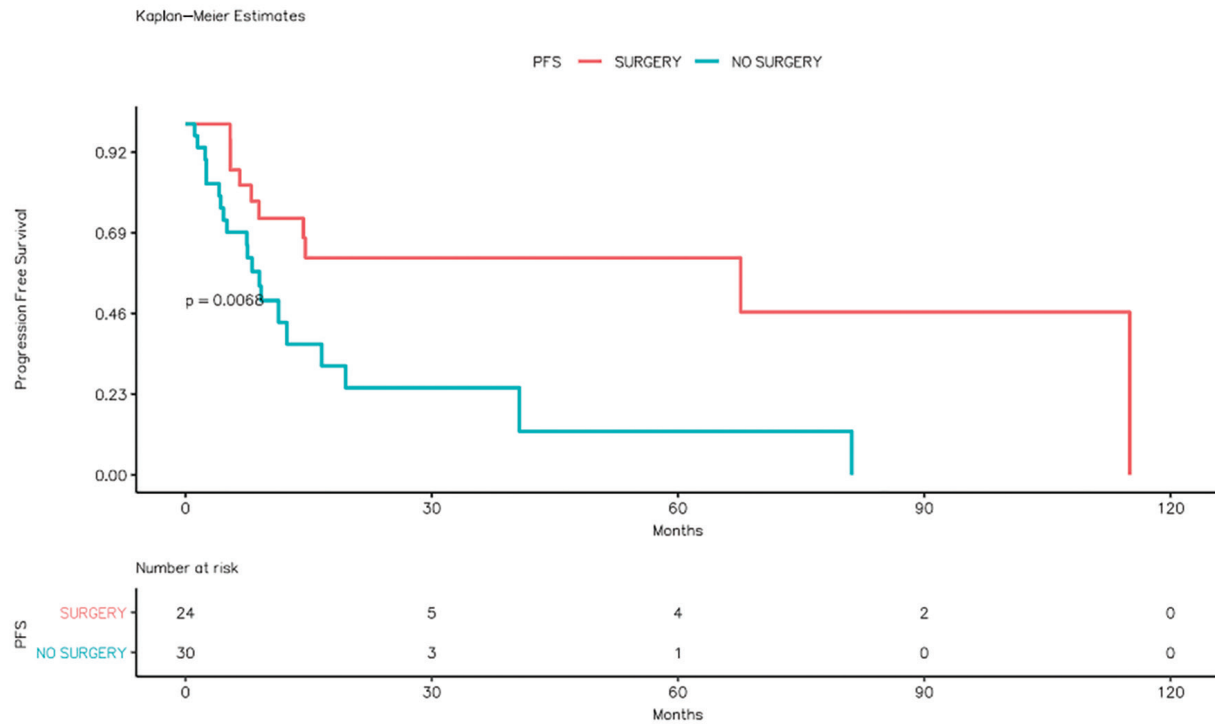


Figure 1. Kaplan-Meier curves for overall survival and PFS in patients with locally advanced esophageal squamous cell carcinoma treated with chemoradiation followed by surgery (blue line) or definitive chemoradiation (red line)
PFS: Progression-free survival

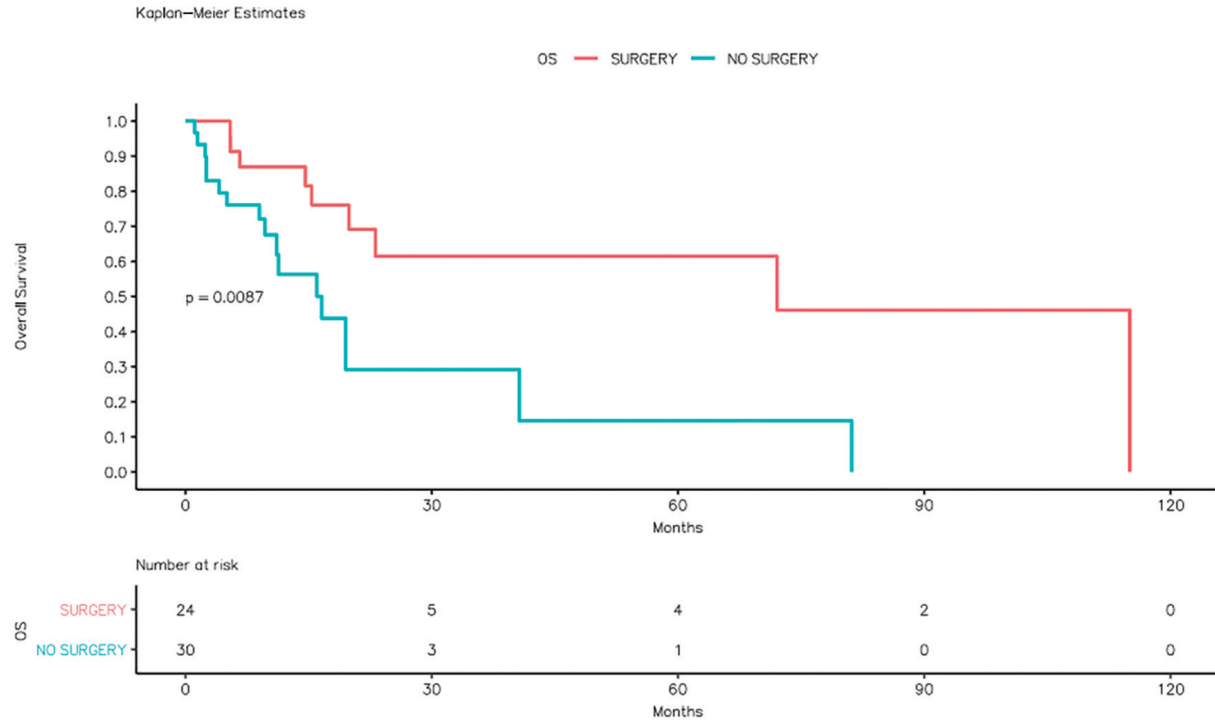


Figure 2. Kaplan-Meier curve for OS in patients with locally advanced ESCC according to treatment modality
OS: Overall survival, ESCC: Esophageal squamous cell carcinoma

Adjusted Survival Analyses

In the 90-day landmark analysis (excluding three early deaths in the definitive CRT group), surgery remained significantly associated with improved survival (landmark OS: median 72.1 vs. 15.8 months, $p=0.012$).

In multivariate Cox regression analysis, after adjustment for age, sex, cT, cN, tumor site, ECOG PS, and concurrent CRT, surgery was an independent predictor of longer OS (HR: 0.41, 95% CI: 0.22-0.78, $p=0.006$) and PFS (HR: 0.44, 95% CI: 0.24-0.81, $p=0.008$).

After weighting, all covariates achieved adequate balance (all SMDs <0.10), confirming successful adjustment for baseline differences between groups (Supplementary Table 1). Weighted analyses confirmed a consistent benefit of surgery, with IPTW-adjusted HRs of 0.46 for OS and 0.49 for PFS (both $p<0.01$). These findings were concordant with the unadjusted Kaplan-Meier results, supporting the robustness of the observed survival advantage. Adjusted analyses are summarized in Table 3.

Discussion

In this real-world cohort of patients with locally advanced ESCC, we found that trimodality therapy was associated with a substantial and consistent survival advantage compared with dCRT. Importantly, this association remained robust across multiple analytical approaches, including multivariable adjustment, propensity score-based weighting, and landmark analysis. These findings suggest that the observed survival benefit is unlikely to be solely explained by baseline imbalances between treatment groups.

A key challenge in interpreting comparative studies between trimodality therapy and dCRT is the presence of treatment selection bias. Patients selected for surgery are inherently more likely to have favorable clinical characteristics, including better performance status and lower tumor burden. As a result, the magnitude of benefit attributed to surgery is often questioned. Our study aimed to minimize this bias and more accurately estimate the independent association between treatment strategy and survival by applying IPTW. Notably, the consistency between unadjusted and weighted analyses conducted in our cohort suggests that the association between surgery and improved survival persisted after adjustment for measured baseline differences (Supplementary Table 1).

While previous randomized and observational studies have suggested a survival benefit with trimodality therapy, most have focused on demonstrating superiority rather than quantifying the magnitude of benefit under real-world conditions. In this context, our findings provide clinically relevant estimates of improvement in survival after adjustment for confounding factors. The observed hazard reductions for both OS and PFS indicate that the effect size associated with surgery is not only statistically significant but also clinically meaningful [13,14].

In our cohort, the median OS for patients treated with surgery after chemoradiotherapy was 72.1 months, compared with 16.6 months for the dCRT group ($p=0.009$). Similarly, the median PFS was significantly longer in the surgical group (67.6 vs. 9.2 months, $p=0.007$). These results indicate that surgical resection, when feasible, provides a substantial survival benefit beyond local control.

The higher proportion of patients with unknown cT stage in the non-surgical group likely reflects diagnostic limitations among patients with poor performance status or advanced local invasion, both of which preclude endoscopic evaluation. Although staging heterogeneity is a limitation of retrospective datasets, our sensitivity analysis confirmed that the survival benefit associated with surgery persisted even after excluding patients with incomplete staging data, thereby supporting the robustness of our findings.

The CROSS trial firmly established neoadjuvant CRT followed by surgery as the standard of care for resectable ESCC, reporting a median OS of 49 months in the CRT-surgery arm compared with 24 months for surgery alone, and an R0 resection rate of 92% [5]. Our study extends these observations by comparing trimodality therapy with definitive chemoradiation, showing that even in a real-world cohort, the addition of surgery remains prognostically favorable. All survival endpoints were measured from the initiation of chemoradiotherapy to ensure consistent time-zero definitions between treatment groups. This approach minimizes bias arising from treatment-related delays or differences in the timing of surgery. A sensitivity analysis using the date of diagnosis as time-zero produced similar results, confirming that the observed survival advantage was not affected by temporal definition bias. This aligns with the findings of Teoh et al. [7], who reported improved long-term outcomes with surgery compared to definitive CRT in resectable squamous carcinoma of the esophagus.

Table 3. Adjusted survival analyses according to treatment approach				
Analysis type	Model adjustment	Endpoint	HR (95% CI)	p value
Multivariate Cox	Adjusted for age, sex, cT, cN, tumor site, ECOG PS, and concurrent CRT	Overall survival	0.41 (0.22-0.78)	0.006
		Progression-free survival	0.44 (0.24-0.81)	0.008
IPTW-weighted Cox	IPTW based on propensity scores	Overall survival	0.46 (0.28-0.75)	<0.01
		Progression-free survival	0.49 (0.30-0.79)	<0.01
90-day Landmark	Early deaths within 90 days excluded	Overall survival	Median 72.1 vs. 15.8 months	0.012
ECOG: Eastern Cooperative Oncology Group, CRT: Chemoradiotherapy, IPTW: Inverse probability of treatment weighting, HR: Hazard ratio, PS: Performance status, CI: Confidence interval, cT: Clinical T, cN: Clinical N				

Conversely, the Fédération Francophone de Cancérologie Digestive 9102 trial reported no significant OS difference between surgery and definitive chemoradiation, although the trial was limited by high treatment-related mortality (11%) and older radiotherapy techniques [6]. More contemporary analyses, including real-world data from Asia, have consistently shown that surgery following CRT leads to better local control and survival outcomes in fit patients [15-17]. Qian et al. [18] reported that among patients with locally advanced ESCC who achieved a clinical CR after chemoradiotherapy, dCRT resulted in OS outcomes comparable to those observed with trimodality therapy. These findings suggest that omission of surgery may represent a reasonable treatment strategy in carefully selected patients who have a favorable response to chemoradiotherapy, supporting a more individualized, response-adapted treatment approach.

A substantial systematic review and meta-analysis, encompassing over 3,000 patients, has indicated that those subjected to neoadjuvant CRT followed by surgery exhibited considerably elevated 3- and 5-year survival rates in comparison with those undergoing definitive CRT (HR=0.63, $p<0.001$) [8]. Similarly, Lin et al. [16] showed that trimodality therapy resulted in superior locoregional control and OS in thoracic ESCC, particularly among younger and fit patients. A more recent Asian multicenter retrospective study by Zhang et al. [17] also demonstrated that patients who were initially unresectable (T4) but downstaged after CRT and underwent surgery achieved significantly improved OS and disease-free survival compared to those who received CRT alone. Collectively, these studies support the concept that surgery remains a key determinant of long-term disease control in operable patients.

Tumor characteristics, especially tumor depth, emerged as significant prognostic determinants in our cohort. Patients with T1-T2 tumors predominated in the surgery group, whereas T4 disease was limited to non-surgical cases, underscoring the role of locoregional invasion in determining operability. The near-significant trend in cN status suggests that extensive nodal involvement may still be compatible with resection, particularly in patients achieving nodal response after CRT. This is consistent with previous data indicating that pCR and nodal downstaging are strong prognostic indicators following neoadjuvant CRT [8,16].

Despite the observed benefits of surgery, definitive CRT remains a crucial curative-intent option for medically inoperable patients or those unwilling to undergo esophagectomy [19,20]. In our study, over half of the cohort received definitive CRT, reflecting the real-world challenges of comorbidities, frailty, and patient preference [21]. Importantly, advances in radiation techniques, such as proton beam therapy and image-guided intensity-modulated radiation therapy, have reduced toxicity while improving local control [22]. Nevertheless, radiation dose escalation beyond standard definitive doses remains controversial. A recent systematic review and meta-analysis by Wang et al. [12] demonstrated that higher radiation doses did not translate into consistent survival benefits, while

being associated with increased treatment-related toxicity, supporting the continued use of standard-dose regimens (approximately 50-50.4 Gy) in dCRT for esophageal cancer. Furthermore, the integration of immune checkpoint inhibitors (e.g., PD-1/PD-L1 blockade) into the CRT setting has shown promising results, potentially redefining the future standard of care for both definitive and neoadjuvant approaches [23,24]. Early-phase trials combining CRT with immunotherapy agents such as nivolumab and camrelizumab have demonstrated encouraging response rates and manageable toxicity profiles [25].

To mitigate potential selection and immortal time biases inherent to retrospective analyses, we performed complementary statistical adjustments, including a 90-day landmark analysis, multivariate Cox regression, and propensity score-based weighting. Each approach consistently confirmed that surgery after chemoradiotherapy remained independently associated with superior OS and PFS. These findings strengthen the validity of our results and suggest that the survival benefit observed for trimodality therapy is not solely attributable to baseline differences in patient fitness or tumor stage.

Importantly, definitive CRT remains a valuable curative-intent option for patients who are medically inoperable or who decline surgery. In our cohort, approximately half of the patients received non-surgical treatment, reflecting real-world constraints related to patient comorbidity and preferences. Technological advances in radiotherapy delivery and immunotherapy combinations may further improve the efficacy of definitive CRT.

Study Limitations

This study has several limitations inherent to its retrospective and single-center design. The relatively small sample size and the specific referral patterns in our region, where ESCC is highly prevalent, may limit the generalizability of the findings. Nevertheless, the inclusion of all consecutive patients and the use of robust adjustment methods (multivariate and IPTW analyses) mitigate selection and information bias to a considerable extent. Moreover, our real-world data reflect clinical practice patterns from a high-incidence area in eastern Turkey and provide unique insights into the management of locally advanced ESCC in resource-limited settings.

These results may inform multidisciplinary decision-making in similar populations, particularly the choice between trimodality therapy and definitive CRT.

Conclusion

After bias-adjusted analyses, surgery was consistently associated with improved survival outcomes in this cohort. Surgical resection should be considered whenever feasible within a multidisciplinary treatment strategy. Definitive CRT remains an appropriate curative-intent option for selected patients who are not surgical candidates. Larger prospective studies are warranted to further refine patient selection and optimize multimodal treatment approaches.

Ethics

Ethics Committee Approval: The study was approved by the University of Health Sciences Türkiye, Ankara Etlik City Hospital Scientific Research Evaluation and Ethics Committee No. 1 (approval no: AEŞH-BADEK1-2025-694, date: 17.12.2025).

Informed Consent: This retrospective study.

Footnotes

Authorship Contributions

Concept: İ.D., E.E.K., O.S., Ö.A.İ., G.K., G.İ.İ., Ş.E., Design: İ.D., E.E.K., G.İ.İ., D.Y., Data Collection or Processing: İ.D., E.E.K., D.Y., M.T.B., S.D.Ş., Ş.E., A.T.A., Y.G., G.Y., D.M.K., Analysis or Interpretation: İ.D., E.E.K., D.Y., F.A., Y.G., D.M.K., Literature Search: İ.D., G.K., Ş.E., Y.G., G.Y., D.M.K., Writing: İ.D., Ö.A.İ., B.A., M.T.B., S.D.Ş., Ş.E.

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Supplementary Tables: <https://d2v96fxpocvxx.cloudfront.net/d363ec1e-9e5e-4591-a00a-d656bfcabb80/content-images/bc33ff9e-87f2-460a-87bb-54435150cb41.pdf>

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Original Article

Diagnostic Contribution of Mucicarmine Staining in TTF-1- and p40-negative Non-small Cell Lung Carcinoma Diagnosed on Small Biopsy Specimens

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ABSTRACT

Aim: Accurate subclassification of non-small cell lung carcinoma (NSCLC) in small biopsy specimens is essential for appropriate therapeutic decision-making and molecular testing. However, accurate subclassification remains challenging in a subset of tumors, particularly in those lacking both thyroid transcription factor-1 (TTF-1) and p40 expression. This study aimed to evaluate the diagnostic contribution of histochemical mucicarmine positivity in the adenocarcinoma-oriented subclassification of TTF-1 and p40-negative NSCLC cases diagnosed from small biopsy specimens.

Methods: This retrospective study included 120 NSCLC cases that were negative for TTF-1 and p40, diagnosed from small biopsy specimens. All cases underwent histochemical mucicarmine staining. Clinicopathological variables, including patient age, sex, biopsy type, mucin staining pattern, initial diagnostic category, and method of final diagnostic confirmation, were evaluated. Statistical analyses were performed using the chi-square and Fisher's exact tests.

Results: The mean age of the patients was 67.75±9.82 years, and 81.7% of the patients were male. Histochemical mucin positivity was observed in 116 of 120 cases (96.7%): focal in 45 cases (37.5%), diffuse in 41 cases (34.2%), and of unspecified extent in 30 cases (25.0%). Only four cases (3.3%) were mucin-negative and remained classified as NSCLC-not otherwise specified (NSCLC-NOS). A statistically significant association was observed between mucin positivity and adenocarcinoma-oriented subclassification ($p<0.001$). No significant association was identified between biopsy modality and mucin positivity ($p=0.296$).

Conclusion: Histochemical mucicarmine positivity provides valuable diagnostic support for adenocarcinoma-oriented subclassification in TTF-1- and p40-negative NSCLC cases diagnosed on small biopsy specimens. Mucicarmine staining is a practical, inexpensive, tissue-preserving adjunctive technique that may help reduce the frequency of NSCLC-NOS diagnoses in diagnostically challenging cases.

Keywords: Adenocarcinoma, biopsy, lung cancer, mucin, pathology

Introduction

Lung cancer remains the leading cause of cancer-related mortality worldwide, and approximately 70% of patients present with advanced-stage disease at the time of diagnosis. In this setting, small biopsy and cytology specimens frequently constitute the only available diagnostic material for histopathological evaluation and molecular testing. Accordingly, accurate subclassification of non-small cell lung

carcinoma (NSCLC) in limited tissue samples has become increasingly important in the era of personalized therapy and predictive biomarker testing [1-4].

Current World Health Organization (WHO) and International Association for the Study of Lung Cancer (IASLC) recommendations emphasize minimizing use of the NSCLC-not otherwise specified (NSCLC-NOS) category and encourage subclassification as adenocarcinoma or squamous cell

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carcinoma whenever possible. Preservation of tissue for molecular testing has become a critical component of modern thoracic pathology practice. Therefore, current diagnostic algorithms advocate the use of limited immunohistochemical panels capable of achieving accurate subclassification while minimizing tissue consumption for downstream molecular analyses, including EGFR, ALK, ROS1, KRAS, and PD-L1 testing [1-6].

In routine practice, thyroid transcription factor-1 (TTF-1) and p40 represent the most widely accepted minimal immunohistochemical panel for subclassification of NSCLC in small biopsy specimens. However, a subset of tumors remains difficult to classify, particularly poorly differentiated carcinomas that lack glandular morphology and are negative for both markers. This challenge is particularly relevant in mucinous and poorly differentiated adenocarcinomas, in which TTF-1 expression may be absent or significantly reduced. Previous studies have demonstrated that invasive mucinous adenocarcinomas frequently exhibit low rates of TTF-1 and Napsin A expression, highlighting the limitations of relying solely on immunohistochemistry in this subgroup [7,8].

Although immunohistochemistry has become the cornerstone of NSCLC subclassification, histochemical mucin stains continue to represent a practical and widely available ancillary diagnostic tool. Current WHO- and IASLC-based recommendations recognize mucin positivity as supportive evidence of glandular differentiation, particularly in small biopsy specimens lacking definitive morphologic or immunophenotypic features. Several studies have demonstrated that histochemical mucin staining may improve subclassification accuracy and reduce the frequency of NSCLC-NOS diagnoses in limited specimens. In particular, the combined use of TTF-1 and mucin staining has been shown to improve adenocarcinoma-oriented subclassification in poorly differentiated NSCLC [2-4,9-11]. Recent review articles have also emphasized that histochemical mucin stains remain valuable ancillary diagnostic tools in selected morphologically and immunophenotypically challenging cases of NSCLC, despite major advances in immunohistochemistry and molecular pathology [12].

Despite these observations, studies specifically evaluating the diagnostic contribution of histochemical mucin positivity in TTF-1- and p40-negative NSCLC diagnosed on small biopsy specimens remain limited. Accordingly, the present study was undertaken to evaluate the diagnostic contribution of histochemical mucicarmine positivity in the adenocarcinoma-oriented subclassification of TTF-1- and p40-negative NSCLC diagnosed on small biopsy specimens and to determine whether this approach could reduce the frequency of NSCLC-NOS diagnoses in routine practice.

Methods

Ethical Statement

This study was approved by the Scientific Studies Ethics Committee of University of Health Sciences Türkiye, Ankara Atatürk Sanatorium Training and Research Hospital (approval

no: 2024-BÇEK/559, date: 06.05.2026). All procedures were performed in accordance with the ethical principles of the Declaration of Helsinki. Due to the retrospective nature of the study, informed consent was waived.

Study Design and Case Selection

This retrospective, single-center, observational study included consecutive patients diagnosed with NSCLC from small biopsy specimens between January 2023 and January 2026. Cases were identified by a retrospective search of the pathology archives in the Department of Pathology at University of Health Sciences Türkiye, Ankara Atatürk Sanatorium Training and Research Hospital.

Inclusion criteria were as follows:

1. Evaluation of small biopsy material, including bronchoscopic biopsy or transthoracic needle biopsy,
2. Absence of TTF-1 and p40 expression on immunohistochemical examination,
3. Availability of histochemical mucin staining,
4. Initial diagnosis rendered as “NSCLC, favor adenocarcinoma” or “NSCLC-NOS,”
5. Final diagnosis of adenocarcinoma established either by examination of resection specimens or clinicoradiologic correlation.

Cases diagnosed with small cell lung carcinoma or squamous cell carcinoma, and those with insufficient biopsy material or technically inadequate mucicarmine staining, were excluded. The study workflow is summarized as follows: eligible TTF-1/p40-negative NSCLC cases diagnosed on small biopsies were identified retrospectively. Histochemical mucicarmine staining results were reviewed, and the final diagnosis was confirmed by resection specimens or by clinicoradiologic correlation, when available.

Clinicopathological Parameters

The following clinicopathological variables were recorded from pathology reports and hospital records:

- patient age and sex,
- biopsy type (bronchoscopic biopsy or transthoracic needle biopsy),
- histochemical mucin status,
- initial diagnostic category,
- method of final diagnosis confirmation.

Initial diagnostic categories were defined as “NSCLC, favor adenocarcinoma” and “NSCLC-NOS.”

The final diagnosis was established either by evaluation of the resection specimens or by clinicoradiologic correlation.

Histochemical Evaluation

Histochemical evaluation of mucin production was performed on formalin-fixed, paraffin-embedded tissue sections using mucicarmine staining as part of the routine diagnostic workup. All stained slides were reviewed by experienced pulmonary

pathologists. Distinct intracellular and/or luminal mucin staining within tumor cells was considered positive.

Based on the extent of staining described in the pathology reports, mucin positivity was categorized as focally positive, diffusely positive, or positive with unspecified extent. Cases lacking detectable staining were classified as mucin negative.

Immunohistochemistry

All cases included in the study showed a complete absence of TTF-1 and p40 expression by routine diagnostic immunohistochemistry. Representative histopathological, immunohistochemical, and histochemical findings are shown in Figure 1.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, version 27.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were expressed as mean $\pm$ standard deviation for continuous variables and as frequency and percentage for categorical variables. Associations between mucin positivity and clinicopathological variables, including initial diagnostic category, biopsy type, and final diagnostic method, were evaluated using the chi-square test or Fisher's exact test, where appropriate. A p value <0.05 was considered statistically significant.

Results

A total of 120 patients with NSCLC negative for TTF-1 and p40, diagnosed on small biopsy specimens were included in the study. All cases underwent histochemical mucin staining, and a final diagnosis of adenocarcinoma was established either by resection specimens or clinicoradiologic correlation. The clinicopathological characteristics of the study cohort are summarized in Table 1.

The mean age of the patients was 67.75 ± 9.82 years (range, 30-87 years). The cohort was predominantly male, comprising 98 men (81.7%) and 22 women (18.3%).

Regarding biopsy type, 78 cases (65.0%) were obtained by transthoracic needle biopsy, whereas 42 cases (35.0%) were obtained by bronchoscopic biopsy.

Histochemical mucin positivity was observed in 116 of 120 cases (96.7%). Among mucin-positive cases, focal positivity was observed in 45 cases (37.5%), diffuse positivity in 41 cases (34.2%), and positivity of unspecified extent in 30 cases (25.0%). Only 4 cases (3.3%) were completely negative for mucin staining.

Based on the initial pathological evaluation of the small biopsy specimens, 116 cases (96.7%) were classified as "NSCLC, favor adenocarcinoma," whereas 4 cases (3.3%) remained categorized as "NSCLC-NOS." Notably, all mucin-negative cases remained within the NSCLC-NOS category.

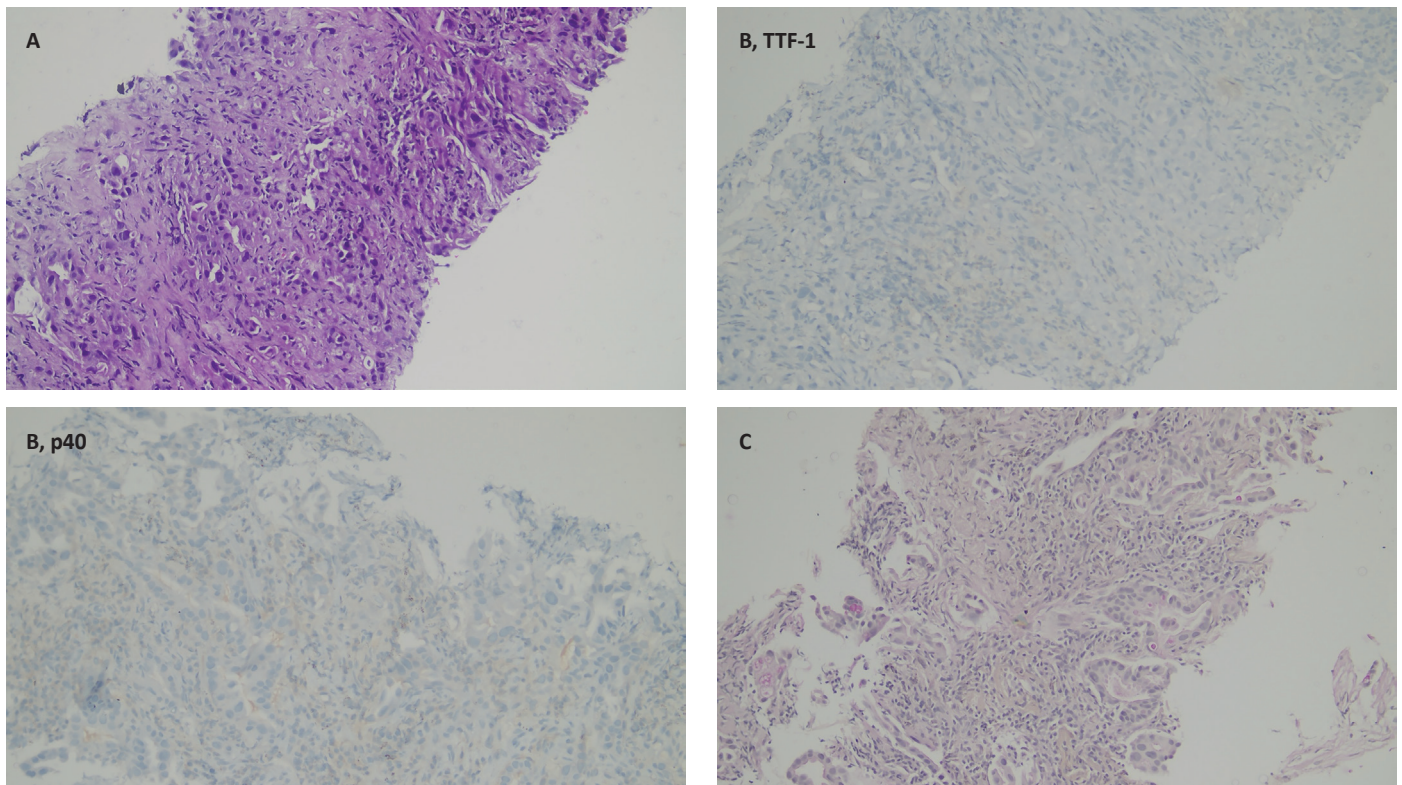


Figure 1. Representative TTF-1-negative and p40-negative NSCLC diagnosed on transthoracic needle biopsy. **(A)** H&E section showing a poorly differentiated NSCLC lacking definitive glandular or squamous differentiation, **(B)** Tumor cells are negative for TTF-1 and p40 immunohistochemical staining, **(C)** Mucicarmine stain demonstrates intracytoplasmic mucin positivity supporting adenocarcinoma-oriented subclassification NSCLC: Non-small cell lung carcinoma, TTF-1: Thyroid transcription factor-1

Table 1. Clinicopathological characteristics of the study cohort

Variable	Value
Age, mean $\pm$ SD (range)	67.75 $\pm$ 9.82 (30-87)
Sex, n (%)	
Male	98 (81.7)
Female	22 (18.3)
Biopsy type, n (%)	
Bronchoscopic biopsy	42 (35.0)
Transthoracic needle biopsy	78 (65.0)
Mucin status, n (%)	
Negative	4 (3.3)
Focal positive	45 (37.5)
Diffuse positive	41 (34.2)
Positive, extent unspecified	30 (25.0)
Initial diagnosis, n (%)	
NSCLC favor adenocarcinoma	116 (96.7)
NSCLC-NOS	4 (3.3)
Final diagnosis method, n (%)	
Resection specimen	13 (10.8)
Clinicoradiologic correlation	107 (89.2)

SD: Standard deviation, NSCLC-NOS: Non-small cell lung carcinoma-not otherwise specified

A statistically significant association was observed between mucin positivity and the adenocarcinoma-oriented subclassification (Fisher's exact test, $p < 0.001$). Nearly all mucin-positive cases could be subclassified as NSCLC favor adenocarcinoma, supporting the diagnostic contribution of histochemical mucin staining in this challenging subgroup of tumors.

No statistically significant association was found between transthoracic needle biopsy and bronchoscopic biopsy specimens with respect to mucin positivity (Fisher's exact test,

$p = 0.296$), suggesting that the utility of mucin staining does not depend on biopsy type.

Final diagnosis was established by clinicoradiologic correlation in 107 cases (89.2%) and by resection specimens in 13 cases (10.8%). A statistically significant association was observed between mucin positivity and the final diagnostic method (Fisher's exact test, $p = 0.004$). Associations between mucin positivity and clinicopathological parameters are presented in Table 2. Mucin-negative cases more frequently required confirmation on resection specimens, whereas most mucin-positive cases were confirmed by clinicoradiologic correlation.

Discussion

Accurate subclassification of NSCLC in small biopsy specimens has become increasingly important in contemporary thoracic pathology because treatment selection, predictive biomarker assessment, and molecular testing are largely dependent on histologic subtype. At the same time, preservation of limited tissue material remains a major concern, particularly in patients with advanced-stage disease in whom small biopsies frequently represent the only available diagnostic specimens. Consequently, current WHO- and IASLC-based approaches advocate the use of minimal ancillary testing while aiming to reduce the use of the NSCLC-NOS category whenever possible [1-6]. Recent studies based on the 5th WHO classification have further confirmed that limited immunohistochemical panels can accurately classify most NSCLCs while preserving tissue for molecular testing [4].

In routine practice, TTF-1 and p40 constitute the most widely accepted minimal immunohistochemical panel for subclassification of NSCLC in small biopsies. Nevertheless, a diagnostically challenging subgroup remains, particularly among poorly differentiated tumors and mucinous adenocarcinomas showing negativity for both markers. In these cases, subclassification may be difficult because classical morphologic features such as gland formation or keratinization are frequently absent in limited tissue samples [2-4,7].

Table 2. Association between mucin positivity and clinicopathological parameters

Variable	Mucin negative n (%)	Mucin positive n (%)	p value
Sex			
Male	4 (100)	94 (81.0)	0.571
Female	0 (0)	22 (19.0)	
Biopsy type			
Bronchoscopic biopsy	0 (0)	42 (36.2)	0.296
Transthoracic needle biopsy	4 (100)	74 (63.8)	
Initial diagnosis			
NSCLC favor adenocarcinoma	0 (0)	116 (100)	<0.001
NSCLC-NOS	4 (100)	0 (0)	
Final diagnosis method			
Resection specimen	3 (75.0)	10 (8.6)	0.004
Clinicoradiologic correlation	1 (25.0)	106 (91.4)	

Statistical analysis was performed using Fisher exact test

NSCLC-NOS: Non-small cell lung carcinoma-not otherwise specified

In the present study, histochemical mucin positivity was identified in 96.7% of TTF-1- and p40-negative NSCLC cases. Only four cases remained mucin-negative and consequently retained the diagnosis of NSCLC-NOS. Furthermore, a statistically significant association was observed between mucin positivity and adenocarcinoma-oriented subclassification. These findings suggest that mucicarmine staining provides meaningful diagnostic support for a subgroup of tumors that often prove problematic when diagnosed on the basis of morphology and immunohistochemistry alone.

Our findings are consistent with previous studies evaluating the contribution of histochemical mucin stains to NSCLC subclassification. Loo et al. [10] demonstrated that the combined use of TTF-1 and mucin staining provided one of the most effective approaches for predicting adenocarcinoma differentiation and substantially reduced the frequency of NSCLC-NOS diagnoses in small biopsy specimens. These observations are also consistent with recent studies showing that the judicious use of ancillary stains in small biopsy specimens improves diagnostic confidence while preserving tissue for molecular profiling [5].

Similarly, Nicholson et al. [9] reported that incorporation of mucin stains into limited diagnostic panels improved the refinement of NSCLC subclassification and increased diagnostic concordance among pathologists.

More recently, WHO 5th edition-based studies have confirmed that a substantial proportion of poorly differentiated NSCLCs can be accurately subclassified using limited ancillary testing, with mucin positivity serving as supportive evidence of glandular differentiation in selected cases [3,4].

Although the increasing availability of immunohistochemical and molecular techniques has transformed thoracic pathology, recent reviews continue to highlight that ancillary histochemical stains retain an important role in selected diagnostically challenging small biopsy specimens, particularly when tissue preservation for molecular testing is a priority [12]. Mucicarmine staining is inexpensive, technically simple, widely available, and requires considerably less tissue than extended immunohistochemical panels. Therefore, it remains an attractive adjunctive method in resource-limited settings and in cases where tissue preservation is a priority for molecular testing [5,9]. Accordingly, contemporary diagnostic recommendations continue to recognize histochemical mucin stains as valuable ancillary tools in selected diagnostically challenging cases.

The biological plausibility of our findings is further supported by the distinctive characteristics of invasive mucinous adenocarcinoma. Previous studies have demonstrated that these tumors frequently exhibit reduced or absent expression of TTF-1 and Napsin A, likely reflecting alterations in lineage-specific differentiation pathways [7,8].

Consequently, reliance solely on immunohistochemical markers may result in diagnostic uncertainty. In such settings, histochemical demonstration of intracellular mucin may provide valuable supportive evidence favoring adenocarcinoma differentiation.

Another important consideration is the growing recognition that TTF-1-negative NSCLCs may represent a biologically distinct subgroup. Recent studies have suggested that loss of TTF-1 expression is associated with less favorable clinical outcomes in advanced non-squamous NSCLC, further emphasizing the importance of accurate characterization of these tumors beyond conventional immunophenotypic classification [13].

Another notable finding in our study was the absence of a significant association between biopsy modality and mucin positivity. The diagnostic contribution of mucicarmine staining appeared comparable between transthoracic needle biopsies and bronchoscopic biopsy specimens, suggesting that the utility of mucin evaluation is not dependent on sampling technique. Similar observations have been reported in cytology-based studies, where mucicarmine staining improved subclassification concordance and reduced NSCLC-NOS diagnoses in bronchial brushing specimens [11].

Study Limitations

The present study has several limitations. First, its retrospective, single-center design may introduce selection bias. Second, molecular data were not available for all cases, precluding assessment of the correlation between mucin positivity and specific genomic alterations. Third, the absence of a control group composed of definitively classified cases of squamous cell carcinoma precluded formal assessment of diagnostic sensitivity and specificity. Finally, mucin positivity alone cannot establish a primary pulmonary origin and should always be interpreted in conjunction with morphologic, immunophenotypic, clinical, and radiologic findings.

Conclusion

Our findings demonstrate that histochemical mucicarmine positivity provides substantial diagnostic support for adenocarcinoma-oriented subclassification in TTF-1- and p40-negative NSCLC cases diagnosed on small biopsy specimens. As a practical, inexpensive, and tissue-preserving ancillary technique, mucicarmine staining may help reduce the frequency of NSCLC-NOS diagnoses while improving diagnostic confidence in diagnostically challenging small biopsy specimens.

Ethics

Ethics Committee Approval: This study was approved by the Scientific Studies Ethics Committee of University of Health Sciences Türkiye, Ankara Atatürk Sanatorium Training and Research Hospital (approval no: 2024-BÇEK/559, date: 06.05.2026).

Informed Consent: This retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: N.G., F.D., M.B.K.Y., Ç.Y.M., H.A., P.A.K., B.B.İ., Concept: N.G., Design: N.G., Data Collection

or Processing: N.G., F.D., M.B.K.Y., Ç.Y.M., H.A., P.A.K., B.B.İ., Analysis or Interpretation: N.G., F.D., M.B.K.Y., Ç.Y.M., Literature Search: N.G., Writing: N.G.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article

Retrospective Analysis of 17p Deletion Status and Treatment Modalities in Patients with Chronic Lymphocytic Leukemia

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ABSTRACT

Aim: This study aimed to examine the demographic characteristics, disease stages, 17p-deletion status, complications, treatment approaches, and survival data of 308 chronic lymphocytic leukemia (CLL) patients followed in the Department of Hematology at Dicle University.

Methods: A retrospective analysis was conducted on 308 patients diagnosed with CLL between January 2015 and December 2019. Data were compiled from patient records, including demographic information, complete blood counts, lactate dehydrogenase levels, lymphocyte counts, flow cytometry results, cytogenetic analysis (17p deletion), and imaging findings. Patients were classified according to the Rai and Binet staging systems, and their treatment protocols and disease complications were examined.

Results: In a retrospective analysis of 308 CLL patients, 61.7% were male, 38.3% were female, and the median age was 63 (28-86). Of the 170 patients studied for 17p deletion, 15.3% were found to be positive for 17p deletion. When patients were staged according to Rai, 8.4% were stage 0, 42.2% were stage I, 31.5% were stage II, 12.3% were stage III, and 5.5% were stage IV. According to the Binet staging, 29.5% of the patients were classified as stage A, 57.1% were classified as stage B, and 13.3% were classified as stage C. Secondary immunodeficiency was observed in 18.8% of the patients, autoimmune hemolytic anemia in 10.1%, and Richter transformation in 2.9%. One hundred ninety-nine patients (64.6%) received treatment, while 109 (35.4%) did not. During a median follow-up of 68.1 months, 38.3% of patients died. Patients with more advanced Rai and Binet stages had significantly shorter survival times. The median overall survival of patients with a positive 17p deletion was 83.4 months, while that of patients with a negative 17p deletion was 116 months ($p=0.015$).

Conclusion: The 17p deletion is associated with poor prognosis in CLL and is an important marker for guiding treatment and monitoring survival. The patient profile, stage, and treatment data from this regional study can contribute to the literature and guide the development of personalized approaches.

Keywords: Chronic lymphocytic leukemia, 17p deletion, leukemia, ibrutinib, venetoclax

Introduction

Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults and is characterized by the clonal proliferation of mature B-lymphocytes. CLL is typically an indolent malignancy [1]. In Western countries, CLL constitutes a substantial proportion of adult leukemias; most patients are elderly, and the disease has a higher incidence in males. Genetic predisposition (such as 17p deletion and TP53 mutations) and environmental factors contribute to its etiology [1,2].

The clinical presentation of CLL is heterogeneous; most patients are asymptomatic and diagnosed incidentally via routine blood tests revealing lymphocytosis. Symptomatic cases may present with lymphadenopathy, splenomegaly, and infections [3]. Prognosis is guided by the Rai and Binet staging systems. Furthermore, genetic abnormalities including 17p deletion, 13q deletion, 11q deletion, and trisomy 12 hold prognostic and predictive significance [4-6]. The 17p deletion is a negative prognostic factor associated with shorter survival. Patients with 17p deletion have a worse response to treatment [7]. CLL

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complications include Richter transformation, autoimmune hemolytic anemia (AIHA), and secondary immunodeficiency [8].

Treatment strategies vary based on disease stage and risk profile; a “watch and wait” approach is employed in early-stage disease, whereas advanced stages are managed with targeted therapies such as Bruton tyrosine kinase inhibitors (ibrutinib, acalabrutinib), B-cell lymphoma 2 inhibitors (venetoclax), and monoclonal antibodies (rituximab) or with chemotherapy-based regimens. In recent years, chemotherapy-free approaches have improved survival; however, outcomes remain poor in relapsed/refractory cases. Emerging therapies, including chimeric antigen receptor T-cell therapy, show promising potential [1,9].

In this single-center retrospective study, we analyzed the demographic characteristics, disease stages, 17p deletion status, complications, treatment modalities, and survival outcomes of 308 CLL patients to characterize the regional patient profile and contribute to the existing literature.

Methods

Patients

This study is a retrospective analysis of 308 patients diagnosed with CLL and/or followed up at the Department of Hematology, Dicle University, between January 2015 and December 2019. This study was conducted with the approval of the Non-Interventional Clinical Research Ethics Committee of Dicle University Faculty of Medicine (approval no: 338, date: 03.06.2021). Data were extracted from the hospital registry system and included the following: demographic characteristics (age, gender); complete blood count at initial presentation; peripheral blood smear examination; lactate dehydrogenase levels; absolute lymphocyte count; immunophenotypic evaluation by flow cytometry (including prognostic surface markers); and cytogenetic analysis results. Organomegaly and lymphadenopathy detected by imaging studies were also documented. Disease staging was performed according to the Rai and Binet systems. Treatment data, including chemotherapy regimens administered, were recorded. Among patients receiving multiple lines of therapy, the first-line treatment, the total number of treatment lines, and subsequent therapies were analyzed. Survival endpoints were defined as overall survival (OS), calculated from the date of diagnosis to the date of last follow-up or death, and treatment-free survival (TFS), calculated from the date of diagnosis to the initiation of first-line therapy.

Inclusion Criteria: Adult patients (≥ 18 years) diagnosed with CLL or small lymphocytic lymphoma between January 2015 and December 2019, with complete demographic, laboratory, and follow-up data available in the hospital registry system, were included.

Exclusion Criteria: Patients with a prior or subsequent diagnosis of another malignancy, incomplete staging or treatment information because of missing data, or a follow-up duration of less than 3 months were excluded from the study.

Statistical Analysis

Categorical variables were analyzed using the chi-square test. Continuous variables that did not follow a normal distribution were evaluated using the Mann-Whitney U and Kruskal-Wallis tests. Survival analyses were performed using the Kaplan-Meier method, and comparisons of survival curves were conducted with the log-rank test. A p value < 0.05 was considered statistically significant.

Results

A total of 308 patients were included in the study, of whom 190 (61.7%) were male and 118 (38.3%) were female. The median age was 63 years (range: 28-86 years). The 17p deletion status was evaluated in 170 patients; results were negative in 144 patients and positive in 26 patients (15.3%). The demographic and laboratory characteristics of the patients are presented in Table 1.

According to the Rai staging system, 26 (8.4%) patients were stage 0, 130 (42.2%) were stage I, 97 (31.5%) were stage II, 38 (12.3%) were stage III, and 17 (5.5%) were stage IV. Regarding Binet staging, 91 patients (29.5%) were stage A, 176 patients

Table 1. Demographic and laboratory characteristics

Characteristic	
Age	
Median (range)-years	63 (28-86)
Gender	
Male	190 (61.7%)
Female	118 (38.3%)
Leukocytes (/μL)	
Median (range)	28.7 (8.9-386)
Lymphocytes (/μL)	
Median (range)	21.2 (5-311)
Neutrophils (/μL)	
Median (range)	4.9 (0.6-16.9)
Hemoglobin (g/dL)	
Median (range)	13.1 (6.3-17.8)
Platelets (μL)	
Median (range)	195 (6.3-17.8)
Erythrocyte sedimentation rate (mm/h)	
Median (range)	11 (1-95)
Lactate dehydrogenase (IU/L)	
Median (range)	226 (95-1056)
Direct coombs	
Positive - n (%)	34 (11%)
Negative - n (%)	217 (70.5%)
Not tested - n (%)	57 (18.5)
Indirect coombs	
Positive - n (%)	8 (2.6%)
Negative - n (%)	217 (78.9%)
Not tested - n (%)	57 (18.5)
17p deletion - n (%)	
Tested	170
Positive	26 (15.3%)
Negative	144 (84.7%)

(57.1%) were stage B, and 41 patients (13.3%) were stage C. Evaluation of secondary complications revealed Richter transformation in 9 patients (2.9%), AIHA in 31 (10.1%), immune thrombocytopenic purpura in 6 (1.9%), and secondary immune deficiency in 58 (18.8%). AIHA was detected in 4 (4.4%) patients in Binet stage A, 22 (12.3%) in stage B, and 5 (13.2%) in stage C ($p=0.10$). Detailed information regarding patient staging and complications is provided in Table 2.

The median follow-up duration was 68.1 months (range: 3.1-186.6 months). Analysis of treatment status and disease stage at diagnosis showed that 109 patients (35.4%) remained untreated (observation), while 199 (64.6%) received treatment. As patients progressed through Binet stages, TFS time decreased significantly ($p<0.001$). Treatment modalities and distribution according to disease stage are summarized in Table 3.

For the 35 patients treated with ibrutinib, the median duration of therapy was 16 months (range: 2-41 months). When patients responses to ibrutinib treatment were examined, 7 showed a complete response, 22 showed a partial response, and 6 had refractory disease. Among the patients, 19 (54%) continued ibrutinib treatment; 10 (28.5%) died due to disease progression, and 6 (17.1%) were switched to venetoclax. Of the 7 patients treated with venetoclax, the median treatment duration was 7 months (range: 3-24 months); 2 (28.5%) continued treatment, while 5 (71.4%) died of disease progression during treatment. When responses to venetoclax treatment were examined, 4 patients showed a partial response and 3 were treatment-refractory. Data concerning patients receiving ibrutinib and venetoclax are shown in Table 4.

Over a median follow-up of 68.1 months (range: 3.4-186.6 months), 118 of the 308 patients (38.3%) died. Since less than 50% of the cohort reached the primary endpoint, the median OS for the total population was not reached. When analyzed by Rai stage, the median OS was 121.9 months [95% confidence interval (CI): 62.3-181.4] for stage 0, 128.7 months (95% CI: 115.5-141.8) for stage I, 105 months (95% CI: 83-127) for stage II, 83.6 months (95% CI: 46-121.1) for stage III, and 84.5 months (95% CI: 36.4-132.6) for stage IV ($p=0.001$). The OS curves by

Table 2. Staging and complications of patients

Characteristic	
Rai staging	
Stage 0 - n (%)	26 (8.4%)
Stage I - n (%)	130 (42.2%)
Stage II - n (%)	97 (31.5)
Stage III - n (%)	38 (12.3)
Stage IV - n (%)	17 (5.5)
Binet staging	
Stage A - n (%)	91 (29.5%)
Stage B - n (%)	176 (57.1%)
Stage C - n (%)	41 (13.3%)
Richter transformation - n (%)	9 (2.9%)
Autoimmune hemolytic anemia - n (%)	31 (10.1%)
Immune thrombocytopenic purpura - n (%)	6 (1.9%)
Secondary immunodeficiency - n (%)	58 (18.8%)

Table 3. Treatment patterns and outcomes

Characteristic	
Binet stage - treated/total patients (%)	
A	34/91 (37.4%)
B	133/179 (74.3%)
C	32/38 (84.2%)
Total	199/308 (64.6%)
Rai stage - treated/total patients (%)	
Stage 0	7/26 (26.9%)
Stage I	66/130 (50.8%)
Stage II	81/97 (83.5%)
Stage III	29/38 (76.3%)
Stage IV	16/17 (94.1%)
Total	199/308 (64.6%)
Number of treatment lines - n (%)	
Untreated	109 (35.4%)
1 line	94 (30.5%)
2 lines	64 (20.8%)
3 lines	22 (7.1%)
4 lines	17 (5.5%)
5 lines	2 (0.6%)
Treatment-free survival duration by Binet stage - median (95% confidence interval) - months	
Stage A	34.1 (22.6-45.6)
Stage B	22.1 (16.5-27.6)
Stage C	2.3 (0-5.5)
Treatment-free survival duration by Rai stage - median (95% confidence interval) - months	
Stage 0	29.4 (18.6-40.2)
Stage I	23.5 (13.3-30.8)
Stage II	27.2 (16.2-38.3)
Stage III	4.1 (0-14.6)
Stage IV	0.8 (0-2)
Treatments received by patients - n (%)	
Untreated	109 (35.4%)
Chlorambucil	95 (30.8%)
R-chlorambucil	6 (1.9%)
FC/R-FC	87 (28.2%)
R-bendamustine	87 (28.2%)
CVP/R-CVP	32 (10.4%)
CHOP/R-CHOP	16 (5.2%)
Ibrutinib	35 (11.4%)
Venetoclax	7 (2.3%)
First-line treatments - n (%)	
Untreated	109 (35.4%)
Chlorambucil	82 (26.6%)
Rituximab-chlorambucil	4 (1.3%)
FC/R-FC	54 (17.5%)
R-bendamustine	36 (11.7%)
CVP/R-CVP	18 (5.8%)
CHOP/R-CHOP	5 (1.6%)

R: Rituximab, FC: Fludarabine + cyclophosphamide, CVP: Cyclophosphamide + vincristine + prednisone, CHOP: Cyclophosphamide + doxorubicin + vincristine + prednisone

Table 4. Patients treated with ibrutinib and venetoclax	
Characteristic	
Patients treated with ibrutinib	
Treatment duration - median (range), months	16 (2-41)
Ongoing treatment - n (%)	19 (54)
Deceased - n (%)	10 (28.5%)
Switched to venetoclax - n (%)	6 (17.1%)
Total	35 (100%)
Patients treated with venetoclax	
Treatment duration - median (range), months	7 (3-24%)
Ongoing treatment - n (%)	2 (28.5%)
Deceased - n (%)	5 (71.4%)
Total	7 (100%)

Rai stage are shown in Figure 1. Regarding Binet staging, the median OS was 145.1 months (95% CI: 114.5-175.7) for stage A; 121.1 months (95% CI: 97.4-126.8) for stage B; and 83.6 months (95% CI: 104.5-129.9) for stage C (p=0.006). Figure 2 also shows the OS curve stratified by Binet stage. Patients with the 17p deletion had a median OS of 83.4 months (95% CI: 60.4-127), whereas patients without the 17p deletion had a median OS of 116 months (95% CI: 89.9-135.9) (p=0.015). The OS curve stratified by 17p deletion status is presented in Figure 3. Comprehensive OS data are summarized in Table 5.

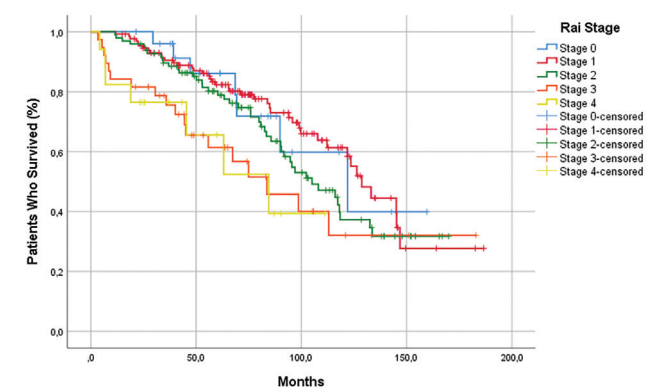


Figure 1. Rai Stage and overall survival

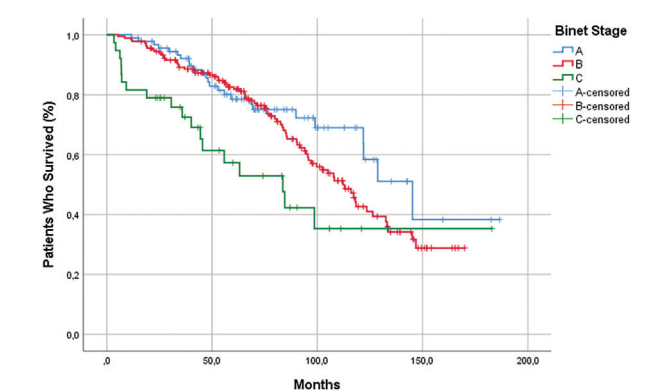


Figure 2. Binet staging and overall survival

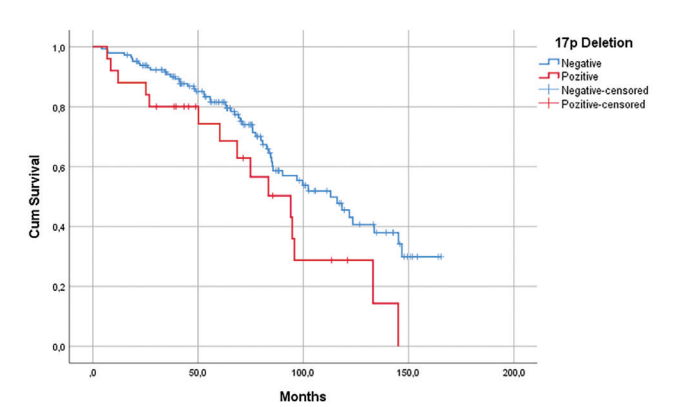


Figure 3. 17p deletion and overall survival

Table 5. Overall survival data	
Characteristic	p
Rai stage - median overall survival, months (95% confidence interval)	
Stage 0	121.9 (62.3-181.4)
Stage I	128.7 (115.5-141.8)
Stage II	105 (83-127)
Stage III	83.6 (46-121.1)
Stage IV	84.5 (36.4-132.6)
Binet stage - median overall survival, months (95% confidence interval)	
Stage A	145.1 (114.5-175.7)
Stage B	121.1 (97.4-126.8)
Stage C	83.6 (104.5-129.9)
17p deletion - median overall survival, months (95% confidence interval)	
Positive	83.4 (60.4-127)
Negative	116 (89.9-135.9)

Discussion

In our study, the male-to-female ratio was 1.6:1. According to 2025 United States statistics [10], this ratio is 1.53:1, while Aslan [11] reported 1.6 in 2013. The median age at diagnosis in our cohort was 63 years, compared with 66 years in the study by Küçük [12] in Aydın, 62 years in the study by Aslan [11] in Ankara, and 71.6 years in the study by Smith et al. [13] in the United Kingdom. The younger median age of CLL patients in Türkiye, compared with Western countries, may be attributed to geographic differences, a higher proportion of younger individuals in Türkiye’s population, and longer life expectancy in Western societies.

Analysis of Rai staging showed that 8.4% of patients were stage 0, 42.2% were stage I, 31.5% were stage II, 12.3% were stage III, and 5.5% were stage IV. In the original 1975 study by Rai et al. [4], the distribution was 17.6% stage 0, 23.2% stage I, 31.2% stage II, 16.8% stage III, and 11.2% stage IV. Aslan [11] reported in 2013 that 18% were stage 0, 21.6% stage I, 27.7% stage II, 12.7% stage III, and 9% stage IV. The lower proportion of stage 0 patients in our cohort may be explained by our center being

the sole hematology referral facility for several provinces for many years, resulting in patients presenting at symptomatic, treatment-requiring stages.

The incidence of AIHA in our study was 10.1%, compared with 11% in the study by Diehl and Ketchum [14] and 4.3% in the study by Mauro et al. [15]. Reported rates of AIHA in the literature range from 4% to 10% [8]. Variations in patient stage, follow-up duration, and demographic characteristics across studies likely account for this heterogeneity. In our study, Richter transformation occurred at a frequency of 2.9%, which is consistent with the 2-9% range reported in the literature [4]. Immune thrombocytopenia was observed in 1.9% of cases, compared with 2% in Diehl and Ketchum [14] and 1.1% in Barcellini et al. [16]. Overall, the frequencies of AIHA and immune thrombocytopenia in our study align closely with published data.

The 17p deletion was detected in 15.3% of tested patients. Hallek et al. [17] reported a rate of 10%, whereas Stilgenbauer et al. [18] found 30%. Sellner et al. [19] noted 3-8% in treatment-naïve patients and up to 30% in treatment-refractory cases. Differences in 17p deletion prevalence across studies may reflect variations in disease stage and prior treatment exposure.

TFS shortened significantly with advancing disease stage, consistent with findings from Rai et al. [4] and Shanafelt et al. [20]. Our results reinforce this established pattern.

OS also decreased significantly with higher Rai stage, mirroring the original observations of Rai et al. [4] in 1975. Shanafelt et al. [20] in 2010 similarly reported stage-dependent reductions in OS, but noted longer survival across all stages compared to the 1975 cohort. Likewise, OS in our study was prolonged compared with historical data, independent of stage. This improvement, seen in contemporary studies including ours and Shanafelt et al. [20], may be attributable to the advent of novel therapies and enhanced access to healthcare.

Patients with 17p deletion exhibited shorter OS, in agreement with the results of Tausch et al. [6] and Stilgenbauer et al. [7]. Thus, our findings are consistent with the literature and validate 17p deletion as a poor prognostic factor for OS. Due to national reimbursement policies during the study period, our patients could not access novel targeted agents such as ibrutinib and venetoclax as first-line therapies; instead, they received these agents in later lines of therapy after chemotherapy failure. The inability to initiate these highly effective targeted therapies in the frontline setting is considered a key factor that prevents their overall efficacy from reaching the high levels reported in the literature.

OS also decreased significantly with higher Rai stage, mirroring the original observations of Rai et al. [4] in 1975. Shanafelt et al. [20] in 2010 similarly reported stage-dependent reductions in OS, but noted longer survival across all stages compared to the 1975 cohort. Likewise, OS in our study was prolonged compared to historical data, independent of stage. This improvement, seen in contemporary studies, including ours and that of Shanafelt et al. [20], may be attributable

to the advent of novel therapies and enhanced access to healthcare.

Patients with 17p deletion exhibited shorter OS, in agreement with Tausch et al. [6] and Stilgenbauer et al. [7]. Thus, our findings confirm the literature and validate 17p deletion as a poor prognostic factor for OS.

Study Limitations

Several limitations of our study should be acknowledged. First, because our study was single-center and retrospective, our findings may primarily reflect the characteristics of a specific geographic region rather than those of a multi-center cohort. Second, the 17p deletion status was not evaluated in all patients; instead, our analysis was based on results available for 55.2% of the study population. Additionally, the median follow-up duration for patients receiving novel therapies, such as ibrutinib and venetoclax, was relatively short, thereby limiting the assessment of long-term treatment response and therapy-related complications. Finally, the absence of data regarding other genetic markers, including immunoglobulin heavy chain variable region mutation status, 11q deletion, and trisomy 12, precluded the establishment of a comprehensive genetic profile for all patients.

Conclusion

In this retrospective analysis of 308 patients with CLL, a significant association was identified between 17p deletion and poor prognosis. The presence of a 17p deletion at any point should prompt consideration of an unfavorable prognosis. As a critical prognostic marker in treatment and survival monitoring, 17p deletion must be carefully considered in the long-term management of CLL. Furthermore, evaluating survival outcomes and treatment efficacy according to disease stage may contribute to the optimization of future therapeutic strategies. These findings will guide the understanding of regional patient characteristics and the development of personalized treatment approaches.

Ethics

Ethics Committee Approval: This study was conducted with the approval of the Non-Interventional Clinical Research Ethics Committee of Dicle University Faculty of Medicine (approval no: 338, date: 03.06.2021).

Informed Consent: The data collection program for this study was approved by ethics committee with a waiver for individual informed consent for this retrospective study.

Footnotes

Authorship Contributions

Concept: M.D.F., A.K., V.D., S.T., M.O.A., Design: M.D.F., A.K., V.D., S.T., M.O.A., Data Collection or Processing: M.D.F., A.K., V.D., S.T., Analysis or Interpretation: M.D.F., A.K., V.D., S.T., M.O.A., Literature Search: M.D.F., A.K., V.D., S.T., M.O.A., Writing: M.D.F., A.K., S.T., M.O.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article

Factor V Leiden Mutation in Women with Recurrent Pregnancy Loss: A Single-center Study

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ABSTRACT

Aim: Recurrent pregnancy loss (RPL) affects approximately 1-2% of women of reproductive age and remains a clinically challenging condition with diverse underlying causes. Inherited thrombophilic abnormalities, particularly Factor V Leiden mutation, have been proposed as potential contributors to its pathogenesis; however, the available evidence remains inconclusive. This study aimed to determine the frequency of the Factor V Leiden mutation in women with RPL and to evaluate whether mutation carrier status was associated with the number of previous pregnancy losses within this cohort. The relationships of maternal age, hemoglobin concentration, and fasting blood glucose levels with the number of previous pregnancy losses were investigated.

Methods: This retrospective cross-sectional study included 103 women aged 18-45 years who had experienced RPL. Demographic, clinical, laboratory, and genetic data were retrieved from hospital records. Associations between thrombophilia mutations and pregnancy loss were assessed using comparative and correlational analyses, followed by multivariate linear regression to determine independent predictors.

Results: Factor V Leiden mutation was identified in 10 patients (9.7%), whereas the Prothrombin G20210A mutation was detected in 5 patients (4.85%). Neither mutation was significantly associated with the number of previous pregnancy losses ($p>0.05$). Maternal age was significantly positively correlated with the number of previous pregnancy losses ($r=0.51$, $p<0.001$). Although fasting blood glucose and hemoglobin levels were associated with the number of previous pregnancy losses in univariate analyses, only hemoglobin and maternal age remained independently associated with the number of previous pregnancy losses after multivariate adjustment (maternal age: $B=0.033$, standardized $\beta=0.459$, $p<0.001$; hemoglobin: $B=0.112$, standardized $\beta=0.293$, $p=0.016$).

Conclusion: Within this cohort of women with RPL, no statistically significant association was observed between Factor V Leiden mutation carrier status and the number of previous pregnancy losses. Maternal age was independently associated with the number of previous pregnancy losses. Larger controlled prospective studies are needed to further clarify the role of inherited thrombophilia in RPL.

Keywords: Recurrent pregnancy loss, Factor V Leiden, prothrombin mutation, thrombophilia, maternal age

Introduction

Recurrent pregnancy loss (RPL) is a complex reproductive disorder that is generally defined as two or more consecutive pregnancy losses before fetal viability. Affecting approximately 1-2% of women of reproductive age, RPL remains an important clinical problem because of its multifactorial etiology and considerable emotional and reproductive consequences. Although advances in reproductive medicine have improved the understanding of this condition, no specific cause can

be identified in a substantial proportion of affected women. Current evidence indicates that genetic, anatomical, endocrine, immunological, and hematological abnormalities may all contribute to the development of RPL [1-4].

Inherited thrombophilia has been extensively investigated as one of the possible mechanisms underlying RPL. Pregnancy itself is associated with a physiological hypercoagulable state, and inherited thrombophilic disorders may further increase the tendency toward thrombosis. Consequently, placental

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microvascular thrombosis, impaired uteroplacental perfusion, and placental insufficiency have been proposed as potential mechanisms linking thrombophilia to pregnancy failure [5,6].

Among inherited thrombophilic disorders, Factor V Leiden mutation is the most prevalent genetic abnormality associated with an increased risk of venous thromboembolism [7,8]. Likewise, the Prothrombin G20210A mutation represents another inherited prothrombotic condition that has been investigated as a possible contributor to adverse pregnancy outcomes [9]. Although numerous observational studies have explored the association between these mutations and RPL, the available evidence remains inconsistent. While some reports have demonstrated a significant relationship, others have failed to confirm a clinically meaningful association, leaving the role of routine thrombophilia screening in women with RPL a matter of ongoing debate [10,11].

Increasing maternal age is consistently recognized as one of the strongest predictors of pregnancy loss. This association is primarily explained by the age-related rise in embryonic chromosomal abnormalities and the progressive decline in oocyte quality. In addition, maternal metabolic disturbances and hematological factors have been suggested to influence reproductive outcomes and pregnancy maintenance [12-15].

Accordingly, the present study was undertaken to determine the frequency of the Factor V Leiden mutation in women with RPL and to evaluate whether carrier status for the Factor V Leiden mutation was associated with the number of previous pregnancy losses in this cohort. The Prothrombin G20210A mutation was evaluated as a secondary inherited thrombophilic variant, and the potential influence of maternal age, hemoglobin concentration, and fasting blood glucose levels was investigated.

Methods

Study Design and Setting

This retrospective cross-sectional study was conducted at the Clinic of Hematology, Ordu State Hospital. Medical records of eligible patients evaluated between January 2023 and December 2025 were retrospectively reviewed to investigate the association between inherited thrombophilic mutations and RPL.

Study Population

A total of 103 women aged between 18 and 45 years with a history of RPL were included in the study. RPL was defined as two or more failed clinical pregnancies, in accordance with the recommendations of the American Society for Reproductive Medicine [3]. Patients with documented uterine anatomical abnormalities, chromosomal abnormalities, uncontrolled endocrine disorders (including diabetes mellitus and thyroid disease), or infectious causes of pregnancy loss, identified in the available medical records, were excluded from the study. Patients with incomplete medical records or lacking thrombophilia test results were also excluded.

Data Collection

Demographic, clinical, laboratory, and genetic information was retrospectively retrieved from the hospital electronic medical record system. The collected variables included maternal age, number of pregnancy losses, hemoglobin concentration, fasting blood glucose level, Factor V Leiden mutation status, and Prothrombin G20210A mutation status. Hemoglobin concentration and fasting blood glucose were obtained from routine laboratory evaluations conducted during the same clinical assessment in which genetic testing for inherited thrombophilia was requested. These laboratory values represented the patients' baseline clinical status at the time of evaluation, rather than reflecting measurements obtained during pregnancy. Hemoglobin and fasting blood glucose were included in the regression analysis because maternal hematologic and metabolic status has previously been associated with adverse pregnancy outcomes, and these measures were therefore considered clinically relevant covariates.

All patient information was anonymized before analysis, and confidentiality was maintained throughout the study.

Genetic Evaluation

The presence of the Factor V Leiden (F5 c.1601G>A, p.(Arg534Gln)) and *Prothrombin G20210A (*F2 c.97G>A) variants was determined by previously performed molecular genetic analyses that were recorded in the hospital laboratory information system. All genetic analyses had been performed in the same molecular genetics laboratory as part of routine clinical care, using standardized laboratory protocols and internal quality-control procedures. Mutation status was interpreted according to the official laboratory reports and recorded as negative, heterozygous, or homozygous. Zygosity information was available for all mutation-positive patients. Because of the retrospective nature of the study, detailed information regarding the specimen type, deoxyribonucleic acid extraction method, molecular assay platform, and assay manufacturer was not consistently available in the medical records.

Ethical Approval

Ethical approval for this study was granted by the Ordu University Non-Interventional Clinical Research Ethics Committee (approval no: 82, date: 11.03.2026). Because of the retrospective design of the study, the requirement for informed consent was waived by the Ethics Committee. All study procedures were conducted in accordance with the ethical principles of the Declaration of Helsinki.

Statistical Analysis

All statistical analyses were carried out using IBM SPSS Statistics version 29.0 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean $\pm$ standard deviation, whereas categorical variables are expressed as frequencies and percentages. The distribution of continuous variables was assessed using the Kolmogorov-Smirnov test.

Comparisons between independent groups were performed using the Independent Samples t-test. Pearson correlation analysis was used to evaluate the relationships between continuous variables. Participants were additionally categorized according to fasting blood glucose levels (<100 mg/dL and ≥ 100 mg/dL), and comparisons between these groups were conducted accordingly.

To identify variables independently associated with the number of pregnancy losses, multivariate linear regression analysis was performed, including maternal age, hemoglobin concentration, fasting blood glucose level, Factor V Leiden mutation status, and Prothrombin G20210A mutation status. A two-sided p value of <0.05 was considered statistically significant. Regression coefficients are reported as unstandardized coefficients (B). Binary mutation variables were coded as 1=present and 2=absent.

Results

During the study period, the medical records of 103 women who were evaluated for RPL and met the study eligibility criteria were retrospectively reviewed. Complete demographic, clinical, laboratory, and genetic data were available for all eligible patients; therefore, all 103 women were included in the final analysis. The mean age of the participants was 34.15 ± 5.74 years. The mean number of previous pregnancy

losses was 2.10 ± 0.44 . The mean hemoglobin level was 11.91 ± 0.99 g/dL. The demographic and clinical characteristics of the study population are presented in Table 1.

Factor V Leiden mutation was detected in 10 (9.7%) patients, all of whom were heterozygous. The Prothrombin G20210A mutation was identified in five (4.85%) patients, of whom four were heterozygous and one was homozygous (Table 1).

The mean number of pregnancy losses was 2.20 among patients with the Factor V Leiden mutation and 2.09 among those without the mutation. There was no statistically significant difference between the groups ($p=0.479$). Similarly, no significant difference was observed between patients with and without the Prothrombin mutation ($p=0.655$) (Table 2).

A moderate positive correlation was found between age and the number of pregnancy losses ($r=0.51$, $p<0.001$). Weak but statistically significant positive correlations were observed between hemoglobin levels and pregnancy loss ($r=0.21$, $p=0.037$) and between fasting blood glucose levels and pregnancy loss ($r=0.25$, $p=0.010$) (Table 3).

When patients were grouped according to fasting blood glucose levels, those with elevated glucose levels (≥ 100 mg/dL) had a significantly higher mean number of pregnancy losses compared to those with normal glucose levels (<100 mg/dL) (2.29 vs. 2.03, $p=0.016$) (Table 4).

Multivariate linear regression analysis demonstrated that

Table 1. Demographic and clinical characteristics of the study population

Variable	Mean $\pm$ SD or n (%)
Age (years)	34.15 ± 5.74
Pregnancy losses (n)	2.10 ± 0.44
Hemoglobin (g/dL)	11.91 ± 0.99
Factor V Leiden heterozygous	10 (9.7%)
Factor V Leiden homozygous	0
Factor V Leiden negative	93 (90.3%)
Prothrombin G20210A heterozygous	4 (3.9%)
Prothrombin G20210A homozygous	1 (0.97%)
Prothrombin G20210A negative	98 (95.15%)
SD: Standard deviation	

Table 2. Comparison of mean number of pregnancy losses according to mutation status

Variable	Mean (mutation ⁺)	Mean (mutation ⁻)	p value
Factor V Leiden	2.20	2.09	0.479
Prothrombin G20210A	2.20	2.10	0.655
Statistical test: Independent Samples t-test			

Table 3. Correlation between clinical variables and number of pregnancy losses

Variables	r	p value
Age vs pregnancy losses	0.51	<0.001
Hemoglobin vs pregnancy losses	0.21	0.037
Blood glucose vs pregnancy losses	0.25	0.010
Statistical test: Pearson correlation analysis		

Table 4. Comparison of pregnancy losses according to blood glucose levels

Glucose level	Mean pregnancy losses	p value
<100 mg/dL	2.03	-
≥100 mg/dL	2.29	0.016

Statistical test: Independent Samples t-test

maternal age was independently associated with the number of previous pregnancy losses within this cohort ($B=0.033$, standardized $\beta=0.459$, $p<0.001$). Hemoglobin level was also independently associated with the number of previous pregnancy losses ($B=0.112$, standardized $\beta=0.293$, $p=0.016$). In contrast, fasting blood glucose level, Factor V Leiden mutation, and Prothrombin G20210A mutation were not independently associated with the outcome (Table 5).

No missing data were identified for the variables included in the final statistical analyses.

Because the number of previous pregnancy losses was not normally distributed, additional non-parametric sensitivity analyses were performed. Spearman correlation analysis confirmed a significant positive correlation between maternal age and the number of previous pregnancy losses ($p=0.475$, $p<0.001$). No significant correlation was observed for fasting blood glucose levels ($p=0.180$, $p=0.069$), whereas hemoglobin levels were weakly correlated with the number of previous pregnancy losses ($p=0.316$, $p=0.021$). Furthermore, Mann-Whitney U tests demonstrated no significant differences in the number of previous pregnancy losses with respect to Factor V Leiden mutation status ($p=0.495$) or Prothrombin G20210A mutation status ($p=0.638$).

Discussion

The present study investigated the relationship between inherited thrombophilic mutations and RPL while simultaneously evaluating maternal demographic and metabolic characteristics. The principal finding was that neither Factor V Leiden nor Prothrombin G20210A mutation showed an independent association with pregnancy loss. In contrast, maternal age and hemoglobin concentration remained independently associated with the number of previous pregnancy losses after multivariate adjustment.

The prevalence of the Factor V Leiden mutation in our cohort was 9.7%, whereas that of the Prothrombin G20210A mutation was 4.85%. These frequencies are comparable with those reported in previous studies involving women with RPL, indicating that our study population is broadly representative of similar cohorts described in the literature [5,10].

Although inherited thrombophilia has long been proposed as a potential contributor to RPL, its exact role remains controversial. Several investigators have suggested that thrombophilic mutations may impair placental circulation through thrombotic mechanisms, thereby increasing the likelihood of miscarriage [9,11]. In addition, an association between Factor V Leiden mutation and unexplained recurrent fetal loss has been reported [16]. Nevertheless, systematic reviews and meta-analyses have produced inconsistent findings, and a definitive relationship between inherited thrombophilia and RPL has not been established [10,17]. Our findings are consistent with the latter reports, as neither of the investigated mutations was independently associated with pregnancy loss.

The clinical relevance of the Prothrombin G20210A mutation remains uncertain. While some studies have linked this mutation to adverse pregnancy outcomes [9], others have failed to demonstrate a significant association with recurrent miscarriage [10]. Consistent with these observations, the Prothrombin G20210A mutation was not identified as an independent predictor of pregnancy loss in the present study.

Among all evaluated variables, maternal age and hemoglobin concentration remained independently associated with the number of previous pregnancy losses in this cohort of women with RPL. The association with maternal age is consistent with previous studies showing that increasing maternal age is associated with a greater cumulative number of pregnancy losses, partly because of increasing embryonic chromosomal abnormalities and reduced oocyte quality [12,13]. However, because the present study included only

Table 5. Multivariate linear regression analysis of factors associated with pregnancy loss

Variable	B	SE	Standardized β	p value
Age	0.033	0.008	0.459	<0.001
Hemoglobin	0.112	0.045	0.293	0.016
Fasting blood glucose	0.002	0.003	0.112	0.352
Factor V Leiden mutation	-0.163	0.148	-0.123	0.277
Prothrombin G20210A mutation	-0.190	0.174	-0.119	0.282

Model: Multivariate linear regression analysis including age, hemoglobin level, fasting blood glucose level, Factor V Leiden mutation, and Prothrombin G20210A mutation

Dependent variable: Number of previous pregnancy losses. Mutation variables were coded as 1=present and 2=absent

B: Unstandardized regression coefficient, β : Standardized regression coefficient, SE: Standard error

women with RPL and lacked a control group, this association should not be interpreted as demonstrating that maternal age is an independent determinant of RPL. Older women have a longer reproductive lifespan and therefore greater cumulative opportunities to experience pregnancy losses, which may also have contributed to the observed association.

Although both fasting blood glucose and hemoglobin levels were associated with the number of previous pregnancy losses in the univariate analyses, only hemoglobin remained independently associated after multivariate adjustment, whereas the association with fasting blood glucose was no longer significant. The observed association between hemoglobin concentration and the number of previous pregnancy losses should be interpreted cautiously because laboratory measurements were obtained during routine clinical evaluation rather than during pregnancy, and because residual confounding cannot be excluded. Previous investigations have shown that impaired glucose metabolism may adversely influence pregnancy outcomes [14]. Furthermore, a recent systematic review and meta-analysis suggested that women with RPL exhibit abnormalities in several markers of glucose metabolism, particularly those reflecting insulin resistance, whereas fasting blood glucose alone is not consistently different between affected women and controls [18]. Accordingly, these findings should be interpreted cautiously because of the retrospective design, the timing of laboratory measurements, and the potential for residual confounding.

The findings of the present study should be interpreted within the context of its limitations, including its retrospective design and the absence of a control group. Although no statistically significant association was observed between Factor V Leiden mutation carrier status and the number of previous pregnancy losses within this cohort, these findings should not be interpreted as evidence against a possible role of inherited thrombophilia in RPL in general. Current recommendations regarding thrombophilia testing are based on the broader body of evidence rather than on the findings of the present study alone [3].

It should also be emphasized that inherited thrombophilia and antiphospholipid syndrome are distinct clinical entities with different levels of evidence and different clinical implications in RPL. Unlike antiphospholipid syndrome, which is an established and treatable cause of RPL, the contribution of inherited thrombophilic variants remains uncertain and is debated in the current literature.

Study Limitations

Several limitations should be acknowledged. First, the retrospective design may have introduced selection bias. Second, the relatively small number of patients carrying thrombophilic mutations may have reduced the statistical power to detect significant associations. Third, because of the retrospective design, several clinically relevant reproductive variables, including gravidity, parity, live birth history, reproductive duration, gestational age at pregnancy loss, and the interval between the first pregnancy and inclusion

in the study, were not consistently available in the medical records, and, therefore, could not be included in the analyses. Consequently, residual confounding cannot be completely excluded. In addition, the limited variability of the outcome variable and the small number of mutation-positive patients should be considered when interpreting the statistical findings. Although additional non-parametric sensitivity analyses supported the primary results, these analyses should be regarded as exploratory, and a type II error cannot be excluded. Furthermore, because genetic testing for inherited thrombophilia was performed according to routine clinical practice, indication bias cannot be completely excluded. The single-center design of the study may limit the generalizability of the findings to other populations.

Conclusion

Despite these limitations, this study provides additional evidence regarding the relationship between inherited thrombophilia and RPL by evaluating genetic, maternal, and metabolic characteristics within the same cohort. Moreover, the application of multivariate regression analysis strengthens the interpretation and reliability of the present findings.

Within this single-center retrospective cohort of women with RPL, maternal age was independently associated with the number of previous pregnancy losses, whereas neither Factor V Leiden nor the Prothrombin G20210A mutation was significantly associated with the number of previous pregnancy losses. Because this study did not include a control group, these findings should not be interpreted as evidence of a role for inherited thrombophilic variants in RPL. Larger prospective controlled studies are warranted to further clarify these associations.

Ethics

Ethics Committee Approval: Ethical approval for this study was granted by the Ordu University Non-Interventional Clinical Research Ethics Committee (approval no: 82, date: 11.03.2026).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Concept: A.O.G., Design: A.O.G., Data Collection or Processing: A.O.G., Ç.D., Analysis or Interpretation: A.O.G., Ç.D., Literature Search: A.O.G., Writing: A.O.G.

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Original Article

Combined Prognostic Value of CEA and Prognostic Nutritional Index in Metastatic Colon Cancer

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ABSTRACT

Aim: To evaluate the combined prognostic value of carcinoembryonic antigen (CEA) and the prognostic nutritional index (PNI) for overall survival (OS) and progression-free survival (PFS) in *de novo* stage IV colon cancer, and to compare their performance at baseline and at the first response assessment during treatment.

Methods: This single-center retrospective study included 102 patients with *de novo* stage IV colon cancer who were diagnosed between 2018 and 2025. CEA and PNI were measured at baseline (T0) and at the first response assessment (T1). CEA cut-offs were derived by receiver operating characteristic analysis, and a literature-based PNI cut-off of 48.0 was applied. Patients were classified into four CEA/PNI groups. Survival was analyzed by Kaplan-Meier and Cox regression methods, with $p < 0.05$ considered significant.

Results: Median OS was 33.0 months, and median PFS was 12.4 months. At baseline, the highest-risk group (high CEA, low PNI) had shorter OS than the reference group (21.6 vs. 37.6 months, $p = 0.01$) and was an independent predictor of death [hazard ratio (HR): 2.76, 95% confidence interval: 1.18-6.44, $p = 0.02$]. The score was more discriminative at T1, where OS ranged from 19.8 to 60.6 months across groups ($p = 0.001$). Bone metastasis (HR: 3.19) and absence of primary tumor surgery were the other independent predictors of poor OS. For PFS, left-sided location and surgery were independent favorable factors.

Conclusion: The combined CEA/PNI score identifies a high-risk subgroup in *de novo* stage IV colon cancer using routine laboratory data. Its prognostic value was greatest when measured early in treatment, supporting serial assessments over a single baseline measurement.

Keywords: Colonic neoplasms, carcinoembryonic antigen, nutritional status, prognosis, neoplasm metastasis

Introduction

Worldwide, colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second leading cause of cancer-related death, accounting for close to 10% of new cancer cases. The 2022 global estimates report about 1.9 million diagnoses and 904,000 deaths [1]. In Türkiye, it ranked third in terms of incidence in 2022, with 21,718 new cases and second among cancer-related deaths, with 11,698 deaths [2]. Between 15% and 30% of patients already have distant metastases at first diagnosis, and even with current treatment the five-year survival in stage IV disease stays below 15% [3]. This poor prognosis points to the need for reliable and readily available prognostic biomarkers to complement the tumor, node, metastasis staging system.

Serum carcinoembryonic antigen (CEA) is the marker most often used for diagnosis and follow-up in CRC [3]. It tracks tumor burden, and its prognostic weight holds across stages: in a pooled analysis of 10,114 patients, raised postoperative CEA predicted worse overall survival (OS) regardless of disease extent [hazard ratio (HR): 2.92] [4]. In stage IV disease, over 75% of patients present with elevated CEA, and high values predict poorer response and survival [5]. However, CEA reflects only tumor burden and does not capture the patient's nutritional or immune status, both of which influence cancer outcomes.

Onodera et al. [6] described the prognostic nutritional index (PNI) in 1984 as serum albumin (g/L) plus five times the peripheral lymphocyte count ($\times 10^9/L$) [6]. It captures host status as a single value by merging a nutritional measure

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(albumin) with an immune measure (lymphocytes). Across 43 studies covering 19,214 CRC patients, a low PNI carried an 89% higher mortality risk independent of stage [HR: 1.89; 95% confidence interval (CI): 1.70-2.10] [7]. A Turkish study of 253 advanced CRC patients reported shorter median OS in the low-PNI group (38.80 vs. 53.06 months; $p=0.039$) [8]. However, since PNI alone is insufficient for predicting progression-free survival (PFS), it is recommended to combine it with a tumor burden marker such as CEA [7,8].

Combining CEA and PNI is clinically rational: CEA quantifies tumor burden, while PNI reflects host disease tolerance. In stage II-III colon cancer, Xu et al. [9] reported that this combination predicted OS superiorly to either marker alone, increasing the area under the curve (AUC) to 0.797 (vs. 0.568 for CEA and 0.427 for PNI). In 512 stage IV CRC patients, Fernández Figueroa et al. [5] confirmed this, with the high-risk profile (CEA-high/PNI-low) carrying the poorest OS (HR: 2.41; 95% CI: 1.19-4.86) and PFS (HR: 2.16; 95% CI: 1.24-3.75). However, that study evaluated a mixed Mexican colorectal population with stage II-III disease, leaving this combined score untested in patients with exclusively advanced (stage IV) colon cancer. Therefore, we investigated the combined prognostic values of CEA and PNI for OS and PFS, comparing the performance at baseline versus the first response assessment during systemic treatment.

Methods

Study Design and Setting

We carried out a single-center retrospective cohort analysis in the Department of Medical Oncology, Aydın Adnan Menderes University Faculty of Medicine Hospital. Records of patients diagnosed with *de novo* stage IV colon cancer between January 2018 and December 2025 were retrieved from the hospital information system and the pathology archives.

Participants

Inclusion criteria: (1) biopsy-proven colon adenocarcinoma with distant metastasis present at first diagnosis, staged by the American Joint Committee on Cancer 8th edition; (2) age ≥ 18 years; (3) baseline serum CEA, albumin, and complete blood count with lymphocyte count, obtained within seven days before systemic treatment; and (4) at least three months of follow-up. Exclusion criteria: rectal cancer; metachronous metastasis; second primary malignancy; conditions affecting baseline albumin or lymphocyte levels (active infection, liver cirrhosis, systemic corticosteroids); and insufficient follow-up data. Of the 235 patients screened, 133 were excluded for the following reasons: rectal cancer ($n=48$), metachronous metastasis ($n=33$), missing laboratory data ($n=22$), second primary malignancy ($n=15$), and insufficient follow-up ($n=15$), leaving 102 for analysis. Baseline (T0) analysis included all 102 patients; at the 2-month assessment (T1), CEA and PNI were available for 88 patients and missing for 14 patients.

Data Collection

All records were captured using a standardized form. The fields covered age, sex, and Eastern Cooperative Oncology Group (ECOG) performance status, primary tumor side (right or left), and the number and sites of metastases (liver, lung, peritoneum, bone, distant lymph nodes, and other sites). Pathological data included histological subtype, mismatch repair/microsatellite instability (MSI) status, and KRAS/NRAS/BRAF mutations. Treatment data included first-line chemotherapy, biological agents (anti-vascular endothelial growth factor or anti-epidermal growth factor receptor), and surgical history. Survival data included dates of diagnosis, progression, and either death or last follow-up. Laboratory values were collected at baseline (T0, <7 days pre-treatment) and at first response assessment (T1, ~ 2 months post-treatment). T1 analyses included the 88 patients with complete data.

Calculation of PNI and CEA/PNI Grouping

The PNI was calculated as: $\text{PNI} = \text{albumin (g/L)} + 5 \times \text{lymphocyte count } (\times 10^9/\text{L})$, as originally described by Onodera et al. [6]. For T0 CEA, the optimal cut-off value was determined by receiver operating characteristic (ROC) curve analysis using the Youden index with OS as the endpoint, yielding 14.28 ng/mL (AUC: 0.621). For T0 PNI, ROC analysis showed limited discrimination (AUC: 0.565); therefore, a literature-based cut-off of 48.0 was adopted from the largest published stage IV CRC cohort validating this threshold and applied to both time points [10]. For T1 CEA, ROC analysis identified an optimal cut-off of 5.42 ng/mL (AUC: 0.682), which approximated the standard clinical threshold of 5 ng/mL; therefore, the standard threshold was applied. The PNI cut-off of 48.0 was used at both time points. Due to these differing CEA cut-off values, no direct statistical comparisons were made between the T0 and T1 groups. Patients were classified into four groups: Group 1 (CEA-high/PNI-low, highest risk); Group 2 (CEA-high/PNI-high); Group 3 (CEA-low/PNI-low); and Group 4 (CEA-low/PNI-high, reference group).

Endpoints Definitions

The two co-primary endpoints, OS and PFS, were measured from diagnosis to death and from diagnosis to progression (response evaluation criteria in solid tumors 1.1), respectively. Event-free patients were censored at their last follow-up. The secondary endpoint was to determine optimal prognostic CEA and PNI cut-offs in *de novo* stage IV colon cancer.

Ethics Committee Approval

This study received ethical approval from the Non-Interventional Clinical Research Ethics Committee of the Faculty of Medicine, Aydın Adnan Menderes University (approval no: 16, date: 14.05.2026). All procedures followed the Declaration of Helsinki. Given the retrospective design and the use of anonymized records, the requirement for informed consent was waived.

Statistical Analysis

Categorical data are shown as counts and percentages. Continuous data departed from normality as assessed by the Kolmogorov-Smirnov test and are presented as medians with interquartile range (IQR). Categorical groups were compared using the chi-square test or Fisher's exact test, and continuous groups were compared using the Kruskal-Wallis test followed by Bonferroni post-hoc testing. To check whether the missing T1 data caused bias, we compared the baseline characteristics of patients with and without T1 measurements using the Mann-Whitney U test and the chi-square or Fisher's exact test. Survival was estimated using the Kaplan-Meier method and compared by the log-rank test, with the comparison between Group 1 and Group 4 pre-specified. Univariable and multivariable Cox proportional hazards models identified independent factors; variables with $p < 0.20$ in univariable testing entered the multivariable model alongside established confounders. Multicollinearity was ruled out by ensuring variance inflation factors < 5 . For each endpoint, Model 1 analyzed the T0 grouping ($n=102$), and Model 2 analyzed the T1 grouping ($n=88$). The number of events per variable was checked for each model to avoid overfitting. The OS models included 82 deaths (T0) and 68 deaths (T1) for 6 parameters; the PFS models included 96 and 82 events for 8 parameters, yielding between 10 and 14 events per variable across models. The proportional hazards assumption was assessed using Schoenfeld residuals and log-log plots. Tests were two-sided ($\alpha=0.05$) and were performed using IBM SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA).

Results

Patient Characteristics

Among the 102 patients, 74.5% were male, 88.2% had ECOG 0-1, and the median age was 65.5 years (IQR: 11.0). Most tumors were left-sided (64.7%), and 54.9% of patients had two or more metastatic sites. The liver was the most frequent metastatic site (72.5%), followed by distant lymph nodes (41.2%), lung (31.4%), peritoneum (21.6%), and bone (11.8%). Mutations in KRAS, NRAS, and BRAF were present in 53.5%, 5.0%, and 7.1% of cases, respectively; 3.0% of cases were MSI-H, and 35.6% of cases had unknown MSI status. FOLFOX was the most common first-line regimen (52.0%). Biological agents were administered to 88.2% of patients, and 45.1% underwent surgery. Median OS was 33.0 months (95% CI: 25.1-40.8) and median PFS was 12.4 months (95% CI: 9.9-14.9). Baseline characteristics are shown in Table 1.

ROC Analysis and Cut-off Determination

Baseline medians were 19.5 ng/mL for CEA (IQR: 141.9) and 49.0 for PNI (IQR: 6.4). Against OS, CEA yielded an AUC of 0.621, and the Youden-optimal cut-off was 14.28 ng/mL. Although higher than the conventional 5.0 ng/mL, this threshold provided superior discrimination in our cohort and was selected for stratification. For PNI, the AUC was 0.565, and no robust data-driven threshold was identified. Therefore, a

literature-supported cut-off of 48.0 was applied, consistent with large-scale stage IV series. ROC curves are shown in Figure 1.

CEA/PNI Score Distribution and Grouping

Based on these cut-offs, the baseline distribution was as follows: 20.6% highest-risk (CEA-high/PNI-low), 34.3% CEA-high/PNI-high, 12.7% CEA-low/PNI-low, and 32.4% reference (CEA-low/PNI-high). Groups were comparable except for sex distribution ($p=0.043$) and a borderline difference in surgery rates ($p=0.070$), with men predominating in the highest-risk group (Table 1). At T1 (~2 months), data were available for 88 patients (86.3%). Median CEA decreased to 5.5 ng/mL (IQR: 31.0), and median PNI decreased to 48.1 (IQR: 6.8). The T1 risk distribution shifted to 26.1% highest risk, 27.3% CEA-high/PNI-high, 22.7% CEA-low/PNI-low, and 23.9% reference (Table 2). Patients with T1 data and those without were similar in most baseline features, including age, baseline CEA, PNI, CEA/PNI score distribution, ECOG status, and tumor location (all $p > 0.05$). The two groups differed only with respect to bone metastasis ($p=0.036$) and primary tumor surgery ($p=0.013$).

Overall Survival

Median follow-up was 33.0 months (95% CI: 25.1-40.8). Overall, 82 patients (80.4%) died, and 20 (19.6%) were alive at the last follow-up (Table 3).

By baseline CEA/PNI score, median OS was 21.6, 30.6, 42.5, and 37.6 months for groups 1-4, respectively (log-rank $p=0.057$, Figure 2A, Table 3). The highest-risk group had shorter OS than the reference group (21.6 vs. 37.6 months; $p=0.010$; Figure 2B).

At T1, median OS was 19.8, 24.8, 31.1, and 60.6 months for groups 1-4, respectively (log-rank $p=0.001$, Figure 2C), representing a 41-month difference between the highest-risk and reference groups ($p < 0.001$) (Table 3).

Progression-free Survival

A total of 96 patients (94.1%) progressed during follow-up, while 6 (5.9%) were censored. The median PFS for the cohort was 12.4 months (95% CI: 9.9-14.9) (Table 3).

Baseline CEA/PNI grouping showed no significant difference in PFS across groups ($p=0.254$) or between highest-risk and reference groups ($p=0.290$); baseline CEA alone was borderline significant ($p=0.050$) (Table 3).

At T1, median PFS across groups 1-4 was 9.4, 8.9, 14.6, and 21.4 months (log-rank $p=0.006$; Figure 2D), representing about a 12-month gap between the highest-risk and reference groups ($p < 0.001$) (Table 3).

Independent Prognostic Factors

Univariable and multivariable Cox analyses were performed for T0 and T1 OS/PFS models (Table 4). In the T0 OS model, univariable analysis identified the highest-risk group (CEA-high/PNI-low; HR: 2.33, 95% CI: 1.04-5.24; $p=0.039$), metastatic site count, bone metastasis, and surgery as significant. In multivariable analysis, only the highest-risk group (HR: 2.75,

Table 1. Baseline clinical and demographic characteristics of patients with *de novo* stage IV colon cancer stratified by CEA/PNI score group (n=102)

CEA/PNI score group (n (%))	Total (102) (%100.0)	CEA-HIGH PNI-LOW Group 1 (21) (%20.6)	CEA-HIGH PNI-HIGH Group 2 (n=35) (%34.3)	CEA-LOW PNI-LOW Group 3 (n=13) (12.7%)	CEA-LOW PNI-HIGH(Ref) Group 4 (n=33) (32.4%)	p value
Demographics						
Age, years, median (IQR)	65.5 (11.0)	67.0 (10.5)	61.0 (15.0)	68.0 (14.0)	66.0 (11.5)	0.019
Sex, n (%)						0.043
Male	76 (74.5)	20 (95.2)	24 (68.6)	11 (84.6)	21 (63.6)	
Female	26 (25.5)	1 (4.8)	11 (31.4)	2 (15.4)	12 (36.4)	
Clinical characteristics						
ECOG-PS, n (%)						0.965
0-1	90 (88.2)	19 (90.5)	31 (88.6)	11 (84.6)	29 (87.9)	
2+	12 (11.8)	2 (9.5)	4 (11.4)	2 (15.4)	4 (12.1)	
Primary tumor location, n (%)						
Right-sided	36 (35.3)	6 (28.6)	11 (31.4)	7 (53.8)	12 (36.4)	0.454
Left-sided	66 (64.7)	15 (71.4)	24 (68.6)	6 (46.2)	21 (63.6)	
Metastatic sites, n (%)						
Single	46 (45.1)	9 (42.9)	13 (37.1)	6 (46.2)	18 (54.5)	0.545
Multiple (2+)	56 (54.9)	12 (57.1)	22 (62.9)	7 (53.8)	15 (45.5)	
Metastatic sites						
Liver, n (%)	74 (72.5)	16 (76.2)	28 (80.0)	9 (69.2)	21 (63.6)	0.475
Lung, n (%)	32 (31.4)	7 (33.3)	13 (37.1)	3 (23.1)	9 (27.3)	0.741
Bone, n (%)	12 (11.8)	3 (14.3)	3 (8.6)	2 (15.4)	4 (12.1)	0.887
Peritoneum, n (%)	22 (21.6)	3 (14.3)	5 (14.3)	4 (30.8)	10 (30.3)	0.273
Distant lymph nodes, n (%)	42 (41.2)	9 (42.9)	17 (48.6)	5 (38.5)	11 (33.3)	0.639
Molecular profile						
KRAS, n (%)						
Wild-type	47 (46.5)	8 (40.0)	15 (42.9)	7 (53.8)	17 (51.5)	0.767
Mutant	54 (53.5)	12 (60.0)	20 (57.1)	6 (46.2)	16 (48.5)	
NRAS, n (%)						
Wild-type	96 (95.0)	19 (95.0)	32 (91.4)	13 (100.0)	32 (97.0)	0.591
Mutant	5 (5.0)	1 (5.0)	3 (8.6)	0 (0.0)	1 (3.0)	
BRAF, n (%)						
Wild-type	92 (92.9)	18 (94.7)	33 (97.1)	12 (92.3)	29 (87.9)	0.519
Mutant	7 (7.1)	1 (5.3)	1 (2.9)	1 (7.7)	4 (12.1)	
MSI-H, n (%)	3 (3.0)	0 (0.0)	1 (2.9)	1 (7.7)	1 (3.0)	0.570
Treatment						
First-line chemotherapy, n (%)						
FOLFOX	53 (52.0)	10 (47.6)	26 (57.1)	6 (46.2)	17 (51.5)	0.282
CAPOX	31 (30.4)	3 (14.3)	11 (31.4)	5 (38.5)	12 (36.4)	
FOLFIRI	14 (13.7)	7 (33.3)	3 (8.6)	1 (7.7)	3 (9.1)	
Other	4 (3.9)	1 (4.8)	1 (2.9)	1 (7.7)	1 (3.0)	
Biological agent, n (%)	90 (88.2)	19 (90.5)	29 (82.9)	11 (84.6)	31 (93.9)	0.517
Surgical intervention, n (%)	46 (45.1)	7 (33.3)	12 (34.3)	9 (69.2)	18 (54.5)	0.070

Group 1: CEA-HIGH/PNI-LOW; **Group 2:** CEA-HIGH/PNI-HIGH; **Group 3:** CEA-LOW/PNI-LOW; **Group 4:** CEA-LOW/PNI-HIGH (reference). Continuous variables were compared by the Kruskal-Wallis test; categorical variables were compared by the chi-square test or Fisher's exact test. Bold p values indicate statistical significance ($p < 0.05$)

BMI: Body mass index, ECOG-PS, Eastern Cooperative Oncology Group performance status, MSI-H: High microsatellite instability, IQR: Interquartile range, CEA: carcinoembryonic antigen, PNI: prognostic nutritional index

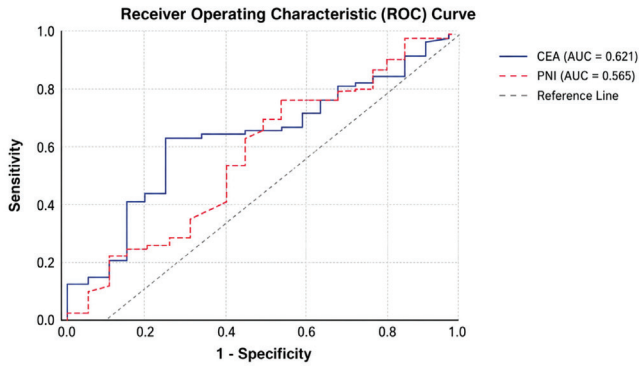


Figure 1. ROC curves for baseline CEA and PNI

CEA: Carcinoembryonic antigen, PNI: Prognostic nutritional index, ROC: Receiver operating characteristic, AUC: Area under the curve

95% CI: 1.18-6.43; $p=0.019$) and bone metastasis (HR: 3.18, 95% CI: 1.05-9.65; $p=0.041$) remained independent predictors (Table 4).

For T1 OS, all three risk groups were significant in univariable analysis. In multivariable analysis, CEA-low/PNI-low (HR: 4.84, 95% CI: 1.43-16.38; $p=0.011$), bone metastasis (HR: 5.45, 95% CI: 1.38-21.55; $p=0.016$), and surgery (HR: 0.45, 95% CI: 0.21-0.95; $p=0.038$) were independent predictors, whereas CEA-high/PNI-low and CEA-high/PNI-high showed borderline significance (Table 4).

Left-sided tumor location and surgery were independent predictors of PFS in both T0 and T1 models, whereas the CEA/PNI score was not significant. In the T1 PFS model, the highest-risk group showed only a nonsignificant trend (HR: 1.72; $p=0.216$, Table 4).

Table 2. CEA, PNI and combined CEA/PNI score distribution at baseline (T0) and first response assessment (T1)

Variable	Baseline (T0) (n=102)	First response assessment (T1) (n=88)
CEA, ng/mL, median (IQR)	19.5 (141.9)	5.5 (31.0)
PNI, median (IQR)	49.0 (6.4)	48.1 (6.8)
CEA category, n (%)		
CEA-high	56 (54.9)	48 (54.5)
CEA-low	46 (45.1)	40 (45.5)
PNI category, n (%)		
PNI-low (<48.0)	34 (33.3)	48 (54.5)
PNI-high (≥ 48.0)	68 (66.7)	40 (45.5)
CEA/PNI combined group, n (%)		
Group 1: CEA-high/PNI-low	21 (20.6)	23 (26.1)
Group 2: CEA-high/PNI-high	35 (34.3)	24 (27.3)
Group 3: CEA-low/PNI-low	13 (12.7)	20 (22.7)
Group 4: CEA-low/PNI-high (ref)	33 (32.4)	21 (23.9)

CEA categorization thresholds differed between time points: at baseline (T0), an ROC-derived cut-off of 14.28 ng/mL (AUC=0.621) was applied, while at the first response assessment (T1), the standard clinical threshold of 5 ng/mL was adopted, as the ROC-derived optimal value at T1 (5.42 ng/mL, AUC=0.682) closely approximated this conventional cut-off. PNI cut-off: 48.0 (adopted from Takamizawa et al. [10]).

A direct comparison between the T0 and T1 groups was not performed because different CEA cut-off values were applied at each time point (T0: 14.28 ng/mL; T1: 5 ng/mL)

CEA: Carcinoembryonic antigen, PNI: Prognostic Nutritional Index, (~2 months after initiation of systemic treatment), AUC: Area under the curve, ROC: Receiver operating characteristic

Table 3. Kaplan-Meier survival outcomes according to CEA/PNI score group at baseline (T0) and first response assessment (T1)

Group	Median OS (months)	95% CI	Log-rank p (OS)	Median PFS (months)	95% CI	Log-rank p (PFS)
Baseline CEA/PNI score (T0, n=102)						
Overall cohort	33.0	25.1-40.8	-	12.4	9.9-14.9	-
Group 1: CEA-high/PNI-low	21.6	14.9-28.4	0.057	12.2	10.4-19.4	0.254
Group 2: CEA-high/PNI-high	30.6	14.6-46.6		9.9	6.3-14.4	
Group 3: CEA-low/PNI-low	42.5	15.4-69.6		17.4	4.9-13.5	
Group 4: CEA-low/PNI-high (ref)	37.6	30.5-44.6		13.8	3.1-28.2	

Table 3. Continued

Group	Median OS (months)	95% CI	Log-rank p (OS)	Median PFS (months)	95% CI	Log-rank p (PFS)
Group 1 vs. Group 4	21.6 vs. 37.6	14.9-28.4 vs. 30.5-44.6	0.010	12.2 vs. 13.8	7.0-17.4 vs. 7.7-20.0	0.290
First response assessment CEA/PNI score (T1, n=88)						
Group 1: CEA-high/PNI-low	19.8	14.9-24.7	0.001	9.4	7.2-11.6	0.006
Group 2: CEA-high/PNI-high	24.8	14.6-35.1		8.9	7.0-10.9	
Group 3: CEA-low/PNI-low	31.1	15.4-46.8		14.6	11.6-17.5	
Group 4: CEA-low/PNI-high (ref)	60.6	33.0-88.2		21.4	8.2-34.7	
Group 1 vs. Group 4	19.8 vs. 60.6	12.0-27.7 vs. 46.6-74.6	<0.001	9.4 vs. 21.4	7.2-11.6 vs. 8.2-34.7	<0.001

OS: Overall survival, PFS: Progression-free survival, CI: Confidence interval, ref: Reference group, CEA: Carcinoembryonic antigen, PNI: Prognostic nutritional index

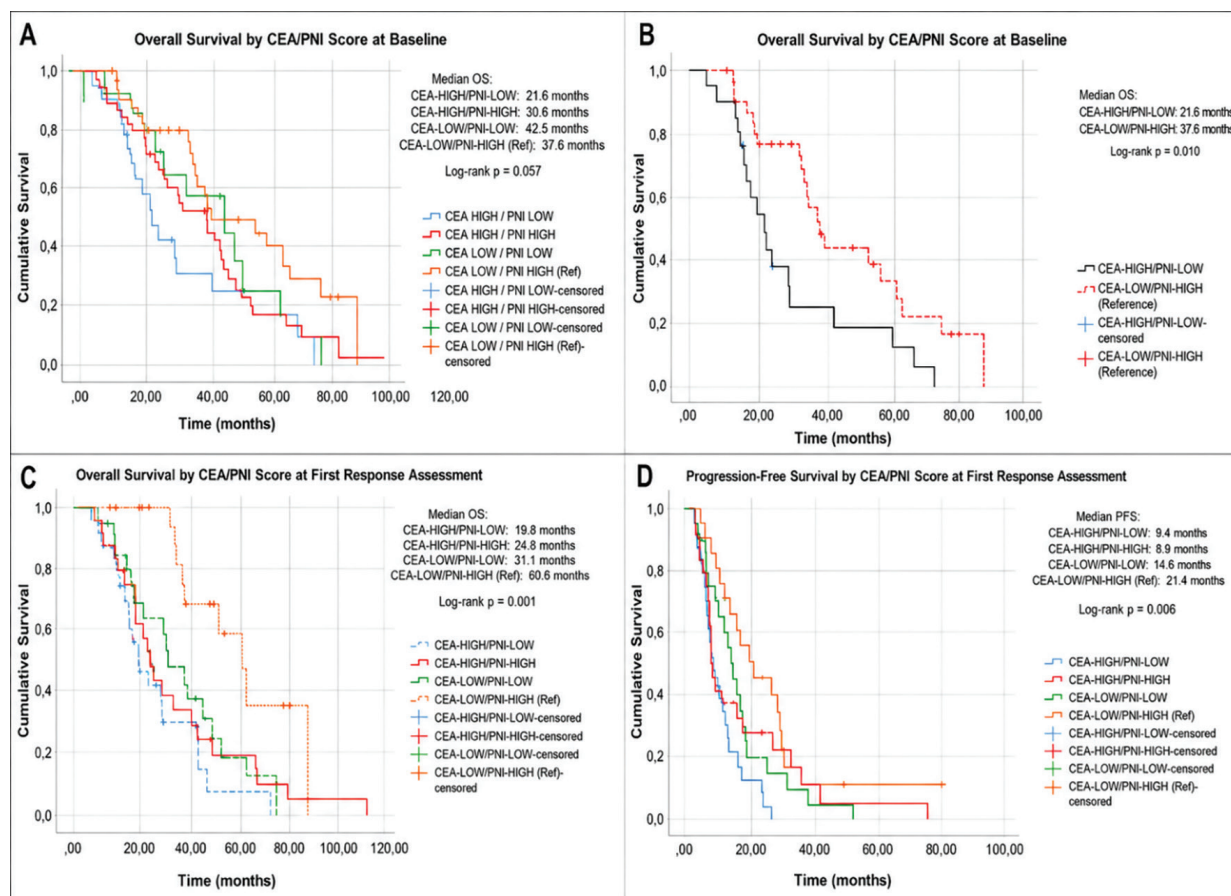


Figure 2. Kaplan-Meier curves for overall and PFS according to CEA/PNI score: (A) OS by baseline CEA/PNI score (T0), (B) OS in the highest-risk group (Group 1) versus the reference group (Group 4) at baseline (T0), (C) OS by CEA/PNI score at first response assessment (T1), (D) PFS by CEA/PNI score at first response assessment (T1)

CEA: Carcinoembryonic antigen, PNI: Prognostic nutritional index, ROC: Receiver operating characteristic, PFS: progression-free survival, OS: Overall survival

Table 4. Univariable and multivariable Cox proportional hazards regression analysis for OS and PFS

Variable	Univariable			Multivariable		
	HR	95% CI	p value	HR	95% CI	p value
OS						
Model 1: baseline CEA/PNI score (T0, n=102)						
CEA/PNI group (ref: Group 4)						
Group 1 (CEA-high/PNI-low)	2.33	1.04-5.24	0.039	2.75	1.18-6.43	0.019
Group 2 (CEA-high/PNI-high)	1.11	0.53-2.31	0.768	1.21	0.56-2.62	0.622
Group 3 (CEA-low/PNI-low)	1.16	0.46-2.90	0.746	1.50	0.56-4.00	0.415
Bone metastasis (ref: absent)	4.12	1.54-10.98	0.005	3.18	1.05-9.65	0.041
Multiple metastatic sites (ref: single)	1.98	1.04-3.79	0.037	1.43	0.72-2.86	0.301
Surgical interven (ref: no surgery)	0.52	0.29-0.95	0.033	0.56	0.28-1.10	0.093
Model 2: first response assessment CEA/PNI score (T1, n=88)						
CEA/PNI group (ref: Group 4)						
Group 1 (CEA-high/PNI-low)	4.28	1.36-13.46	0.013	3.48	0.97-12.4	0.055
Group 2 (CEA-high/PNI-high)	3.20	1.01-10.16	0.048	3.16	0.96-10.37	0.057
Group 3 (CEA-low/PNI-low)	4.30	1.35-13.69	0.013	4.84	1.43-16.38	0.011
Bone metastasis (ref: absent)	4.12	1.54-10.98	0.005	5.45	1.38-21.55	0.016
Multiple metastatic sites (ref: single)	1.98	1.04-3.79	0.037	1.33	0.57-3.11	0.506
Surgical interven (ref: no surgery)	0.52	0.29-0.95	0.033	0.45	0.219-0.9	0.038
PFS						
Model 1: baseline CEA/PNI score (T0, n=102)						
CEA/PNI group (ref: Group 4)						
Group 1 (CEA-high/PNI-low)	1.35	0.67-2.70	0.394	1.40	0.68-2.86	0.350
Group 2 (CEA-high/PNI-high)	1.13	0.59-2.16	0.707	1.09	0.54-2.20	0.808
Group 3 (CEA-low/PNI-low)	0.54	0.22-1.30	0.173	0.63	0.24-1.61	0.337
Primary tumor location (ref: right)	0.83	0.71-0.97	0.021	0.42	0.23-0.74	0.003
Multiple metastatic sites (ref: single)	1.92	1.12-3.29	0.016	1.45	0.78-2.69	0.228
Lung metastasis (ref: absent)	2.00	1.16-3.45	0.013	1.69	0.93-3.09	0.084
Bone metastasis (ref: absent)	2.07	0.82-5.24	0.122	1.14	0.39-3.25	0.811
Surgical interven (ref: no surgery)	0.45	0.25-0.78	0.005	0.49	0.25-0.93	0.031
Model 2: first response assessment CEA/PNI Score (T1, n=88)						
CEA/PNI group (ref: Group 4)						
Group 1 (CEA-high/PNI-low)	2.64	1.19-5.82	0.016	1.72	0.72-4.10	0.216
Group 2 (CEA-high/PNI-high)	1.23	0.53-2.85	0.625	1.14	0.46-2.85	0.770
Group 3 (CEA-low/PNI-low)	1.69	0.74-3.85	0.211	1.61	0.68-3.81	0.277
Primary tumor location (ref: right)	0.56	0.33-0.96	0.035	0.47	0.25-0.89	0.021
Multiple metastatic sites (ref: single)	1.92	1.12-3.29	0.016	1.49	0.75-2.98	0.250
Lung metastasis (ref: absent)	2.00	1.16-3.45	0.013	1.54	0.77-3.06	0.213
Bone metastasis (ref: absent)	2.07	0.82-5.24	0.122	1.14	0.30-4.31	0.837
Surgical interven (ref: no surgery)	0.45	0.25-0.78	0.005	0.43	0.21-0.87	0.019
Bold values indicate statistical significance (p<0.05). T0 model: n=102; T1 model: n=88 (patients with available T1 laboratory data) HR: Hazard ratio, CI: Confidence interval, PFS: Progression-free survival, OS: Overall survival, CEA: Carcinoembryonic antigen, PNI: Prognostic nutritional index						

Discussion

Among our patients with *de novo* stage IV colon cancer, the combination of CEA and PNI had greater prognostic value than either marker alone. At baseline, membership in the

highest-risk group (high CEA/low PNI) was associated with a median survival of 21.6 months compared with 37.6 months in the reference group, and remained an independent predictor of mortality after multivariable adjustment (HR: 2.75). The prognostic value of the score increased at the first

response assessment (T1), when the median OS ranged from 19.8 months in the highest-risk group to 60.6 months in the reference group. Among clinical variables, bone metastasis was the strongest independent predictor of poor survival, whereas primary tumor resection was associated with longer survival.

Combining a marker of tumor burden with a marker of host status outperformed either marker alone, echoing earlier work predominantly in earlier-stage disease. In stage II-III colon cancer, Xu et al. [9] reported an AUC of 0.797 for the combined score, well above CEA (0.568) or PNI (0.427) singly, and a larger metastatic series found the high-CEA/low-PNI profile again carried the shortest survival [5]. Composite indices pairing CEA with inflammatory or immune markers, such as the C-systemic inflammation response index score validated by Cai et al. [11] and the inflammation-nutrition score proposed by Li et al. [12], consistently retain independent prognostic value in multivariable models where individual markers frequently lose statistical significance. Our colon-specific, *de novo*, stage IV Turkish cohort adds to this evidence.

The primary finding of our study is the gain in prognostic power from baseline (T0) to the first response assessment (T1). While the four-group survival stratification was only borderline significant at baseline ($p=0.057$), it became clearly significant at T1 ($p=0.001$). A similar pattern was observed for CEA and PNI when evaluated separately, suggesting that a single baseline measurement may underestimate the value of serial assessment. The early treatment measurement likely reflects not only initial tumor burden and host reserve, but also treatment response. The individual group results at T1 should be interpreted with caution. Because each group was small, the confidence intervals were wide; the highest-risk group reached only borderline significance ($p=0.055$), and the CEA-low/PNI-low group had a particularly wide interval (95% CI: 1.43-16.39). The clear result of the overall four-group comparison ($p=0.001$) is, therefore, the stronger finding.

This interpretation is supported by previous studies on biomarker dynamics. Konishi et al. [13] found that postoperative CEA normalization brought outcomes in line with patients who started with normal CEA, while CEA that stayed high tracked with worse prognosis. Similarly, Muñoz-Montaño et al. [14] reported that failure to normalize CEA identified the group with the worst outcomes. Regarding nutritional status, Lee and Kang [15] found that combining pre- and postoperative PNI provided better prognostic performance than either value alone, and PNI has been shown to change during chemotherapy [16]. Our findings emphasize the added prognostic value of serial CEA and PNI measurements when this dynamic assessment is extended from the surgical setting to systemic treatment for metastatic colon cancer.

Our baseline CEA cut-off of 14.28 ng/mL sits well above the usual 5 ng/mL; this is consistent with work showing that a single fixed threshold performs poorly across different stages. Jeon et al. [17] found mean CEA rising from 3.0 ng/mL at stage I to 9.2 at stage III and argued for stage-specific thresholds; Jia et al. [18] used cut-offs of 4.9 and 27.2 ng/

mL tied to nodal status; and Cai et al. [11] adopted 13.0 ng/mL, close to ours. When every patient is at stage IV and has a high tumor burden, a higher cut-off is expected, and the conventional 5 ng/mL loses its value when most exceed it. At T1, however, we applied the standard 5 ng/mL because the ROC-derived 5.42 ng/mL closely matched it and preserved the clinical practicality of the measurement. Because the two time points used different CEA thresholds, the T0 and T1 groups are not directly comparable, so we did not treat them as a single variable over time. Each time point was analyzed on its own. The two measurements also convey different information: the baseline value reflects the tumor burden before treatment, while the T1 value reflects an early response. The stronger separation at T1 should therefore be interpreted as indicating that an early on-treatment check adds prognostic information, rather than as a direct comparison of the same score over time. Because this cut-off came from a single center, it should be considered exploratory and should be tested in other independent groups before wider use.

PNI behaved differently. Weak at baseline ($p=0.121$), it became significant at the first response assessment ($p=0.009$), in line with Xu et al. [7] meta-analysis showing PNI a robust predictor of OS but weaker for PFS. Reported PNI cut-offs vary: 46.6 in the Turkish series of Kesinkilic et al. [8], 48.0 in the Japanese cohort of Takamizawa et al. [10], and 47.05 by Li et al. [12]. Our analysis did not yield a stable threshold; therefore, we adopted 48.0 from the largest stage IV series. The 46-48 clustering suggests that this value is reasonable for advanced disease. Only the CEA cut-off came from our own data; the PNI cut-off was taken from the literature. The combined score is therefore better regarded as a practical, ready-to-use tool rather than as a score specific to our population.

Bone metastasis was our strongest single clinical predictor, raising the risk of death about threefold at baseline and more than fivefold at T1. This fits the literature: survival after skeletal involvement is short, around 5 to 18 months, and high CEA adds to the risk [19,20]. In the surveillance, epidemiology, and end results nomogram by Guan et al. [21], CEA positivity, absence of primary resection, and bone involvement were all associated with worse survival, consistent with our results.

Primary tumor resection was associated with longer survival, and left-sided tumor location predicted better PFS. This matches large series that tie resection to lower mortality and several months of added survival, with the benefit mainly in left-sided tumors [22-24]. However, this association should be interpreted with caution, as patients selected for surgery often have lower disease burden and better clinical status; therefore, part of the observed survival advantage may reflect selection bias rather than the direct effect of surgery. In this context, the poorer outcomes observed in the high-CEA/low-PNI group may reflect the combined effect of higher tumor burden and impaired host nutritional-inflammatory status.

Study Limitations

A few limitations apply. The study was retrospective, single-center, and included 102 patients; the small size of the low-

CEA/low-PNI group (n=13) limited the precision of subgroup estimates. T1 data were missing for 14 patients, and the PNI cut-off was based on previous literature rather than being derived from the present cohort. The T1 models were based on 88 patients across four groups, so some subgroups were small and their individual estimates were less stable. The overall four-group comparison, rather than any single group's HR, should therefore be considered the primary evidence of the score's value. Patients missing T1 data did not differ from the remaining patients with respect to baseline CEA, PNI, or the CEA/PNI score, which argues against systematic bias in the on-treatment analyses. The associations observed for primary tumor resection and tumor sidedness should also be interpreted cautiously, as patients selected for surgery are often fitter and have lower disease burden, which may introduce selection bias [22,24]. Although the first-line treatment regimen was not associated with survival and was therefore not included in the models, treatment heterogeneity remains a potential confounder. Finally, MSI status was unavailable in more than one-third of patients, thereby limiting molecular interpretation.

Conclusion

In patients with *de novo* stage IV colon cancer, the combined CEA/PNI score identifies a high-risk group with markedly shorter survival using laboratory values already collected in routine care. The score was more informative when measured early in treatment than at diagnosis alone, suggesting that a second assessment after initiation of systemic therapy provides additional prognostic information. Bone metastasis and the absence of primary tumor surgery were the other main determinants of poor outcome. These results require confirmation in prospective multicenter studies before the score can be recommended for routine clinical use, but they support its potential as a low-cost tool for risk stratification in advanced colon cancer.

Ethics

Ethics Committee Approval: This study received ethical approval from the Non-Interventional Clinical Research Ethics Committee of the Faculty of Medicine, Aydın Adnan Menderes University (approval no: 16, date: 14.05.2026).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Concept: M.T., M.N.B., Design: M.T., M.N.B., Data Collection or Processing: M.T., M.N.B., Analysis or Interpretation: M.T., M.N.B., Literature Search: M.T., M.N.B., Writing: M.T., M.N.B.

Conflict of Interest: No conflict of interest was declared by the authors.

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Declaration Regarding the Use of AI and AI-Assisted Technologies: During the preparation of this article, the authors used artificial intelligence for language correction and text editing. The authors are fully responsible for the accuracy, completeness, and scientific content of the final article.

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Original Article

Global Research Trends in Paroxysmal Nocturnal Hemoglobinuria: A Bibliometric and Scientometric Analysis (2015-2025)

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ABSTRACT

Aim: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired hematologic disorder characterized by complement-mediated intravascular hemolysis, thrombosis, and bone marrow failure. Over the past decade, advances in complement-inhibition therapies have significantly transformed disease management. This study aimed to provide a comprehensive bibliometric and scientometric analysis of global PNH research published between 2015 and 2025 and to identify research trends, thematic evolution, and collaborative networks.

Methods: Publications indexed in the Web of Science Core Collection between 2015 and 2025 were retrieved using a topic-based search strategy. After manual screening of records, 1,352 English language articles and reviews were included. VOSviewer and CiteSpace were used to perform collaboration, keyword co-occurrence, and temporal cluster analyses.

Results: Annual PNH-related publications generally demonstrated an increasing trend, particularly after 2018, coinciding with the clinical expansion of complement-inhibition strategies. Keyword analysis revealed four principal thematic clusters: (1) complement-mediated hemolysis and therapy; (2) bone marrow failure syndromes; (3) diagnostics and molecular biology; and (4) clinical outcomes and complications. A temporal analysis demonstrated a shift from mechanistic and diagnostic studies (2015-2018) to therapeutic innovation and long-term outcomes research (2022-2025). The United States emerged as the leading contributor and central hub of international collaboration, followed by Japan, England, and Italy. Research activity from Asian countries has increased in recent years.

Conclusion: PNH research over the past decade has shifted from foundational disease characterization toward therapeutic optimization and personalized management strategies. Complement inhibition remains the central focus of investigation. Continued international collaboration and research on emerging therapies and real-world outcomes are essential to address unmet clinical needs.

Keywords: Paroxysmal nocturnal hemoglobinuria, bibliometric analysis, scientometrics, VOSviewer, complement inhibitors

Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired hematologic disorder characterized primarily by complement-mediated intravascular hemolysis, thrombosis, and bone marrow failure. The disease arises from a somatic mutation in the *PIGA* gene, which leads to the loss of glycosylphosphatidylinositol-anchored proteins on hematopoietic cells, rendering red blood cells highly susceptible to complement-mediated lysis. PNH can present

with fatigue, hemoglobinuria, abdominal pain, thrombosis, and varying degrees of anemia, which contribute to significant morbidity and mortality if untreated [1].

The discovery and implementation of complement inhibition therapies have transformed the clinical management of PNH. The first targeted therapy, eculizumab, is a humanized monoclonal antibody directed against complement protein C5. Eculizumab has been shown to dramatically reduce intravascular hemolysis, decrease transfusion requirements,

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improve quality of life, and markedly reduce the risk of thrombotic events, which historically were the leading cause of death in untreated PNH patients [2].

Building upon the success of eculizumab, second-generation C5 inhibitors such as ravulizumab have been developed, offering extended dosing intervals and improved pharmacokinetics, thereby enhancing treatment convenience without compromising efficacy [3]. In addition, more recent advances include the development of proximal complement inhibitors such as pegcetacoplan (targeting C3), and oral inhibitors targeting complement factors B and D, which aim to address both intravascular and extravascular hemolysis and further improve patient outcomes [4].

Despite significant therapeutic advancements, PNH remains an ultra-rare and clinically heterogeneous condition, with ongoing research addressing unmet needs such as breakthrough hemolysis, suboptimal responses to existing therapies, and optimal long-term disease management strategies [5]. Over the past decade, the volume of scientific literature on PNH has increased substantially, encompassing mechanistic insights, therapeutic developments, clinical outcomes, and real-world treatment experiences.

Bibliometric and scientometric analyses provide quantitative and visual insights into research trends, influential authors, institutional collaborations, and thematic focuses within a scientific field. These methods are especially valuable in rare diseases like PNH, where a comprehensive understanding of research activity can guide future investigations and identify knowledge gaps. Therefore, this study aims to conduct a bibliometric and scientometric analysis of PNH research published between 2015 and 2025, using data from the Web of Science database and performing network visualization with VOSviewer. To our knowledge, no previous study has comprehensively evaluated the global intellectual structure, thematic evolution, and collaboration patterns of PNH research using combined bibliometric and scientometric approaches.

Methods

Data Source and Search Strategy

The bibliometric dataset was retrieved from the Web of Science Core Collection on 18 December 2025. The search was conducted within the Science Citation Index Expanded and the Emerging Sources Citation Index.

Publications related to PNH published between 2015 and 2025 were identified using the following topic-based search strategy:

TS=(“paroxysmal nocturnal hemoglobinuria” or “PNH”)

Both the full disease name and its commonly used abbreviation were included in the search strategy to maximize retrieval sensitivity. Only English language articles and reviews were included in the analysis. Editorials, meeting abstracts, corrections, letters, and non-English publications were excluded.

Because searches using the isolated abbreviation “PNH” may retrieve unrelated publications, all retrieved records were manually screened by title and abstract to ensure relevance to PNH. Irrelevant records were excluded from the final dataset.

All records were exported in plain text (.txt) format, including full records and cited references, for bibliometric and scientometric analyses. Duplicate records were identified and removed prior to bibliometric analysis. Data from 2025 represent a partial year and should therefore be interpreted cautiously.

Ethical Considerations

This study did not involve human participants or animal subjects and was based exclusively on publicly available bibliographic data; therefore, ethical approval was not required.

Bibliometric Network Analysis

VOSviewer (version 1.6.20) was used to construct keyword co-occurrence, country collaboration, and author collaboration networks. Full counting was applied. The minimum keyword occurrence threshold was set at 5. Node size represented publication or keyword frequency, whereas link thickness reflected the total link strength.

Country- and author-level collaboration analyses were performed to identify the most productive countries, institutions, and influential researchers in PNH research.

Temporal and Cluster Analysis

Temporal evolution and cluster analyses were conducted using CiteSpace (version 6.3.R1). The analysis was performed using time slicing with one-year intervals from 2015 to 2025. The node selection criterion was set to the top 25 most-cited or most frequently occurring items per slice. Pathfinder pruning was applied to simplify the network structure and improve visualization clarity.

Network quality and clustering validity were evaluated using modularity (Q) and mean silhouette (S) scores. Higher Q and S values indicated robust and well-defined thematic clusters.

Visualization and Data Interpretation

All visualizations were generated using the default layout and clustering algorithms of VOSviewer and CiteSpace. Network maps were interpreted according to node size, color distribution, and link strength. Results obtained from both software tools were compared to improve thematic consistency and interpretability.

Results

Global Publication Trends

A total of 1,352 publications related to PNH, published between 2015 and 2025, were retrieved from the Web of Science Core Collection and were included in the analysis. The annual number of publications increased over the study period,

particularly after 2018, coinciding with the introduction and broader clinical adoption of novel complement inhibitors such as ravulizumab and pegcetacoplan. However, modest year-to-year fluctuations were observed (Figure 1).

Keyword Co-occurrence Analysis

The keyword co-occurrence network generated using VOSviewer revealed a well-organized and interconnected

thematic structure. The most frequent keyword, PNH formed the central node of the network and was surrounded by closely related concepts such as hemolysis, aplastic anemia, complement system, and flow cytometry (Figure 2).

Several major thematic clusters were identified:

- Cluster 1: Complement-mediated hemolysis and therapy: Included terms such as eculizumab, ravulizumab, complement inhibitor, pegcetacoplan, and extravascular hemolysis.

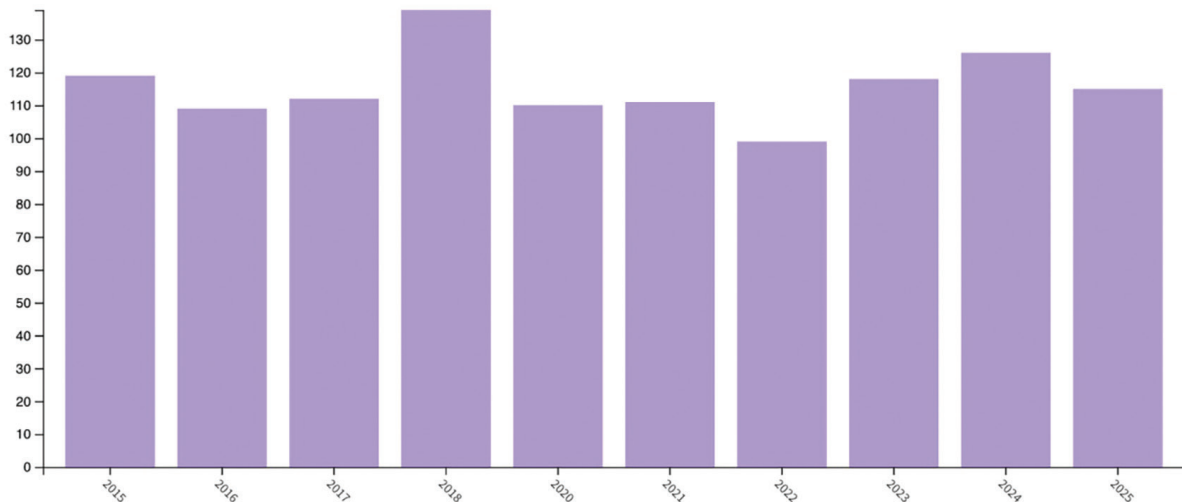


Figure 1. Annual number of publications on paroxysmal nocturnal hemoglobinuria between 2015 and 2025, illustrating the temporal trend and growth in research activity

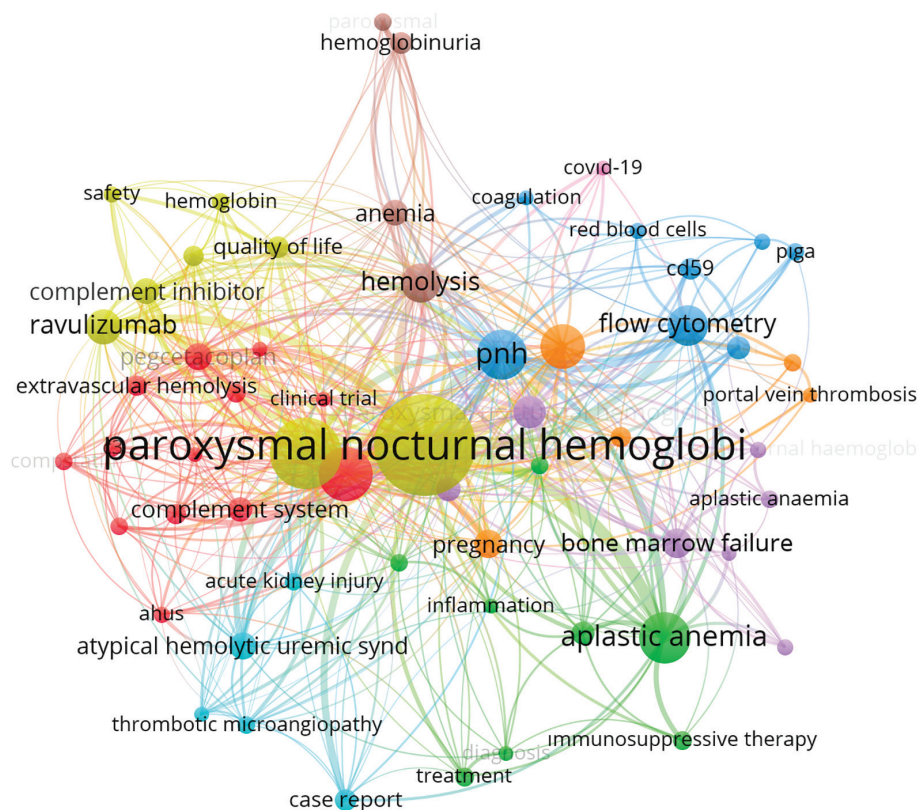


Figure 2. Keyword co-occurrence network visualization of paroxysmal nocturnal hemoglobinuria research generated using VOSviewer. Node size reflects keyword frequency, while link thickness indicates co-occurrence strength between keywords. Different colors represent thematic clusters related to complement inhibition, bone marrow failure syndromes, diagnostics, and clinical outcomes

This cluster reflects the dominance of therapeutic research centered on complement inhibition strategies.

- Cluster 2: Bone marrow failure syndromes contained aplastic anemia, bone marrow failure, and stem cell transplantation, underscoring the biological overlap between PNH and immune-mediated marrow disorders.
- Cluster 3: Diagnostics and molecular biology included flow cytometry, CD55, CD59, PIGA mutation, and biomarkers, highlighting sustained attention to diagnostic advancements and pathogenesis.
- Cluster 4: Clinical outcomes and complications: contains thrombosis, quality of life, and safety, emphasizing patient-centered research directions.

Overall, the keyword network demonstrates that recent PNH research integrates pathophysiology, diagnostics, and therapeutic innovation into a cohesive, multidisciplinary field.

Temporal Evolution of Research Themes

CiteSpace analysis demonstrated a well-structured temporal progression of research themes from 2015 to 2025 (modularity Q=0.584; mean silhouette=0.918) (Figure 3).

- 2015-2018: Early studies focused on disease mechanisms, diagnostic confirmation, and hemolysis-related concepts.
- 2019-2021: Research attention shifted toward complement-targeted therapies, particularly eculizumab and ravulizumab, and toward the intersection between PNH and aplastic anemia.

- 2022-2025: New clusters emerged around complement-mediated hemolysis, narrative reviews, novel complement inhibitors, and real-world clinical data, indicating the maturation of clinical research and the expansion of therapeutic options.

Country Collaboration Analysis

The country co-authorship network demonstrated that PNH research is globally distributed but concentrated in a limited number of highly productive countries (Table 1, Figure 4). The United States emerged as the principal hub for international collaboration, demonstrating the highest publication volume and the strongest collaborative linkages.

Other leading contributors included Japan, England, and Italy, which formed a major triad of transcontinental research partnerships. China, South Korea, and India have shown rapid growth in publication output since 2020, reflecting expanding global participation and access to complement inhibitors.

Peripheral yet notable contributions were observed from Germany, France, and Brazil, suggesting a gradual diversification of PNH-related research beyond traditional centers.

The institutional productivity analysis demonstrated that major contributions originated from specialized hematology and complement research centers, located primarily in Europe and North America (Table 4). Assistance Publique-Hôpitaux de Paris, Université Paris Cité, and Johns Hopkins University were among the most productive institutions in the field.

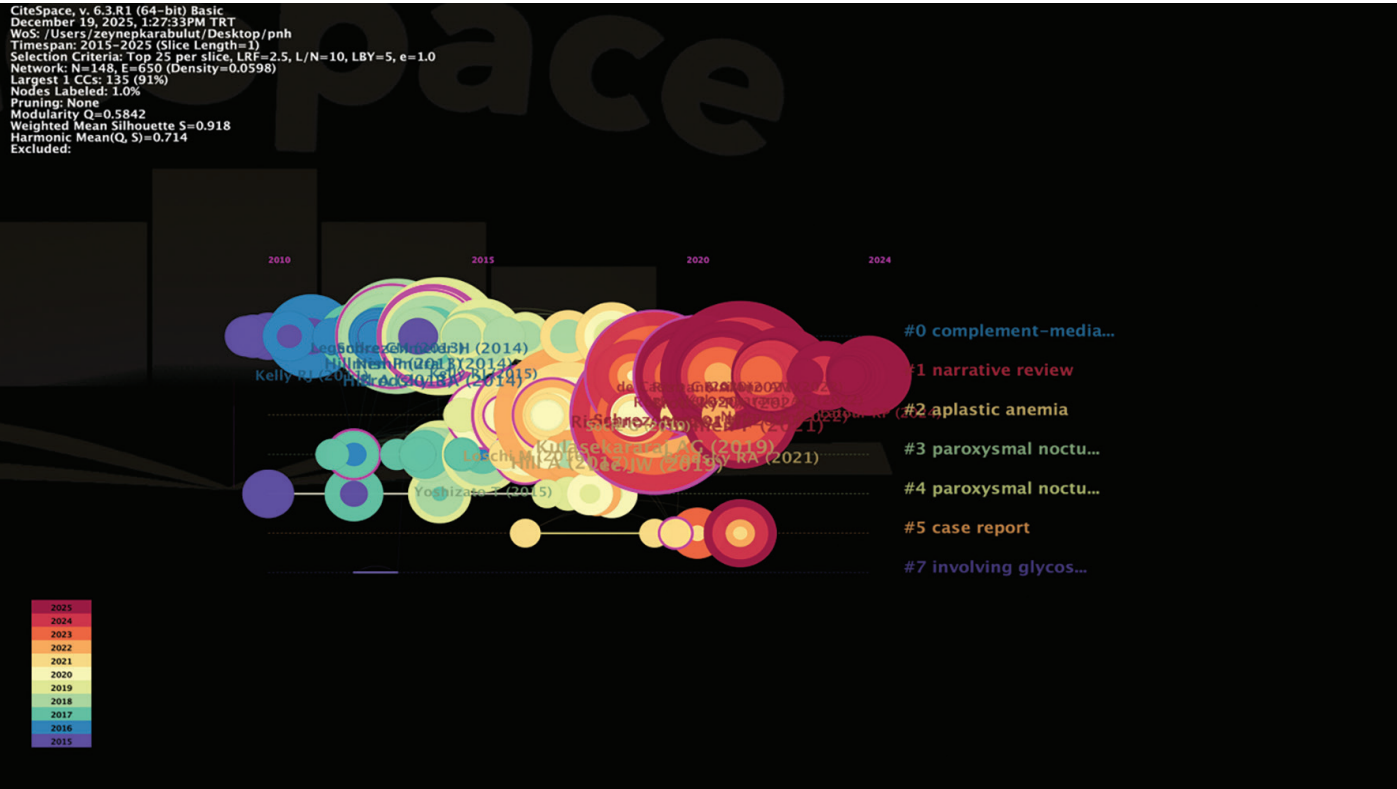


Figure 3. Timeline visualization of thematic evolution in paroxysmal nocturnal hemoglobinuria research between 2015 and 2025 generated using CiteSpace. Node size represents keyword frequency, and color gradients indicate publication year. The figure demonstrates the temporal transition from mechanistic and diagnostic studies toward complement inhibition therapies, real-world clinical outcomes, and personalized treatment strategies

Table 1. Top contributing countries in paroxysmal nocturnal hemoglobinuria research

Rank	Country	Publications
1	United States	520
2	Italy	156
3	China	153
4	Japan	147
5	England	144
6	Germany	136
7	France	101
8	Canada	69
9	Netherlands	66
10	Switzerland	66

Author Collaboration Network

The author co-authorship network analysis identified several influential researchers who serve as central nodes in the field (Table 2, Figure 5). Key contributors included de Latour et al. [6], Kulasekararaj et al. [7], Brodsky [8], Lambris et al.[9], and Obara N. [10], who represent core nodes with dense interconnections.

Distinct author clusters corresponded to subfields such as:

- clinical trial design and complement therapy (de Latour et al. [6], Brodsky [8]),
- immunopathogenesis (Lambris et al. [9]),
- and long-term patient management (Kulasekararaj et al. [7], Obara et al. [10]).

This demonstrates a highly collaborative, multidisciplinary research structure that combines clinical and translational expertise.

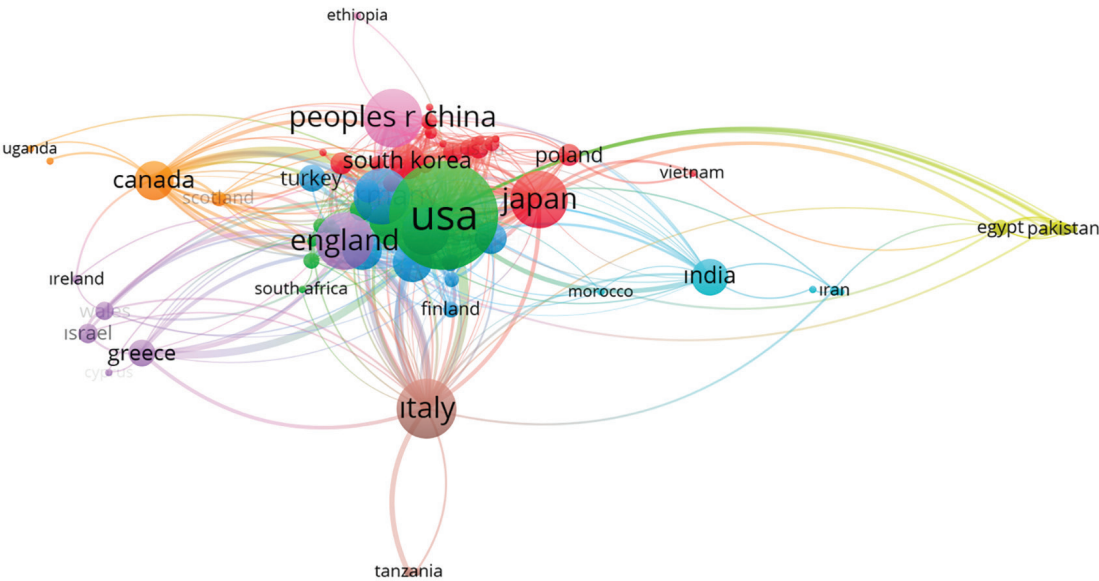


Figure 4. International country collaboration network in paroxysmal nocturnal hemoglobinuria research generated using VOSviewer. Node size reflects publication output, while link thickness represents collaboration strength between countries. The United States occupies the central position within the global collaboration network, with strong connections to major research contributors including Japan, England, Italy, and China

Table 2. Most influential authors in paroxysmal nocturnal hemoglobinuria research

Rank	Author	Publications
1	de Latour et al. [6]	43
2	Brodsky [8]	39
3	Kulasekararaj et al. [7]	38
4	Schrezenmeier et al. [11]	36
5	Lee et al. [12]	33
6	Risitano et al. [13]	31
7	Kulasekararaj et al. [14]	30
8	Fattizzo et al. [15]	26
9	Schrezenmeier et al. [11]	26
10	Höchsmann et al. [16]	25

Network Characteristics

The global keyword co-occurrence network comprised 148 nodes and 650 links, with a network density of 0.0598. The largest connected component contained 91% of items, which confirms a well-integrated and cohesive field. The high silhouette score indicates that thematic clusters are internally consistent and conceptually meaningful. The

relatively high proportion of interconnected nodes suggests that PNH research has evolved into a highly collaborative and interdisciplinary field that integrates hematology, immunology, molecular biology, and translational therapeutics. Citation analysis identified several landmark publications that substantially shaped the contemporary understanding and therapeutic management of PNH (Table 3).

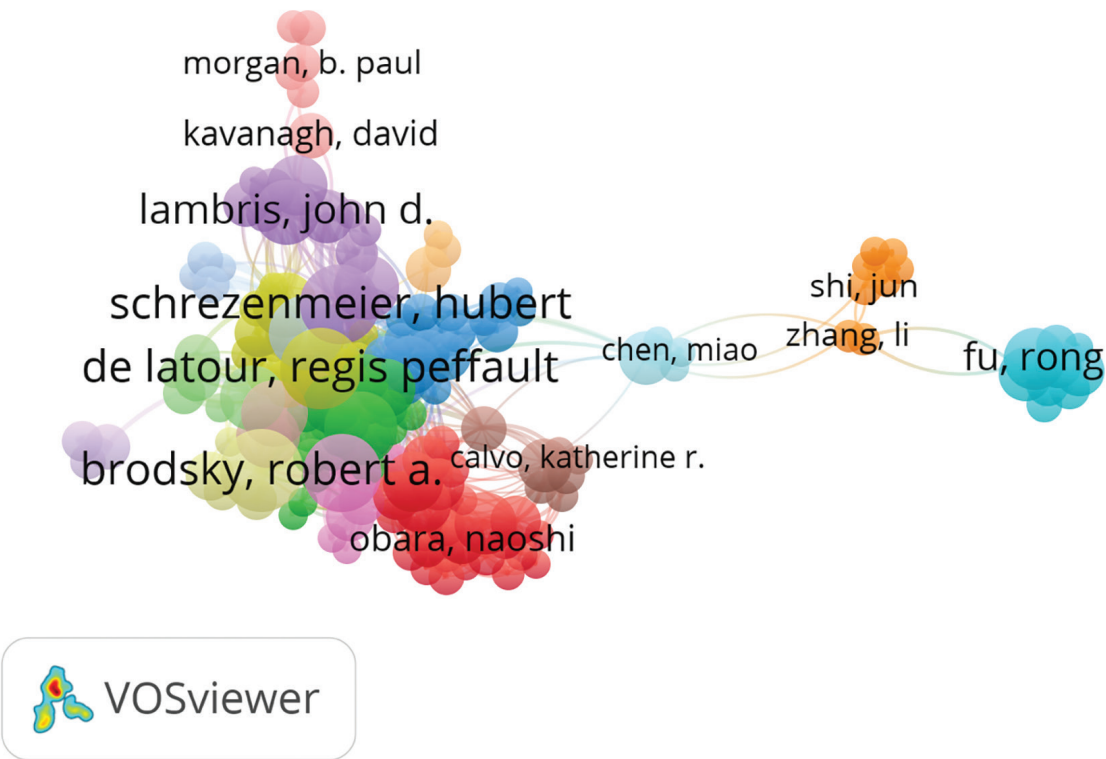


Figure 5. Author collaboration network in PNH research (2015-2025) generated by VOSviewer. Node size represents publication volume, and link thickness indicates collaboration strength. Prominent hubs include de Latour et al. [6], Kulasekararaj et al. [7], Brodsky [8], Lambris et al. [9], and Obara et al. [10], forming distinct but interconnected research clusters

Table 3. Most influential PNH-related publications identified in the bibliometric analysis (2015-2025)				
Rank	First Author	Year	Journal	Citations
1	Hill et al. [17]	2017	Nature Reviews Disease Primers	367
2	Lee et al. [12]	2019	Blood	340
3	Hillmen et al. [18]	2021	New England Journal of Medicine	329
4	Kulasekararaj et al. [14]	2019	Blood	311
5	Kelly et al. [19]	2015	New England Journal of Medicine	221

PNH: Paroxysmal nocturnal hemoglobinuria

Table 4. Leading institutions contributing to PNH research		
Rank	Institution	Publications
1	Assistance Publique Hôpitaux Paris	75
2	Université Paris Cité	75
3	Hospital Universitaire Saint Louis APHP	57
4	Johns Hopkins University	54
5	King’s College Hospital NHS foundation trust	53

PNH: Paroxysmal nocturnal hemoglobinuria, APHP: Assistance Publique-Hôpitaux de Paris

Discussion

This bibliometric and scientometric analysis provides a comprehensive overview of the intellectual structure and temporal evolution of PNH research between 2015 and 2025. By integrating keyword co-occurrence, temporal clustering, and collaboration networks, this study highlights how research priorities have progressively shifted from foundational disease mechanisms toward therapeutic innovation and long-term clinical management.

Our results indicate that complement-mediated hemolysis and complement-inhibition therapies are a central research theme in contemporary PNH literature. This finding is consistent with the pivotal role of complement dysregulation in PNH pathophysiology and the transformative clinical impact of complement inhibitors. Since the introduction of eculizumab, a monoclonal antibody targeting complement component C5, multiple studies have demonstrated significant reductions in intravascular hemolysis, thromboembolic events, and transfusion requirements, along with improved survival outcomes [6,7]. These therapeutic advances are clearly reflected in the prominence of keywords related to complement inhibition in our co-occurrence network.

The temporal analysis further revealed a transition in research focus. Early studies primarily emphasized diagnostic confirmation, hemolytic mechanisms, and disease characterization, whereas later publications increasingly concentrated on long-acting and next-generation complement inhibitors, including ravulizumab and pegcetacoplan. Ravulizumab, engineered to allow extended dosing intervals while maintaining sustained complement inhibition, has been shown to provide efficacy comparable to eculizumab with improved treatment convenience [8,9]. The emergence of ravulizumab-related keywords in recent years underscores the growing emphasis on optimizing long-term disease control and patient quality of life.

Importantly, recent literature has expanded beyond terminal complement blockade to explore proximal complement inhibition, particularly targeting C3 and factor B. Pegcetacoplan, a C3 inhibitor, has demonstrated superior hemoglobin stabilization and reduced transfusion dependence in selected patient populations, especially those with persistent extravascular hemolysis despite C5 inhibition [2,10]. The increasing visibility of terms related to extravascular hemolysis and proximal complement inhibition in our analysis reflects this evolving therapeutic paradigm.

Another prominent theme identified in this study is the close association between PNH and bone marrow failure syndromes, particularly aplastic anemia. This relationship has long been recognized, with immune-mediated bone marrow injury playing a central role in the clonal expansion of PNH cells [11,12]. The persistence of aplastic anemia-related keywords across multiple time slices suggests that this overlap remains a critical area of investigation, particularly regarding treatment sequencing and hematopoietic stem cell transplantation.

Our findings demonstrate that, geographically, PNH research is dominated by a small number of highly productive countries: the United States, followed by Japan and several European countries. These regions have historically hosted major clinical trials and centers of excellence in complement biology and rare hematologic diseases [7,13]. The increasing contribution from Asian countries, such as China and South Korea, in recent years likely reflects broader access to complement inhibitors and growing participation in multinational clinical studies.

The author collaboration network further highlights the importance of multidisciplinary and multicenter cooperation in advancing PNH research. Influential researchers identified in our analysis have contributed substantially to clinical trials, translational studies, and consensus guidelines, reinforcing the collaborative nature of progress in this rare disease field [13,14].

Despite these advances, unmet clinical needs remain. Breakthrough hemolysis, residual anemia, and long-term treatment burden continue to affect a subset of patients receiving complement inhibitors [6,15]. The recent focus on novel therapeutic targets and real-world outcome studies, as reflected in our temporal clusters, suggests that future research will increasingly address treatment personalization, long-term safety, and cost-effectiveness. The growing visibility of real-world outcome studies in recent years suggests an increasing emphasis on long-term treatment safety, healthcare utilization, and patient-reported outcomes beyond controlled clinical trial settings.

Research Gaps and Future Directions

Despite substantial advances in complement inhibition therapies, several important research gaps remain in the PNH literature. Our analysis demonstrated that current research is heavily concentrated in North America, Europe, and selected Asian countries, whereas data from low- and middle-income regions remain limited. Although therapeutic studies dominate recent publications, relatively few studies focus on long-term real-world outcomes, pediatric PNH populations, quality-of-life measures, and cost-effectiveness analyses.

The emergence of keywords related to proximal complement inhibition and personalized treatment strategies suggests that future research will increasingly focus on individualized disease management. Further integration of genomic profiling, biomarker-driven approaches, and real-world registry data may improve long-term therapeutic optimization. Expanded international collaboration will also be essential to address disparities in access to novel complement inhibitors and to improve global understanding of rare hematologic disorders such as PNH.

Overall, this bibliometric analysis mirrors the clinical evolution of PNH management, underscores the central role of complement inhibition, and highlights emerging therapeutic strategies. By mapping the development of research themes and collaborations, this study provides valuable insights into past achievements and future directions in PNH research.

Study Limitations

This study has several limitations. First, the analysis was limited to the Web of Science Core Collection database and English-language publications, which may have excluded relevant studies indexed in other databases or published in other languages. Second, bibliometric analyses are inherently influenced by database coverage, citation practices, and temporal citation bias. Third, because data for 2025 cover only a partial year, recent publication trends should be interpreted with caution. Despite these limitations, the present study provides a comprehensive overview of the global research landscape and thematic evolution of PNH research.

Conclusion

This bibliometric and scientometric analysis provides a comprehensive overview of global research trends in PNH from 2015 to 2025. Complement-mediated hemolysis and complement inhibition therapies remain the central focus, reflecting their pivotal role in PNH pathophysiology and management. Temporal trends highlight a shift from foundational mechanistic studies toward next-generation complement inhibitors, real-world outcomes, and personalized treatment strategies. The study also emphasizes the importance of international and multidisciplinary collaborations in advancing PNH research. These insights not only map past achievements but also guide future directions, particularly in optimizing long-term patient outcomes, addressing unmet clinical needs, and evaluating cost-effectiveness of emerging therapies. The findings of this study may help guide future collaborative research efforts and support strategic prioritization in the rapidly evolving field of complement-mediated hematologic disorders.

Ethics

Ethics Committee Approval: This study did not involve human participants or animal subjects and was based exclusively on publicly available bibliographic data; therefore, ethical approval was not required.

Informed Consent: The requirement for informed consent has been removed.

Footnotes

Financial Disclosure: The authors declared that this study received no financial support.

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Original Article

Are the Neutrophil-to-lymphocyte Ratio and Lymphocyte-to-monocyte Ratio Prognostic Markers in Elderly Patients with Diffuse Large B-cell Lymphoma?

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ABSTRACT

Aim: Neutrophil-to-lymphocyte ratio (NLR) and lymphocyte-to-monocyte ratio (LMR) are inflammatory markers that have been studied as prognostic factors in diffuse large B-cell lymphoma (DLBCL). However, the prognostic value of these markers in elderly patients is unclear.

Methods: A retrospective analysis was conducted on 82 *de novo* DLBCL patients aged ≥65 years who received rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) or R-CHOP-like treatment. Kaplan-Meier and Cox regression analyses were used to evaluate overall survival (OS) and progression-free survival (PFS). The discriminatory power of NLR and LMR was examined using receiver operating characteristic (ROC) analysis. Restricted cubic spline analysis was used to evaluate possible nonlinear associations between these biomarkers and survival.

Results: The median follow-up time was 35.5 months. Analyses based on median values showed no significant association between NLR and LMR and OS and PFS. In ROC analysis, both NLR [area under the curve (AUC): 0.575, $p=0.289$] and LMR (AUC: 0.517, $p=0.815$) showed low discriminatory power. Multivariate analysis revealed that NLR was an independent prognostic factor for OS (hazard ratio: 1.11, 95% confidence interval: 1.01-1.22, $p=0.032$), whereas LMR was not significant. The restricted cubic spline analysis did not reveal a significant nonlinear association or a threshold effect between NLR and survival outcomes.

Conclusion: Although NLR was statistically associated with OS when analyzed as a continuous variable, no distinct threshold value was identified. These results show that, in older DLBCL patients, NLR may function as a weak, continuous biomarker rather than as a clinically significant risk indicator.

Keywords: Aged, lymphoma, lymphocyte-to-monocyte ratio, neutrophil-to-lymphocyte ratio, prognosis

Introduction

Diffuse large B-cell lymphoma (DLBCL) is responsible for one-third of all non-Hodgkin lymphomas [1]. The International Prognostic Index (IPI) [lactate dehydrogenase (LDH), stage, extranodal region, >60 years, Eastern Cooperative Oncology Group (ECOG)]; Revised-IPI (R-IPI) (stage, ECOG, LDH); and National Comprehensive Cancer Network (NCCN) IPI are risk scores used to estimate patient prognosis.

Prognosis in DLBCL also varies by subtype and biological characteristics. Due to difficulties in standardization, a comprehensive risk scoring system incorporating these

factors is not yet available, thereby limiting clinicians' ability to accurately assess patient prognosis and tailor treatment strategies. There is still a need for simple scoring systems in daily practice [2]. Furthermore, the prognosis of DLBCL is poorer in elderly patients than in younger ones. Their tumor microenvironment, tumor immunology, and genetic mutations are thought to differ, which may contribute to the observed differences in prognosis and treatment response between elderly and younger patients.

Recently, several studies have suggested that the neutrophil-to-lymphocyte ratio (NLR) and lymphocyte-to-monocyte ratio (LMR), which are frequently measured in clinical practice, may

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be prognostic markers in DLBCL [3-6]. However, because the prognosis of elderly lymphoma patients differs, the effects of these ratios in this population remain unknown. This study aimed to characterize elderly patients with DLBCL and to evaluate whether LMR and NLR have prognostic significance in this population.

Methods

Patients newly diagnosed with DLBCL between 2012 and 2022 at the university hematology outpatient clinic or inpatient ward who received at least 4 cycles of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) or R-CHOP-like chemotherapy and had complete clinical data were included in the study. Patients who had previously received radiotherapy or chemotherapy were excluded. Before beginning any treatment, lymphocyte, neutrophil, and monocyte counts were measured using standard CBC testing. The absolute neutrophil count was divided by the absolute lymphocyte count to determine the NLR. The LMR was determined by dividing the absolute lymphocyte count by the absolute monocyte count. The study was designed retrospectively. This study was approved by the Ethics Committee of the Başkent University Medical and Health Sciences Research Board (approval no: KA23/260, date: 19.07.2023).

Statistical Analysis

Overall survival (OS) was defined as the time from diagnosis to death or last follow-up, and progression-free survival (PFS) was defined as the time from diagnosis to disease progression, death from any cause, or last follow-up. The associations of OS and PFS with NLR and LMR were evaluated using Kaplan-Meier curves and compared using the log-rank test. Univariate Cox regression analyses, followed by multivariate Cox proportional hazards regression analyses, were performed to assess the prognostic significance of the variables.

The cut-off values of NLR and LMR were determined based on their median values. Continuous variables were primarily analyzed without categorization to avoid loss of information.

In addition, restricted cubic spline functions were applied to Cox regression models adjusted for IPI to explore potential non-linear relationships between NLR/LMR and survival outcomes.

A two-sided p value of less than 0.05 was considered statistically significant, and all analyses included 95% confidence interval (CI). R software (version 4.5.3) and IBM SPSS Statistics 24.0 (IBM Corporation, Armonk, NY, USA) were used for statistical analyses.

Results

Between 2012 and 2022, 111 patients over the age of 65 were diagnosed with DLBCL. Thirteen patients were lost to follow-up, nine died before receiving therapy, and seven patients were excluded from the study because they refused treatment. The study included a total of 82 patients. The median age was 73.5 years, and 53.7% were female. 22% of

patients were aged 80 years or older. 78% of patients had an ECOG performance status of 0-1. Extranodal involvement was detected in 69.5% (n=57) of the cases, while central nervous system (CNS) involvement was present in 4.9% (n=4). Bone marrow involvement was present in only 4.9% (n=4) of the patients. C-myc positivity was found in 25.5% (n=12), BCL6 positivity in 75.4% (n=52), and BCL2 positivity in 58.2% (n=39). 6 (7%) patients had a double hit, and 3 (3.6%) had a triple hit. The mean Ki-67 proliferation index was 75.44±14.67 (median: 80.00; minimum-maximum: 20.00-100.00) (Table 1).

Bulky masses were present in 12.2% (n=10) of patients. According to the IPI score, 18.5% were in the high-risk group [4,5]. According to the NCCN-IPI, 29.6% were in the high-risk group [6-8].

The median follow-up time was 35.53 months (0.13-237.07). At the end of the follow-up, the recurrence rate was 15.9% (n=13), and 29.3% (n=24) of patients had died.

The discriminative ability of NLR and LMR to predict mortality was evaluated using receiver operating characteristic (ROC) analysis. The analysis showed that the area under the curve (AUC) for NLR was 0.575 (95% CI: 0.422-0.727), which was not statistically significant (p=0.289). For the NLR ≥3.07 cut-off point, the sensitivity was calculated as 54.2% and the specificity as 55.2%. Similarly, for LMR, the AUC was 0.517 (95% CI: 0.360-0.673), indicating that LMR also lacked significant discriminative power in predicting mortality (p=0.815). For the LMR ≤2.45 cut-off point, the sensitivity was 54.2% and the specificity was 55.3%. The cut-off values of NLR and LMR were determined based on their median values.

As shown in Table 2, the overall median OS (months) was not reached. Median OS (months) differed significantly across ECOG (p=0.021) and extranodal involvement (p=0.013) groups. The median NLR and LMR values were not significant for OS.

As shown in Table 3, the overall median PFS could not be reached. Median PFS (months) differed significantly between gender groups (p=0.030). Median NLR and LMR values were not significantly associated with PFS.

ECOG and extranodal involvement were found to be significant in the univariate analysis, as shown in Table 2. The multivariate Cox regression model contained these variables, which were determined to be significant in univariate analysis. Extranodal involvement was found to be an independent negative prognostic factor for OS in the multivariate Cox regression analysis including LMR [hazard ratio (HR): 4, 95% CI: 1.16-14.29, p=0.028]. However, LMR was not found to be significant when included as a continuous variable in the model (p=0.209) (Table 4).

In multivariate analysis including NLR, NLR was determined to be an independent prognostic factor for OS (HR: 1.11, 95% CI: 1.01-1.22, p=0.032). Extranodal involvement approached statistical significance in this model (p=0.051) (Table 5).

Restricted cubic spline analyses, adjusted for IPI, did not demonstrate a clear non-linear relationship between baseline NLR and survival outcomes. Although a gradual trend was observed, no distinct threshold effect was identified, and CIs were wide, particularly at higher NLR values (Figures 1 and 2).

Table 1. Distribution of sociodemographic and clinical variables

Variables	N	%
Age		
Mean $\pm$ SD	74.72 $\pm$ 7.42	
Median (min-max)	73.5 (65-95)	
Gender		
Female	44	53.7
Male	38	46.3
ECOG		
0	30	36.6
1	34	41.5
2	15	18.3
3	3	3.7
NLR		
Mean $\pm$ SD	3.87 $\pm$ 3.20	
Median (min-max)	2.91 (0.10-16.57)	
LMR		
Mean $\pm$ SD	3.29 $\pm$ 5.13	
Median (min-max)	2.58 (0.39-46.40)	
Stage		
1	22	27.5
2	9	11.3
3	12	15.0
4	37	46.3
Extranodal involvement		
Absent	25	30.5
Present	57	69.5
CNS involvement		
Absent	78	95.1
Present	4	4.9
Bone marrow involvement		
Absent	78	95.1
Present	4	4.9
c-MYC		
Negative	35	74.5
Positive	12	25.5
BCL6		
Negative	17	24.6
Positive	52	75.4
BCL2		
Negative	28	41.8
Positive	39	58.2
Ki67		
Mean $\pm$ SD	75.44 $\pm$ 14.67	
Median (min-max)	80.00 (20.00-100.00)	
Bulky disease		
Negative	72	87.7

Table 1. Continued

Variables	N	%
Positive	10	12.2
IPI score		
1	22	27.2
2	22	27.2
3	22	27.2
4	8	9.9
5	7	8.6
R-IPI		
1	3	75.0
2	1	25.0
NCCN-IPI		
1	2	2.5
2	7	8.6
3	16	19.8
4	14	17.3
5	18	22.2
6	14	17.3
7	6	7.4
8	4	4.9
Treatment response		
Complete response	60	73.2
Partial response	6	7.3
Refractory	4	4.9
Not evaluable	12	14.7
Relapse		
Absent	69	84.1
Present	13	15.9
Mortality		
Alive	58	70.7
Deceased	24	29.3
Follow-up time (months)		
Mean $\pm$ SD	53.02 $\pm$ 51.24	
Median (min-max)	35.53 (0.13-237.07)	
ECOG: Eastern Cooperative Oncology Group, NLR: Neutrophil-to-lymphocyte ratio, LMR: Lymphocyte-to-monocyte ratio, CNS: Central nervous system, NCCN-IPI: National Comprehensive Cancer Network IPI		

Discussion

The most popular clinical forecasting models for DLBCL patients receiving R-CHOP are IPI, R-IPI, and NCCN-IPI. Even though IPI has been developed across cohorts that include all age groups, it does not fully address factors particularly significant to older patients, and it does not account for the increased age-related heterogeneity among individuals over 60, including comorbidities and the effects of polypharmacy on treatment outcomes. Accumulating evidence suggests that prognostic factors change with age, and the importance of non-lymphoma-related factors increases [7,8]. The change in

Table 2. Patient OS comparisons				
Variables	2 year survival %	5 year survival %	Median (95% CI)	p
Overall survival	76.2	73.6	- (-)	
Gender				
Female	76.5	76.5	- (-)	0.452
Male	75.9	68.3	94.23 (29.51-158.95)	
ECOG				
0	83.1	83.1	- (-)	0.021
1	81.8	75.9	- (-)	
2	57.8	57.8	66.20 (0.00-174.54)	
3	33.3	33.3	2.33 (0.00-4.74)	
Stage				
1	72.7	72.7	- (-)	0.096
2	88.9	88.9	- (-)	
3	100.0	100.0	- (-)	
4	66.6	60.6	94.23 (-)	
Extranodal involvement				
Absence	91.7	91.7	- (-)	0.013
Present	69.6	65.7	94.23 (-)	
CNS involvement				
Absence	60.7	60.7	- (-)	0.630
Present	78.0	71.5	94.23 (25.31-163.14)	
Bone marrow involvement				
Absence	77.5	74.9	- (-)	0.102
Present	50.0	-	4.96 (-)	
c-MYC				
Negative	86.9	86.9	- (-)	0.098
Positive	66.7	66.7	- (-)	
BCL6				
Negative	81.4	81.4	- (-)	0.830
Positive	78.3	78.3	134.00 (-)	
BCL2				
Negative	78.6	78.6	134.00 (-)	0.487
Positive	83.1	83.1	- (-)	
Bulky disease				
Absence	75.9	73.1	- (-)	0.775
Present	78.8	78.8	- (-)	
IPI score				
Low risk (0-2)	77.3	77.3	- (-)	0.483
Intermediate-high risk (3-5)	74.5	69.5	134.00 (44.69-223.31)	
NCCN-IPI				
Low risk (0-3)	88.0	88.0	- (-)	0.114
Intermediate-high risk (4-8)	70.6	67.2	134.00 (-)	
NLR				
>2.91	79.4	79.4	- (-)	0.305
≤2.91	63.2	55.3	- (-)	
LMR				
<2.58	73.6	69	- (-)	0.430
≥2.58	79.2	72.6	- (-)	

Kaplan-Meier: Log-rank test: p<0.05 statistically significant

ECOG: Eastern Cooperative Oncology Group, CNS: Central nervous system, R-IPI: Revised International Prognostic Index, NCCN-IPI: National Comprehensive Cancer Network IPI, NLR: Neutrophil-to-lymphocyte ratio, LMR: Lymphocyte-to-monocyte ratio, CI: Confidence interval

Table 3. Patient PFS comparisons

Variables	2 year survival %	5 year survival %	Median (95% CI)	p
Overall	85.5	82.4	- (-)	
Gender				
Female	95.0	90.2	- (-)	0.030
Male	74.0	74.0	94.23 (-)	
ECOG				
0	82.3	82.3	94.23 (-)	0.735
1	83.3	83.3	- (-)	
2-3	76.9	76.9	- (-)	
Extranodal involvement				
Absence	90.2	90.2	- (-)	0.082
Present	82.8	77.9	- (-)	
CNS involvement				
Absence	76.5	76.5	- (-)	0.302
Present	89.0	81.6	- (-)	
Bone marrow involvement				
Absence	86.5	83.4	- (-)	0.177
Present	50.0	-	14.90 (-)	
c-MYC				
Negative	84.6	84.6	- (-)	0.673
Positive	75.8	75.8	- (-)	
BCL6				
Negative	93.8	93.8	- (-)	0.218
Positive	81.2	75.4	- (-)	
BCL2				
Negative	91.8	91.8	- (-)	0.190
Positive	79.9	72.6	- (-)	
Bulky disease				
Absence	85.2	81.9	- (-)	0.790
Present	88.9	88.9	- (-)	
IPI score				
Low risk (0-2)	86.6	86.6	- (-)	0.205
Intermediate-high risk (3-5)	83.6	77.2	- (-)	
NCCN-IPI				
Low risk (0-3)	85.9	85.9	- (-)	0.393
Intermediate-high risk (4-8)	84.8	80.4	- (-)	
NLR				
>2.91	79.5	75.1	- (-)	0.227
≤2.91	63.2	54.1	- (-)	
LMR				
<2.58	72.5	66.9	- (-)	0.423
≥2.58	79.7	73	- (-)	

Kaplan-Meier: Log-rank test: $p < 0.05$ statistically significant
 ECOG: Eastern Cooperative Oncology Group, CNS: Central nervous system, R-IPI: Revised International Prognostic Index, NCCN-IPI: National Comprehensive Cancer Network, NLR: Neutrophil-to-lymphocyte ratio, LMR: Lymphocyte-to-monocyte ratio, CI: Confidence interval

Table 4. Multivariate Cox regression results on the mortality risk of various clinical variables in LMR patients

Variables	HR (95% CI)	p
ECOG		0.224
0	ref	
1	0.39 (0.14-1.14)	0.086
2 and 3	0.43 (0.16-1.22)	0.113
Extranodal involvement		
Absence	ref	0.028
Present	4.00 (1.16-14.29)	
LMR (continuous)	1.03 (0.98-1.08)	0.209

-2 Log log likelihood: 179.467; $p = 0.025$

ECOG: Eastern Cooperative Oncology Group, LMR: Lymphocyte-to-monocyte ratio, HR: Hazard ratio, CI: Confidence interval

Table 5. Multivariate Cox regression results on the mortality risk of NLR and various clinical variables in patients

Variables	HR (95% CI)	p
ECOG		0.117
0	ref	
1	0.43 (0.14-1.33)	0.964
2 and 3	0.42 (0.14-1.23)	0.251
Extranodal involvement		
Absence	ref	0.051
Present	3.57 (0.99-12.50)	
NLR (continuous)	1.11 (1.01-1.22)	0.032

-2 Log log likelihood: 172.371; $p = 0.022$

ECOG: Eastern Cooperative Oncology Group, NLR: neutrophil-to-lymphocyte ratio, HR: Hazard ratio, CI: Confidence interval

tumor microenvironment and immunogenicity with aging has led to the search for immunological markers for prognostic factors in elderly lymphoma patients [9]. NLR and LMR have previously been studied as potential prognostic markers in several lymphoma subtypes. However, there is a lack of research specifically focused on older patients with DLBCL. This study adds to the existing research by being one of the few to examine the prognostic value of NLR and LMR in elderly patients with DLBCL.

Previous studies have suggested that the normal range for the NLR is between 2.1 and 5.54 [10-12]. For LMR, the values span from 2.2 to 3 [13-15]. Koh et al. [15] reported that an LMR of 2.36 or lower was a significant predictor of OS and PFS in older patients with DLBCL. However, no specific studies have examined the relationship between the NLR and DLBCL in elderly patients. A universally accepted cut-off value for these ratios has not yet been established.

In our study, no significant cut-off value could be determined using ROC analysis. Analyses based on median values did not demonstrate significant prognostic effects of NLR and LMR on OS and PFS. However, in multivariate analysis, NLR was identified as a statistically significant continuous predictor. Furthermore, restricted cubic spline analyses did not demonstrate a clear non-linear relationship or threshold effect

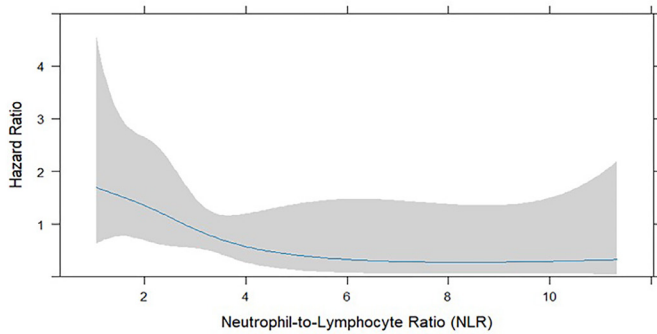


Figure 1. Restricted cubic spline for NLR and PFS Restricted cubic spline curve showing the association between baseline neutrophil-to-lymphocyte ratio and PFS adjusted for IPI. Shaded areas represent 95% confidence intervals

NLR: Neutrophil-to-lymphocyte ratio, IPI: International Prognostic Index, PFS: Progression-free survival

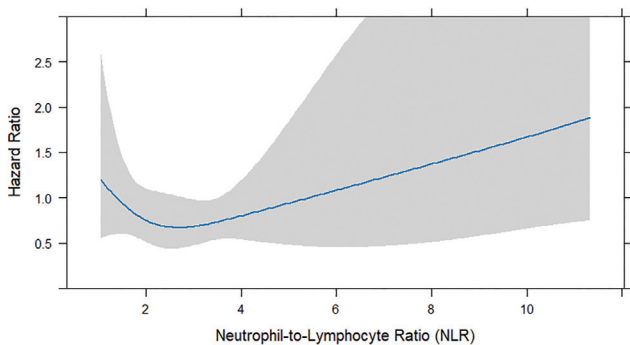


Figure 2. Restricted cubic spline for NLR and OS

Restricted cubic spline curve illustrating the association between baseline NLR and OS adjusted for IPI. Shaded areas represent 95% confidence intervals

NLR: Neutrophil-to-lymphocyte ratio, IPI: International Prognostic Index, OS: Overall survival

between NLR and survival outcomes. Although a gradual trend was observed, the absence of a distinct cut-off point supports the use of NLR as a continuous variable.

However, the low discriminative performance observed in ROC analyses suggests that, although NLR is statistically associated with survival, it provides limited clinical discrimination and functions as a relatively weak continuous biomarker.

The complete response rate (73.2%) and 5-year OS and PFS rates (73.6% and 82.4%, respectively) obtained in our study are consistent with the elderly DLBCL series reported in the literature [16,17]. A high ECOG performance score ($p=0.021$) and the presence of extranodal involvement ($p=0.013$) were associated with a statistically significant reduction in median OS, consistent with the existing literature. Results regarding the prognostic value of LMR are heterogeneous in the literature. While some studies show that low LMR is associated with OS and PFS, other studies show that LMR is not an independent prognostic marker [18,19]. Several factors may explain the lack of prognostic significance of LMR in our study. First, age-

related alterations in immune function may impair the extent to which lymphocyte and monocyte counts accurately reflect tumor biology in elderly patients. Second, the statistical power to detect weaker relationships may have been compromised by the relatively small sample size and limited occurrences. Finally, the effect of LMR may be overshadowed by stronger clinical variables and other inflammatory markers, such as NLR, in multivariate models, thereby leading to an underestimation of its significance in predicting outcomes in elderly patients with cancer.

Study Limitations

The limitations of this study include a retrospective design, a single-center setting, a small sample size, a lack of immunotherapy-based medications, and incomplete molecular data.

Conclusion

NLR was associated with OS when analyzed as a continuous variable; however, it did not demonstrate meaningful discriminatory ability or a clinically useful threshold. These findings suggest that NLR functions as a weak, continuous biomarker rather than a robust risk-stratification tool in elderly patients with DLBCL. LMR was also not prognostically significant. To further understand the clinical role of inflammatory biomarkers in this cohort, larger prospective studies are required.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of the Başkent University Medical and Health Sciences Research Board (approval no: KA23/260, date: 19.07.2023).

Informed Consent: Because the study was designed retrospectively no written informed consent form was obtained from the patients.

Footnotes

Authorship Contributions

Surgical and Medical Practices: B.T.T., D.A., E.K., S.K., Ş.Z.A., Concept: B.T.T., Ş.Z.A., Design: B.T.T., Ş.Z.A., Data Collection or Processing: B.T.T., Analysis or Interpretation: B.T.T., Literature Search: B.T.T., Ş.Z.A., Writing: B.T.T.

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Original Article

Thromboembolic Risk and Survival Outcomes in Multiple Myeloma Patients Following Autologous Stem Cell Transplantation

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ABSTRACT

Aim: To assess risk factors for venous thromboembolism (VTE) in patients with multiple myeloma (MM) who underwent autologous hematopoietic cell transplantation (HCT).**Methods:** This single-center retrospective study included 380 MM patients who underwent autologous HCT. Patients developed VTE were identified by reviewing patients' medical data. In all patients, chemotherapy regimen, remission status, comorbid conditions, age at time of HCT, number of autologous HCTs, disease stage, follow-up duration, immunoglobulin type, and mortality were assessed, and their effects on VTE development were evaluated.**Results:** Of the patients included, 63.2% (n=240) were male while 36.8% (n=140) were female. VTE occurred in 40 patients (10.5%). Mean follow-up was found as 41.63±33.84 months. Overall, 55.8% (n=212) of the patients died after autologous HCT. A significant correlation was found between older age at the time of HCT and VTE development (p=0.046). The rate of VTE-free patients was higher in patients in remission than in those not in remission (p=0.045).**Conclusion:** Remission status and older age at the time of HCT increased the risk of VTE in MM patients who underwent autologous HCT.**Keywords:** Venous thromboembolism, multiple myeloma, autologous stem cell transplantation

Introduction

Multiple myeloma (MM) is a malignancy characterized by the neoplastic proliferation of monoclonal plasma cells within the bone marrow. This proliferation often results in osteolytic lesions, osteopenia, or pathological fractures, alone or in combination. It is the second most common hematological malignancy following non-Hodgkin lymphoma.

In recent decades, advancements in MM treatment, including the introduction of novel therapies such as autologous hematopoietic cell transplantation (HCT), immunomodulatory drugs (IMiDs), and proteasome inhibitors, have significantly improved survival rates. Despite these improvements, MM patients who develop venous thromboembolism (VTE) face a threefold increase in mortality risk within the first year after diagnosis [1,2].

The onset of VTE in MM patients is influenced by both intrinsic and extrinsic factors. Tumor cells release prothrombotic factors, while external contributors such as medications, immobility, and the use of indwelling catheters further exacerbate the risk [3]. Among all hematological malignancies, MM patients exhibit the highest rates of VTE, with 4-10% developing VTE at some stage of their disease [4].

The risk of VTE is particularly elevated among MM patients receiving proteasome inhibitors and monoclonal antibodies combined with IMiDs (thalidomide, lenalidomide, and pomalidomide). The thrombogenic potential of IMiDs is attributed to their multifactorial mechanisms of action, and the risk further increases when these agents are used alongside multi-drug chemotherapy and/or high-dose dexamethasone [5,6]. *In vitro* studies have demonstrated that these agents

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elevate plasma levels of factor VIII and von Willebrand factors, induce protein C resistance, and decrease thrombomodulin levels, all of which contribute to an increased risk of thrombotic events [7,8]. Consequently, several guidelines recommend thromboprophylaxis for MM patients undergoing such therapies.

Numerous studies have investigated the VTE risk associated with drugs used in MM treatment, leading to the development of guidelines to mitigate this risk in patients receiving chemotherapy regimens or allogeneic bone marrow transplantation. However, data regarding VTE rates specifically in MM patients undergoing autologous HCT remain limited.

In this study, we aimed to evaluate the incidence of embolic events in MM patients who underwent autologous HCT, thereby contributing to the understanding of thrombotic complications in this population and to informing thromboprophylaxis strategies.

Methods

Patient Selection

We retrospectively reviewed 380 patients with MM (aged 18-65 years) who underwent autologous HCT and were followed at the Hematology and Bone Marrow Transplant Clinic, Erciyes University Faculty of Medicine, between December 2008 and December 2022.

The study was approved by the Erciyes University Clinical Research Ethics Committee (approval no: 2021/622, date: 22.09.2021). Because the study was designed retrospectively no written informed consent form was obtained from the patients. The study was conducted in accordance with the tenets of the Declaration of Helsinki and applicable patients' rights legislation.

Data were extracted from patients' files and the hospital electronic database. In all patients undergoing autologous HCT, a central venous catheter was inserted into the upper extremity before transplantation. The catheter was left in place until hematopoietic recovery unless an infection was suspected or confirmed. The central venous catheter was flushed weekly, and dressing changes were performed weekly or as needed.

Catheter-related thrombotic events were included in the study. Patients considered eligible for autologous HCT received primary thromboprophylaxis during hospitalization according to institutional standard practice. In addition, patients receiving immunomodulatory agents were administered appropriate anticoagulant or antiplatelet prophylaxis based on their individual thromboembolic risk.

The study included patients aged ≥ 18 years with available clinical data, who were diagnosed with MM according to the 18th International Myeloma Workshop criteria and underwent autologous HCT [9]. Patients with a history of long-term anticoagulation use due to thrombosis or cardiac arrhythmia, those receiving antiplatelet therapy, those with coagulopathy, and those with a history of VTE were excluded. Data on comorbid conditions, including obesity, cardiac disorders, diabetes mellitus (DM), hypertension (HT), renal failure, and

chronic obstructive pulmonary disease (COPD), were extracted from patient files. In addition, information on immunoglobulin type, response evaluation, number of transplants performed, chemotherapy regimen administered, disease stage, remission status, duration of follow-up, age at time of HCT, and mortality was assessed.

The disease response status before autologous HCT was assessed. Patients who achieved at least a partial response ($\geq PR$) before transplantation were classified as good responders, whereas those with a response less than a PR were classified as poor responders.

Identification of Cases with VTE

In this study, the primary endpoint was acute symptomatic VTE, including pulmonary embolism and deep venous thrombosis of the upper or lower extremities. Data were analyzed up to treatment completion or death for all patients.

Imaging studies and reports obtained following the first autologous HCT were screened for thrombosis or embolism. Additionally, we reviewed contrast-enhanced thoracic computed tomography images, ventilation-perfusion scintigraphy, and Doppler sonography of the upper and lower extremities. In all VTE cases, disease status was confirmed using medical records. The secondary endpoint was mortality.

Statistical Analysis

All data analyses were performed using IBM SPSS (SPSS Inc., Chicago, IL, USA), version 22. The normality of the variables was assessed using Kolmogorov-Smirnov tests and graphical analysis. When the study data were analyzed, normally distributed variables were expressed as mean and standard deviation; non-normally distributed variables were expressed as median and interquartile range, and categorical variables were expressed as percentages and counts. Independent t-tests were used to compare quantitative data that were normally distributed, while the Mann-Whitney U test was employed for data with skewed distributions. Qualitative data were compared using Pearson's chi-square test and the Fisher-Freeman-Halton exact test. The presence of DVT and PTE was analyzed using Kaplan-Meier survival analysis, according to Vadborlen 2. A p value < 0.05 was considered statistically significant.

Results

A total of 380 patients were included in the study, 63.2% (n=140) of whom were female. No significant difference in gender distribution was observed between the groups (p=0.068). The mean age of the patients was 63.48 ± 9.02 years, while their mean age at the time of HCT was 56.66 ± 8.84 years. VTE occurred in 40 patients (10.5%). The mean age at the time of HCT for the group that developed VTE was 59.30 ± 7.64 years, compared to 56.35 ± 8.93 years for the group that did not develop VTE. This difference was statistically significant (p=0.046).

Among the 40 patients who developed VTE, 9 experienced pulmonary embolism and 3 had catheter-related thrombosis.

One patient developed recurrent VTE; however, this patient was counted as a single case in the analysis. VTE events occurred from the pre-transplant conditioning period through the second month after transplantation, and the majority developed within the first 100 days following transplantation. Only one patient developed VTE during the first year after transplantation, and one additional patient did so during the second year.

While 63.2% of the patients had no comorbidities, 5.8% had DM, 4.7% had HT, 2.9% had heart failure, 2.4% had COPD, 11.3% had renal failure, and 9.7% had two or more comorbidities. The average follow-up duration of the patients was 41.63±33.84 months. Among patients in the VTE group, 80% were in remission, compared with 65% in the non-VTE group. This difference was statistically significant ($p=0.045$) (Table 1).

Table 1. Demographic and clinical characteristics of the patients

	All patients	Non-VTE (n:340)	VTE (n:40)	p value
Sex (male/female)	240/140 (63.2/36.8)	220/120	20/20	^a 0.068
Age	63.48±9.02	63.24±9.06	65.53±8.49	^b 0.130
Age at time of HCT	56.66±8.84	56.35±8.93	59.30±7.64	^b 0.046*
Follow-up (n=374)	41.63±33.84	41.89±33.97	39.45±33.13	^b 0.557
Comorbidity				
None	240 (63.2)	211 (62.1)	29 (72.5)	^c 0.666
DM	22 (5.8)	21 (6.2)	1 (2.5)	
HT	18 (4.7)	15 (4.4)	3 (7.5)	
Heart failure	11 (2.9)	10 (2.9)	1 (2.5)	
COPD	9 (2.4)	8 (2.4)	1 (2.5)	
Renal	43 (11.3)	41 (12.1)	2 (5.0)	
≥2	37 (9.7)	34 (10.0)	3 (7.5)	
Melphalan dose				
200 mg	322 (84.7)	290 (90.1)	32 (9.9)	^a 0.378
140 mg	58 (15.3)	50 (86.2)	8 (13.8)	
Ig type				
IgG/Kappa	132 (35.5)	114 (86.4)	18 (13.6)	^c 0.063
IgG/Lambda	73 (19.6)	70 (95.9)	3 (4.1)	
IgA/Kappa	51 (13.7)	43 (84.3)	8 (15.7)	
IgA/Lambda	34 (9.1)	30 (88.2)	4 (11.8)	
Kappa	50 (13.4)	48 (96)	2 (4)	
Lambda	31 (8.3)	28 (90.3)	3 (9.7)	
Number of autologous HCT				
Two autologous HCT	93 (24.5)	86 (92.5)	7 (7.5)	^a 0.278
One autologous HCT	287 (75.5)	254 (88.5)	33 (11.5)	
Treatment regimens				
VAD	75 (18.4)	70 (94.3)	5 (5.7)	^c 0.113
Bortezomib	260 (67.1)	233 (89.8)	27 (10.2)	
Lenalidomide	45 (10.5)	37 (82.5)	8 (17.5)	
Renal failure				
No	325 (85.5)	289 (88.9)	36 (11.1)	^a 0.395
Yes	55 (14.5)	51 (92.7)	4 (7.3)	
Remission status				
Poor responder (<PR)	82 (16.3)	68 (20%)	14 (35%)	^c 0.045*
Good responder (≥PR)	298 (73.2)	272 (80%)	26 (65%)	
Mortality				
Non-survivor	212 (55.8)	188 (88.7)	24 (11.3)	^a 0.571
Survivor	168 (44.2)	152 (90.5)	16 (9.5)	

^a: Pearson chi-square test, ^b: Student's test, ^c: Fisher freeman halton test, *: $p<0.05$

VTE: Venous thromboembolism, DM: Diabetes mellitus, HT: Hypertension, COPD: Chronic obstructive pulmonary disease, HCT: Hematopoietic cell transplantation, VAD: Vincristine, adriamycin, dexamethasone, PR: Partial response, IgG: Immunoglobulin G

It was observed that 44.7% of the patients who developed VTE survived, with an average survival time of 64.23 ± 2.69 months, while 40% of the patients who did not develop VTE survived, with an average survival time of 58.20 ± 6.93 months. The last death in the VTE group occurred in the 146th month, whereas in the non-VTE group the last death occurred in the 114th month. At this point, the cumulative survival rate was 0%, with a standard error of 0%. When survival rates were evaluated using the log-rank test, no statistically significant difference in survival was observed between the groups ($p=0.305$) (Table 2).

Regarding treatment regimens, 53.3% of patients who received vincristine, adriamycin, dexamethasone (VAD) survived, with an average survival time of 91.37 ± 5.52 months. Among those who received bortezomib, 41.5% survived, with an average survival time of 57.09 ± 2.87 months. In the group receiving lenalidomide, 51.1% survived, with an average survival time of 38.47 ± 6.45 months. The last death occurred in the VAD group in the 146th month, in the bortezomib group in the 129th month, and in the lenalidomide group in the 89th month. At these time points, the cumulative survival rate was 0%, with a standard error of 0%. When survival rates were evaluated using the log-rank test, no statistically significant difference was found between the treatment groups ($p=0.56$) (Table 3).

Discussion

In this study, a significant relationship was found between the development of VTE and the age at which patients underwent autologous HCT. The mean age of patients who developed VTE was significantly lower than that of patients who did not develop VTE. A nother important finding in our study was that patients who developed VTE had a significantly higher remission rate. This may be related to increased life expectancy and greater care requirements.

VTE is a common complication in patients with hematologic and non-hematologic malignancies and is an important cause of morbidity and mortality. With advances in MM treatment and prolongation of the chronic process, VTE has emerged as a common cause of death in this population [10]. In this study, the age at HCT administration was significantly lower

in the VTE group than in the VTE-free group. This finding may have developed due to younger patients being exposed to an increased thrombotic risk associated with intensive chemotherapy and immunosuppressive treatments [11-13]. Increased endothelial reactivity, changes in coagulation profiles, or more aggressive treatment approaches applied at a younger age may be considered mechanisms contributing to this. Furthermore, exposure of younger patients to higher cumulative doses of chemotherapy and immunosuppressive agents, due to their longer life expectancy, may contribute to endothelial dysfunction and prothrombotic conditions. More frequent catheterization and invasive procedures in younger patients may also increase the risk of vascular injury and thrombosis [14]. Therefore, early implementation of thromboprophylaxis measures may be recommended in this population.

Another important finding is the higher remission rate among patients who develop VTE. This suggests that patients who achieve remission may face an increased thrombotic risk due to their longer survival and need for continuous treatment [15,16]. To prevent vascular events that may develop in this group, more stringent monitoring and prophylactic strategies may be useful. In addition, it should be considered that remission is associated with ongoing maintenance treatments and that these treatments may affect coagulation pathways [17,18]. In particular, the effects of immunomodulatory agents and corticosteroids on thrombotic risk should be investigated in more detail in future studies.

Although there were no significant differences in survival rates or mean survival times between the VTE and non-VTE groups, the survival rates of patients who developed VTE were observed to be slightly higher than those of patients who did not. This trend can be explained by effective anticoagulant treatment and close clinical monitoring, which reduce long-term mortality from thrombotic events. The survival advantage observed in the VTE group may also be related to the increased frequency of supportive care and monitoring. Among patients diagnosed with VTE, improved survival may be attributable to more comprehensive medical follow-up, including anticoagulant therapy, cardiovascular monitoring,

Table 2. Survival analysis according to VTE

	Non-survivor	Survivor	Survival rate	Mean survival time
Non-VTE (n: 340)	188	152	44.7%	64.23 ± 2.69
VTE (n: 40)	24	16	40.0%	58.20 ± 6.93

Kaplan-Meier analysis
VTE: Venous thromboembolism

Table 3. Survival analysis according to chemotherapy regimens employed

Last treatment before auto-HCT	Non-survivor	Survivor	Survival rate	Mean survival time
VAD (n: 75)	35	40	53.3%	91.37 ± 5.52
Bortezomib (n: 260)	152	108	41.5%	57.09 ± 2.87
Lenalidomide (n: 45)	22	23	51.1%	38.47 ± 6.45

Kaplan-Meier analysis
VAD: Vincristine, adriamycin, dexamethasone, HCT: Hematopoietic cell transplantation

and lifestyle modifications. Based on this, the effects of intensive monitoring protocols on survival warrant further investigation.

In this study, no significant difference was found in survival outcomes between chemotherapy regimens. No statistically significant association was found between the last chemotherapy regimen administered before transplantation and the development of VTE. However, patients receiving VAD had the longest mean survival time, while those receiving lenalidomide had the shortest. This situation highlights the importance of careful risk assessment and supportive measures in high-risk groups. In particular, lenalidomide is known to increase thrombotic risk when used in combination with corticosteroids and this may explain the shorter survival times observed [19-21]. In our cohort, lenalidomide was used as second-line treatment prior to autologous HCT for patients whose disease was refractory to first-line VAD- or bortezomib-based regimens. Accordingly, the shorter mean survival observed among patients treated with lenalidomide most likely reflects the adverse prognosis associated with treatment-refractory disease rather than an independent detrimental effect of lenalidomide. C hemotherapy can induce local inflammation by causing irritation of the vascular endothelium and an imbalance between procoagulant and anticoagulant mechanisms. Studies on the effect of antithrombotic prophylaxis in patients receiving high-risk treatment regimens will provide more guidance in this regard.

The findings of this study highlight the importance of individualized risk assessment in patients undergoing HCT, especially for those who are younger or in remission. Implementation of routine thromboprophylaxis and strict monitoring protocols may reduce morbidity and improve survival outcomes in high-risk groups. Furthermore, larger prospective studies are needed to confirm these associations and elucidate the underlying pathophysiologic mechanisms.

Study Limitations

Some limitations of this study include the relatively small sample size of the group that did not develop VTE, which may limit statistical power and generalizability. Furthermore, the retrospective design poses challenges in establishing causal relationships, and factors such as genetic predispositions, medication compliance, and lifestyle influences could not be fully accounted for. Incorporating such variables into prospective analyses may enhance risk estimation models and improve prevention strategies.

The retrospective and single-center design of our study inherently limits the generalizability of our findings and introduces potential selection bias. We were unable to fully evaluate the impact of unmeasured confounding factors such as patients' genetic thrombophilia profiles, lifestyle habits, and adherence to prophylactic medications outside the hospital setting. Although the overall patient cohort was adequately sized, the relatively small number of patients who developed VTE (n=40) may have limited the statistical power to detect more subtle differences in survival or risk among specific

subgroups. Well-designed, multicenter, prospective studies are required to validate our findings and comprehensively identify the independent risk factors for thromboembolic events in this patient population.

Conclusion

Our study highlights the complex relationship among age, remission status, and development of VTE in patients undergoing autologous HCT. Specifically, older age at transplantation and achievement of remission were associated with a higher risk of VTE, emphasizing the need for careful monitoring and individualized treatment strategies. These observational findings advance understanding of thrombotic complications in hematologic malignancies and underscore the importance of proactive management approaches to improve patient outcomes. Future research focusing on prospective studies and incorporating genetic and inflammatory biomarkers may further refine risk stratification and guide targeted interventions.

Ethics

Ethics Committee Approval: The study was approved by the Erciyes University Clinical Research Ethics Committee (approval no: 2021/622, date: 22.09.2021).

Informed Consent: Because the study was designed retrospectively no written informed consent form was obtained from the patients.

Footnotes

Authorship Contributions

Surgical and Medical Practices: G.A., Concept: Ş.E.Ü., Design: Ş.E.Ü., H.S., Data Collection or Processing: H.S., Analysis or Interpretation: G.A., H.S., Literature Search: Ş.E.Ü., Writing: Ş.E.Ü.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article

A Retrospective Investigation of Microsatellite Instability (MSI) Testing in a Tertiary Centre Genetic Laboratory

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ABSTRACT

Aim: Microsatellite instability (MSI) is an important biomarker associated with mismatch repair (MMR) deficiency, hereditary cancer syndromes, and response to immunotherapy. This study aimed to evaluate the distribution of MSI status in a heterogeneous cancer cohort and to investigate the relationship between MSI findings and accompanying germline pathogenic or likely pathogenic variants.

Methods: This retrospective, single-center cohort study included 240 cancer patients who simultaneously underwent MSI analysis (n=240) and germline genetic testing (n=101). Patients were classified as MSI-high (MSI-H), MSI-low (MSI-L), or microsatellite stable (MSS). Demographic features, tumor types, and germline variant profiles were evaluated. Pathogenic and likely pathogenic variants were classified according to American College of Medical Genetics and Genomics criteria. Statistical comparisons between MSI-H and MSS groups were performed where applicable.

Results: The cohort consisted of 126 females (52.5%) and 114 males (47.5%), with a mean age of 55.8±13.5 years. The most common malignancies were colon cancer, pancreatic cancer, endometrial cancer, gastric cancer, and ovarian cancer. MSI-H status was detected in 34 patients (14.2%), MSI-L status in 3 patients (1.2%), and MSS status in 203 patients (84.6%). Pathogenic or likely pathogenic MMR-related germline variants were identified in 7 individuals (31.8% of those tested; n=22), predominantly involving *MSH2*, *MSH3*, and *MSH6* genes. Most of these variants demonstrated clinical concordance with Lynch syndrome-associated tumor types. In contrast, MSS patients more frequently harbored pathogenic variants in non-*MMR* genes, including *BRCA1*, *BRCA2*, *ATM*, *RAD51D*, and *MUTYH*.

Conclusion: MSI-H tumors appeared to have a higher frequency of MMR-associated germline variants, particularly in Lynch syndrome-related cancers. However, clinically actionable germline variants were also identified in MSS tumors, highlighting the importance of combined MSI and germline genetic evaluation in hereditary cancer assessment.

Keywords: Microsatellite instability, Lynch syndrome, mismatch repair, hereditary cancer, germline variant

Introduction

Microsatellite instability (MSI) results from defects in the deoxyribonucleic acid (DNA) mismatch repair (MMR) system and represents one of the most important molecular hallmarks in hereditary and sporadic cancers. Deficiency in *MMR* genes such as *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM* deletion leads to the accumulation of insertion-deletion errors within microsatellite regions, ultimately contributing to tumorigenesis [1].

MSI testing has gained increasing clinical importance over the past decades due to its diagnostic, prognostic, and therapeutic

implications. MSI-high (MSI-H) tumors are strongly associated with Lynch syndrome, the most common hereditary colorectal cancer syndrome, but may also occur in sporadic cancers through somatic epigenetic mechanisms, particularly *MLH1* promoter hypermethylation [2].

Beyond colorectal cancer, MSI-H status has been observed in endometrial, gastric, ovarian, pancreatic, small bowel, and several other malignancies [3]. Identification of MSI-H tumors may prompt further germline investigation and guide immunotherapy decisions, particularly with immune checkpoint inhibitors [4].

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Although the association between MSI-H and Lynch syndrome is well established, the overall spectrum of germline variants encountered in routine MSI-tested patient cohorts remains incompletely characterized in many populations. Moreover, the relationship between tumor type, MSI status, and clinically actionable germline variants continues to evolve.

In this study, we aimed to evaluate the demographic and molecular characteristics of a real-world cancer cohort undergoing MSI analysis. We additionally investigated the frequency of pathogenic or likely pathogenic germline variants and assessed whether these variants were concordant with the clinical tumor phenotype.

Methods

This retrospective, single-center study included 240 patients with various malignancies who underwent MSI testing (n=240) and hereditary cancer panel analysis (n=101) at our institution between 2022 and 2026. Clinical and molecular data were collected retrospectively from laboratory records and patient files. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from the patients or their legal guardians. Ethical approval for this study was obtained from the Scientific Research Evaluation and Ethics Committee No. 1 of University of Health Sciences Türkiye, Ankara Etlik City Hospital, (AEŞH-BADEK1-2026-435/date: 06.05.2026).

MSI analysis was performed using fragment analysis methodology with the Seqline Multiplex MSI Analysis Kit (EYS Medikal ve Laboratuvar Projeleri, Ankara, Türkiye) and run on an Applied Biosystems 3500xL Genetic Analyzer platform (Applied Biosystems, Thermo Fisher Scientific, South San Francisco, CA, USA) using somatic tissues obtained from cancer patients.

Fragment analysis data were evaluated according to the manufacturer's recommendations. MSI status was classified as follows: microsatellite stable (MSS): no unstable markers detected; MSI-low (MSI-L): instability detected in less than 30% of analyzed markers (1 marker); MSI-high (MSI-H): instability detected in more than 30% of analyzed markers (2-5 markers).

Hereditary cancer panel analysis was performed using a custom hereditary cancer panel (60 genes), CUSTOM SOLUTION (CHCS_C_v2) v2 by SOPHiA GENETICS (SOPHiA GENETICS SA, Saint-Sulpice, Switzerland) (Supplementary Material 1). Sequencing quality metrics were assessed as part of the routine analytical pipeline. A minimum read depth of 30× was required for reliable variant calling; regions with insufficient coverage were flagged for additional review or confirmatory testing when clinically indicated (excluding copy-number changes). Variants within coding exons and the flanking intronic regions (±20 bp from exon-intron boundaries) were evaluated. Sequence alignment, variant calling, annotation, and quality control were performed using the SOPHiA DDM platform according to the manufacturer's recommendations. Variant interpretation followed the American College of

Medical Genetics and Genomics (ACMG)/ association for molecular pathology guidelines using evidence from population databases (gnomAD), disease-specific databases (ClinVar and Franklin Genoox), published literature, and multiple in silico prediction tools. Clinically relevant variants were confirmed by Sanger sequencing when appropriate as part of routine diagnostic practice. As this was a retrospective study, germline genetic testing was available only to patients who had undergone testing as part of routine clinical care. The decision to perform germline testing was based on individual clinical indications such as personal or family history, tumor characteristics (if available), and suspicion of a hereditary cancer predisposition, rather than on the study protocol. Libraries were sequenced on the NextSeq platform (Illumina, San Diego, CA, USA). Sequence data were analyzed using the SOPHiA DDM platform according to the manufacturer's bioinformatics workflow. Variants were classified according to the ACMG guidelines [5]. The standard workflow of the cohort is displayed in Figure 1.

Statistical Analysis

Descriptive statistics were used to summarize the clinical and demographic characteristics of the cohort. Categorical variables are presented as frequencies and percentages. For continuous variables, such as age at diagnosis, data are expressed as median values.

Results

Patient Characteristics

A total of 240 patients who underwent MSI testing were included in the analysis. The mean age of the cohort was 55.8±13.5 years. Females constituted 52.5% of the cohort (n=126), while males represented 47.5% (n=114).

Clinical classification of the primary malignancies revealed that colorectal cancer was the most frequent diagnosis, occurring in 58 (24.1%) cases; gastric cancer was identified in 19 (7.9%) cases, while rectal cancer, breast cancer, and glioblastoma represented smaller proportions of the cohort.

MSI Status Distribution

Molecular screening for microsatellite status categorized the patients into three groups, identifying 34 cases (14.2%) as MSI-H, 3 cases (1.2%) as MSI-L, and 203 cases (84.6%) as MSS. Within the MSI-H subgroup, colorectal and endometrial tumors were most common. Colon cancer represented the largest proportion of MSI-H tumors, followed by endometrial and rectal cancers.

The mean age of patients with MSI-H tumors was 59.5±11.3 years, compared with 55.1±13.8 years among MSS patients. The sex distribution in the MSI-H group was relatively balanced (18 males, 16 females).

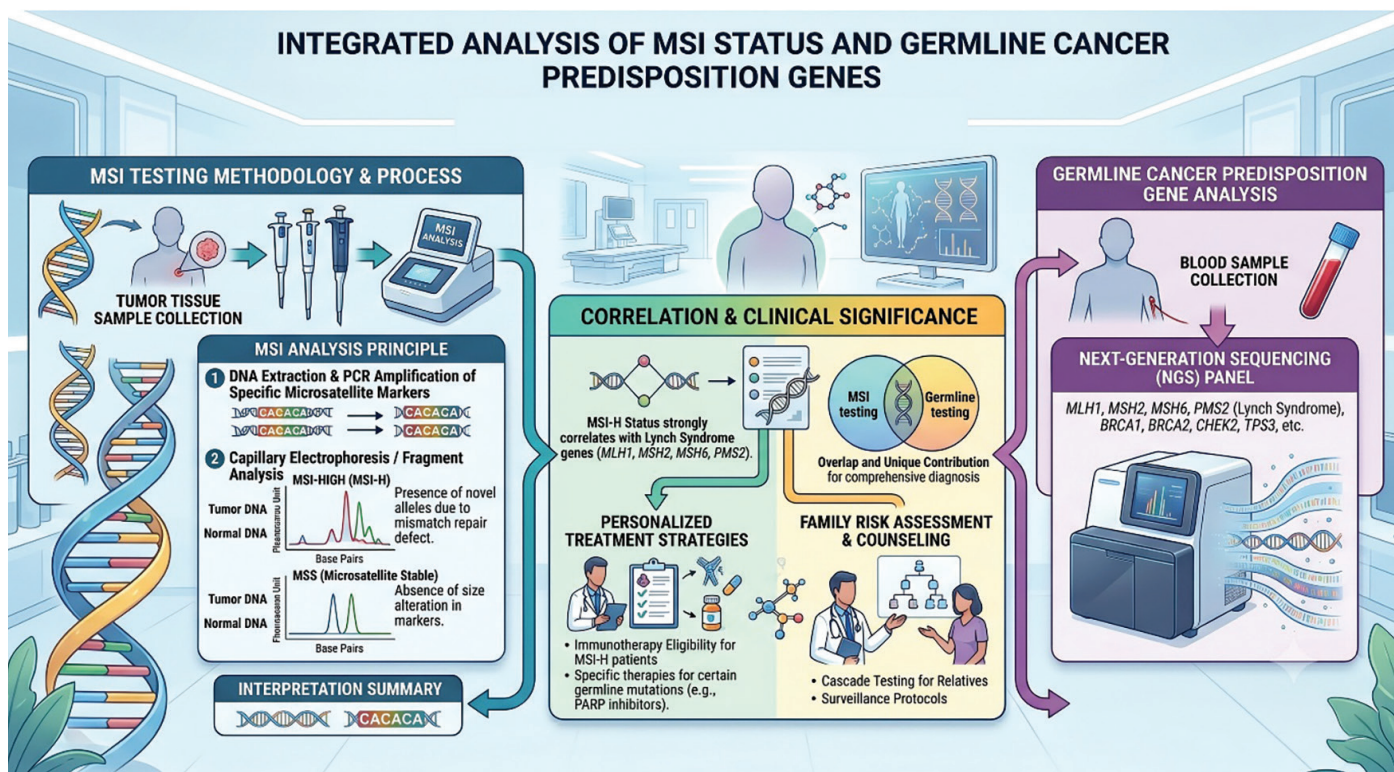


Figure 1. Generalized workflow of the study cohort
MSI: Microsatellite instability, DNA: Deoxyribonucleic acid, PCR: Polymerase chain reaction

Germline Variant Analysis

A total of 101 patients underwent germline testing simultaneously. Pathogenic or likely pathogenic germline variants were identified in both the MSI-H and MSS groups; however, the molecular spectra differed substantially between the two groups.

Among MSI-H patients, 7 of 34 individuals (31.8% among those tested; $n=22$) harbored pathogenic or likely pathogenic variants involving MMR-associated genes. These included pathogenic or likely pathogenic variants in *MSH2*, *MSH3*, and *MSH6*. Notably, the identified variants were predominantly associated with tumors within the Lynch syndrome spectrum. *MSH2* and *MSH6* variants were identified in patients with colorectal, rectal, and endometrial cancers, supporting the clinical concordance between germline findings and tumor phenotype.

One MSI-H patient with colon cancer carried both a pathogenic *MUTYH* variant and a pathogenic *MSH6* variant, suggesting the possibility of overlapping hereditary cancer susceptibility mechanisms (P#44). In the MSI-L group ($n=3$), no patients were tested for germline variants.

In contrast, MSS patients demonstrated a broader spectrum of non-MMR hereditary cancer-related variants. Pathogenic or

likely pathogenic variants identified in MSS tumors included alterations in *BRCA1*, *BRCA2*, *ATM*, *RAD51D*, *ERCC2*, and *MUTYH*.

Several variants identified in MSS patients were clinically concordant with the presenting tumor type. For example, pathogenic variants in *BRCA1* and *BRCA2* were observed in ovarian and breast cancer patients, respectively, while pathogenic variants in *MUTYH* were identified in patients with gastrointestinal malignancies.

Variant Classification Overview

Across the entire cohort, pathogenic/likely pathogenic variants were identified in 17/101 (16.8%) patients; one patient had dual variants. Variants of uncertain significance (VUS) were observed in 36 patients. All of the detected variants were in a heterozygous state.

Overall, MMR-associated pathogenic or likely pathogenic variants were significantly enriched in the MSI-H subgroup, whereas MSS tumors more commonly demonstrated pathogenic variants involving alternative hereditary cancer predisposition pathways. Detailed characteristics of the cohort are depicted in Table 1.

Table 1. Detailed characteristics of the cohort

Patient ID	Age	Sex	Cancer type	MSI status	Hereditary cancer panel (variant-if detected/zygosity/ACMG classification)
P1	66	Male	Stomach	MSS	NA
P2	45	Female	Colon	MSS	NA
P3	67	Male	Colon	MSS	APC (NM_000038.5) c.3920T>A p.(Ile1307Lys)/Het/VUS
P4	50	Female	Pancreatic	MSS	N
P5	76	Female	Ovarian	MSS	N
P6	45	Female	Colon	MSS	NA
P7	71	Female	Sarcoma	MSS	NA
P8	65	Female	Biliary tract	MSS	N
P9	60	Female	Ovarian	MSS	NA
P10	41	Male	Brain (glioblastoma)	MSS	NA
P11	33	Male	Sarcoma	MSS	NA
P12	82	Female	Stomach	MSS	NA
P13	25	Male	Testicular	MSS	NA
P14	71	Male	Colon	MSS	MLH1 (NM_000249) c.1480T>C p.(Cys494Arg), VHL (NM_000551) c.340+657C>G/Het (both)/VUS (both)
P15	59	Female	Ovarian	MSS	BRCA2 (NM_000059.3) c.1587_1590delTAAA p.(Phe529Leufs)/het/pathogenic
P16	54	Male	Biliary tract	MSS	NA
P17	71	Male	Colon	MSS	RAD51C (NM_058216) c.810C>G p.(Ile270Met)/Het/VUS
P18	55	Female	Ovarian	MSS	RET (NM_020975.6) c.394C>A p.(Leu132Ile)/Het/VUS
P19	45	Male	Pancreatic	MSS	NA
P20	45	Female	Colon	MSI-H	NA
P21	59	Female	Colon	MSI-H	POLE (NM_006231) c.2466G>C p.(Lys822Asn)/Het/VUS
P22	65	Male	Colon	MSS	NA
P23	46	Female	Breast	MSS	NA
P24	60	Female	Biliary tract	MSS	NA
P25	73	Female	Pancreatic	MSS	NA
P26	63	Female	Stomach	MSS	NA
P27	41	Female	Lung	MSS	PALB2 (NM_024675) c.3058_3069dup p.(Gln1020_Gln1023dup), TSC1 (NM_000368) c.3436G>A p.(Asp1146Asn)/Het (both)/VUS (both)
P28	50	Male	Thyroid (papillary)	MSS	NA
P29	34	Female	Sarcoma	MSS	NA
P30	67	Male	Prostate	MSS	NA
P31	51	Female	Endometrial	MSI-H	N
P32	70	Female	Endometrial	MSI-H	STK11 (NM_000455.5) c.238C>G p.Leu80Val/Het/VUS
P33	51	Female	Breast/endometrium (MSI tested in endometrial tissue)	MSI-H	MSH2 (NM_000251.2) c.1222dup p.(Tyr408Leufs*9)/ Het/Pathogenic
P34	59	Male	Pancreatic	MSS	N
P35	43	Male	Rectum	MSS	NA
P36	44	Female	Biliary tract	MSS	NA
P37	62	Male	Colon	MSS	N
P38	58	Female	Ovarian	MSS	NA
P39	62	Male	Colon	MSI-H	MSH6 (NM_000179) c.3740C>T p.(Thr1247Ile)/Het/VUS

Table 1. Continued					
Patient ID	Age	Sex	Cancer type	MSI status	Hereditary cancer panel (variant-if detected/zygosity/ACMG classification)
P40	64	Male	Rectum	MSI-H	<i>MSH6 (NM_000179) c.3556+1G>C/het/pathogenic</i> CDH1 (NM_001317184) c.1583A>G p.(Asn528Ser)/Het/VUS
P41	67	Female	Hepatocellular	MSS	PALB2 (NM_024675) c.2882T>C p.(Leu961Pro)/Het/ VUS
P42	64	Male	Colon	MSI-H	NA
P43	75	Female	Rectum	MSS	NA
P44	53	Male	Colon	MSI-H	MUTYH (NM_001048171) c.1395_1397del p.(Glu466del), MSH6 (NM_000179) c.691del p.(Val231Tyrfs*15)/ Het (both)/Pathogenic (both)
P45	31	Female	Stomach	MSS	POLE (NM_006231) c.5244G>A p.(Met1748Ile)/Het/VUS
P46	37	Male	Brain (glioblastoma)	MSS	N
P47	57	Female	Endometrial	MSS	NA
P48	78	Female	Colon	MSI-H	N
P49	58	Male	Colon	MSS	NA
P50	72	Female	Biliary tract	MSS	NA
P51	42	Male	Stomach	MSS	NA
P52	61	Male	Colon	MSS	NA
P53	25	Female	Lung	MSS	NA
P54	67	Male	Prostate	MSS	NA
P55	48	Male	Colon	MSS	NA
P56	73	Male	Pancreatic	MSS	NA
P57	60	Male	Colon	MSS	N
P58	56	Male	Colon	MSS	NA
P59	58	Male	Biliary tract	MSI-H	N
P60	53	Female	Urinary tract (bladder)	MSS	NA
P61	61	Male	Colon	MSS	NA
P62	68	Female	Colon	MSI-H	NA
P63	80	Male	Breast	MSS	NA
P64	72	Male	Colon	MSS	NA
P65	72	Female	Rectum	MSS	NA
P66	42	Female	Rectum	MSS	NA
P67	54	Female	Cervix	MSS	NA
P68	47	Female	Stomach	MSS	NA
P69	37	Male	Colon	MSI-H	N
P70	32	Female	Sarcoma	MSS	NA
P71	49	Female	Biliary tract	MSS	RAD51D (NM_001142571) c.540+1G>A/Het/Pathogenic
P72	67	Female	Endometrial	MSS	NA
P73	38	Female	Ovarian	MSI-H	NA
P74	52	Male	Colon	MSS	N
P75	48	Female	Rectum	MSS	N
P76	26	Male	Stomach	MSS	BLM (NM_000057) c.2474C>T p.(Pro825Leu)/Het/VUS
P77	47	Female	Stomach	MSS	NA
P78	66	Male	Melanoma	MSS	NA
P79	70	Female	Endometrial	MSS	N
P80	50	Male	Oesophageal	MSI-H	NA

Table 1. Continued					
Patient ID	Age	Sex	Cancer type	MSI status	Hereditary cancer panel (variant-if detected/zygosity/ACMG classification)
P81	57	Male	Pancreatic	MSI-H	N
P82	76	Female	Breast	MSS	SMAD4 (NM_005359) c.977T>C p.(Ile326Thr), POLE (NM_006231) c.6124G>A p.(Gly2042Arg)/Het (both)/ VUS (both)
P83	23	Male	Colon	MSS	N
P84	53	Male	Colon	MSI-H	NA
P85	32	Male	Colon	MSI-H	MSH2 (NM_000251) c.1784T>C p.(Leu595Pro)/Het/Likely pathogenic
P86	64	Male	Stomach	MSI-H	NA
P87	26	Female	Brain (glioblastoma)	MSS	NA
P88	57	Female	Pancreatic	MSS	GALNT12 (NM_024642) c.5G>T p.(Trp2Leu)/Het/VUS
P89	71	Female	Breast	MSS	NA
P90	53	Female	Melanoma	MSS	N
P91	58	Male	Pancreatic	MSS	ERCC3 (NM_000122) c.1606A>T p.(Met536Leu)/Het/VUS
P92	80	Female	Colon	MSS	NA
P93	51	Male	Thyroid (medullary)	MSS	NA
P94	58	Male	Brain (glioblastoma)	MSS	NA
P95	72	Female	Lung	MSS	NA
P96	54	Male	Sarcoma	MSS	NA
P97	39	Female	Stomach	MSS	N
P98	69	Female	Biliary tract	MSS	N
P99	55	Male	Colon	MSS	NA
P100	58	Female	Ovarian	MSS	BRCA1 pathogenic variant (previous external testing)
P101	14	Female	Nerve sheat	MSS	NA
P102	64	Female	Pancreatic	MSS	N
P103	51	Male	Malign melanoma	MSS	NA
P104	54	Male	Biliary tract	MSS	NA
P105	62	Male	Colon	MSS	NA
P106	56	Male	Pancreatic	MSS	N
P107	64	Female	Endometrial	MSS	N
P108	44	Male	Brain (glioblastoma)	MSS	NA
P109	68	Male	Urinary tract	MSS	NA
P110	23	Female	Thyroid (medullary)	MSS	N
P111	28	Female	Biliary tract	MSS	NA
P112	62	Male	Urinary tract (bladder)	MSS	NA
P113	49	Male	Colon	MSS	NA
P114	76	Female	Colon	MSS	NA
P115	76	Female	Colon	MSS	N
P116	44	Male	Wilms tumour	MSS	NA
P117	55	Male	Malignant mesenchymal tumour of the salivary gland	MSI-L	NA
P118	59	Male	Pancreatic/ prostate (MSI performed on prostate tissue)	MSS	<i>MUTYH</i> (NM_001048171.) c.1395_1397del p.(Glu466del)/het/pathogenic ATM (NM_000051.3) c.6497T>C p.(Val2166Ala)/Het/VUS
P119	48	Male	Colon	MSS	NA

Table 1. Continued					
Patient ID	Age	Sex	Cancer type	MSI status	Hereditary cancer panel (variant-if detected/zygosity/ACMG classification)
P120	40	Female	Breast	MSS	BRCA2 (NM_000059.3) c.67+1G>A/Het/Pathogenic
P121	68	Male	Colon	MSI-L	NA
P122	74	Male	Urinary tract (bladder)	MSS	NA
P123	51	Female	Ovarian	MSS	N
P124	43	Female	Biliary tract	MSS	NA
P125	64	Female	Endometrial	MSS	N
P126	77	Male	Colon	MSS	N
P127	49	Female	Biliary tract	MSS	NA
P128	31	Male	Urinary tract (bladder)	MSS	CDH1 (NM_001317184) c.1592C>A p.(Ala531Asp)/ Het/VUS
P129	53	Female	Ovarian	MSS	N
P130	40	Male	Biliary tract	MSS	N
P131	57	Female	Breast	MSS	N
P132	51	Female	Rectum	MSS	NA
P133	60	Male	Lung	MSS	NA
P134	72	Male	Pancreatic	MSS	NA
P135	49	Male	Colon	MSS	NA
P136	45	Female	Pancreatic	MSS	PTEN (NM_001304717) c.176C>A p.(Pro59Gln)/ Het/VUS
P137	46	Female	Pancreatic	MSS	MUTYH (NM_001048171) c.1395_1397del p.(Glu466del)/ Het/Likely pathogenic
P138	54	Male	Stomach	MSS	NA
P139	52	Male	Pancreatic	MSS	N
P140	58	Female	Pancreatic	MSS	NA
P141	58	Male	Colon	MSI-H	MSH3 (NM_002439) c.2179C>T p.(Arg727*)/ Het/Pathogenic
P142	59	Male	Pancreatic	MSS	APC (NM_000038.5) c.4364A>G p.(Asn1455Ser)/Het/VUS
P143	50	Male	Colon	MSS	APC (NM_000038)c.8120G>A p.(Gly2707Asp)/Het/VUS
P144	46	Male	Brain (glioblastoma)	MSS	NA
P145	34	Female	Brain (glioblastoma)	MSS	NA
P146	52	Female	Breast	MSS	RB1 (NM_000321) c.74C>T p.(Pro25Leu)/Het/VUS
P147	55	Female	Biliary tract	MSS	MSH3 (NM_002439.4) c.1307C>T p.(Ile945Met)/Het/VUS
P148	77	Female	Hepatocellular	MSS	NA
P149	61	Male	Biliary tract	MSS	NA
P150	47	Female	Sarcoma	MSS	MUTYH (NM_001048171) c.892-2A>G, POLE (NM_006231) c.5794C>T p.(Arg1932Cys)/Het (both)/VUS (both)
P151	53	Male	Colon	MSS	N
P152	44	Female	Sarcoma	MSS	N
P153	85	Female	Unknown primary	MSS	NA
P154	64	Female	Sarcoma	MSS	NA
P155	61	Male	Biliary tract	MSS	NA
P156	46	Female	Breast	MSS	MUTYH (NM_001048171) c.1395_1397del p.(Glu466del)/het/likely pathogenic NF1 (NM_000267) c.2629A>G p.(Met877Val)/Het/VUS
P157	65	Male	Pancreatic	MSS	NA
P158	51	Female	Sarcoma/ breast (MSI tested in sarcoma tissue)	MSS	NA

Table 1. Continued

Patient ID	Age	Sex	Cancer type	MSI status	Hereditary cancer panel (variant-if detected/zygosity/ACMG classification)
P159	63	Female	Ovarian	MSS	NA
P160	59	Female	Endometrial	MSS	NA
P161	70	Male	Stomach	MSS	NA
P162	63	Female	Endometrial	MSS	BARD1 (NM_000465.3) c.827C>G p.(Thr276Ser), MSH6 (NM_000179) c.3763G>A p.(Asp1255Asn)/Het (both)/VUS (both)
P163	72	Female	Brain (glioblastoma)	MSS	NA
P164	59	Male	Unknown primary	MSS	NA
P165	43	Female	Pancreatic	MSS	NA
P166	59	Male	Oesophageal	MSI-L	NA
P167	63	Female	Colon	MSS	N
P168	50	Female	Endometrial	MSS	N
P169	80	Female	Biliary tract	MSS	NA
P170	51	Male	Pancreatic	MSS	NA
P171	49	Female	Ovarian	MSS	RAD51D (NM_001142571) c.862T>C p.(Trp288Arg)/Het/VUS
P172	33	Female	Ovarian	MSS	N
P173	60	Female	Endometrial	MSI-H	NA
P174	38	Male	Biliary tract	MSS	NA
P175	72	Male	Colon	MSI-H	N
P176	67	Male	Stomach	MSS	N
P177	70	Female	Ovarian	MSS	NA
P178	43	Male	Stomach	MSS	N
P179	49	Female	Colon	MSS	EPCAM (NM_002354.2) c.491+5G>A/Het/VUS
P180	72	Female	Colon	MSI-H	N
P181	41	Female	Stomach	MSS	N
P182	70	Female	Colon	MSS	NA
P183	73	Female	Biliary tract	MSS	N
P184	67	Male	Lung	MSS	NA
P185	57	Male	Pancreatic	MSS	ATM (NM_000051) c.3576G>A p.(Lys1192=)/Het/Pathogenic
P186	55	Male	Gastrointestinal stromal tumour	MSS	NA
P187	61	Female	Pancreatic	MSS	NA
P188	32	Female	Ovarian	MSS	NA
P189	57	Female	Colon	MSS	NA
P190	41	Male	Lung	MSS	NA
P191	53	Female	Colon	MSS	N
P192	62	Female	Endometrial	MSI-H	N
P193	51	Male	Pancreatic	MSS	N
P194	79	Female	Stomach	MSS	NA
P195	33	Male	Rectum	MSS	NA
P196	56	Male	Pancreatic	MSS	MUTYH (NM_001048171) c.692G>A p.(Arg231His)/Het/Likely Pathogenic
P197	62	Female	Cervix	MSS	NA
P198	69	Female	Thyroid (papillary)	MSS	NA

Table 1. Continued					
Patient ID	Age	Sex	Cancer type	MSI status	Hereditary cancer panel (variant-if detected/zygosity/ACMG classification)
P199	39	Female	Breast	MSS	N
P200	48	Female	Breast	MSS	MSH2 (NM_000251.2) c.70C>T p.(Gln24*)/ Het/Pathogenic
P201	65	Male	Colon	MSS	NA
P202	73	Male	Prostate	MSS	N
P203	60	Female	Endometrial	MSI-H	MSH2 (NM_000251.2) c.70C>T p.(Gln24*)/Het/Pathogenic
P204	65	Female	Endometrial	MSS	RAD51C (NM_058216) c.1039A>G p.(Arg347Gly)/ Het/VUS
P205	59	Female	Breast	MSS	EPCAM (NM_002354.2) c.-9_7dup (p.Pro3Argfs*33), TSC2 (NM_000548.4) c.4678G>A (p.Ala1560Thr)/Het (both)/VUS (both)
P206	65	Male	Stomach	MSS	NA
P207	61	Female	Pancreatic	MSS	N
P208	47	Male	Pancreatic	MSS	NA
P209	67	Male	Lung	MSS	NA
P210	81	Male	Colon	MSS	NA
P211	37	Female	Lung	MSS	NA
P212	37	Male	Brain (glioblastoma)	MSS	NA
P213	50	Male	Colon	MSS	TSC1 (NM_000368.4) c.106+16T>C , AXIN2 (NM_004655.3) c.203G>A p p.(Arg68Gln)/Het (both)/ VUS (both)
P214	35	Female	Thyroid	MSS	NA
P215	61	Female	Endometrial	MSS	N
P216	62	Male	Pancreatic	MSS	N
P217	48	Male	Colon	MSS	NA
P218	77	Male	Stomach	MSI-H	BRIP1 (NM_032043.2) c.2284C>T p.(Arg762Cys), FH (NM_000143.3) c.730C>T p.(Leu244Phe), MSH3 (NM_002439.4) c.3382A>G p.(Met1128Val), TSC2 (NM_000548.4) c.4664G>A p.(Ser1555Asn) /Het (all)/VUS (all)
P219	70	Female	Rectum	MSS	NA
P220	65	Male	Renal cell cancer/ rectum (MSI tested in both tissues)	MSS	NA
P221	33	Female	Colon	MSS	APC (NM_000038) c.5528C>T p.(Pro1843Leu)/Het/VUS
P222	46	Male	Unknown primary	MSS	NA
P223	63	Male	Small bowel	MSI-H	N
P224	45	Female	Small bowel	MSI-H	MLH1 (NM_000249) c.1832T>C p.Ile611Thr/Het/VUS
P225	35	Male	Brain (astrocytoma)	MSS	NA
P226	56	Female	Colon	MSS	NA
P227	73	Male	Colon	MSS	NA
P228	70	Female	Endometrial	MSI-H	NA
P229	58	Female	Brain (glioblastoma)	MSS	NA
P230	47	Male	Lung	MSS	NA
P231	52	Male	Colon	MSS	NA
P232	53	Female	Colon	MSS	NA
P233	62	Female	Endometrial	MSI-H	NA

Table 1. Continued

Patient ID	Age	Sex	Cancer type	MSI status	Hereditary cancer panel (variant-if detected/zygosity/ACMG classification)
P234	32	Female	Colon	MSS	EPCAM (NM_002354.2) c.28G>C p.(Gly10Arg), CHEK2 (NM_001005735.1) c.551A>C p.(Lys184Thr)/Het (both)/VUS (both)
P235	42	Male	Colon	MSS	NA
P236	65	Female	Ovarian	MSS	FLCN (NM_144997.7) c.203G>A p.Ser68Asn, POLD1 (NM_002691.4) c.2048G>A p.Arg683His/Het (both)/VUS (both)
P237	67	Male	Colon	MSI-H	N
P238	62	Male	Stomach	MSI-H	NA
P239	61	Male	Rectum	MSI-H	MSH2 (NM_000251.2) c.942+3A>T/Het/Pathogenic
P240	77	Female	Endometrial	MSI-H	NA

N: Hereditary cancer panel performed but no causative variant was found, MSI-H results were written in bold, pathogenic alterations were written in bold and italic

ACMG: American College of Medical Genetics and Genomics, MSI: Microsatellite instability, MSS: Microsatellite stabil, MSI-L: MSI-low, MSI-H: MSI-high NA: Not available, Het: Heterozygous, VUS: Variant of unknown significance

Discussion

In this retrospective study, we evaluated MSI status and accompanying germline variant profiles in a heterogeneous cancer population. Our findings demonstrate that MSI-H tumors were strongly enriched for pathogenic or likely pathogenic variants affecting MMR-associated genes, particularly among tumors classically associated with Lynch syndrome.

The observed MSI-H rate of 14.2% in our cohort is generally consistent with previously reported frequencies in mixed gastrointestinal and endometrial cancer populations [3,6]. MSI-H tumors are known to occur most frequently in colorectal and endometrial cancers, a finding that was similarly reflected in our dataset. Although previous studies in the Turkish population have investigated MSI in relatively modest patient cohorts and predominantly within specific tumor groups, the present study represents, to our knowledge, the first analysis conducted in such a large cohort encompassing a broad tumor spectrum in which MSI could be observed with correlation to germline variants [7-9].

Importantly, pathogenic or likely pathogenic MMR-related variants were identified in approximately a third of MSI-H cases. Variants involving *MSH2*, *MSH3* and *MSH6* demonstrated particularly strong clinicopathological concordance with Lynch syndrome-associated tumor types. These findings support the clinical utility of integrating germline testing with MSI analysis, especially in patients with colorectal, rectal, and endometrial malignancies. However, it should be treated carefully since this does not prove that MSI-H is a highly sensitive standalone marker for germline MMR deficiency [10].

One patient harbored concurrent pathogenic alterations in both *MUTYH* and *MSH6*. Although the clinical implications of multiple inherited cancer susceptibility variants remain incompletely understood, recent studies suggest that multi-locus inherited neoplasia syndromes may contribute to variable phenotypic presentations and modified cancer risk profiles.

Another notable observation was the detection of clinically actionable germline variants in MSS tumors. Although MSS status generally lowers the suspicion for Lynch syndrome, hereditary cancer predisposition cannot be excluded solely on the basis of MSI negativity [11]. Pathogenic variants involving *BRCA1*, *BRCA2*, *ATM*, *RAD51D*, and *MUTYH* identified in MSS tumors highlight the genetic heterogeneity of hereditary cancer syndromes and emphasize the importance of phenotype-driven germline evaluation.

The identification of *BRCA*-associated variants in breast and ovarian cancer patients further supports the internal clinical consistency of the dataset. Similarly, the detection of *MUTYH* pathogenic variants in gastrointestinal malignancies aligns with the established association between *MUTYH*-associated polyposis and colorectal tumorigenesis.

Our study has several strengths. First, it reflects real-world clinical practice by including multiple tumor types rather than a single disease-focused cohort. Second, the simultaneous evaluation of MSI status and germline findings allowed assessment of genotype-phenotype concordance in routine diagnostic settings.

Study Limitations

Several limitations must be acknowledged in the interpretation of these findings. First, approximately 20 samples from the initial cohort were excluded due to insufficient DNA quality, preventing reliable MSI analysis and possibly affecting the overall sample size. In addition, detailed family history, immunohistochemistry data, somatic testing results, treatment response information, and comprehensive histopathological findings were not uniformly available for all patients, limiting detailed genotype-phenotype correlation analyses. Furthermore, genetic testing of hereditary cancer genes was not systematically validated for copy-number variations using gold-standard methods, which may have resulted in an underestimation of large genomic rearrangements. Segregation studies and functional validation assays could not

be performed for several variants, particularly those classified as VUS. Because the study was conducted at a tertiary referral center, sampling bias is likely; such centers often receive more complex or high-risk cases. Additionally, the relatively limited number of MSI-H cases may have reduced the statistical power for subgroup analyses. Another limitation is the differential availability of germline testing across study groups, which may have introduced verification bias and affected comparisons of germline variant frequencies among MSI categories. The absence of advanced epigenetic investigations, such as DNA methylation profiling of *MMR* genes, limited our ability to fully characterize the mechanisms underlying deficient MMR in cases without detectable germline mutations. Despite these limitations, our cohort reflects real-world clinical practice and provides valuable insight into the relationship between MSI status and hereditary cancer predisposition across a broad and heterogeneous cancer population.

Conclusion

This study supports the clinical utility of MSI-H as an indicator of germline MMR deficiency, while emphasizing the importance of comprehensive germline sequencing for a complete diagnostic evaluation. The discovery of discordant cases and a significant number of non-MMR hereditary mutations underscores the necessity of a multifaceted testing strategy in oncology. Future clinical guidelines should continue to promote a combination of phenotypic and genotypic testing to optimize the detection of hereditary syndromes and enhance personalized therapeutic interventions.

Ethics

Ethics Committee Approval: Ethical approval for this study was obtained from the Scientific Research Evaluation and Ethics Committee No. 1 of Ankara Etlik City Hospital, University of Health Sciences Türkiye (AEŞH-BADEK1-2026-435/date: 06.05.2026).

Informed Consent: Written informed consent was obtained from the patients or their legal guardians.

Footnotes

Authorship Contributions

Concept: A.K., Design: A.K., Data Collection or Processing: Ö.B.Ç.Ö., Analysis or Interpretation: A.K., Ö.B.Ç.Ö., Literature Search: A.K., Writing: A.K., Ö.B.Ç.Ö.

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Declaration Regarding the Use of AI and AI-Assisted Technologies: During the preparation of this article, the authors used artificial intelligence to design Figure 1. The authors are fully responsible for the accuracy, completeness, and scientific content of the final article.

Supplementary Material 1 Link: <https://d2v96fxpocvxx.cloudfront.net/f6118de3-f656-440d-95f2-50c620149951/content-images/292dc4b9-04a8-41c0-ae1e-79fe411ee295.pdf>

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A Descriptive Molecular Case Series of Novel Variants in APC-associated Polyposis

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ABSTRACT

Aim: Familial adenomatous polyposis (FAP) is the most common hereditary adenomatous polyposis syndrome caused by pathogenic variants in the APC gene. This study aimed to describe the clinical and molecular characteristics of four unrelated patients harboring previously unreported APC variants.

Methods: Four unrelated patients referred for suspected hereditary cancer predisposition were included in the study. Genomic deoxyribonucleic acid extracted from peripheral blood samples was analyzed using a hereditary cancer syndrome multigene panel via next-generation sequencing.

Results: Four previously unreported heterozygous APC variants were identified: NM_000038.6:c.1621del (p.Gln541SerfsTer8), c.2194_2195del (p.Asn732Ter), c.3804_3808del (p.Ile1269PhefsTer5), and c.4066_4067dup (p.Gly1357GlnfsTer59). All variants were predicted to be loss-of-function and were expected to result in premature protein truncation. Only one variant was located within the mutation cluster region (MCR), whereas the remaining three were identified outside the MCR in patients with classical FAP.

Conclusion: This case series expands the molecular spectrum of APC-associated polyposis by describing four previously unreported protein-truncating variants. Our findings further illustrate the clinical heterogeneity associated with APC-related disorders and underscore the value of comprehensive molecular characterization in individuals with suspected hereditary cancer predisposition.

Keywords: APC, FAP, protein-truncating variants, hereditary cancer predisposition

Introduction

Adenomatous polyposis (AP) syndromes are hereditary cancer-predisposition disorders characterized by the development of multiple colorectal adenomatous polyps and a markedly increased lifetime risk of colorectal cancer (CRC). In addition to colorectal involvement, affected individuals may develop a broad spectrum of extraintestinal manifestations, including both benign and malign lesions involving the upper gastrointestinal tract and other organs, based on the underlying genetic defect [1].

Familial AP (FAP) is the most common and best-characterized form of AP and results from heterozygous germline pathogenic variants in the APC gene. APC functions as a key tumor

suppressor and a negative regulator of the Wnt/ β -catenin signaling pathway, thereby playing a critical role in the regulation of cellular proliferation and differentiation [2]. In accordance with Knudson's two-hit hypothesis, tumor initiation occurs following somatic inactivation of the remaining wild-type APC allele (the "second hit"), leading to disruption of the β -catenin destruction complex and constitutive activation of Wnt signaling, a hallmark of early colorectal tumorigenesis [3]. Consistent with this central role, somatic APC alterations are detected in approximately 80% of sporadic CRCs [4,5].

FAP exhibits considerable clinical heterogeneity. The classical phenotype is characterized by the development of hundreds to thousands of colorectal adenomas, beginning in childhood or adolescence, and, if left untreated, confers an almost

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inevitable risk of CRC. In contrast, attenuated FAP is typically associated with a lower adenoma burden and a later age of disease onset. In addition to colorectal polyposis, affected individuals may present with a variety of extraintestinal manifestations, including duodenal adenomas, desmoid tumors, osteomas, congenital hypertrophy of the retinal pigment epithelium, dental abnormalities, and epidermoid cysts. Among these manifestations, upper gastrointestinal neoplasms and desmoid disease represent major contributors to disease-related morbidity and mortality [6].

Accumulating evidence has demonstrated that the location of pathogenic variants within the *APC* gene influences disease severity and specific clinical manifestations. Variants located within the mutation cluster region (MCR) in exon 15 are generally associated with a more severe polyposis phenotype, whereas variants toward the 5' and 3' ends of the gene tend to be associated with attenuated disease. Nevertheless, substantial phenotypic variability has been observed even among individuals harboring identical pathogenic variants, suggesting that additional genetic, epigenetic, and environmental factors contribute to disease expression beyond variant location alone [1].

The widespread implementation of next-generation sequencing (NGS) has substantially expanded the mutational spectrum of *APC*, leading to the identification of numerous previously unreported variants. Defining the clinical significance of these variants is essential for accurate molecular diagnosis, genetic counseling, risk assessment, and clinical management. In the present study, we describe the clinical and molecular characteristics of four unrelated individuals carrying previously unreported *APC* variants and thereby contribute to the expanding molecular spectrum of *APC*-associated polyposis.

Methods

Genetic Analysis

Four unrelated patients who were referred to University of Health Science Türkiye, Ankara Etlik City Hospital between 2022 and 2025 for the evaluation of suspected hereditary cancer predisposition were included in this retrospective, descriptive molecular case series. Patients with molecularly confirmed *APC*-associated polyposis and sufficient clinical and molecular data for retrospective review were included in the study. The study was conducted in accordance with the Declaration of Helsinki. This has been approved by the Ethics Committee of the University of Health Science Türkiye, Ankara Etlik City Hospital (approval no: AEŞH-BADEK-2024-845, date: 25.09.2024). Written informed consent was obtained from all participants or their legal guardians before genetic testing.

All patients underwent hereditary cancer syndrome (HCS) multigene panel testing based on their personal and/or family histories suggestive of an inherited cancer predisposition syndrome. Genomic deoxyribonucleic acid was extracted from peripheral blood samples and analyzed by NGS using the SOPHiA GENETICS™ Custom Hereditary Cancer Solution (CHCS_C_v2) panel on the Illumina NextSeq 2000 platform.

The panel comprised the following 60 cancer-predisposition genes: *APC*, *ATM*, *AXIN2*, *BAP1*, *BARD1*, *BLM*, *BMPR1A*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CDK4*, *CDKN2A*, *CHEK2*, *DDB2*, *EPCAM*, *ERCC2*, *ERCC3*, *ERCC4*, *ERCC5*, *FANCA*, *FANCC*, *FH*, *FLCN*, *GALNT12*, *HDAC2*, *HOXB13*, *MEN1*, *MET*, *MITF*, *MLH1*, *MSH2*, *MSH3*, *MSH6*, *MUTYH*, *NBN*, *NF1*, *NF2*, *NTHL1*, *PALB2*, *PMS2*, *PMS2CL*, *POLD1*, *POLE*, *POLH*, *PTCH1*, *PTEN*, *RAD51C*, *RAD51D*, *RB1*, *RET*, *SMAD4*, *STK11*, *TP53*, *TSC1*, *TSC2*, *VHL*, *WT1*, *XPA*, and *XPC*. Sequence data were aligned to the GRCh38 human reference genome and analyzed using the SOPHiA DDM™ platform.

Variants were filtered according to the validated quality control criteria of the SOPHiA DDM™ pipeline, including read depth, variant allele fraction, mapping quality, and coverage metrics, and were subsequently reviewed manually using the Integrative Genomics Viewer (IGV). Across all samples, the average sequencing depth in the targeted regions was approximately 500×, and approximately 100% of the target bases achieved a sequencing depth of at least 25×. Orthogonal confirmation by Sanger sequencing was not routinely performed. However, all reported variants were supported by high sequencing depth, appropriate variant allele fractions, and manual review.

Variant interpretation was performed according to the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) guidelines using the *APC*-specific recommendations provided by the ClinGen InSiGHT Hereditary CRC/Polyposis Variant Curation Expert Panel (VCEP; version 1.0). Variant novelty was assessed by systematically reviewing ClinVar, LOVD, gnomAD (v4.1.1), Franklin, and PubMed. In addition to exact Human Genome Variation Society (HGVS) matches, alternative HGVS representations were also evaluated to minimize the possibility of overlooking previously reported variants. Variants were considered novel when no exact match was identified in any of the queried resources at the time of analysis. All databases were accessed in July 2026.

The corresponding Binary Alignment/Map (BAM) file alignments, visualized with IGV, are provided in Figure 1.

Statistical Analysis

This study is descriptive; no statistical analysis was performed because the dataset did not require comparative or inferential evaluation.

Results

Patients

Case 1: A 51-year-old man was evaluated for FAP. He had undergone total proctocolectomy with permanent ileostomy in 2008 because of extensive colorectal polyposis. His family history was remarkable for FAP, affecting his father, siblings, and nephews. During follow-up, he was diagnosed with primary lung adenocarcinoma. Although definitive concurrent chemoradiotherapy was recommended, he declined this treatment and discontinued therapy after receiving five cycles

of cisplatin plus pemetrexed. Approximately two months after discontinuation of treatment, he presented with a brain metastasis and underwent metastasectomy, after which second-line immunotherapy was planned.

Molecular genetic analysis identified a heterozygous APC variant, NM_000038.6: c.1621del (p.Gln541SerfsTer8) in exon 13 (385x, 45% variant allele fraction). This frameshift variant introduces a premature termination codon, resulting in truncation of the APC protein, and has not been previously reported in the literature. According to the ACMG/AMP variant interpretation guidelines, this variant fulfilled the PVS1, PM2, and PP4 criteria and was therefore classified as likely pathogenic. The patient's phenotype, including extensive colorectal polyposis requiring proctocolectomy and a strong family history, was highly consistent with APC-associated FAP.

Case 2: A 44-year-old patient was referred for genetic evaluation after diagnosis of CRC at age 42. Histopathological examination revealed a well-differentiated adenocarcinoma (pT3N0M0) together with multiple tubular adenomas in the setting of colorectal polyposis. The patient subsequently underwent panproctocolectomy with permanent ileostomy.

The family history was notable for colorectal polyposis in the patient's mother, who was undergoing regular surveillance and had not required colectomy because of a relatively limited polyp burden. In addition, the patient's maternal aunt had a history of colorectal polyposis and had undergone a partial colectomy. Molecular confirmation was not available for either relative.

Follow-up abdominal imaging demonstrated hepatomegaly, a left adrenal nodular lesion, mesenteric panniculitis, parastomal herniation, and right renal malrotation. A soft-tissue lesion measuring 24x46 mm was identified within the left rectus abdominis muscle and initially considered suggestive of a desmoid tumour. However, histopathological examination revealed chronic inflammatory changes with fat necrosis, foreign body-type granulomatous inflammation, haemorrhage, congestion, and fibromuscular and adipose tissue fragments, with no evidence of a desmoid tumour.

Molecular genetic analysis identified a heterozygous novel APC variant, NM_000038.6:c.3804_3808del (p.Ile1269PhefsTer5), in exon 16 (916x depth, 46% variant allele fraction). This deletion results in a frameshift and introduces a premature termination codon, which is predicted to result in loss of normal APC protein function. According to the ACMG/AMP variant interpretation guidelines, the variant fulfilled the PVS1_Strong, PM2, and PP4 criteria and was therefore classified as likely pathogenic.

Case 3: A 39-year-old woman with a clinical diagnosis of FAP was referred for genetic evaluation. She was initially diagnosed at age 25 and subsequently underwent total colectomy because of extensive colorectal polyposis. During follow-up, numerous gastric and duodenal polyps were identified, and further surgical intervention was planned because of recurrent polyposis.

Her family history was strongly suggestive of hereditary polyposis. Her father died of CRC at the age of 45 years. One of her sisters died of CRC at 25 years of age. Another sister was diagnosed with FAP at age 25 and underwent total colectomy with ileostomy. However, molecular genetic data were not available for any of the affected family members.

Molecular genetic analysis revealed a heterozygous novel APC variant, NM_000038.6:c.2194_2195del (p.Asn732Ter), in exon 16 (471x depth, 43% variant allele fraction). This deletion introduces a premature termination codon and is predicted to result in the loss of normal APC protein function. According to the ACMG/AMP variant interpretation guidelines, the variant fulfilled the PVS1_Strong, PM2, and PP4 criteria and was therefore classified as likely pathogenic.

Case 4. A 27-year-old woman was referred for HCS testing because of a remarkable family history of early-onset CRC. She had a personal history of endometrioid intraepithelial neoplasia (EIN).

A colonoscopic examination performed at 27 years of age revealed dozens of polyps measuring 3-5 mm throughout the colon. Three polyps from the ascending colon, three from the transverse colon, three from the descending colon, two from the sigmoid colon, and one from the rectum were removed endoscopically. Histopathological examination demonstrated that these lesions were tubular adenomas. First-degree internal haemorrhoids were also identified.

Upper gastrointestinal endoscopy revealed findings consistent with pangastritis and duodenitis. Biopsy specimens were obtained from the antrum and duodenum, and *Helicobacter pylori* infection was detected. Thyroid ultrasonography was unremarkable. Magnetic resonance imaging of the brain demonstrated findings suggestive of idiopathic intracranial hypertension, a probable arachnoid cyst, and a small vascular lesion.

Her family history was notable for early-onset CRC. Her mother died of rectal cancer at age 34, and her maternal grandfather died of CRC at approximately age 50. In addition, her maternal aunt had undergone a colectomy for polyposis, and her maternal great-aunt had a history of breast cancer. There was no known consanguinity between her parents.

Molecular genetic analysis identified a heterozygous APC variant, NM_000038.6:c.4066_4067dup (p.Gly1357GlnfsTer59), in exon 16 (565x depth, 45% variant allele fraction). This duplication results in a frameshift and introduces a premature termination codon, leading to loss of normal APC protein function. According to the ACMG/AMP variant interpretation guidelines, the variant fulfilled the PVS1_Strong, PM2, and PP4 criteria and was therefore classified as likely pathogenic.

Based on the clinical findings, family histories, and molecular results, the identified variants were responsible for the observed disease phenotypes. Genetic counselling and cascade testing were recommended for at-risk family members.

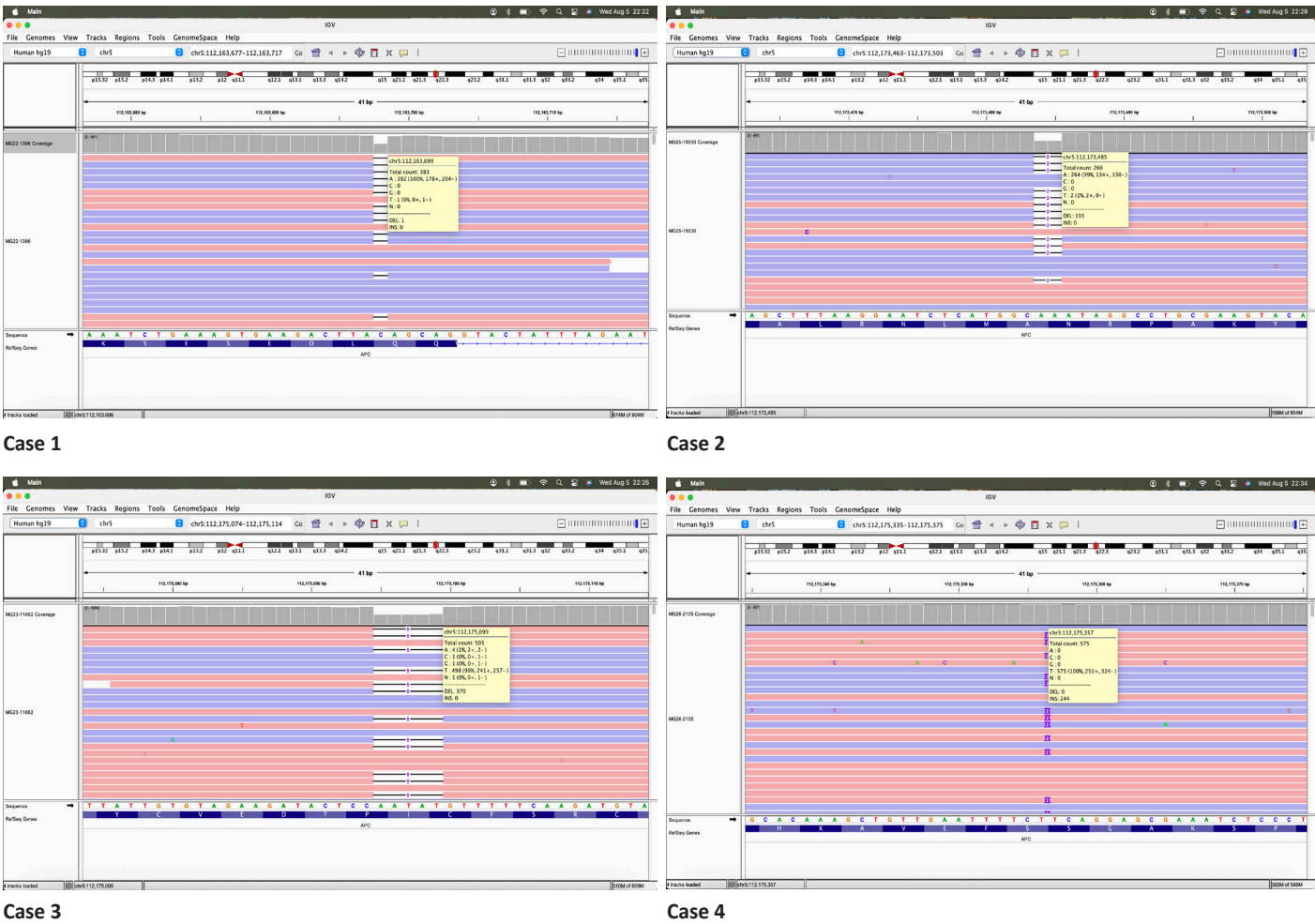


Figure 1. IGV alignments of the identified APC variants. The panels show the genomic location of each variant together with the corresponding sequencing coverage and VAF. The position of the variant is indicated by the blue arrow

IGV: Integrative genomics viewer, VAF: Variant allele fraction

The clinical characteristics, family histories, and molecular findings of the four unrelated patients carrying novel APC variants are summarized in Table 1.

Discussion

FAP is the most common hereditary adenomatous polyposis syndrome and is predominantly caused by germline loss-of-function variants in the APC gene. Although the mutational spectrum of APC continues to expand with the identification of previously unreported variants, characterization of these novel variants remains essential for accurate variant interpretation, genetic counseling, risk assessment, and clinical management. Pathogenic APC variants are predominantly protein-truncating alterations, with frameshift and nonsense variants accounting for the majority of reported disease-causing variants, whereas missense variants are encountered less frequently [7]. According to the Leiden Open Variation Database, the most frequently reported APC variants worldwide, c.3927_3931del (p.Glu1309Aspfs) and c.3183_3187del (p.Gln1062Ter), are also truncating variants, further emphasizing that loss of

APC function represents the principal molecular mechanism underlying FAP (LOVD, <https://www.lovd.nl/>). Consistent with this distribution, all four APC variants identified in our cohort were predicted to result in loss of function. However, their molecular consequences are not expected to be identical. The c.1621del (p.Gln541SerfsTer8) variant is predicted to undergo nonsense-mediated mRNA decay (NMD), whereas the remaining three variants, located within the terminal coding exon, are predicted to escape NMD and give rise to truncated protein products. Accordingly, the variants fulfilled either the PVS1 or PVS1_Strong criteria and were classified as likely pathogenic according to the ACMG/AMP guidelines.

Previous studies have consistently associated pathogenic variants located within the APC MCR (MCR; codons 1286-1513) with a more severe polyposis phenotype and an earlier age at disease onset [8]. Among the four novel variants identified in our cohort, only p.Gly1357GlnfsTer59 was located within the MCR (Figure 2). Although this patient has not yet developed a classical FAP phenotype, her relatively young age may preclude full phenotypic expression. Therefore, close

Table 1. Clinical characteristics and molecular findings of the four patients carrying novel APC variants

Case	Age/ sex	Clinical features	Age at diagnosis	Extracolonic manifestations associated with FAP	Family history	APC variant (NM_000038.6)	Variant type	Exon	ACMG evidence	Classification
1	51/M	Classical FAP, extensive colorectal polyposis, total proctocolectomy, lung adenocarcinoma	~30y	-	Father, siblings, and nephews with FAP	c.1621del (p.Gln541SerfsTer8)	PTV	13	PVS1, PM2, PP4	Likely pathogenic
2	44/F	FAP, total colectomy	24y	-	Non-extensive colonic polyps in the mother and colectomy for polyposis in the maternal aunt	c.3804_3808del (p.Ile1269PhefsTer5)	PTV	16	PVS1, Strong, PM2, PP4	Likely pathogenic
3	39/F	FAP, prophylactic colorectal colectomy, recurrent polyposis	25y	-	Father and two sisters with FAP; father and one sister died of colorectal cancer	c.2194_2195del (p.Asn732Ter)	PTV	16	PVS1, Strong, PM2, PP4	Likely pathogenic
4	27/F	FAP, colonic tubular adenoma, EIN, family history of early-onset rectal cancer	27y	-	Mother died of rectal cancer at 34 years; maternal great-aunt with breast cancer	c.4066_4067dup (p.Gly1357GlnfsTer59)	PTV	16	PVS1, Strong, PM2, PP4	Likely pathogenic

The table summarizes the demographic characteristics, major clinical manifestations, family history, molecular findings, variant type, exon location according to the RefSeq transcript NM_000038.6, and ACMG/AMP classification of the novel APC variants identified in this study
 FAP: Familial adenomatous polyposis; PTV: Predicted truncating variant, EIN: Endometrioid intraepithelial neoplasia, ACMG: American College of Medical Genetics and Genomics, AMP: Association for Molecular Pathology

endoscopic surveillance and long-term clinical follow-up have been recommended. In contrast, three of the four protein-truncating variants identified in our cohort were located outside the MCR.

Data on the molecular spectrum of APC variants in the Turkish population remain limited. Erdem and Bahsi [9]. reported three missense APC variants, all of which were classified as variants of uncertain significance. In contrast, Ceylan and Ceylan [10]. identified seven pathogenic protein-truncating APC variants, including three nonsense and four frameshift alterations, the majority of which were located in exon 16. Consistent with these observations, three of the four novel variants identified in our cohort were in exon 16. However, given the limited sample size, these findings should not be interpreted as representative of the variant distribution in the Turkish population.

Case 4 presented with EIN. Current evidence does not support a definitive association between APC-associated polyposis and gynecological malignancies, although rare cases have been reported in the literature [11,12]. Therefore, in our patient, the coexistence of EIN and an APC pathogenic variant was considered an uncertain finding rather than an established disease manifestation. Although Case 1 developed lung adenocarcinoma during follow-up, lung cancer is not currently regarded as part of the established extracolonic tumor spectrum of APC-associated polyposis. Given the patient's substantial smoking history, the finding was considered more likely to represent an independent, coexisting malignancy.

Taken together, these findings expand the molecular spectrum of APC-associated polyposis and further emphasize the importance of integrating molecular findings with clinical evaluation and longitudinal follow-up. Larger multicenter studies will be necessary to improve our understanding of genotype-phenotype relationships in APC-associated disease.

Study Limitations

The present study has several limitations. First, this was a small, single-center case series comprising only four unrelated patients; therefore, our findings should not be considered representative of the Turkish population. In addition, functional studies and comprehensive segregation analyses were not available for all cases. In some families, segregation analyses could not be completed because potentially informative relatives were deceased, declined genetic testing, or were unavailable for follow-up. Orthogonal validation by Sanger sequencing was not routinely performed, although all variants were supported by high sequencing depth, appropriate variant allele fractions, and manual IGV review. Larger multicenter studies are needed to further define the molecular spectrum of APC-associated polyposis and to clarify genotype-phenotype relationships.

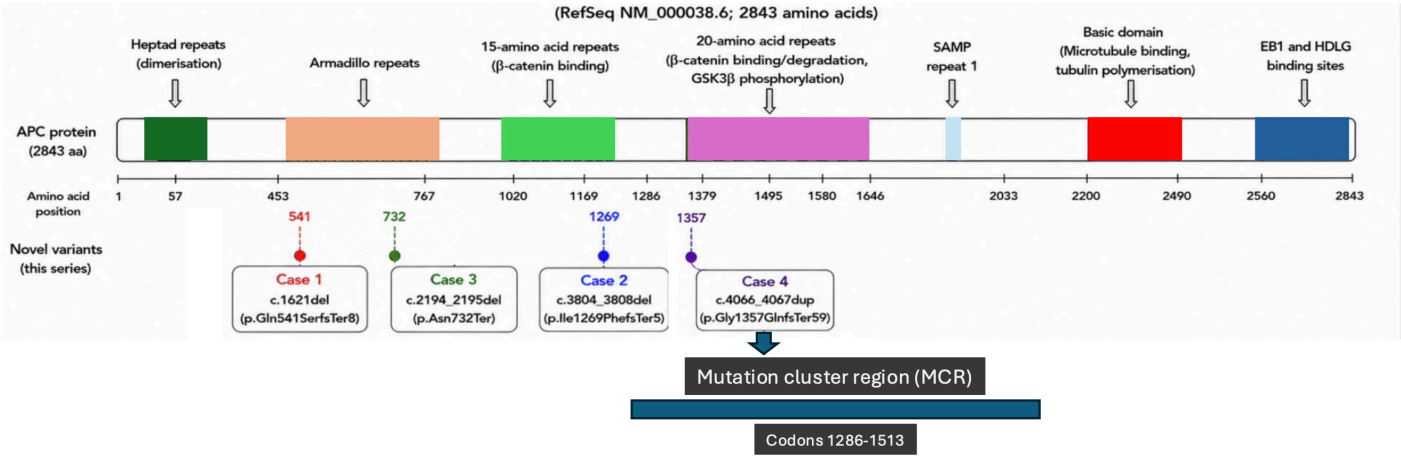


Figure 2. Schematic representation of the APC protein domains and the localization of the novel variants identified in this study. The protein structure is shown according to the RefSeq transcript NM_000038.6 and the RefSeq protein NP_000029.2. The APC protein consists of 2,843 amino acids and contains multiple functional domains, including the N-terminal heptad repeats, armadillo repeats, 15-amino acid β -catenin-binding repeats, 20-amino acid β -catenin-binding/degradation repeats, the SAMP repeat, the basic microtubule-binding domain, and the EB1/HDLG-binding region. The positions of the four novel APC variants identified in this study are indicated according to their amino acid coordinates. The MCR (codons 1286-1513), which has been associated with a more severe polyposis phenotype, is highlighted. The domain organization shown in this figure was inspired by the schematic representation reported by Zhang et al. [13]
Aa: Amino acid, MCR: Mutation cluster region, SAMP: Serine-alanine-methionine-proline repeat, GSK3 β : Glycogen synthase kinase 3 beta, EB1: End-binding protein 1, HDLG: Human discs large homolog

Conclusions

This study describes four previously unreported APC variants identified in Turkish patients with APC-associated polyposis. All variants were predicted to result in loss of APC function and classified as likely pathogenic according to the ACMG/AMP guidelines, thereby expanding the mutational spectrum of APC. Overall, our findings provide additional data that may contribute to future variant interpretation studies. Further multicenter studies are needed to improve our understanding of the molecular spectrum of APC-associated polyposis.

Ethics

Ethics Committee Approval: This has been approved by the Ethics Committee of the University of Health Science Türkiye, Ankara Etlik City Hospital (approval no: AEŞH-BADEK-2024-845, date: 25.09.2024).

Informed Consent: Written informed consent was obtained from all participants or their legal guardians before genetic testing.

Footnotes

Authorship Contributions

Surgical and Medical Practices: L.D., Concept: E.T., L.D., Design: E.T., Data Collection or Processing: E.T., Analysis or Interpretation: E.T., L.D., Literature Search: E.T., Writing: E.T.

Conflict of Interest: No conflict of interest was declared by the authors.

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Declaration Regarding the Use of AI and AI-Assisted Technologies: During manuscript preparation, the authors used OpenAI's ChatGPT (GPT-5, San Francisco, CA, USA) as a language-support tool to improve the clarity, grammar, and fluency of the text. The tool was not used for data analysis, figure preparation, or the generation of scientific content. All outputs produced by the AI tool were carefully reviewed, edited, and verified by the authors. The authors take full responsibility for the integrity and accuracy of the entire manuscript.

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Original Article

Development of a Composite Risk Score and Real-world Outcomes in Advanced Pancreatic Adenocarcinoma: Prognostic Value of Baseline ¹⁸F-FDG PET/CT Metabolic Parameters

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ABSTRACT

Aim: Reliable prognostic biomarkers are needed to improve risk stratification in patients with advanced pancreatic adenocarcinoma. We developed and validated a simple composite risk score that integrates clinical, laboratory, and ¹⁸F-fluorodeoxyglucose (¹⁸F-FDG) positron emission tomography/computed tomography (PET/CT) derived parameters to predict survival.

Methods: This retrospective single-center study included 50 patients with advanced pancreatic adenocarcinoma who underwent a baseline ¹⁸F-FDG PET/CT before systemic treatment. Receiver operating characteristic (ROC) analysis was used to determine cut-off values for total lesion glycolysis (TLG), metabolic tumor volume (MTV), and serum carbohydrate antigen 19-9 (CA19-9). A composite risk score (0-4) was created by assigning one point each for liver metastasis, MTV above the cut-off, TLG above the cut-off, and CA19-9 >1000 U/mL. Overall survival (OS) and progression-free survival (PFS) were estimated using the Kaplan-Meier method and compared using the log-rank test.

Results: The median OS and PFS were 13.73 and 7.23 months, respectively. ROC analysis identified cut-off values of 15.55 cm³ for MTV, 93.88 for TLG, and 1000 U/mL for CA19-9. Twenty-three patients (46.0%) were classified as low risk (score 0-1) and 27 (54.0%) as high-risk (score ≥2). High-risk patients had significantly shorter OS (10.35 vs. 14.62 months, p=0.018) and PFS (6.01 vs. 7.29 months, p=0.048) than low-risk patients. One-year OS rates were 41.7% and 81.0%, respectively.

Conclusions: The proposed composite risk score was associated with distinct survival outcomes in patients with advanced pancreatic adenocarcinoma. By integrating routinely available clinical, laboratory, and PET/CT-derived variables, this pragmatic model may assist baseline prognostic stratification. These findings require validation in larger prospective multicenter cohorts before clinical implementation.

Keywords: Pancreatic adenocarcinoma, metabolic tumor volume, total lesion glycolysis, CA19-9, composite risk score

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive solid malignancies and is expected to become a leading cause of cancer-related mortality worldwide. Despite advances in systemic therapy, most patients are diagnosed

with unresectable locally advanced or metastatic disease, and long-term survival remains poor [1,2]. Although multi-agent chemotherapy regimens, such as FOLFIRINOX and gemcitabine plus nab-paclitaxel, have improved outcomes in selected patients, median overall survival (OS) for metastatic PDAC remains below 12-15 months in real-world practice. Therefore,

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identifying reliable prognostic biomarkers that can improve risk stratification and facilitate individualized treatment decisions continues to be a major clinical challenge [3,4].

Current prognostic assessment in metastatic PDAC is primarily based on clinical performance status, metastatic burden, and serum carbohydrate antigen 19-9 (CA19-9) [5]. Among these, CA19-9 is the most widely used biomarker for disease monitoring; however, its prognostic performance is limited by substantial interpatient variability, Lewis antigen negativity, biliary obstruction, and inflammatory conditions. Consequently, additional imaging- and tumor biology-based biomarkers are needed to improve prognostic accuracy beyond conventional clinical parameters [6,7].

^{18}F -fluorodeoxyglucose (^{18}F -FDG) positron emission tomography/computed tomography (PET/CT) provides quantitative information regarding tumor glucose metabolism and has emerged as a valuable imaging modality in pancreatic cancer. While the maximum standardized uptake value (SUV_{max}) has historically been the most frequently reported PET parameter, volumetric metabolic indices—including metabolic tumor volume (MTV) and total lesion glycolysis (TLG)—have gained increasing attention because they more comprehensively reflect whole-tumor metabolic burden [8,9]. Several studies have demonstrated that MTV and TLG may correlate more closely with tumor aggressiveness and patient survival than SUV_{max} alone; however, published results remain heterogeneous owing to differences in patient populations, treatment strategies, and PET acquisition protocols [10-12].

Because pancreatic cancer progression is driven by both tumor biology and metastatic dissemination, prognostic evaluation based on a single biomarker is unlikely to capture the complexity of disease behavior. Composite prognostic models integrating clinical, laboratory, and imaging biomarkers have therefore attracted growing interest across multiple malignancies. Such multidimensional models may outperform individual biomarkers by combining complementary prognostic information and enabling more robust patient stratification [13]. Nevertheless, few studies have specifically evaluated integrated PET/CT-based composite risk scores in metastatic pancreatic adenocarcinoma, and most available reports have focused on either isolated PET parameters or conventional clinical variables rather than their combined prognostic utility [14-16].

Liver metastasis represents the most frequent site of distant spread in PDAC and is consistently associated with poorer survival. Likewise, elevated CA19-9 reflects increased tumor burden and biologically aggressive disease, whereas high MTV and TLG indicate extensive metabolically active tumor volume. These variables represent distinct but complementary aspects of tumor behavior, and therefore constitute rational candidates for incorporation into a clinically applicable prognostic model. A simple composite score derived from routinely available baseline PET/CT and laboratory findings could facilitate early identification of patients at particularly high-risk of adverse outcomes without increasing cost or requiring additional molecular testing.

Therefore, we developed and validated a novel composite risk score integrating the presence of baseline liver metastasis, MTV, TLG, and CA19-9 to predict survival in patients with advanced pancreatic adenocarcinoma who were treated in routine clinical practice. In addition, we investigated the prognostic value of baseline PET/CT metabolic parameters for progression-free survival (PFS) and OS, and assessed whether combining these variables into a simple composite score improves risk stratification compared with individual biomarkers.

Methods

Patient Data

We retrospectively enrolled 50 patients with histologically confirmed locally advanced or metastatic pancreatic adenocarcinoma who underwent baseline ^{18}F -FDG PET/CT before receiving first-line systemic chemotherapy at Pamukkale University. Approval was granted by the Pamukkale University Non-Interventional Clinical Research Ethics Committee (approval no: 12, date: 30.06.2026). All procedures adhered to the Declaration of Helsinki.

Eligible patients had an Eastern Cooperative Oncology Group performance status 0-2 and were considered suitable candidates for systemic chemotherapy. All patients had baseline ^{18}F -FDG PET/CT imaging, pretreatment laboratory data, including serum tumor markers, and complete clinical follow-up records. Patients with pancreatic neuroendocrine tumors, with non-adenocarcinoma histology, with unavailable baseline PET/CT imaging, or with insufficient baseline clinical data were excluded.

Outcome Measures

OS was measured from the date of diagnosis to death from any cause or, for survivors, to the last follow-up. PFS was measured from the start of first-line systemic chemotherapy to the first radiologically documented progression or death, whichever occurred first.

PET/CT Acquisition and Image Analysis

Baseline whole-body ^{18}F -FDG PET/CT was obtained in all patients before first-line systemic chemotherapy, according to the institutional protocol, with images reconstructed using the manufacturer's standard algorithm. All baseline ^{18}F -FDG PET/CT examinations were reviewed by two experienced nuclear medicine physicians according to the institutional imaging protocol. Quantitative metabolic parameters were obtained from pretreatment PET/CT images. The highest voxel-based SUV within the primary tumor was recorded as SUV_{max} . MTV was measured using a semi-automatic three-dimensional volume-of-interest segmentation method based on a 41% SUV_{max} isocontour threshold, in accordance with the institutional imaging protocol. TLG was calculated as the product of MTV and the corresponding mean SUV (SUV_{mean}), representing the total metabolically active tumor burden.

Composite Risk Score

A composite risk score ranging from 0 to 4 was developed using four baseline variables: liver metastasis, MTV above the ROC-derived cut-off, TLG above the ROC-derived cut-off, and serum CA19-9 >1000 U/mL. One point was assigned for each adverse factor present. Patients were subsequently classified into a low-risk group (score 0-1) and a high-risk group (score ≥ 2) for survival analyses. Each component was assigned one point to preserve transparency and ease of use. Coefficient-based weighting was not used because the modest sample size could have produced unstable, sample-dependent weights and increased the risk of overfitting. Equal weighting was therefore chosen as a pragmatic, exploratory approach and should not be interpreted as evidence that the four variables have identical biological effect sizes.

Statistical Analysis

Continuous data were reported as median and interquartile range and categorical data were reported as counts and percentages. ROC curve analysis, with 12-month mortality as the reference outcome, was used to determine optimal thresholds for SUV_{max} , MTV, TLG, and CA19-9; the cut-off was set at the maximum Youden index, and discriminative capacity was expressed as the area under the curve (AUC) with 95% confidence intervals (CIs). Continuous variables included in the composite score were dichotomized at these ROC-derived thresholds to enable a simple and clinically applicable point-based classification without requiring a regression formula. The ROC-derived thresholds were generated in the present cohort to explore the prediction of 12-month mortality. They are data-driven and cohort-specific, and should not be interpreted as universally applicable clinical cut-offs. OS and PFS were estimated by Kaplan-Meier analysis and compared with the log-rank test. Candidate predictors of survival were first screened by univariable Cox proportional hazards regression. All tests were two-sided, with $p < 0.05$ taken as significant. Analyses were performed using IBM SPSS Statistics v.26.0 (IBM Corp., Armonk, NY, USA), and supplementary

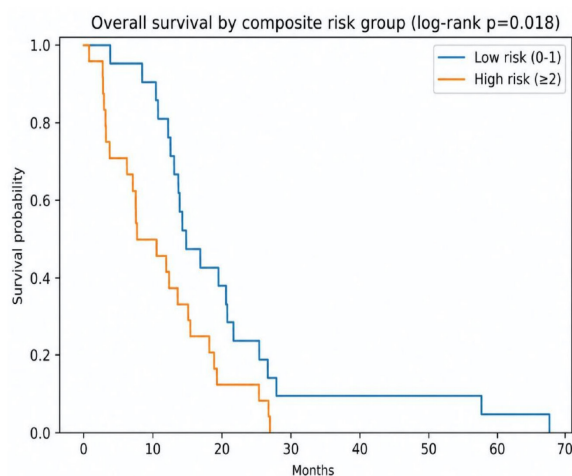


Figure 1. Kaplan-Meier overall survival curves according to the composite risk group

exploratory analyses and figures were produced using Python-based packages.

Results

Fifty patients with advanced pancreatic adenocarcinoma were analyzed. At the data cut-off, 49 of 50 patients (98.0%) had died, and the median follow-up from diagnosis was 13.73 months. The median age was 68.0 years (IQR, 59.2-74.0); 60.0% were men. The primary tumor arose in the pancreatic body in 38.0% of cases, in the head in 34.0%, and in the tail in 28.0; 76.0% of cases presented at stage IV. Liver metastases were present in 44.0% of patients, lung metastases in 14.0%, bone metastases in 6.0%, and lymph node involvement in 82.0%. Median baseline PET/CT values were SUV_{max} 5.21, MTV

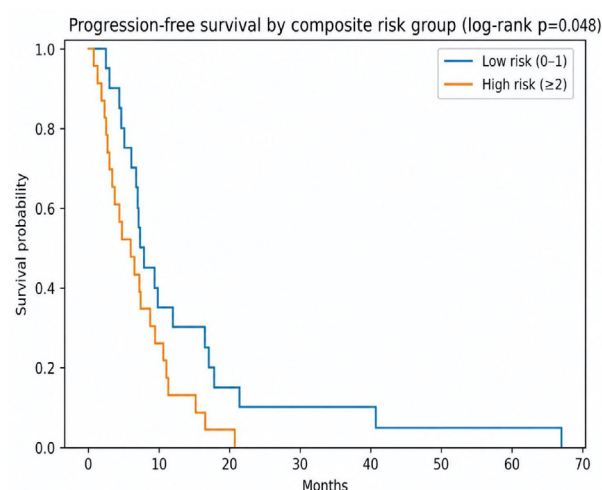


Figure 2. Kaplan-Meier progression-free survival curves according to the composite risk group

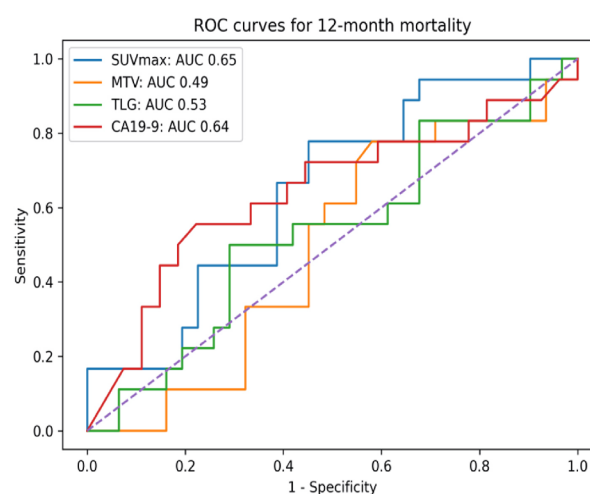


Figure 3. ROC curves of baseline PET/CT parameters and CA19-9 for 12-month mortality

ROC: Receiver operating characteristic, SUV_{max} : Maximum standardized uptake value, AUC: Area under the curve, MTV: Metabolic tumor volume, TLG: Total lesion glycolysis, CA19-9: Carbohydrate antigen 19-9, PET/CT: Positron emission tomography/computed tomography

Table 1. Baseline clinicopathological and imaging characteristics

Variable	Overall (n=50)
Age, years	68.0 (59.2-74.0)
Male sex	30 (60.0)
Primary tumor location: body	19 (38.0)
Primary tumor location: head	17 (34.0)
Primary tumor location: tail	14 (28.0)
Stage IV	38 (76.0)
Liver metastasis	22 (44.0)
Lung metastasis	7 (14.0)
Bone metastasis	3 (6.0)
Lymph node/LAP involvement	41 (82.0)
Other metastasis	5 (10.0)
SUV _{max}	5.21 (4.29-7.11)
MTV, cm ³	19.55 (12.55-37.98)
TLG	68.68 (38.37-110.77)
CA19-9, U/mL	273.60 (23.02-1541.75)
CEA, ng/mL	6.14 (4.01-19.03)
SUV _{max} : Maximum standardized uptake value, MTV: Metabolic tumor volume, TLG: Total lesion glycolysis, CA 19-9: Carbohydrate antigen 19-9, CEA: Carcinoembryonic antigen, LAP: Lymphadenopathy	

19.55 cm³, and TLG 68.68; median serum CA19-9 and CEA were 273.6 U/mL and 6.14 ng/mL, respectively (Table 1).

ROC analysis with respect to 12-month mortality showed that among PET-derived measures, SUV_{max} demonstrated the greatest discriminative ability (AUC=0.645), with CA19-9 close behind (AUC=0.642). Optimal thresholds were 4.96 for SUV_{max}, 15.55 cm³ for MTV, 93.88 for TLG, and 1000 U/mL for CA19-9. MTV (AUC=0.492) and TLG (AUC=0.532) discriminated only weakly individually; however, their thresholds were retained in the composite score because of their biological relevance and potential complementary contribution (Table 2).

Metabolic activity was higher in patients with liver metastasis than in those without. SUV_{max} was higher in the liver metastasis group (6.21 vs. 4.55, p=0.003), as was TLG (96.22 vs. 48.24, p=0.025), whereas MTV showed no significant difference (p=0.249).

Patients with bone metastases likewise carried a heavier metabolic burden, with higher MTV (54.59 vs. 19.13 cm³, p=0.027) and TLG (142.07 vs. 51.84, p=0.041); SUV_{max} was not significantly associated with bone involvement.

No significant links emerged between PET metabolic parameters and either lung metastases or lymph node involvement (all p>0.05), indicating that the volumetric indices were mainly associated with liver and bone dissemination (Table 3). The composite score distribution is shown in Table 4.

Table 2. ROC analysis for 12-month mortality

Variable	AUC	95% CI	Cut-off	Sensitivity, %	Specificity, %
SUV _{max}	0.645	0.474-0.800	4.96	77.8	54.8
MTV	0.492	0.337-0.660	15.55	77.8	41.9
TLG	0.532	0.355-0.713	93.88	50.0	71.0
CA19-9	0.642	0.460-0.826	1000.00	55.6	77.8

AUC confidence intervals were obtained by bootstrap resampling. Cut-offs were selected using the maximum Youden index

SUV_{max}: Maximum standardized uptake value, MTV: Metabolic tumor volume, TLG: Total lesion glycolysis, CA 19-9: Carbohydrate antigen 19-9, AUC: Area under the curve

Table 3. PET parameters according to metastatic site

Metastatic site	PET parameter	Absent	Present	p
Liver metastasis	SUV _{max}	4.55 (4.09-5.43)	6.21 (5.02-8.77)	0.003
Liver metastasis	MTV	15.80 (12.06-30.57)	21.54 (14.36-38.59)	0.249
Liver metastasis	TLG	48.24 (36.72-92.07)	96.22 (44.20-152.63)	0.025
Lung metastasis	SUV _{max}	5.17 (4.17-7.10)	5.56 (4.86-7.04)	0.442
Lung metastasis	MTV	21.31 (12.98-38.75)	13.95 (12.06-19.36)	0.166
Lung metastasis	TLG	75.31 (39.03-122.25)	42.13 (38.34-80.31)	0.410
Bone metastasis	SUV _{max}	5.26 (4.31-7.10)	4.23 (4.05-6.12)	0.567
Bone metastasis	MTV	19.13 (12.38-34.52)	54.59 (47.14-58.78)	0.027
Bone metastasis	TLG	51.84 (37.51-106.82)	142.07 (125.69-191.47)	0.041
Lymph node/LAP involvement	SUV _{max}	4.96 (3.85-5.89)	5.39 (4.45-7.80)	0.174
Lymph node/LAP involvement	MTV	19.13 (12.79-34.52)	19.77 (12.57-38.39)	0.936
Lymph node/LAP involvement	TLG	82.17 (28.48-93.70)	51.84 (39.86-132.94)	0.484

SUV_{max}: Maximum standardized uptake value, MTV: Metabolic tumor volume, TLG: Total lesion glycolysis, LAP: Lymphadenopathy, PET: Positron emission tomography

During follow-up, median OS for the whole cohort was 13.73 months, with OS of 83.7%, 63.3%, and 22.4% at 6, 12, and 24 months, respectively. Median PFS was 7.23 months; PFS at the same time points were 66.0%, 23.4%, and 6.4%.

Survival was better in the low-risk group (score 0-1) than in the high-risk group (score ≥ 2). Median OS was 14.62 months versus 10.35 months (log-rank $p=0.018$); median PFS was 7.29 months versus 6.01 months (log-rank $p=0.048$). One-year OS was 81.0% in the low-risk group and 41.7% in the high-risk group; the corresponding one-year PFS rates were 30.0%

and 13.0%, demonstrating clear separation by composite risk (Table 5).

In univariable Cox proportional hazards regression analysis, a baseline CA19-9 >1000 U/mL was significantly associated with worse OS (HR=2.41, 95% CI: 1.23-4.69; $p=0.010$). Likewise, patients in the high composite risk group (score ≥ 2) had significantly higher mortality than those in the low-risk group (HR=1.91, 95% CI: 1.04-3.51; $p=0.036$). No significant associations were observed for age, sex, tumor location, stage, metastatic sites, SUV_{max}, MTV, TLG, or CEA (Table 6).

Table 4. Distribution of the composite risk score

Composite risk score	Number of patients	Percentage
0	14	28.0%
1	9	18.0%
2	10	20.0%
3	16	32.0%
4	1	2.0%

The composite score assigned one point each for liver metastasis, MTV >15.55 , TLG >93.88 , and CA19-9 >1000
MTV: Metabolic tumor volume, TLG: Total lesion glycolysis, CA19-9: Carbohydrate antigen 19-9

Table 5. Kaplan-Meier survival estimates

Endpoint	Group	Median, months	6 month, %	12 month, %	24 month, %	Log-rank p
Overall survival	All valid cases	13.73	83.7	63.3	22.4	-
Overall survival	Low-risk (0-1)	14.62	95.2	81.0	-	0.018
Overall survival	High-risk (≥ 2)	10.35	70.8	41.7	-	0.018
Progression-free survival	All valid cases	7.23	66.0	23.4	6.4	-
Progression-free survival	Low-risk (0-1)	7.29	75.0	30.0	-	0.048
Progression-free survival	High-risk (≥ 2)	6.01	52.2	13.0	-	0.048

Low-risk group (composite risk score 0-1); high-risk group (composite risk score ≥ 2)

Table 6. Univariable Cox regression analysis for overall survival

Variable	Comparison	HR (95% CI)	p value
Age	Per 1-year increase	1.015 (0.989-1.041)	0.261
Sex	Male vs. female	1.711 (0.927-3.156)	0.086
Primary tumor location	Body vs. head	1.241 (0.620-2.485)	0.542
Primary tumor location	Tail vs. head	1.876 (0.876-4.017)	0.105
Stage	IV vs. III	1.252 (0.696-2.252)	0.452
Liver metastasis	Present vs. absent	1.352 (0.757-2.413)	0.308
Lung metastasis	Present vs. absent	1.246 (0.553-2.807)	0.596
Bone metastasis	Present vs. absent	0.529 (0.127-2.201)	0.381
Lymph node involvement	Present vs. absent	0.624 (0.298-1.307)	0.211
SUV _{max}	>4.96 vs. ≤ 4.96	1.313 (0.736-2.343)	0.356
MTV	>15.55 vs. ≤ 15.55 cm ³	1.452 (0.802-2.629)	0.218
TLG	>93.88 vs. ≤ 93.88	1.230 (0.664-2.280)	0.510
CA19-9	>1000 vs. ≤ 1000 U/mL	2.407 (1.234-4.693)	0.010
CEA	>6.14 vs. ≤ 6.14 ng/mL	1.125 (0.598-2.118)	0.715
Composite risk group	High (≥ 2) vs. low (0-1)	1.914 (1.043-3.512)	0.036

SUV_{max}: Maximum standardized uptake value, MTV: Metabolic tumor volume, TLG: Total lesion glycolysis, CA 19-9: Carbohydrate antigen 19-9, CEA: Carcinoembryonic antigen, HR: Hazard ratio, CI: Confidence interval

Discussion

This real-world study evaluated the prognostic significance of baseline ^{18}F -FDG PET/CT-derived metabolic parameters and explored a simple composite risk score integrating liver metastasis, MTV, TLG, and CA19-9 in patients with advanced pancreatic adenocarcinoma. The principal findings of this study can be summarized as follows. First, patients classified as high risk according to the composite score experienced significantly shorter OS and PFS than those classified as low risk. Second, baseline CA19-9 concentrations above 1000 U/mL were associated with inferior OS in a univariable Cox regression analysis. Third, although MTV and TLG demonstrated relatively modest discriminatory performance when evaluated individually by ROC analysis, their incorporation into a composite model that included liver metastasis and CA19-9 appeared to improve prognostic stratification. Taken together, these findings suggest that integrating routinely available clinical, laboratory, and metabolic imaging variables may provide complementary prognostic information beyond that obtained from individual biomarkers.

Among PET-derived metabolic parameters, SUV_{max} has historically been the most widely reported index because of its simplicity and reproducibility. However, SUV_{max} represents the single voxel with the highest glucose uptake and, therefore, reflects only a limited portion of the tumor. It does not account for tumor volume, spatial heterogeneity, or total metabolically active disease burden [17]. In contrast, MTV quantifies the volume of metabolically active tumor tissue, whereas TLG combines MTV with average glucose uptake, thereby providing a more comprehensive estimate of whole-tumor metabolic burden [18].

Growing evidence suggests that volumetric PET parameters may be more closely associated with patient outcomes than SUV_{max} alone. In a systematic review and meta-analysis including multiple studies of pancreatic carcinoma, Zhu et al. [19] demonstrated that elevated MTV and TLG were consistently associated with poorer survival, although substantial heterogeneity existed regarding patient selection, disease stage, treatment modality, PET acquisition protocols, and segmentation methods. Similarly, Lee et al. [20] reported that both MTV and TLG were significant prognostic markers in patients undergoing surgery for pancreatic cancer and suggested that volumetric PET parameters better reflected tumor biology than conventional SUV measurements [20]. Mohamed and colleagues subsequently observed that increased TLG was associated with inferior OS in patients with pancreatic adenocarcinoma and remained prognostically relevant after adjustment for selected clinical variables [21].

Our findings are generally consistent with previous observations, while also emphasizing several important practical considerations. In the present study, MTV and TLG individually demonstrated only modest discriminatory ability for predicting 12-month mortality. Their ROC-derived AUC values were relatively low and numerically inferior to those observed for CA19-9 and SUV_{max} . Rather than indicating

that volumetric PET parameters lack prognostic value, these findings likely reflect the considerable biological and clinical heterogeneity encountered in routine oncology practice.

Methodological variability may also contribute to the heterogeneous performance of volumetric PET biomarkers reported in the literature. MTV and TLG measurements are influenced by scanner characteristics, reconstruction algorithms, uptake time, blood glucose levels, lesion delineation techniques, and segmentation thresholds [17,18]. Consequently, absolute cut-off values reported across studies vary considerably. Previous investigators have employed fixed SUV thresholds, percentage-based thresholds, adaptive segmentation techniques, and liver-background methods, making direct comparison challenging [18-21]. In the present study, MTV was measured using a standardized semi-automatic 41% SUVmax isocontour threshold according to institutional imaging protocols. Although this approach ensured methodological consistency within our cohort, the proposed cut-off values should not be considered universally applicable and require validation across different PET/CT platforms and imaging protocols.

Another noteworthy observation was the relationship between metabolic parameters and metastatic distribution. Patients with liver metastases exhibited significantly higher SUV_{max} and TLG, whereas patients with bone metastases exhibited significantly higher MTV and TLG. Although the number of patients with bone metastases was limited, these findings are biologically plausible because increasing metastatic dissemination would be expected to increase the total metabolically active tumor burden. By contrast, no significant associations were observed between PET-derived metabolic parameters and lung metastases or lymph node involvement. These observations suggest that different metastatic sites may reflect distinct biological patterns of disease spread, rather than uniformly increasing the metabolic tumor burden.

Collectively, these findings support the concept that PET-derived volumetric parameters provide clinically relevant information regarding tumor burden; however, they also indicate that their prognostic utility may be limited when interpreted in isolation. The modest discriminatory performance observed for MTV and TLG individually suggests that metabolic tumor burden represents only one dimension of disease biology. Consequently, integrating PET-derived metabolic information with complementary clinical and laboratory variables may provide a more comprehensive assessment of prognosis than reliance on a single biomarker. This concept formed the rationale for developing the exploratory composite risk score evaluated in the present study.

Although neither MTV nor TLG demonstrated strong discriminatory performance in ROC analyses, patients classified as high-risk according to the composite score experienced significantly shorter OS and PFS than those classified as low risk. MTV and TLG were nevertheless retained because they reflect whole-body metabolic tumor burden, a biologically relevant dimension intended to provide complementary rather than stand-alone prognostic information. These

observations suggest that integrating multiple baseline variables reflecting different biological aspects of advanced pancreatic adenocarcinoma may provide complementary prognostic information. However, because the score was developed and evaluated within the same cohort, its apparent performance should be interpreted cautiously until confirmed in independent populations.

This concept is consistent with the current understanding that prognosis in advanced pancreatic adenocarcinoma is determined by multiple interacting biological processes. Patients with apparently similar tumor, node, metastasis stage frequently demonstrate markedly different clinical outcomes, suggesting that anatomical staging alone is insufficient for accurate prognostic assessment [13]. Increasing attention has therefore been directed toward multidimensional prognostic models incorporating clinical, laboratory, and imaging biomarkers rather than relying on isolated parameters [22,23].

The present findings also support the biological relevance of liver metastasis as an important component of the composite score. Liver involvement is the most common pattern of distant dissemination in pancreatic adenocarcinoma and has consistently been associated with unfavorable survival outcomes [24]. Beyond simply representing metastatic spread, liver metastasis is frequently considered a surrogate marker of aggressive tumor biology, increased systemic tumor burden, and impaired hepatic reserve. Consistent with these observations, patients with liver metastases in the present study demonstrated significantly higher SUV_{max} and TLG values, suggesting that hepatic dissemination was accompanied by increased metabolic tumor activity.

Similarly, CA19-9 remains the most extensively investigated serum biomarker in pancreatic adenocarcinoma despite its well-recognized limitations. Elevated baseline CA19-9 concentrations have repeatedly been associated with increased tumor burden and inferior survival across different treatment settings [5]. In a meta-analysis including more than 6,000 patients, elevated pretreatment CA19-9 was consistently associated with shorter OS irrespective of disease stage [25]. Furthermore, Pelzer et al. [26] demonstrated that patients with baseline CA19-9 concentrations above 1000 U/mL experienced substantially shorter survival than those with lower values. The optimal ROC-derived threshold identified in the present study was identical to the previously reported value, providing indirect support for the biological plausibility of the selected cut-off.

The relatively stronger association between the composite score and OS, compared with that for PFS, also deserves consideration. Although the score significantly separated Kaplan-Meier curves for both endpoints, the association appeared more robust for OS. Progression represents only one event during the disease course, whereas OS reflects the cumulative influence of baseline disease burden, treatment sensitivity, subsequent therapies, supportive care, and patient-related factors. Consequently, a prognostic model based on

baseline tumor burden would be expected to demonstrate a more stable relationship with OS than with the timing of first radiological progression.

Study Limitations

Several limitations of this study should be considered. The retrospective single-center design and relatively small sample size may have reduced the statistical robustness and limited the generalizability of the results. In addition, post-progression treatment regimens were heterogeneous, which may have influenced OS. Although 49 of the 50 patients experienced the study endpoint, multivariable Cox regression was not performed because the cohort was limited and the analysis was exploratory, which could have resulted in unstable estimates and overfitting. For the same reasons, internal validation was considered unreliable, and external validation could not be performed because an independent cohort was unavailable.

Conclusion

In conclusion, this real-world study suggests that a simple composite risk score integrating liver metastasis, MTV, TLG, and serum CA19-9 may improve baseline prognostic stratification in patients with advanced PDAC. While individual PET-derived volumetric parameters showed limited discriminatory performance, their integration with clinical and laboratory variables may provide complementary prognostic information. Given the exploratory nature of this study, external validation of the proposed risk score in larger prospective multicenter cohorts is essential to confirm its reproducibility and clinical utility before routine clinical implementation.

Ethics

Ethics Committee Approval: Approval was granted by the Pamukkale University Non-Interventional Clinical Research Ethics Committee (approval no: 12, date: 30.06.2026).

Informed Consent: Because the study was designed retrospectively no written informed consent form was obtained from the patients.

Footnotes

Authorship Contributions

Surgical and Medical Practices: T.G.K., A.A.K., A.Y., Concept: T.G.K., A.A.K., B.Y.T., A.G.D., T.Ş., G.G.D., Design: T.G.K., T.D., S.T., B.Y.T., A.G.D., A.Y., T.Ş., G.G.D., Data Collection or Processing: T.G.K., T.D., S.T., Analysis or Interpretation: T.G.K., T.D., S.T., A.Y., T.Ş., G.G.D., Literature Search: T.G.K., B.Y.T., A.G.D., Writing: T.G.K., A.A.K.

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