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ORIGINAL ARTICLE

Casual Association between Circulating White Blood Cell Traits and Colorectal Cancer: A Mendelian Randomization Study

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Abstract

Background: The relationship between circulating white blood cell (WBC) traits and colorectal cancer (CRC) risk remains unclear. This study employs Mendelian randomization (MR) to investigate whether genetic variants associated with WBC traits are causally linked to the development of CRC.

Methods: We utilized summary statistics from large-scale genome-wide association studies (GWAS) to identify single nucleotide polymorphisms (SNPs) associated with WBC traits, including total WBC count, neutrophils, lymphocytes, monocytes, and eosinophils. We then employed two-sample MR to assess the causal relationships between these WBC traits and CRC risk using publicly available GWAS data for CRC.

Results: Our analysis identified several SNPs significantly associated with WBC traits and CRC risk. The findings suggest a significant inverse causal relationship between colorectal cancer (CRC) and white blood cell count (Odds Ratio = 0.888, 95% Confidence Interval = 0.800 − 0.980, p = 0.026), as well as eosinophil count (Odds Ratio = 0.831, 95% Confidence Interval = 0.710 − 0.973, p = 0.022). However, no significant associations were seen between monocyte cell count, lymphocyte cell count, or neutrophil cell count and CRC.

Conclusion: This MR study suggests that higher circulating total white blood cell count and eosinophil count may be causally linked to decrease risk of colorectal cancer, highlighting the importance of immune regulation in cancer pathogenesis. Further investigations are warranted to elucidate the underlying mechanisms and assess the potential for WBC traits as biomarkers for CRC risk.

Keyword: Colorectal cancer, white blood cell, Mendelian randomization, GWAS

Introduction

Colorectal cancer (CRC) poses a significant global health issue, accounting for more than 10% of all cancer cases around the world and being the second leading cause of cancer-related deaths internationally [1–2]. The occurrence of this disease is on the rise, with epidemiological data indicating a persistent growth in CRC diagnoses [3], compounded by an alarming surge in cases

among younger populations (individuals under 50 years of age) [4–5]. Given these trends and the projection that nearly 50% of CRC cases may be preventable through modifiable risk factors or early interventions [6], there is an immediate necessity to emphasize investigation into new risk factors, preventive measures, and creative treatment methods to reduce the increasing healthcare challenge posed by this illness.

White blood cells (WBCs), commonly evaluated in routine blood examinations, consist of five unique subtypes: basophils, eosinophils, lymphocytes, monocytes, and neutrophils [7]. Alterations in the levels of circulating WBCs have been associated with disease vulnerability, severity, progression, and outcomes, including the development and mortality of colorectal cancer (CRC) [8–10]. Notably, increased levels of circulating basophils and eosinophils—cells involved in IgE-mediated immunological responses—have been linked to a lower risk of CRC and enhanced survival rates [11–12]. Similarly, higher absolute counts of lymphocytes (which include T cells, B cells, and natural killer [NK] cells) are connected to improved overall survival among CRC patients [13-14]. In contrast, elevated monocyte levels are correlated with worse survival outcomes in CRC, consistent with their proposed role in facilitating tumor progression and metastasis [13-14]. Lastly, neutrophils, essential components of innate immunity [15], are also associated with diminished overall survival in CRC, highlighting their intricate role in cancer biology [13-14].

Conventional observational studies investigating the connection between characteristics of circulating blood cells and colorectal cancer (CRC) frequently face challenges from confounding factors and reverse causality, where the presence of cancer may influence blood cell profiles. To overcome these issues, Mendelian randomization (MR) provides a strong methodological approach for determining causal links. By employing genetic variants as instrumental variables, MR enables researchers to evaluate the effect of exposures (in this context, blood cell characteristics) on outcomes (the risk of CRC) while reducing biases typical of observational studies [16].

This research seeks to investigate the causal link between traits of circulating blood cells and the risk of colorectal cancer through a Mendelian randomization framework. By utilizing genetic information, we aim to shed light on the role of inflammatory processes in the development of colorectal cancer and offer insights into the potential mechanisms behind this connection. Gaining a better understanding of these associations could help shape future prevention and treatment strategies, emphasizing the significance of managing inflammation in the context of colorectal cancer risk.

2. Materials and Methods

2.1 Study Design

The relationship between PCBCs and colorectal cancer was evaluated using two-sample Mendelian Randomization (MR) analyses. MR utilizes genetic variations as proxies for risk factors. To ensure accurate interpretation of cause-and-effect relationships, three essential assumptions regarding instrumental variables (IVs) in MR must be fulfilled: (1) The genetic variation must be directly linked to the exposure; (2) The genetic variation should not be associated with any potential confounding factors between the exposure and the outcome; (3) The genetic variation must influence outcomes solely through the exposure, without any influence from other pathway**s Figure 1**.

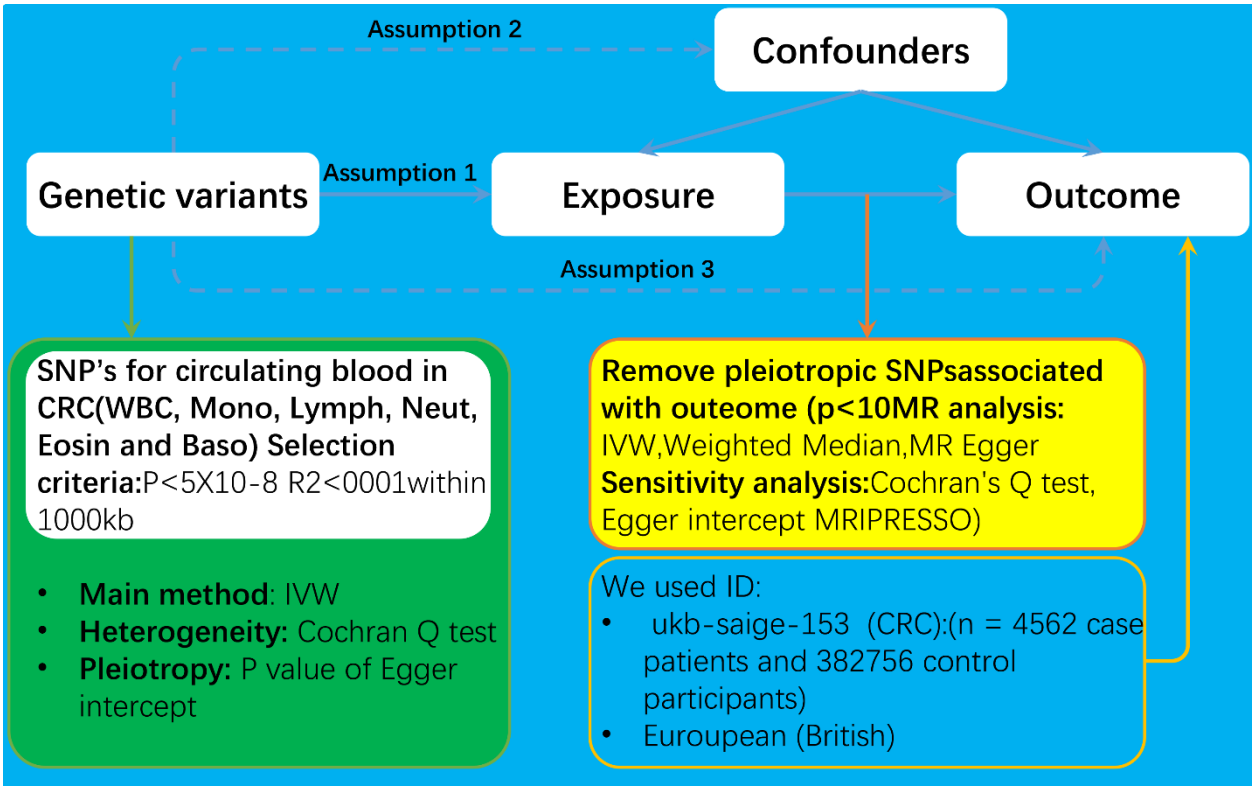

Figure 1 Study Design Flowchart.

2.2 Data Sources for Exposure and Outcome

A comprehensive summary of GWAS statistics for each trait associated with PCBCs is available in the GWAS catalog (accession numbers: GCST0001391 to GCST0002121). We utilized the specific keywords related to each cancer to locate the ID from (https://gwas.mrcieu.ac.uk/). The analysis relied on pooled statistics from the latest extensive blood cell Characterization Genome Study (BCX), organized by the blood cell consortium (BCC), and included participants of European descent. From this GWAS, we identified genetic variants linked to the levels of circulating Leukocyte, Lymphocyte, Monocyte, Neutrophil, Eosinophil, and Basophil counts. Referring to the ID of colorectal cancer, 387318 European individuals (n = 4562 case patients and 382756 control participants) for CRC-related data were downloaded from the UK Biobank pheweb database (https://pheweb.org/UKB-SAIGE/).

2.3 Instrument Selection

Given the extensive array of single-nucleotide polymorphisms (SNPs) that demonstrate genome-wide significance ($p < 5 \times 10^{-8}$) for traits related to PCBCs, we adopted more rigorous correlation thresholds ($p < 5 \times 10^{-9}$) for the selection of genetic instrumental variables (IVs). These IVs were categorized using the reference panel of Linkage Disequilibrium (LD) derived from the 1000 Genomes Project, applying an R^2 threshold of < 0.001 over a distance of 1,000 kilobases (kb). Considering the relatively small GWAS dataset for peripheral circulating blood cells, we utilized a p-value cutoff of 5×10^{-8} alongside a less stringent clustering threshold ($R^2 < 0.001$ at a distance of 1000 kb). To ensure the dependability of our tools, we focused on IVs with F-statistics greater than 10, designating them as strong instruments for further analyses. We subsequently extracted these IVs from the summary statistics relating to CRC outcomes, omitting any that displayed potential pleiotropy ($p < 10^{-5}$) regarding CRC, in accordance with methods from earlier studies. To maintain uniformity in our analysis, we aligned the SNPs between the exposure and outcome datasets to guarantee consistent effect estimates for the same effect allele. Our analysis excluded any alleles with intermediate effect frequencies (EAFs > 0.42) or SNPs that were inconsistent with the allele.

2.4 Statistical Analysis

In our study, we employed a range of Genetic Variants as Instrumental Variables instead of depending solely on an allele score. This strategy was selected to thoroughly investigate critical

assumptions, identify possible pleiotropy, and enable more robust sensitivity and multivariable MR analyses. To evaluate the consistency of our results under varying assumptions regarding heterogeneity and pleiotropy, we implemented four different MR techniques: Inverse Variance Weighted (IVW), Weighted Median, MR-Egger, and MR-PRESSO. The IVW method, utilizing a random-effects model, acted as the primary analytical framework for all four sets of instrumental variables. We applied Cochran's Q statistics and relevant p-values to assess heterogeneity among the chosen IVs.

Our research also involved analyses under more rigorous conditions. The Inverse Variance Weighted (IVW) method operates on the assumption that all genetic variants are effective; however, if numerous SNPs are affected by horizontal pleiotropy, this may introduce bias. On the other hand, the weighted median method is effective when fewer than 50% of the variants are subject to horizontal pleiotropy, as it assumes that the majority of genetic variants are valid [17]. In scenarios where more than 50% of the variants are impacted by horizontal pleiotropy, we assessed the robustness of our genetic instruments using F statistics, with a mean F value below 10 indicating weak Instrumental Variables.

Moreover, to exclude the influence of horizontal multidimensionality, we employed a commonly used method (MR-Egger), which indicates the presence of horizontal multidimensionality if the intercept term is statistically significant. We also applied the robust MR Pleiotropy Residual Sum and Outlier (MR-PRESSO) technique to identify and remove potential horizontal pleiotropic outliers that might have impacted the results from the MR-PRESSO package. Additionally, we performed Steiger-filtering analyses to identify and discard genetic variants that were more strongly linked to the outcome than to the exposure, highlighting potential reverse causation.

All statistical computations were carried out using R software version 4.3.2 (R Foundation) along with specific R packages ("TwoSampleMR" and "Mendelian Randomization") designed for MR analysis.

3. Results

Figure 2 summarizes the causal effects of circulating blood counts on the risk of colorectal cancer (CRC). Our analysis revealed a significant negative association between CRC and white blood cell count (Odds Ratio = 0.888, 95% Confidence Interval = 0.800 − 0.980, p = 0.026). Additionally, results from the inverse variance weighted (IVW) method indicated that eosinophils act as a protective factor against CRC (Odds Ratio = 0.831, 95% Confidence Interval = 0.710 − 0.973, p = 0.022) (refer to **Figure 2**).

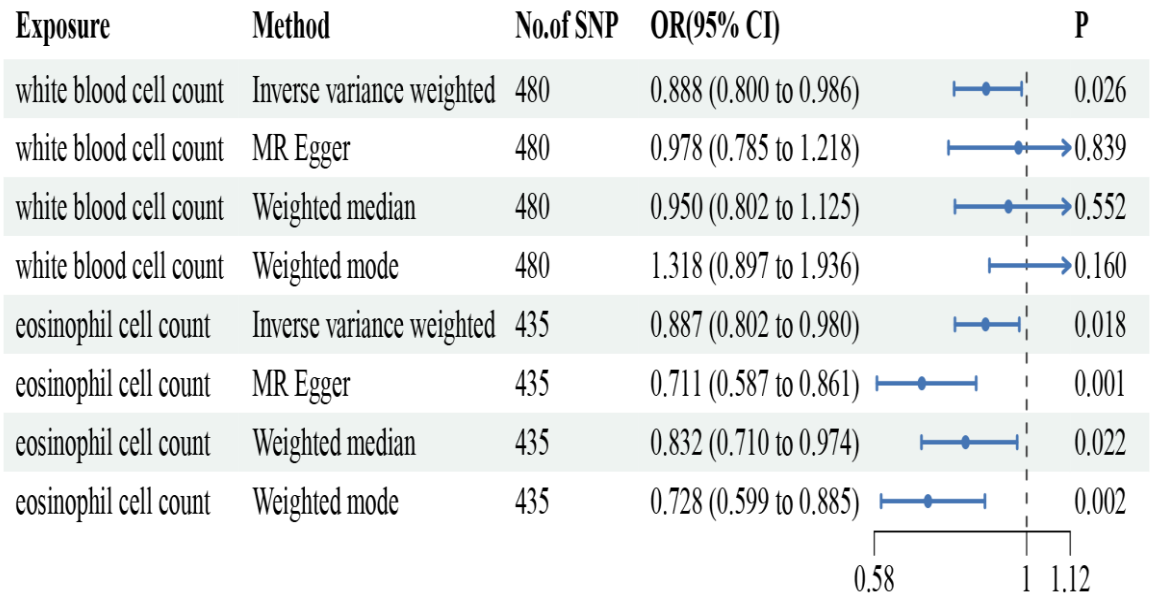

Figure 2. The causal association between white blood cell traits and colorectal cancer. We selected Inverse variance weighted (IVW) as a primary method p<0.05 showed statistically significant; OR value >1 indicated a risk factor; OR value <1 indicated a protective factor.

However, no notable relationships were found between monocyte, lymphocyte, and neutrophil counts and the susceptibility to CRC. Furthermore, we utilized scatter plots (**Figure 3A-B**) and funnel plots (**Figure 3C-D**) to assess the data, which helped minimize the chances of potential outliers and horizontal pleiotropy influencing the identified relationships with white blood cells.

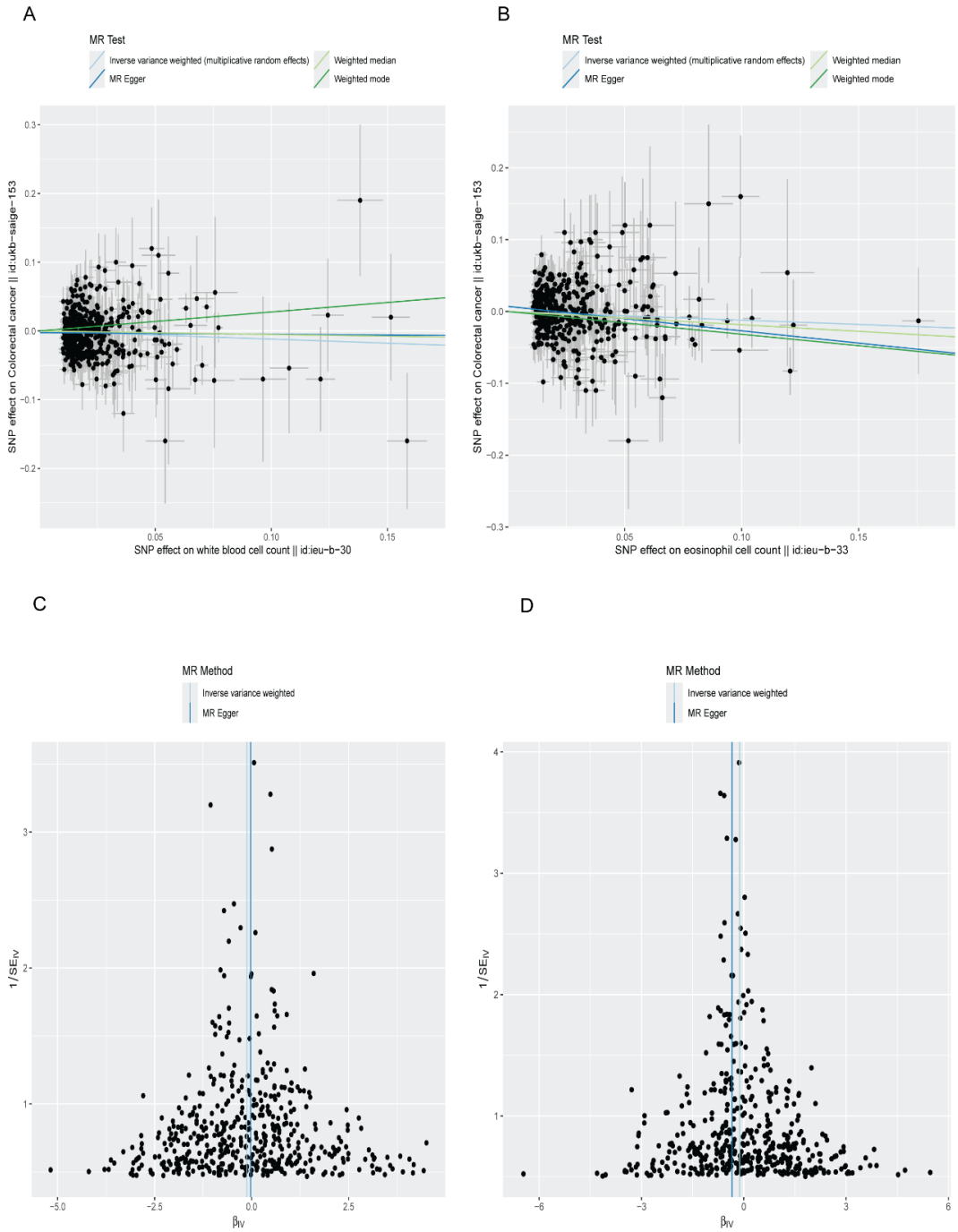

Figure 3. Scatter plot showing the relationship of white blood cell traits with the risk of colorectal cancer (CRC); (A) white blood cell; (B) Eosinophil. Funnel plot showing instrumental variables for each significant causal association between white blood cell traits and CRC. (C) white blood cell in CRC; (D) Eosinophil in CRC.

4. Discussion

White blood cells, also known as leukocytes, make up a varied group of cells capable of carrying different antigens on their surfaces. Antigens are specific molecules or structures recognized by the immune system as either foreign or self. In the context of the immune

system, leukocytes present particular antigens on their surfaces, which are crucial for the identification and reaction of the immune response. The human leukocyte antigen (HLA) class II is produced by the HLA-DR, DQ, and DP genes, and is found within the HLA-D region [18]. Its roles encompass the uptake, processing, and display of antigens to helper T cells [19-20]. The tissue distribution of HLA class II molecules is more restricted compared to HLA class I molecules, as they are primarily present on cells engaged in the immune response, including B lymphocytes, activated T lymphocytes, monocytes/macrophages, and dendritic cells that present antigens [21].

Numerous studies have established a link between white blood cells and cancer. For instance, research by Wei et al. indicated that the counts of leukocytes, neutrophils, and monocytes were elevated in patients with breast cancer when compared to healthy controls [22]. Additionally, Okutural et al. discovered that higher neutrophil levels were associated with an increased risk of breast cancer [23]. Research has indicated that elevated preoperative white blood cell counts correlate with poorer outcomes and are linked to the immunosuppressive environments present in colorectal cancer (CRC) [24]. These results differ from our current findings, suggesting that further validation through larger clinical studies is necessary. Eosinophils play a crucial role in eliminating pathogens and parasites and are essential for immune responses and hypersensitivity reactions. They also play a role in tissue damage and the onset of inflammation by discharging granular substances. Additionally, they are significantly linked to the emergence and progression of cancer. Eosinophils react to different environmental signals and influence tumor development by infiltrating the tumors, directly engaging with tumor cells, and/or indirectly modifying the tumor's immune microenvironment [25]. Research shows that patients with a reduced baseline neutrophil to eosinophil ratio tend to have improved overall survival in melanoma [26]. In a substantial cohort investigation focused on nasopharyngeal carcinoma, individuals displaying reduced eosinophil levels post-radical treatment experienced worse progression-free survival (PFS) and overall survival (OS). Conversely, patients who had elevated eosinophil counts prior to treatment benefited less from radical synchronous radiotherapy and chemotherapy [27]. Constantinescu et al. noted that higher numbers of circulating eosinophils and lymphocytes were associated with a lower risk of colorectal cancer (CRC) [28], aligning with our research results. These studies reinforce our assertions that fluctuations in eosinophil counts could be useful in predicting CRC outcomes. Aligned with our results, Prizment et al. found that higher eosinophil levels (tertiles Q3 and Q2 compared to Q1) showed a negative correlation with the risk of colon cancer, although no significant link was noted for rectal cancer. Similar trends have been noted in studies of other types of cancer. For instance, Wong et al. reported an inverse relationship between escalating eosinophil counts in quartiles and the risk of lung adenocarcinoma in a UK Biobank (UKBB) analysis [29]. Likewise, a study on prostate cancer indicated a reduced risk associated with higher eosinophil levels in quintiles (Q3–5) and for each 1-standard deviation (SD) rise in eosinophil counts (hazard ratio [HR] 0.96), echoing the protective effect seen in our study for colorectal cancer (odds ratio [OR] 0.93) [30].

Eosinophils are known to be significant in allergic conditions, including asthma and allergic rhinitis [31]. Analyses using Mendelian randomization (MR) have reinforced this connection by showing that levels of eosinophil counts affect the likelihood of developing allergies [32-33]. Furthermore, a recent systematic review investigating the association between allergies and cancer found indications that individuals with allergic diseases may have a lower risk of colorectal cancer (CRC) [34]. Our research indicates that immune responses mediated by eosinophils may provide a protective effect against the development of tumors. In various forms of neoplasia, including colorectal cancer (CRC), eosinophils have demonstrated anti-tumor effects, in part through the release of molecules that inhibit tumor growth, such as eosinophil-derived neurotoxin (EDN) [35-36]. Experimental investigations have also shown that immunoglobulin E (IgE), closely associated with eosinophil functions, possesses properties that protect against tumors [37]. Moreover, a higher influx of eosinophils to sites of CRC tumors has been linked to better survival rates, even when considering the influence of CD8+ T-cells [37].

Additionally, the release of granzyme A from eosinophil-specific granules has been associated with the elimination of CRC cells [38].

5. Limitation

In the context of this MR analysis, the genetic tools employed in this research represent long-term variations in white blood cell (WBC) counts. Therefore, this MR analysis is not suitable for drawing conclusions about how significant short-term changes may affect the onset of colorectal cancer (CRC). Furthermore, caution should be exercised when using the multivariable Mendelian randomization (MVMR) approach to examine traits connected to weak instruments, as this method might not effectively adjust for those traits. As a result, while the weak-MVMR analysis indicates a potentially greater harmful effect than the primary MVMR analysis, the odds ratios (ORs) obtained from this analysis should be interpreted carefully, given the challenges related to weak instruments.

6. Conclusion

In conclusion, the results from this research indicate that higher levels of circulating eosinophils and white blood cell counts may have a protective impact on the likelihood of developing nasopharyngeal carcinoma. Further investigations are necessary to clarify the underlying biological mechanisms and to pinpoint the specific pathways through which eosinophils and white blood cells might provide protection against the onset of CRC.

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Availability of Data and Materials

All the data for the present article can be found on the GWAS (http://www.ebi.ac.uk/gwas/) and yunshang Gwas (https://gwas.medicineitlab.com/).

Authors' Contributions

Pengkhun Nov, and Arzoo Prasai collected, analyzed, and interpreted the data. Ying Li wrote the manuscript. Other authors, and JL designed, revised, and supervised the study. All authors had reviewed and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

Patient Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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