Osteomyelitis and immune cell phenotypes: a study based on a Mendelian randomisation approach

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Article

Keywords: Osteomyelitis, Immune cell phenotype, Mendelian randomization, Genome-wide association study, Causal relationship

Posted Date: December 26th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3763384/v1

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Additional Declarations: No competing interests reported.
Abstract

**Background:** Osteomyelitis is a severe bone marrow infection, whose pathogenesis is not fully understood. This study aims to explore the causal relationship between immune cell characteristics and osteomyelitis, in hopes of providing new insights for the prevention and treatment of osteomyelitis.

**Methods:** Based on two independent samples, this study employed a two-sample Mendelian randomization (MR) analysis to assess the causal relationship between 731 immune cell characteristics (divided into 7 groups) and osteomyelitis. Genetic variants were used as proxies for risk factors to ensure the selected instrumental variables meet the three key assumptions of MR analysis. GWAS data for immune characteristics came from the public GWAS catalog, while data for osteomyelitis was sourced from the Finnish database.

**Results:** At a significance level of 0.05, 21 immune phenotypes were identified as having a causal relationship with the development of osteomyelitis. In the B cell group, phenotypes such as Memory B cell %B cell, CD20- %B cell, and Memory B cell %lymphocyte showed a positive causal relationship with osteomyelitis, while Naive-mature B cell %B cell and IgD- CD38- AC phenotypes showed a negative causal relationship. In addition, specific immune phenotypes in the cDC cell group, Myeloid cell group, TBNK cell group, T cell maturation stage, and Treg cell group also showed significant associations with osteomyelitis. Through reverse MR analysis, it was found that osteomyelitis had no significant causal impact on these immune phenotypes, suggesting that the occurrence of osteomyelitis might not in turn affect these immune cell phenotypes.

**Conclusion:** This study reveals for the first time the causal relationship between specific immune cell characteristics and the development of osteomyelitis, providing a new perspective for understanding the immune mechanism of osteomyelitis. These findings are significant for formulating targeted prevention and treatment strategies, and hold promise for improving the clinical treatment outcomes of patients’ osteomyelitis.

Introduction

Osteomyelitis, an infectious disease of bone and bone marrow, has epidemiological characteristics that vary significantly across the globe. Although its incidence is relatively low in developed countries, the incidence of this disease and associated mortality remain high in resource-limited areas (1). Treatment of osteomyelitis usually requires long-term use of antibiotics and possibly surgical intervention, and the issue of delayed diagnosis and treatment resistance is becoming a major clinical challenge (2). In addition, the high rate of recurrence of osteomyelitis, the high cost of treatment, and the impact on patients’ quality of life make this disease a serious public health problem (3, 4).

During the pathophysiological process of osteomyelitis, invasion of the pathogen triggers an immune response in the host involving a variety of immune cells and cytokines. Studies have shown that the host immune response not only plays a role in infection control, but may also lead to increased tissue damage
and inflammation (5). Understanding the details of this process is critical to developing more effective therapeutic strategies. For example, immunomodulatory strategies that control the inflammatory response and promote tissue repair may be important for improving the prognosis of patients with osteomyelitis (6).

In recent years, immune cell characteristics, such as cell counts, surface marker expression and cell activation status, have been found to play an important role in the development of several diseases (7). In the context of osteomyelitis, specific immune cell phenotypes may be associated with pathogen clearance and modulation of the inflammatory response, thereby influencing disease progression and therapeutic response (8, 9). Therefore, a deeper understanding of how these immune cell characteristics are associated with osteomyelitis development may reveal new biomarkers and therapeutic targets (10).

MR analysis provides a unique approach to investigate the causal relationship between immune cell characteristics and osteomyelitis. By utilising genetic variation as an instrumental variable in the relationship between exposure (e.g., immune cell traits) and outcome (e.g., osteomyelitis), MR analysis can help to overcome the problem of confounding factors in traditional observational studies (6). In this study, by analysing data from a large-scale genome-wide association study (GWAS), we assessed potential causal associations between specific immune cell profiles and osteomyelitis risk (11). The application of this approach not only enhances our understanding of the etiology of osteomyelitis, but also provides a scientific basis for the development of new therapeutic strategies (12).

The results of this study are expected to provide important new insights into the prevention, diagnosis and treatment of osteomyelitis. By identifying immune cell characteristics associated with osteomyelitis risk, we can better understand the biological basis of the disease and thus provide new guidance for clinical practice. In addition, these findings may contribute to the development of new biomarkers for early diagnosis and risk assessment of the disease, as well as provide new ideas for the design of targeted therapeutic strategies. Future studies will further explore the specific mechanisms of action between these immune cell signatures and osteomyelitis, and how this knowledge can be applied to clinical practice to improve outcomes and quality of life for patients with osteomyelitis.

Materials and methods

Study design

Based on MR analyses of two samples, we assessed causal associations between 731 immune cell traits (7 groups) and osteomyelitis. MR uses genetic variation to represent risk factors, and therefore valid instrumental variables in causal inference must satisfy three key assumptions: (1) genetic variation is directly associated with exposure; (2) genetic variation is not associated with confounders between exposure and outcome; and (3) genetic variation will not influence outcome through pathways other than exposure (13). Data on osteomyelitis were obtained from the Finnish database, which tested 210,417
Europeans for GWAS, of which 842 were GWAS and 209,575 were controls. The GWAS consisted of 16,380,449 single nucleotide polymorphisms (SNPs) (14).

**Immunity-wide GWAS data sources**

Total GWAS statistics for each immunological profile are publicly available from the GWAS catalogue (registry numbers GCST90001391 to GCST90002121). A total of 731 immunophenotypes were included, including absolute cell count (AC) \((n = 118)\), median fluorescence intensity (MFI) reflecting surface antigen levels \((n = 389)\), morphology parameter [MP] \((n = 32)\) and relative cell count (RC) \((n = 192)\). Specifically, the MFI, AC and RC features contained B cells, CDCs, mature T cells, monocytes, myeloid cells, TBNK (T cells, B cells, natural killer cells) and Treg cells, while the MP feature contained CDCs and TBNK cells (15). The original immune signature GWAS used data from 3757 Europeans with no overlap. SNPS were calculated for approximately 22 million high-density array genotypes using a reference panel based on Sardinian sequences, and correlations were examined after adjusting for covariates (i.e., sex, age, and age) (16).

**Selection of instrumental variables (IVs)**

The significance level of IVs for each immune characteristic was set at \(5 \times 10^{-6}\) because genetic variation is directly related to exposure (17). To obtain site-independent IVs, we used the "TwoSampleMR" packet data with a linkage disequilibrium (LD) threshold set at \(R^2 < 0.001\) and an aggregation distance of 10,000 kb (18). For osteomyelitis, we adjusted the significance level to \(1 \times 10^{-5}\), which is typically used to indicate genome-wide significance in GWAS, with an LD threshold of \(R^2 < 0.001\) and an aggregation distance of 10000kb.

**Statistical analysis**

In the statistical analysis part of our study of the causal effect of immune cell phenotypes on the risk of osteomyelitis, all analyses were performed using the R software version 4.2.1, a widely used software for statistical calculations and graphical environments available at (http://www.Rproject.org) (19). In order to determine the causal relationship between 731 immunophenotypes and osteomyelitis, we mainly used inverse variance weighting (IVW) and weighted median (20). These analyses were provided by the "TwoSampleMR" package (version 0.5.7) in the R software environment (21). This package is specifically designed for performing MR analyses and provides tools for estimating, testing and sensitivity analyses of causal effects. The IVW method is a standard method in MR that combines Wald analysis (ratio of snp outcome associations to snp exposure associations) from multiple genetic variants, weighted by the inverse variance of each snp outcome association (22). Weighted median and model-based approaches were used as complementary methods to provide reliable causal estimates even when some instrumental variables were invalid, provided certain assumptions were met (23). These analyses were supported by rigorous sensitivity analyses, including Cochran's Q tests to test for heterogeneity among instrumental variables (24). This thorough statistical assessment ensured that findings regarding the relationship between immunophenotypes and osteomyelitis were as reliable and accurate as possible given the data. The whole process is shown in Fig. 1.
Results

Exploration of the causal effect of immunophenotypes on Osteomyelitis risk

A total of 21 immunophenotypes were identified to be causally associated with the development of osteomyelitis at a significance level of 0.05. There were 5 cases in the B-cell group, 5 cases in the cDC cell group, 3 cases in the Myeloid cell group, 5 cases in the TBNK cell group, 2 cases in the Maturation stages of T cell group, and 1 case in the Treg cell group (as shown in Fig. 2).

In the B cell group, Memory B cell %B cell (P = 0.035, OR = 1.137, 95%CI = 1.008 ~ 1.281), CD20- %B cell (P = 0.037, OR = 1.171, 95%CI = 1.009 ~ 1.359) and Memory B cell % lymphocyte (P = 0.013, OR = 1.105, 95%CI = 1.021 ~ 1.196) showed a positive causal relationship with the development of osteomyelitis, while Naive-mature B cell %B cell (P = 0.014, OR = 0.863, 95%CI = 0.776 ~ 0.971) and IgD- CD38- AC (P = 0.024, OR = 0.831, 95%CI = 0.708 ~ 0.976) showed a negative causal relationship with the development of osteomyelitis. In cDC cells, HLA DR on myeloid DC (P = 0.005, OR = 1.134, 95%CI = 1.037 ~ 1.240) and HLA DR on plasmacytoid DC (P = 0.004, OR = 1.139, 95%CI = 1.041 ~ 1.246) showed a positive causal relationship, while CD62L on CD62L + plasmacytoid DC (P = 0.034, OR = 0.805, 95%CI = 0.658 ~ 0.984), CD62L- monocyte %monocyte (P = 0.042, OR = 0.854, 95%CI = 0.734 ~ 0.994) and CD86 + plasmacytoid DC %DC (P = 0.006, OR = 0.800, 95%CI = 0.682 ~ 0.939) showed a negative causal relationship with the development of osteomyelitis. In the Myeloid cell group, CD45 on lymphocyte (P = 0.021, OR = 1.214, 95%CI = 1.029 ~ 1.432) showed a positive causal relationship with the development of osteomyelitis, while CD33br HLA DR + CD14dim AC (P = 0.036, OR = 0.920, 95%CI = 0.851 ~ 0.994) and CD11b on CD66b + + myeloid cell (P = 0.013, OR = 0.779, 95%CI = 0.638 ~ 0.950) showed a negative causal relationship with the development of osteomyelitis. In the TBNK cell group, all immune features showed a positive causal relationship with the development of osteomyelitis: CD4 + AC (P = 0.016, OR = 1.163, 95%CI = 1.028 ~ 1.316), HLA DR + CD4+ %T cell (P = 0.032, OR = 1.316, 95%CI = 1.023 ~ 1.692), and HLA DR + CD4+ %lymphocyte (P = 0.046, OR = 1.228, 95%CI = 1.003 ~ 1.504), and HLA DR + CD8br AC (P = 0.010, OR = 1.146, 95%CI = 1.032 ~ 1.273) CD3 on HLA DR + T cell (P = 0.025, OR = 1.184, 95%CI = 1.021 ~ 1.373).

In Maturation stages of T cell group, HVEM on T cell (P = 0.028, OR = 1.161, 95%CI = 1.016 ~ 1.328) showed a positive causal relationship with the development of osteomyelitis, and CD3 on TD CD4+ (P = 0.047, OR = 0.873, 95%CI = 0.764 ~ 0.998) showed a negative causal relationship with the development of osteomyelitis. In the Treg cell group, CD127 on CD4+ (P = 0.031, OR = 0.654, 95%CI = 0.445 ~ 0.962) showed a negative causal relationship with the development of osteomyelitis. The results of sensitivity analyses showed the robustness of the observed causal associations (Supplementary Fig. 1). Scatter plots and funnel plots also showed the stability of the results (Supplementary Figs. 2 and 3).

Exploration of the causal effect of Osteomyelitis risk on immunophenotypes
To investigate the causal relationship between osteomyelitis and immune phenotype, two-sample MR analysis was used, with the IVW method as the primary analysis method and other methods as auxiliary. Then, we used inverse MR to study the effect of osteomyelitis on immune phenotype cells. The results showed that there was no causal relationship between osteomyelitis and any of the 21 immune cells mentioned above.

**Discussion**

In this study, we explored the causal link between 731 immune cell traits (divided into seven groups) and osteomyelitis using two-sample MR analysis, an approach whose application is innovative in the field of genetic epidemiology (25). By analysing genome-wide association study (GWAS) data from a large number of European populations, we identified 21 immune phenotypes that are causally associated with the development of osteomyelitis (26). In particular, in the B cell group, phenotypes such as Memory B cell %B cell, CD20- %B cell and Memory B cell %lymphocyte showed positive causality with the development of osteomyelitis, whereas phenotypes such as Naive-mature B cell %B cell and IgD- CD38-AC showed negative causality.

The development of osteomyelitis is closely linked to the complex interactions of the immune system, and our study reveals a clear link between specific immune cell characteristics and osteomyelitis risk (27). For example, an increased proportion of Memory B cells may reflect an enhanced immune response to osteomyelitis pathogens, whereas a decreased proportion of Naive-mature B cells may indicate a weaker response to this infection (28). These findings suggest that the dynamics of specific immune cells may play a key role in the development of the disease, particularly in recognising and clearing pathogens and modulating inflammatory responses (26).

An in-depth understanding of these immune cell characteristics will help us better grasp the pathophysiological processes of osteomyelitis. For example, B cells play a key role in humoral immunity by producing antibodies to neutralise pathogens or to label them for clearance by other immune cells. An increase in Memory B cells may indicate a strengthened immune memory response, which may be a response to previous exposure to a pathogen (29). This enhanced immune memory may contribute to faster recognition and response to the same or similar pathogens, thus providing a defence mechanism. On the other hand, the reduction in Naive-mature B cells may suggest a relatively weak initial immune response, which may lead to more efficient spread of pathogens in the early stages (30).

The findings of this study provide new perspectives on the prevention, diagnosis, and treatment of osteomyelitis. For example, by monitoring changes in specific immune cell phenotypes, physicians may be able to recognise the risk of osteomyelitis earlier and develop more effective treatment strategies. For example, if a particular patient has an increased percentage of Memory B cells, more aggressive interventions may be needed to prevent the development of osteomyelitis (31). In addition, these immune cell profiles may also serve as new biomarkers for early diagnosis of osteomyelitis and assessment of treatment efficacy (32). Future studies should further explore the specific mechanisms of action between
these immune cell traits and osteomyelitis, which includes understanding how these traits affect pathogen clearance, modulation of the inflammatory response, and other aspects of the host immune system (31). For example, exploring the role of specific immune cell phenotypes in different types of osteomyelitis may reveal unique patterns of immune responses in specific disease subtypes (33). At the same time, these findings need to be validated in different ethnicities and different clinical settings to ensure their general applicability and effectiveness. For example, populations from different geographic regions and ethnic backgrounds may differ in their genetic make-up and environmental exposures, which may influence the relationship between immune cell profiles and osteomyelitis risk (34).

Although this study provides new insights into the relationship between osteomyelitis and immune cell characteristics, there are some limitations. For example, MR analyses, while reducing the influence of confounding factors in observational studies, cannot completely exclude the co-mingling of genetic background and environmental factors. In addition, this study relied primarily on data from a European population, and its results may not be fully applicable to other ethnicities or populations (35). Therefore, future studies need to further validate these findings in a wider range of populations and explore the role of different immune cell profiles in different types of osteomyelitis to ensure wider applicability and relevance of our findings. In summary, our study has shed some light on the relationship between specific immune cell characteristics and osteomyelitis through MR analysis methods. Inflammation in a causal relationship. These findings not only enhance our understanding of the pathophysiology of osteomyelitis, but also provide new ideas for clinical practice, especially in early diagnosis and personalised treatment. Future studies should be devoted to further exploring the specific mechanisms of these immune cell features in osteomyelitis and exploring how these findings can be translated into practical clinical applications to improve the outcome and quality of life of osteomyelitis patients.

**Declarations**

**ACKNOWLEDGEMENTS**

We extend our heartfelt thanks to all authors for their invaluable contributions to this study.

**AUTHOR’ CONTRIBUTIONS**

Kehan Long, Zhendong Ying and Sumiao Dong were pivotal in conceptualization, supervision, and the review and editing process. Dou Yu was instrumental in formal analysis and drafting the original manuscript. Kehan Long, Sumiao Dong, and Ao Gong played crucial roles in data acquisition. Ao Gong and Dou Yu carried out the statistical analysis. All authors have actively contributed to the article and have approved the final version submitted for publication.

**FUNDING SOURCES**

The research was financially supported by the Natural Science Foundation of Shandong Province under grant number ZR2023QH517.
DATA AVAILABILITY

Summary data from GWAS for immune cells are available at https://gwas.mrcieu.ac.uk/ GWAS id:ebi-a-GCST90001391-ebi-a-GCST90002121 (accessed on 4 November 2023); summary data for Osteomyelitis are from https://gwas.mrcieu.ac.uk/datasets/finn-b-M13_OSTEOMYELITIS/ (accessed on 4 November 2023)

Ethics Approval and Consent to Participate

The data for this study were obtained exclusively from publicly accessible databases containing anonymised participant information. As the dataset was pre-anonymised and publicly available, this study did not directly involve human participants and therefore did not require traditional ethical review. The design and use of data in this study followed all relevant data use guidelines and policies, ensuring sound and ethical use of data.

Consent for publication

Not applicable.

Competing Interests

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Figures
Figure 1

Flow diagram for quality control of the instrumental variables (IVs) and the entire Mendelian Randomization (MR) analysis process. *Abbreviations: SNPs, single-nucleotide polymorphisms; IVW, inverse variance weighted; MR, Mendelian Randomization; MR Presso, Mendelian Randomization Pleiotropy RESidual Sum and Outlier.
**Figure 2**

Forest plots depicting the causal associations between osteomyelitis and specific immune cell traits.

*Abbreviations: IVW, inverse variance weighting; CI, confidence interval.

**Supplementary Files**

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