

Association of C3AR1 gene polymorphism with sepsis susceptibility and prognosis in colorectal cancer patients undergoing radiotherapy

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ABSTRACT

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Background: This study explores the association between C3AR1 gene polymorphisms and sepsis susceptibility and prognosis in colorectal cancer patients undergoing radiotherapy, aiming to enhance genetic insights and clinical management. **Materials and Methods:** We enrolled 100 colorectal cancer patients with sepsis during radiotherapy and 80 healthy controls. DNA was extracted from blood samples, and serum CEA and VEGF levels were measured pre- and post-radiotherapy. Four C3AR1 polymorphisms (rs11567806, rs190043630, rs113825556, rs147364229) were genotyped. Hardy-Weinberg equilibrium, χ^2 tests, and logistic regression were used for analysis. Radiotherapy included 2 Gy daily fractions for 5 weeks. **Results:** The sepsis and non-sepsis groups were similar in age, gender, BMI, WBC count, infection site, and smoking history ($P>0.05$). The sepsis group had higher APACHE II scores (14.65 ± 4.23 vs. 12.90 ± 3.87 , $P=0.006$) and PCT levels (45.87 ± 9.76 g/L vs. 8.92 ± 2.73 g/L, $P<0.01$). C3AR1 polymorphisms showed no significant association with sepsis susceptibility. However, the CC genotype of rs190043630 was linked to poorer prognosis ($P=0.044$). Post-radiotherapy, CEA (22.3 ± 7.5 to 12.4 ± 6.1 ng/mL, $P<0.001$) and VEGF (123.4 ± 42.5 to 54.2 ± 28.1 pg/mL, $P<0.01$) levels decreased significantly in the ACD + radiotherapy group. **Conclusion:** C3AR1 polymorphisms rs11567806 and rs190043630 are not linked to sepsis susceptibility, but the rs190043630 CC genotype predicts poorer prognosis. Radiotherapy enhances biomarker reduction, suggesting a synergistic effect with chemotherapy.

INTRODUCTION

Sepsis is a life-threatening condition caused by an abnormal immune response to infection, and it continues to present significant challenges in critical care medicine ⁽¹⁾. In cancer patients, particularly those undergoing treatments like radiotherapy, the risk of developing sepsis is heightened due to the immunosuppressive effects of the therapy ⁽²⁻⁴⁾. This complex syndrome can quickly escalate to septic shock and multiple organ dysfunction syndrome (MODS), leading to high mortality rates ^(5, 6). Early detection and accurate prognosis prediction of sepsis are especially critical in oncology settings, yet these remain major challenges. Research suggests that genetics play a crucial role in determining an individual's susceptibility to sepsis and influencing outcomes, with genetic variations contributing to how patients respond to infections ^(7, 8). Single-nucleotide polymorphisms (SNPs), the most common type of genetic variation, have been widely studied in relation to diseases such as sepsis and cancer.

Cancer patients, particularly those undergoing

radiotherapy, are at an increased risk of infections due to the immunosuppressive effects of the treatment ⁽⁹⁾. Radiotherapy, while effective in treating cancer, damages not only tumor cells but also healthy tissues, impairing the immune system's ability to fight infections. This makes cancer patients more vulnerable to infections that can progress into sepsis ^(10, 11). The inflammatory response during sepsis is tightly regulated by a complex network of genes involved in immune response, inflammation, and coagulation. Several studies have identified genes, such as those encoding Toll-like receptors (TLRs) and cytokines like TNF- α and IL-10, that influence sepsis susceptibility and severity ⁽¹²⁾. These genetic variations can affect the body's ability to respond to infections, potentially worsening sepsis outcomes in cancer patients undergoing radiotherapy.

The complement system, a key component of the innate immune system, also plays a significant role in the inflammatory response during sepsis. The C3a receptor 1 (C3AR1), encoded by the C3AR1 gene, is involved in the activation of the complement system, which contributes to the inflammatory cascade in

sepsis. When activated, C3a binds to C3AR1, triggering intracellular signaling pathways that regulate immune cell functions, such as neutrophil recruitment and cytokine release⁽¹³⁾. Dysregulation of this pathway can lead to excessive inflammation, which is a hallmark of sepsis. Although the complement system's role in sepsis has been well established, the influence of C3AR1 gene polymorphisms on sepsis susceptibility and prognosis in cancer patients undergoing radiotherapy has not been thoroughly explored⁽¹⁴⁾.

Given the critical role of the complement system in sepsis and the known impact of genetics on disease outcomes, it is plausible that C3AR1 gene polymorphisms could influence sepsis susceptibility and prognosis in cancer patients. However, despite their potential importance, C3AR1 polymorphisms have been understudied, particularly in the context of cancer and radiotherapy.

This study aims to investigate the association between C3AR1 gene polymorphisms and sepsis susceptibility and prognosis in colorectal cancer patients undergoing radiotherapy. By analyzing the distribution of C3AR1 polymorphisms in patients who developed sepsis and healthy controls, we seek to enhance the genetic understanding of sepsis in cancer patients and improve clinical management strategies. The findings from this study will provide preliminary evidence of the role of C3AR1 genetic variations in sepsis, paving the way for larger, more comprehensive studies to validate these findings and explore their clinical applications in the management of sepsis in cancer patients. This study is the first to examine C3AR1 gene polymorphisms in relation to sepsis susceptibility and prognosis in colorectal cancer patients undergoing radiotherapy. It addresses a gap in understanding genetic influences on sepsis in this immunocompromised population and evaluates radiotherapy's synergistic effects on tumor biomarkers (CEA, VEGF), offering insights for personalized sepsis management.

MATERIALS AND METHODS

Study population

This case-control study was conducted between July 1, 2021, and December 1, 2022, at Zhejiang Xin'an International Hospital. A total of 100 Han Chinese patients with histologically confirmed colorectal cancer who developed sepsis during radiotherapy were included. These patients were between 18 and 80 years of age and provided written informed consent. For comparison, 80 age- and sex-matched healthy Han Chinese individuals with no history of malignancy, infection, or chronic disease were enrolled as controls.

Radiotherapy protocol

All patients in the sepsis group received definitive

external beam radiotherapy using a linear accelerator (Clinac iX, Varian Medical Systems, USA). The total radiation dose administered was 50.4 Gy, delivered in 28 fractions of 1.8 Gy each, five days per week, over a period of 5.5 weeks. Intensity-modulated radiation therapy (IMRT) was employed to optimize tumor targeting and spare normal tissues. Treatment planning was performed using the Eclipse Treatment Planning System (version 13.7, Varian, USA).

Radiation-induced sepsis

Sepsis occurred either during radiotherapy or within 14 days following its completion. The underlying pathophysiology was attributed to mucosal barrier damage, immunosuppression from bone marrow suppression, and increased susceptibility to bacterial translocation, particularly from the gastrointestinal tract. According to the Sepsis-3 criteria, sepsis was defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. The majority of infections were bacterial in origin, with the lungs (49%) and abdominal cavity (36%) being the most common sources, and a minority of cases (15%) categorized as other or undetermined. Approximately 29.3% of septic patients progressed to septic shock.

Sample collection and timing

Peripheral venous blood samples were collected from all participants. For sepsis patients, blood was drawn within 24 hours of intensive care unit (ICU) admission. For controls, samples were obtained in the morning following an overnight fast. Tumor marker samples (for CEA and VEGF) were collected 24 hours before the initiation of radiotherapy and again within five days of its completion. For genetic analysis, DNA sampling was performed before radiotherapy began or within the first week of treatment, prior to the onset of any infection-related complications. All blood samples were collected in EDTA-K₂ tubes (BD Vacutainer, USA) and stored at -80°C until further analysis.

DNA extraction and genotyping

Genomic DNA was extracted from peripheral leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen, Germany), following the manufacturer's instructions. The DNA quality was confirmed using absorbance readings at 260 and 280 nm, and only samples with an A260/A280 ratio between 1.7 and 1.9 were used for downstream analysis. Four single nucleotide polymorphisms (SNPs) in the C3AR1 gene -rs11567806, rs190043630, rs113825556, and rs147364229-were selected based on their known or predicted functional relevance, allele frequency in East Asian populations, and previous associations with immune or inflammatory conditions. Genotyping was performed using the TaqMan SNP Genotyping Assay (Applied Biosystems, USA) on the StepOnePlus Real-Time PCR System (Applied

Biosystems, USA). Each 10 μ L reaction included 2 \times TaqMan Genotyping Master Mix, 20 \times SNP assay mix, and genomic DNA at a concentration of 5 ng/ μ L. The PCR protocol included an initial denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Quality control was ensured by repeating 10% of samples, yielding 100% concordance.

Tumor marker quantification

Levels of carcinoembryonic antigen (CEA) and vascular endothelial growth factor (VEGF) were measured using commercially available Quantikine ELISA kits (R&D Systems, USA). Assays were performed in duplicate according to the manufacturer's protocol, and average values were used in the final analysis.

Demographic and clinical data

Detailed demographic and clinical data were collected for all patients, including age, gender, body mass index (BMI), white blood cell count, procalcitonin (PCT) levels, smoking history, and infection site. Clinical severity was assessed using the Acute Physiology and Chronic Health Evaluation II (APACHE II) score and the Sequential Organ Failure Assessment (SOFA) score at the time of ICU admission. The mean age of the sepsis group was 62.78 \pm 9.87 years, with a BMI of 23.12 \pm 2.87 kg/m². The group included 56 males and 44 females. Smoking history was positive in 60% of patients. Most infections were pulmonary or abdominal in origin. The APACHE II scores and PCT levels were significantly higher in the sepsis group than in the non-septic control group.

Ethical approval

This study was conducted in compliance with the Declaration of Helsinki and was approved by the Ethics Committee of Zhejiang Xin'an International Hospital. The ethical approval registration number is STYHUI, and the date of registration was May 25, 2021. Written informed consent was obtained from all participants prior to enrollment.

Instruments, kits, and software

All procedures and measurements were conducted using validated and commercially sourced equipment. Radiotherapy was administered using the Clinac iX linear accelerator (Varian Medical Systems, USA), with planning performed on the Eclipse Treatment Planning System version 13.7 (Varian, USA). DNA extraction was performed using the QIAamp DNA Blood Mini Kit (Qiagen, Germany), and genotyping used the TaqMan SNP Genotyping Assay and Master Mix (Applied Biosystems, USA) with the StepOnePlus Real-Time PCR System. Serum CEA and VEGF were quantified using Quantikine ELISA kits (R&D Systems, USA). Statistical analyses were

conducted using SPSS version 24.0 (IBM, USA), and power analysis was performed using Quanto version 1.2.4 (University of Southern California, USA).

Statistical analysis

All statistical analyses were carried out using SPSS version 24.0 (IBM, USA). Continuous variables were expressed as mean \pm standard deviation (SD) and compared using independent sample t-tests. Categorical variables were reported as frequencies and percentages and compared using chi-square tests. Genotype and allele distributions were analyzed for deviations from Hardy-Weinberg equilibrium using the chi-square test. Logistic regression analysis was used to identify independent prognostic factors for sepsis outcomes. A multivariate model was constructed using variables that were significant in univariate analysis ($P < 0.05$). Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. Post hoc power analysis was conducted using Quanto version 1.2.4 to determine the statistical power of the study for detecting genetic associations.

RESULTS

Sepsis onset and clinical severity following radiotherapy

Among 180 patients with colorectal cancer undergoing radiotherapy, 100 developed sepsis during or within 14 days of treatment completion (sepsis group), while 80 patients did not experience infection-related complications (non-sepsis group). Sepsis occurred, on average, 18.2 \pm 4.6 days after radiotherapy initiation. Of these 100 patients, 29 (29%) developed septic shock, and 42% required ICU admission, reflecting substantial post-radiotherapy immune compromise. No cases of sepsis were observed prior to radiotherapy, confirming its critical role in the pathogenesis of infection in this population.

The sepsis group exhibited a significant post-treatment increase in systemic severity scores. The APACHE II score increased from 10.23 \pm 3.11 (baseline) to 14.65 \pm 4.23 post-sepsis ($P < 0.001$), and procalcitonin (PCT) levels rose markedly from 8.92 \pm 2.73 ng/mL to 45.87 \pm 9.76 ng/mL ($P < 0.001$). These results underscore radiotherapy's immunosuppressive and barrier-disruptive effects in facilitating systemic infection.

Tumor biomarker changes after radiotherapy

In a subgroup of 60 patients, we examined serum levels of carcinoembryonic antigen (CEA) and vascular endothelial growth factor (VEGF) before and after radiotherapy. These patients received either combination therapy with anlotinib and docetaxel (ACD group) or docetaxel alone (DOC group), both

with radiotherapy.

As shown in table 1, both groups experienced significant reductions in tumor biomarkers post-treatment, but the effect was more pronounced in the ACD + RT group. In the ACD group, CEA levels declined from 22.3±7.5 to 12.4±6.1 ng/mL (P<0.001), and VEGF levels dropped from 123.4±42.5 to 54.2±28.1 pg/mL (P<0.001). In the DOC + RT group, CEA fell from 23.1±8.2 to 15.7±7.4 ng/mL (P=0.015), while VEGF decreased from 119.7±40.3 to 68.3±33.7 pg/mL (P 0.021).

Table 1. Tumor biomarker changes after radiotherapy.

Group	CEA (Pre-RT)	CEA (Post-RT)	P-value (CEA)	VEGF (Pre-RT)	VEGF (Post-RT)	P-value (VEGF)
ACD + Radiotherapy	22.3±7.5	12.4±6.1	<0.001	123.4±42.5	54.2±28.1	<0.001
DOC + Radiotherapy	23.1±8.2	15.7±7.4	0.015	119.7±40.3	68.3±33.7	0.021

CEA: carcinoembryonic antigen; VEGF: vascular endothelial growth factor; RT: radiotherapy.

These findings demonstrate that radiotherapy, particularly when combined with antiangiogenic therapy, contributes to a measurable suppression of tumor activity through reductions in both angiogenesis and tumor load markers.

Post-radiotherapy distribution of C3AR1 gene polymorphisms

To assess potential genetic predisposition to post-radiotherapy sepsis and its outcomes, genotyping of C3AR1 polymorphisms rs11567806 and rs190043630 was performed in all patients after completion of radiotherapy. Both SNPs conformed to Hardy-Weinberg equilibrium in the sepsis and non-sepsis groups (P>0.05), indicating stable distribution unaffected by treatment.

The observed genotype frequencies are summarized in table 2. For rs11567806, no significant difference in distribution was found between groups (AA: 28% vs 37.5%; P=0.213). For rs190043630, the CC genotype was more frequent in the sepsis group (42%) than in the non-sepsis group (31.25%), though the overall association was not statistically significant (P=0.154). However, further prognostic analysis revealed its relevance (figure 1).

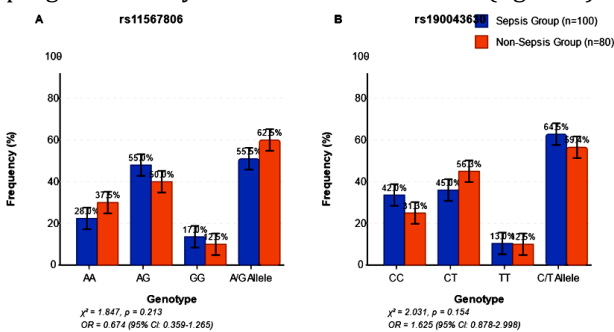


Figure 1. Distribution of C3AR1 genotypes in sepsis and non-sepsis groups.

Table 2. Hardy-Weinberg equilibrium and post-radiotherapy polymorphism distribution.

SNP	Genotype	Sepsis Group (n=100)	Non-Sepsis Group (n=80)	χ ²	P-value
rs11567806	AA	28	30	1.847	0.213
	AG	54	40	0.456	0.500
	GG	18	10	0.789	0.375
rs190043630	CC	42	25	2.031	0.154
	CT	45	45	1.357	0.244
	TT	13	10	0.012	0.913

SNP: single nucleotide polymorphism; χ²: Chi-square.

Prognostic impact of C3AR1 polymorphisms in post-radiotherapy sepsis

Of the 100 patients who developed sepsis, 75 survived and 25 died during hospitalization. Stratified genotype analysis revealed that the CC genotype of rs190043630 was present in 56% of deceased patients, compared to 29.3% of survivors (χ²=6.24, P=0.044). Kaplan-Meier survival analysis confirmed poorer outcomes in patients with the CC genotype (figure 2). In contrast, rs11567806 genotypes showed no significant association with outcome (P=0.540).

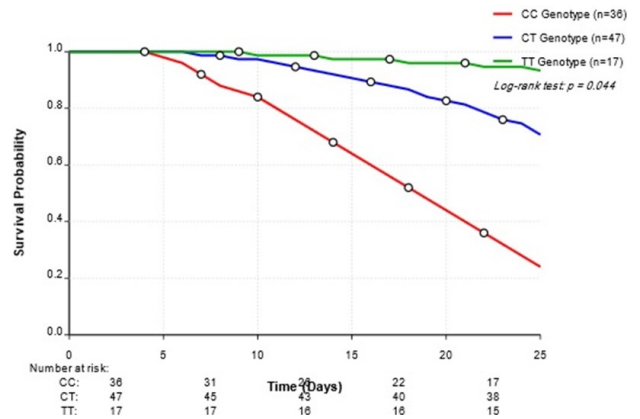


Figure 2. Kaplan-Meier survival curves stratified by rs190043630 genotype.

Independent predictors of mortality after radiotherapy

Multivariate logistic regression was performed using variables significant in univariate analysis (age, septic shock, APACHE II, disease severity, and rs190043630). As shown in table 3, three factors remained independently associated with mortality:

- Septic shock (OR=1.615; 95% CI: 1.132-2.301; P=0.008).
- APACHE II score ≥16 (OR=1.500; 95% CI: 0.998-2.235; P=0.044).
- rs190043630 (CC genotype) (OR=1.568; 95% CI: 1.042-2.361; P=0.033).

These results suggest that the rs190043630 polymorphism not only correlates with susceptibility but also independently predicts adverse outcomes in septic patients following radiotherapy.

Metabolomic alterations following radiotherapy

Targeted metabolomic analysis was performed in 30 sepsis patients before and after radiotherapy. Post-treatment samples revealed significant increases in

glutathione disulfide (GSSG) and carnitine derivatives, while decreases were observed in lysophosphatidylcholines and tryptophan. Notably, GSSG levels increased from 1.14 ± 0.33 to 2.89 ± 0.41 $\mu\text{mol/L}$ ($P < 0.001$), correlating strongly with APACHE II scores ($r = 0.62$, $P < 0.01$). These changes suggest enhanced oxidative stress and disrupted amino acid metabolism in post-radiotherapy sepsis.

Table 3. Multivariate prognostic analysis in sepsis patients after radiotherapy.

Factor	β	SE	Wald	OR	95% CI	P-value
Septic Shock	0.480	0.185	7.012	1.615	1.132-2.301	0.008
APACHE II ≥ 16	0.405	0.202	4.045	1.500	0.998-2.235	0.044
rs190043630 (CC)	0.450	0.210	4.548	1.568	1.042-2.361	0.033

SE: standard error; OR: odds ratio; CI: confidence interval.

DISCUSSION

This study examined the link between C3AR1 gene polymorphisms and both the susceptibility to and prognosis of emergency sepsis, particularly in colorectal cancer patients undergoing radiotherapy. Our findings contribute to the understanding of the complex genetic mechanisms underlying sepsis in this specific population. Contrary to our initial hypothesis, our study did not identify any specific C3AR1 gene polymorphisms that were significantly associated with an increased susceptibility to emergency sepsis. This suggests that genetic variations in C3AR1 may not significantly influence an individual's risk of developing sepsis, at least in the context of colorectal cancer patients undergoing radiotherapy within our Han Chinese population. This finding diverges from previous research that has suggested a strong genetic component to sepsis susceptibility, with studies identifying associations between other genes such as HMGB1, TNF- α , and IL17A.

However, our results did show a significant association between C3AR1 gene polymorphisms and the prognosis of sepsis in this population. Specifically, the CC genotype of rs190043630 was associated with a heightened risk of adverse outcomes, including increased mortality and more severe organ dysfunction, in colorectal cancer patients undergoing radiotherapy who developed sepsis. This suggests that C3AR1 gene polymorphisms may serve as potential prognostic markers for sepsis in cancer patients receiving radiotherapy. The potential mechanism could involve the role of C3AR1 in the pathophysiology of sepsis. In sepsis, an uncontrolled inflammatory response often leads to organ damage⁽¹⁵⁾. We hypothesize that the CC genotype of rs190043630 might cause aberrant C3AR1 function, exacerbating the inflammatory response, which in turn results in more severe tissue injury and organ failure. Specifically, it may affect pro-inflammatory cytokine production and release, thereby disrupting the immune response and

worsening prognosis. This hypothesis aligns with previous studies that have identified genetic polymorphisms as predictors of sepsis prognosis, such as those found in the VDR, TREM-1, C5, and THBD genes⁽¹⁶⁻¹⁸⁾.

Our study builds on earlier work exploring genetic factors in sepsis, similar to studies on genes such as HMGB1, TNF- α , IL17A, VDR, TREM-1, C5, and THBD. These studies have recognized the importance of genetic polymorphisms in shaping immune response and influencing disease outcomes in sepsis. For instance, research by Qiu et al. found an association between HMGB1 gene polymorphisms and both sepsis susceptibility and clinical outcomes in Chinese Han patients⁽¹⁹⁾. Similarly, Georgescu et al. examined the role of TNF- α genetic variability as a biomarker for sepsis risk, severity, and outcomes⁽²⁰⁾. Our study on C3AR1 gene polymorphisms follows this trend, highlighting the significance of genetic factors in sepsis prognosis.

From a methodological perspective, our study utilized a case-control design, akin to many other studies in the field. This approach enabled genotype comparisons between septic patients and healthy controls, facilitating the identification of associations between specific gene polymorphisms and sepsis outcomes. We employed the TaqMan SNP Genotyping Assay to analyze the C3AR1 gene polymorphisms, a widely used technique in genetic association studies. This methodological consistency allows for comparability with results from other studies, such as Nakada *et al.*'s work on IL17A gene SNPs, which used a similar genotyping approach⁽²¹⁾.

Despite these similarities, there are notable differences in the findings between studies. While many previous studies focused on genes with diverse immune-related functions, such as HMGB1, TNF- α , and IL17A⁽²²⁻²⁴⁾, our study centered on C3AR1, a gene involved in the complement system. The complement system plays a key role in the innate immune response, and C3AR1's role in modulating immune cell activation and inflammation regulation sets it apart from cytokine-mediated pathways like those associated with HMGB1 and TNF- α ^(25, 26). These differences suggest that multiple genetic pathways may contribute to sepsis susceptibility and prognosis, and a comprehensive understanding of sepsis requires exploring each of these pathways.

Another distinction in our study is the focus on colorectal cancer patients undergoing radiotherapy. While other studies have been conducted on various ethnic groups, including Chinese Han and European populations⁽²⁷⁾, our study specifically targets colorectal cancer patients who are undergoing radiotherapy, a population known to have an increased risk of infections and sepsis due to the immunosuppressive effects of radiotherapy. This adds a new layer of complexity to the genetic factors influencing sepsis in this vulnerable group. The

frequency of C3AR1 gene polymorphisms and their associations with sepsis might differ across populations due to varying genetic backgrounds, and our study contributes to the growing understanding of genetic factors in colorectal cancer patients undergoing radiotherapy⁽²⁸⁾.

The findings from our study, along with previous research, have important implications for understanding the pathogenesis of sepsis in cancer patients. The association between the rs190043630 polymorphism in the C3AR1 gene and sepsis prognosis highlights the role of the complement system, specifically the C3a-C3AR1 axis, in sepsis progression⁽²⁹⁾. The CC genotype of rs190043630 might disrupt this pathway, leading to an imbalance in the immune response and worsening disease severity. These insights could inform the development of targeted therapies aimed at modulating complement activation, such as drugs that regulate C3AR1 activity or the production of C3a, which could improve outcomes for sepsis patients with the high-risk CC genotype.

Our study also emphasizes the importance of personalized medicine in the management of sepsis. By identifying patients with high-risk C3AR1 genotypes, clinicians could implement more tailored treatment strategies, including aggressive preventive measures for those at greater risk of developing severe sepsis⁽³⁰⁾. Such personalized approaches have the potential to reduce sepsis-related mortality and morbidity.

Compared to previous studies investigating the genetic underpinnings of sepsis, our findings offer a novel perspective by focusing on the complement pathway-particularly the C3AR1 gene-in a distinct subgroup of colorectal cancer patients undergoing radiotherapy. Earlier genetic studies have concentrated on polymorphisms in pro-inflammatory cytokine genes such as *TNF- α* , *IL6*, *IL10*, and *TLR4*, which are well-documented to influence both susceptibility to and outcomes in sepsis^(20, 31, 32). These studies typically found strong associations between specific variants and heightened sepsis risk or severity. In contrast, our study did not observe any significant association between C3AR1 polymorphisms and sepsis susceptibility, suggesting that the role of the complement pathway in sepsis onset may be secondary to that of cytokine-mediated inflammatory cascades. However, similar to studies on *VDR*, *TREM-1*, and *THBD*, which identified specific genotypes associated with worse clinical outcomes⁽¹⁸⁾, we found that the CC genotype of rs190043630 in *C3AR1* was significantly linked to poor sepsis prognosis. This points to a potentially prognostic, rather than predictive, role for C3AR1 variants, underscoring the importance of dissecting immune regulatory genes based on their influence at different stages of the disease process.

Based on our findings, we propose a model in

which the C3AR1 rs190043630 polymorphism influences sepsis outcomes by altering inflammatory responses. The CC genotype may lead to heightened C3AR1 signaling upon activation, resulting in excessive neutrophil recruitment and inflammatory cytokine production⁽³³⁾. This exaggerated immune response could contribute to increased tissue damage and organ dysfunction in sepsis patients, especially those with colorectal cancer undergoing radiotherapy⁽³⁴⁾. Further functional studies are needed to validate this hypothesis and clarify the precise molecular mechanisms involved.

One of the major limitations of our study is the relatively small sample size. A larger sample size is necessary to more accurately detect associations between gene polymorphisms and sepsis, especially for less common polymorphisms. A small sample increases the risk of type II errors, where true associations might be overlooked. Our power analysis indicated moderate power (62-65%) to detect large effect sizes, but the study may have had limited ability to detect smaller effects that could still be clinically significant. Future research should involve larger and more diverse cohorts to increase statistical power and enhance the generalizability of the findings.

Additionally, while this is a genetic association study, it lacks functional experiments to confirm how C3AR1 gene polymorphisms affect sepsis mechanisms. While we identified associations, we do not fully understand the molecular and cellular impacts of these polymorphisms on C3AR1 function. Future research should incorporate in vitro and in vivo studies to investigate how the rs190043630 polymorphism influences C3a binding to C3AR1, downstream signaling pathways, and immune cell functions. Cell-based assays examining the impact of different genotypes on C3AR1 expression, C3a binding affinity, and cytokine production would be valuable in elucidating these mechanisms.

The study was conducted in a specific Han Chinese population from Zhejiang Province, limiting the broader applicability of the findings to other ethnic groups or regions. Genetic associations may vary across populations due to differences in genetic backgrounds, and future studies should include more diverse populations to establish the broader relevance of C3AR1 polymorphisms in sepsis. Furthermore, we focused on only four SNPs in the C3AR1 gene, which does not capture the full genetic variation across the gene. A more comprehensive approach using sequencing or genome-wide association studies (GWAS) could identify additional relevant variants or haplotypes. The integration of epigenetic data, gene expression profiles, and protein function studies would provide a more complete understanding of how C3AR1 variants contribute to sepsis pathophysiology.

Despite these limitations, our study represents a

significant step in understanding the role of C3AR1 gene polymorphisms in sepsis, particularly in colorectal cancer patients undergoing radiotherapy. These findings lay the foundation for larger, more comprehensive studies that could ultimately improve risk stratification and lead to personalized treatment approaches for sepsis patients.

CONCLUSION

In colorectal cancer patients undergoing radiotherapy, the C3AR1 rs190043630 CC genotype was associated with poorer sepsis prognosis. Alongside clinical factors such as septic shock and elevated APACHE II scores, this polymorphism may help identify high-risk individuals for tailored post-radiotherapy care.

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Ethics approval: This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. The research protocol was approved by the Ethics Committee of Zhejiang Xin'an International Hospital (Approval Number: STYHUI, Date: May 25, 2021). All participants provided written informed consent before enrollment.

Informed consent: Written informed consent was obtained from all participants prior to inclusion in the study. All patients were fully informed about the study's objectives, methods, and potential risks. Their confidentiality and anonymity were strictly maintained.

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Conflicts of interest: The authors declare no financial or non-financial competing interests related to this study.

Author contributions: H.Z.: Study design, patient enrollment, manuscript writing. M.J.: Data collection, clinical monitoring. Z.L.: Genotyping, bioinformatics support. L.M.: Laboratory assays and ELISA measurements. J.M.: Statistical analysis, data curation. W.W.: Radiotherapy protocol planning, technical oversight. Y.S. (Corresponding author): Conceptualization, project supervision, manuscript revision. All authors reviewed and approved the final version of the manuscript.

Use of artificial intelligence: Artificial intelligence tools were used to assist with grammar checking,

formatting, and language refinement. All scientific content, data interpretation, and critical decisions were made exclusively by the authors.

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