Effect of immune cells and plasma metabolites on osteomyelitis: a two-sample Mendelian randomization and mediation analysis

Guang Yang*, Chengzhang Tie, Jin Wang, Heng Zhang, Yonghui Yang

Department of Orthopedics and Traumatology, Shuguang-Anhui Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Western Area of the First Affiliated Hospital of Anhui University of Chinese Medicine, Hefei, Anhui, China

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***Corresponding author:**
Guang Yang MD
Department of Orthopedics and Traumatology
Shuguang-Anhui Hospital Affiliated to Shanghai University of Traditional Chinese Medicine
Western Area of the First Affiliated Hospital of Anhui University of Chinese Medicine
Hefei, Anhui, China
E-mail: 2134661062@qq.com

Abstract

Introduction: Osteomyelitis (OM) is an infectious bone disease characterized by complex immune and metabolic features. Previous studies have found that immune cells play an important role in the development and progression of OM. However, the causal relationship between immune cells, plasma metabolites, and OM remains undetermined.

Material and methods: Instrumental variables (IVs) for 1400 plasma metabolite features (N = 7,824) and 731 immunophenotypes (N = 3,757) were sourced from genome-wide association studies (GWAS). The IVs for OM were derived from a comprehensive GWAS meta-analysis dataset of European ancestry. The relationship between exposure and outcome was assessed using two-sample Mendelian randomization (MR) analysis. The robustness of the results was evaluated through heterogeneity tests, sensitivity analyses, and pleiotropy analyses. Additionally, mediation analysis was employed to identify pathways through which immune characteristics and metabolites mediate OM.

Results: MR analysis revealed a genetic causal relationship between three immunophenotypes and nine plasma metabolites with OM. Reverse MR was used to identify the directionality of the causal relationship between CD27 on switched memory B cells, CD127 on $CD8^+$ T cells, and OM. Lastly, mediation analysis confirmed that three plasma metabolites have a significant mediating effect on the association between two immune phenotypes and OM.

Conclusions: Through MR analysis, this study demonstrated that plasma metabolites can mediate the causal effects of immune phenotypes on OM, providing new insights into the development mechanisms of OM and potential biomarkers, which hold promising value for the clinical diagnosis and treatment of OM.

Key words: osteomyelitis, immunophenotypes, metabolites, Mendelian randomization.

Introduction

Osteomyelitis (OM) is a bone-destructive disease primarily caused by pathogenic microbial infection. It is characterized by its complexity and high recurrence rate, which have long posed significant challenges in orthopedic infection control [1]. OM not only results in chronic pain and

functional disability but also imposes substantial healthcare costs and necessitates prolonged therapeutic interventions [2]. In addition to thorough debridement of the lesion, anti-infection measures remain the primary treatment approach [3]. Despite advancements in antibiotic and surgical treatments, the high recurrence rate persists, driven by bacterial resistance that reduces antibiotic efficacy [4, 5]. Routine laboratory tests and clinical indicators such as white blood cell count, C-reactive protein levels, and erythrocyte sedimentation rate have limited diagnostic value due to their lack of specificity [6, 7]. Therefore, understanding the pathogenesis of OM and identifying novel biomarkers are crucial for the development of improved diagnostic and therapeutic strategies.

From a pathogenesis perspective, the development of OM involves intricate immune responses and regulatory processes [8]. Specifically, the host response mediated by bacteria and immune escape mechanisms underscores the critical role of the immune system within the osteomyelitis microenvironment. Innate immune cells, such as macrophages and neutrophils, serve as the first line of defense against bacterial invasion in OM. However, bacteria can survive within immune cells for extended periods, contributing to the persistent nature of the disease [9, 10]. T cells also play a key role in host defense, aiding in the mobilization of macrophages and neutrophils to the site of infection [11]. Moreover, bacteria can manipulate adaptive immune cells to achieve immune escape, and they may inhibit the differentiation and activation of T cells [12]. During pathogen invasion, immune cells exhibit pro-inflammatory characteristics, such as M1 macrophage polarization, which further promotes bone resorption and loss [13]. Therefore, immune cells play a significant role in the onset and progression of OM; however, the precise regulatory mechanisms underlying these processes remain unclear.

Recently, the role of plasma metabolites in inflammatory diseases has gained significant attention [14]. Metabolomics, the comprehensive analysis of all low-molecular-weight chemicals in an organism, has led to the identification of numerous biomarkers associated with various diseases [15]. Plasma metabolites are thought to play a key role in regulating immune responses and the inflammatory process, potentially influencing the onset and progression of OM both directly and indirectly. Isogai *et al.* [16], using a mouse bacterial OM model, identified 279 metabolites through plasma metabolomics that could serve as biomarkers for OM. Although previous studies have confirmed the crucial role of immunophenotypes and metabolites in OM, the precise causal relationship and regulatory mechanisms remain unclear. The Mendelian randomization (MR) method, which utilizes genetic variations as instrumental variables, helps to explore the causal link between exposure factors and outcomes [17]. This approach effectively reduces the impact of confounding factors and circumvents reverse causation, making it a powerful tool for uncovering complex biological mechanisms [18].

This study integrates summary data from genome-wide association studies (GWAS), utilizing MR analysis and mediation analysis to investigate the genetic causality between immune cells, plasma metabolites, and OM, along with potential mediating mechanisms. We aim to uncover the roles of immune cells and metabolites in the pathogenesis of OM, providing scientific evidence to support their potential as therapeutic targets. In conclusion, this study not only aims to offer new insights into the prevention and treatment of OM but also presents a new approach for deciphering the causal relationships of complex diseases using genetic epidemiological methods.

Material and methods

Study design

This study employed a two-step MR analysis to ascertain the causal impact of immunophenotypes on OM and the mediating role of plasma metabolites. Specifically, the analysis was divided into three parts: (1) investigating the causality and correlation between immunophenotypes and OM; (2) exploring the causality and correlation between plasma metabolites and OM; (3) further examining through mediation analysis whether plasma metabolites act as a mediator affecting the association between immunophenotypes and OM. Figure 1 displays the workflow of this study.

Exposure and outcome GWAS data

The summary data for immunophenotypes were derived from previously published articles and the Open GWAS database, with study IDs ranging from GCST90001391 to GCST90002121. This study included 731 immunophenotypes from 3,757 Europeans, with no overlap of participants in terms of immunophenotypes [19].

The summary dataset for plasma metabolites was obtained from a previous study [20] and the GWAS catalog, with study IDs ranging from GCST90199621 to GCST90201020. This study includes 1,091 plasma metabolites and 309 metabolite ratios from 8,299 Europeans. Of the 1,091 plasma metabolites, 850 can be categorized into eight super pathways: lipids, amino acids, xenobiotics, nucleotides, cofactors and vitamins, carbohydrates, peptides, and energy, with the remaining 241 classified as unknown or "partially" characterized molecules. OM data as an outcome

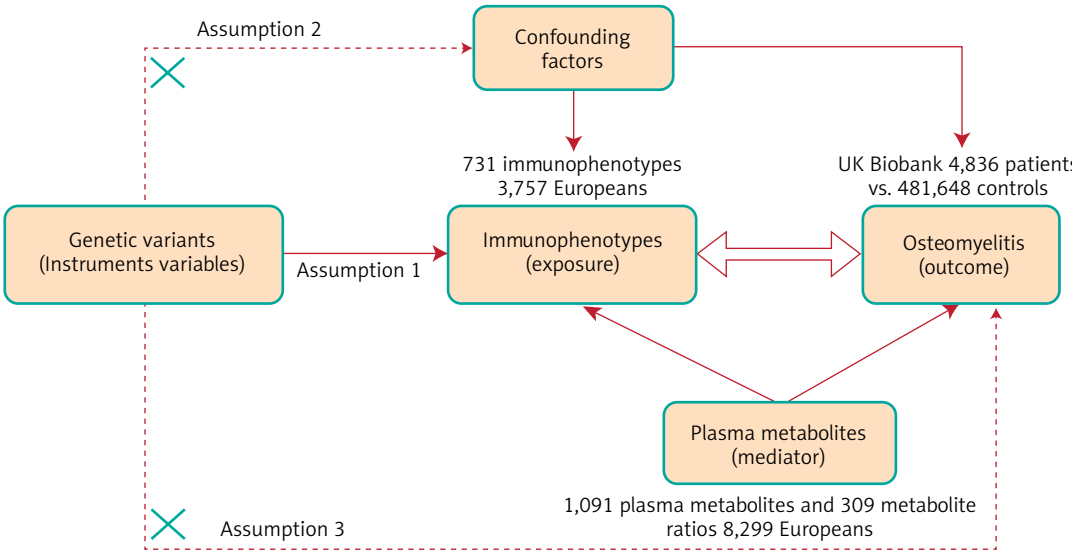


Figure 1. Flowchart of this study. Assumption 1: Genetic variants are associated with immunophenotypes (exposure); Assumption 2: Genetic variants are not associated with confounding factors; Assumption 3: Genetic variants are associated with osteomyelitis (outcome), and genetic variants determine osteomyelitis (outcome) solely through immunophenotypes (exposure)

originated from a GWAS of European samples, consisting of 4,836 patients and 481,648 healthy controls from the UK Biobank [21]. After adjusting for genotyping, age, gender, and 10 principal components, 12,243,512 genetic loci were obtained.

Instrument variables selection

When estimating genetic variations for causal effects, three fundamental assumptions must be followed to ensure the validity of instrumental variables (IVs): first, IVs must be significantly associated with the exposure under study; second, IVs should not be associated with any potential confounding variables; lastly, IVs should influence the outcome only through the exposure. In this study, given the limitation on the number of SNPs under certain conditions, we adjusted the significance level threshold for IVs of immunophenotypes and serum metabolites to $p < 1 \times 10^{-5}$, aiming to identify SNPs closely related more accurately to the traits under investigation. Furthermore, we excluded SNPs significantly related to the outcome variable ($p < 0.05$) and reduced the impact of linkage disequilibrium (LD) through clustering, specifically setting $R^2 < 0.001$ within a 10,000 kb window size. We also selected IVs with an F-statistic greater than 10 for analysis to ensure that the chosen genetic instrumental variables have sufficient strength.

Two-sample MR analysis

In exploring the genetic causal links between immunophenotypes, plasma metabolites, and OM, the MR method assesses the presence of causal relationships among these biomarkers by utilizing genetic variants as instrumental variables (IVs). When only one IV was available, the Wald ratio method was used to estimate the causal effect of the exposure on the outcome. In scenarios with multiple IVs, various statistical methods were used to enhance the accuracy and reliability of causal inference, including the inverse variance weighted (IVW) method, maximum likelihood estimation, MR-Egger regression, the weighted median method, and the mode-based estimation. The MR-Egger method offered an assessment tool for pleiotropic effects by introducing an intercept term to test for the presence of systemic pleiotropy among IVs [22]. If the intercept of the MR-Egger regression significantly deviates from zero, it may indicate the presence of horizontal pleiotropy, where some IVs may affect the outcome through pathways other than just the exposure under study. If the intercept was close to zero, the results of MR-Egger were similar to those of the IVW method, indicating a minimal impact of pleiotropy. The weighted median method calculated the median based on the ranked effect values of IVs and their weights, providing a robust estimate even when more than half of the IVs were affected by pleiotropy [23]. The Cochran Q statistic of IVW was used to detect heterogeneity among IVs. Furthermore, a leave-one-out sensitivity analysis was performed to assess the stability of MR results after the removal of any specific IV.

Reverse MR analysis

To identify false-positive results, reverse MR analysis was utilized to assess whether OM has

a causal relationship with identified immunophenotypes. Specifically, with OM as the exposure and immunophenotypes as the outcome, analysis was conducted using the "TwoSampleMR" package (version 0.5.11). For the IVW method, a filtering threshold of *p*-value > 0.05 was set, and results with a *p*-value < 0.05 were visualized.

Mediation analysis

Mediation analysis is commonly used to assess and explain causal relationships between variables, aiding in our understanding of how immunophenotypes affect the risk of OM occurrence through one or several mediator variables [24]. First, MR analysis was used to assess the causal relationship between immunophenotypes and plasma metabolites, calculating the β1 value, which represents the magnitude of the causal effect from immunophenotypes to the mediator variable (plasma metabolites). Next, the causal relationship between plasma metabolites and OM was further assessed through MR analysis, calculating the β2 value, which represents the magnitude of the causal effect from the mediator variable to the outcome. The mediating effect was then estimated using the two-step MR method, with the formula: mediating effect = β1 × β2, quantifying the impact of exposure transmitted through the mediator variable on the outcome. The direct effect was determined by subtracting the mediating effect from the total effect, reflecting the impact of exposure on the outcome aside from the mediating pathway. The mediation proportion was calculated using the formula (mediating effect/total effect) ×100%, quantifying the relative importance of the mediating variable in the overall causal pathway. The 95% confidence intervals (CI) for mediating effect and mediation proportion were estimated using the delta method, providing a statistical evaluation for the accuracy and reliability of the results. Based on the results of the mediation analysis, this study classified mediating factors according to the level of evidence. When a variable simultaneously meets the following three conditions, then the variable is considered to have a potential mediating role in the association between immunophenotypes and OM: 1) There is a causal relationship between exposure and outcome. 2) There is a causal relationship between the mediator and the outcome. 3) There is a causal relationship between exposure and the mediator.

Statistical analysis

We performed two-sample Mendelian randomization (MR) analyses using the "TwoSampleMR" package in R (version 4.3.2). Primary causal estimates were derived via inverse-variance weighted (IVW) regression, supplemented by MR-Egger, weighted median, simple mode, and maximum likelihood methods. Horizontal pleiotropy was assessed through MR-Egger intercept tests, while heterogeneity was evaluated using Cochran's Q statistic. Reverse MR analyses were conducted to exclude reverse causation by treating osteomyelitis as exposure. For mediation analysis, the mediating effect was then estimated using the two-step MR method, with the formula: mediating effect = β1 × β2. A significance level of $p < 0.05$ was considered statistically significant.

Results

The causal effect of immunophenotypes on OM

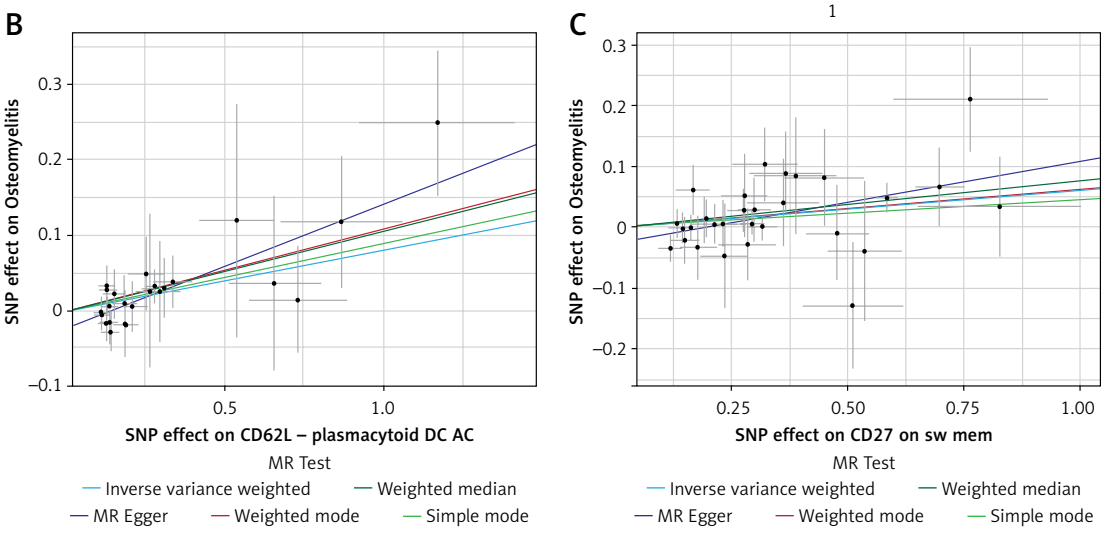
All SNPs related to immunophenotypes had an *F*-statistic > 10, excluding weak IVs. Extracted immune cells with consistent OR value directions across five methods, and IVW method analysis showed that three out of 731 immunophenotypes have a causal relationship with OM ($p < 0.05$). Specifically, CD27 on switched memory B cells (OR = 1.063, 95% CI = 1.016–1.112, $p = 0.008$) and CD62L plasmacytoid dendritic cell absolute count (OR = 1.084, 95% CI = 1.022–1.150, $p = 0.007$) are risk factors for OM, while CD127 on $CD8^+$ T cells (OR = 0.925, 95% CI = 0.876–0.977, $p = 0.005$) acts as a protective factor for OM (Figures 2 A–D). Furthermore, pleiotropy tests confirmed that there is no pleiotropy in the causal relationship between the three immunophenotypes and OM (Supplementary Table SI): CD27 on switched memory B cells (intercept = –0.024, $p = 0.09$), CD62L plasmacytoid dendritic cell absolute count (intercept = 0.011, $p = 0.40$) and CD127 on $CD8^+$ T cells (intercept = –0.010, $p = 0.35$). Sensitivity analysis suggested the reliability and stability of MR analysis outcomes (Supplementary Figure S1). Lastly, the Q statistics showed no evidence of heterogeneity (Supplementary Table SII).

Reverse causality analysis

Reverse MR analysis was then used to assess whether OM has a causal relationship with identified immunophenotypes, further excluding potential causality (Figure 3). Our analysis did not identify a significant causal relationship between OM and CD27 on switched memory B cells ($p = 0.053$) or CD127 on CD8+ T cells ($p = 0.119$). However, a causal relationship was found between OM and absolute count of CD62L-negative plasmacytoid dendritic cells ($p = 0.012$), with sensitivity analysis indicating no heterogeneity or horizontal pleiotropy (Supplementary Figure S2). Therefore, we excluded $CD62L^-$ plasmacytoid dendritic cell absolute count from subsequent analysis.

A

Exposure	Outcome	nsnp	Method	*P*-value	OR (95% CI)
CD62L – plasmacytoid DC AC	Osteomyelitis	24	Inverse variance weighted	**0.007**	1.084 (1.022 to 1.149)
CD27 on sw mem	Osteomyelitis	30	Inverse variance weighted	**0.008**	1.063 (1.016 to 1.112)
CD127 on CD8br	Osteomyelitis	22	Inverse variance weighted	**0.005**	0.925 (0.876 to 0.977)



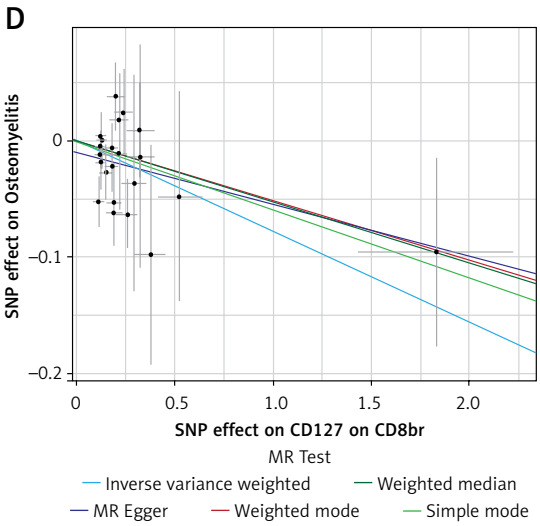


Figure 2. MR analysis reveals the causal relationship between immunophenotypes and OM. **A** – Forest plot displaying three immunophenotypes with a causal relationship on OM. Scatter plots showing the causal relationship between (**B**) $CD62L^-$ plasmacytoid dendritic cell absolute count, (**C**) CD27 on switched memory B cells, and (**D**) CD127 on $CD8^+$ T cells and OM using different methods

Exposure	Outcome	nsnp	Method	*P*-value	OR (95% CI)
II id:ieu-b-4975	CD62L – plasmacytoid DC AC	10	MR Egger	0.277	0.799 (0.549 to 1.164)
		10	Weighted median	0.159	0.850 (0.679 to 1.065)
		10	Inverse variance weighted	**0.012**	0.812 (0.690 to 0.956)
		10	Simple mode	0.092	0.749 (0.554 to 1.011)
		10	Weighted mode	0.284	0.863 (0.670 to 1.112)
II id:ieu-b-4975	CD27 on sw mem	10	MR Egger	0.564	0.889 (0.605 to 1.305)
		10	Weighted median	0.134	1.195 (0.947 to 1.508)
		10	Inverse variance weighted	0.053	1.188 (0.998 to 1.414)
		10	Simple mode	0.115	1.321 (0.966 to 1.805)
		10	Weighted mode	0.227	1.200 (0.911 to 1.583)
II id:ieu-b-4975	CD127 on CD8br	10	MR Egger	0.220	0.763 (0.512 to 1.137)
		10	Weighted median	0.078	0.807 (0.636 to 1.024)
		10	Inverse variance weighted	0.119	0.871 (0.732 to 1.036)
		10	Simple mode	0.529	0.908 (0.680 to 1.213)
		10	Weighted mode	0.127	0.809 (0.632 to 1.036)

Figure 3. Forest plot showing the reverse MR analysis between OM and three immunophenotypes

Exposure	Outcome	nsnp	Method	*P*-value	OR (95% CI)
Kynurenine levels	Osteomyelitis	25	Inverse variance weighted	**0.003**	1.176 (1.056 to 1.310)
Docosapentaenoate n3 DPA; 22:5n3 levels	Osteomyelitis	22	Inverse variance weighted	**0.009**	1.177 (1.041 to 1.331)
1-linolenoyl-GPC (18:3) levels	Osteomyelitis	29	Inverse variance weighted	**0.009**	0.870 (0.784 to 0.965)
Etiocholanolone glucuronide levels	Osteomyelitis	22	Inverse variance weighted	**0.002**	1.125 (1.045 to 1.210)
Myristoyl dihydrosphingomyelin (d18:0/14:0) levels	Osteomyelitis	30	Inverse variance weighted	**0.004**	1.157 (1.046 to 1.280)
Heneicosapentaenoate (21:5n3) levels	Osteomyelitis	16	Inverse variance weighted	**0.009**	1.184 (1.044 to 1.344)
Ximenoylcarnitine (C26:1) levels	Osteomyelitis	29	Inverse variance weighted	**0.002**	0.885 (0.818 to 0.957)
Palmitoyl-sphingosine-phosphoethanolamine (d18:1/16:0) levels	Osteomyelitis	26	Inverse variance weighted	**0.002**	0.838 (0.751 to 0.935)
Uridine to cytidine ratio	Osteomyelitis	21	Inverse variance weighted	**0.007**	0.835 (0.733 to 0.952)

Figure 4. Forest plot showing the causal relationship between nine blood metabolites and OM assessed using the IVW method

The causal effect of plasma metabolites on OM

Based on the IVW method, MR analysis revealed a causal relationship between nine plasma metabolites and OM (Figure 4). Elevated levels of kynurenine (OR = 1.176, 95% CI = 1.056–1.310, p = 0.003), docosapentaenoic acid (22:5n-3) (OR = 1.177, 95% CI = 1.041–1.331, p = 0.009), etiocholanolone glucuronide (OR = 1.125, 95% CI = 1.045–1.210, p = 0.002), myristoyl dihydrosphingomyelin (d18:0/14:0) (OR = 1.157, 95% CI = 1.046–1.280, p = 0.004), and heneicosapentaenoate (21:5n3) (OR = 1.184, 95% CI = 1.044–1.344, p = 0.009) are potential risk factors for OM. 1-linolenoyl-GPC (18:3) levels (OR = 0.870, 95% CI = 0.784–0.965, p = 0.009), ximenoylcarnitine (C26:1) levels (OR = 0.885, 95% CI = 0.818–0.957, p = 0.002), palmitoyl-sphingosine-phosphoethanolamine (d18:1/16:0) levels (OR = 0.838, 95% CI = 0.751–0.935, p = 0.002), and uridine to cytidine ratio (OR = 0.835, 95% CI = 0.733–0.952, p = 0.007) were identified as protective factors against OM. Furthermore, pleiotropy tests confirmed the absence of pleiotropy in the causal relationship between the nine plasma metabolites and OM (Supplementary Table SIII). The Q statistics and sensitivity analysis suggested the reliability and stability of MR analysis outcomes (Supplementary Table SIV).

Mediation analysis of immunophenotypes and OM

To explore the mechanisms of OM development, mediation analysis was conducted integrating immunophenotypes and plasma metabolites, with mediator and direct effects calculated to assess the mediator's role and importance. The analysis indicated a negative causal relationship between the increase in CD27 on switched memory B cells and palmitoyl–sphingosine–phosphoethanolamine (d18:1/16:0) levels (OR = 0.962, 95% CI = 0.928–0.997, p = 0.033), implying that an increase in CD27 expression on switched memory B cells is associated with a decrease in palmitoyl–sphingosine–phosphoethanolamine (d18:1/16:0) levels. Palmitoyl–sphingosine–phosphoethanolamine (d18:1/16:0) levels have a significant positive mediating effect on CD27 on switched memory B cells and OM (β = 0.00687, 95% CI: 0.000407–0.0133, p = 0.0372), with the percentage of mediation being 11.2% (Figure 5 A). CD127 on $CD8^+$ T cells may influence the risk of OM occurrence through two metabolites. Specifically, an increase in CD127 on $CD8^+$ T cells has a negative causal relationship with docosapentaenoic acid (22:5n-3) levels (OR = 0.942, 95% CI = 0.899–0.988, p = 0.014), and docosapentaenoic acid (22:5n-3) levels have a significant negative mediating effect between CD127 on $CD8^+$ T cells and OM (β = –0.00966, 95% CI: –0.0179 to –0.00143, p = 0.0213), with the percentage of mediation being 12.4% (Figure 5 B). Conversely, an increase in CD127 on $CD8^+$ T cells has a positive causal relationship with ximenoylcarnitine (C26:1) levels (OR = 1.047, 95% CI = 1.005–1.091, p = 0.028), and ximenoylcarnitine (C26:1) levels have a significant negative mediating effect between CD127 on $CD8^+$ T cells and OM (β = –0.00562, 95% CI: –0.011 to –0.000251, p = 0.0402), with the percentage of mediation being 7.19% (Figure 5 C and Supplementary Table SV).

Discussion

This study investigated the potential causal effects in the occurrence and development of OM by integrating data on immunophenotypes and circulating metabolites. MR analysis identified potential causal relationships between three immunophenotypes (CD27 on switched memory B cells, $CD62L^-$ plasmacytoid dendritic cell absolute count, and CD127 on CD8+ T cells) and nine plasma metabolites with OM. Further mediation analysis revealed that three metabolites may mediate the causal relationship between two immunophenotypes and OM. Specifically, palmitoyl–sphingosine–phosphoethanolamine (d18:1/16:0) levels exhibited a significant positive mediating effect on the relationship between CD27 on switched memory B cells and OM, with a mediation percentage of 11.2%. For CD127 on $CD8^+$ T cells, docosapentaenoic acid (22:5n-3) levels demonstrated a significant negative mediating effect between CD127 on $CD8^+$ T cells and OM, with a mediation percentage of 12.4%. Additionally, ximenoylcarni-

A

Exposure	Outcome	nsnp	Method	*P*-value	OR (95% CI)
CD27 on switched memory B cell	Palmitoyl-sphingosine-phosphoethanolamine (d18:1/16:0) levels	29	MR Egger	0.075	0.933 (0.866 to 1.004)
		29	Weighted median	0.498	0.984 (0.940 to 1.031)
		29	Inverse variance weighted	**0.033**	0.962 (0.928 to 0.997)
		29	Simple mode	0.190	0.939 (0.857 to 1.029)
		29	Weighted mode	0.164	0.966 (0.922 to 1.013)
Palmitoyl-sphingosine-phosphoethanolamine (d18:1/16:0) levels	Osteomyelitis	26	MR Egger	0.063	0.767 (0.587 to 1.001)
		26	Weighted median	0.033	0.846 (0.725 to 0.986)
		26	Inverse variance weighted	**0.002**	0.838 (0.751 to 0.935)
		26	Simple mode	0.715	0.942 (0.686 to 1.294)
		26	Weighted mode	0.582	0.915 (0.671 to 1.248)
CD27 on switched memory B cell	Osteomyelitis	30	MR Egger	**0.008**	1.140 (1.042 to 1.247)
		30	Weighted median	0.023	1.078 (1.011 to 1.150)
		30	Inverse variance weighted	**0.008**	1.063 (1.016 to 1.112)
		30	Simple mode	0.407	1.046 (0.941 to 1.163)
		30	Weighted mode	0.071	1.066 (0.997 to 1.139)

B

Exposure	Outcome	nsnp	Method	*P*-value	OR (95% CI)
CD27 on CD8br	Docosapentaenoate n3 DPA; 22:5n3 levels	22	MR Egger	0.223	0.949 (0.875 to 1.030)
		22	Weighted median	0.075	0.939 (0.876 to 1.006)
		22	Inverse variance weighted	**0.014**	0.942 (0.899 to 0.988)
		22	Simple mode	0.197	0.938 (0.853 to 1.031)
		22	Weighted mode	0.102	0.944 (0.884 to 1.008)
Docosapentaenoate n3 DPA; 22:5n3 levels	Osteomyelitis	22	MR Egger	0.806	1.034 (0.793 to 1.350)
		22	Weighted median	0.053	1.194 (0.998 to 1.429)
		22	Inverse variance weighted	**0.009**	1.177 (1.041 to 1.331)
		22	Simple mode	0.509	1.115 (0.812 to 1.531)
		22	Weighted mode	0.214	1.195 (0.910 to 1.569)
CD127 on CD8br	Osteomyelitis	22	MR Egger	0.322	0.956 (0.876 to 1.043)
		22	Weighted median	0.231	0.949 (0.871 to 1.034)
		22	Inverse variance weighted	**0.005**	0.925 (0.876 to 0.977)
		22	Simple mode	0.489	0.942 (0.799 to 1.112)
		22	Weighted mode	0.239	0.950 (0.875 to 1.032)

C

Exposure	Outcome	nsnp	Method	*P*-value	OR (95% CI)
CD127 on CD8br	Ximenoylcarnitine (C26:1) levels	22	MR Egger	0.085	1.066 (0.995 to 1.143)
		22	Weighted median	0.109	1.054 (0.988 to 1.124)
		22	Inverse variance weighted	**0.028**	1.047 (1.005 to 1.091)
		22	Simple mode	0.370	1.047 (0.949 to 1.154)
		22	Weighted mode	0.123	1.053 (0.989 to 1.122)
Ximenoylcarnitine (C26:1) levels	Osteomyelitis	29	MR Egger	**0.042**	0.827 (0.694 to 0.985)
		29	Weighted median	0.192	0.928 (0.830 to 1.038)
		29	Inverse variance weighted	**0.002**	0.885 (0.818 to 0.957)
		29	Simple mode	0.770	0.975 (0.827 to 1.151)
		29	Weighted mode	0.249	0.928 (0.820 to 1.051)
CD127 on CD8br	Osteomyelitis	22	MR Egger	0.322	0.956 (0.876 to 1.043)
		22	Weighted median	0.235	0.949 (0.870 to 1.035)
		22	Inverse variance weighted	**0.005**	0.925 (0.876 to 0.977)
		22	Simple mode	0.437	0.942 (0.814 to 1.091)
		22	Weighted mode	0.247	0.950 (0.873 to 1.034)

Figure 5. Mediation analysis between immunophenotypes and OM. **A** – Forest plot shows that palmitoyl–sphingosine–phosphoethanolamine (d18:1/16:0) levels have a significant positive mediating effect on CD27 on switched memory B cells and OM. **B** – Forest plot indicates thatdocosapentaenoate n3 DPA; 22:5n3 levels have a significant negative mediating effect between CD127 on CD8⁺ T cells and OM. **C** – Forest plot demonstrates that ximenoylcarnitine (C26:1) levels have a significant negative mediating effect between CD127 on CD8⁺ T cells and OM

tine (C26:1) levels showed a significant negative mediating effect between CD127 on CD8+ T cells and OM, with a mediation percentage of 7.19%. These results suggest that docosapentaenoic acid (22:5n-3) levels may play a more prominent role in mediating the causal relationship between CD127 on CD8+ T cells and OM.

Circulating immune cells, as key components of the immune system, may play a role in defending against OM infections and related virulence factors [25]. Our study identified a significant genetic causal link between CD127 on $CD8^+$ T cells and OM, suggesting that CD127 may act as a protective factor against OM. Kumar *et al*. [26] characterized T cells in patients with chronic OM using flow cytometry and found that T cells in infected bones were activated, as indicated by an increased number of $CD8^+$ T cells compared to uninfected bones. Furthermore, Sokhi *et al*. [27] performed a cellular composition analysis in a mouse tibia bone infection model and observed that multiple components of innate and adaptive immunity were strongly activated. This included persistent neutrophil infiltration in infected joints and bone tissues, along with activation of the feedback mechanism of regulatory T cells, which promoted the continuous proliferation of T cells during the infection phase. In clinical research, a histopathological study on osteomyelitis patients found that plasmacytic and neutrophilic inflammation exhibited similar specificity and positive predictive values for OM [28]. Additionally, Wagner *et al*. [29] observed evidence of CD8+ T cell expansion in the peripheral blood of patients with implant-associated post-traumatic OM, along with their infiltration into the infection site. In pediatric chronic recurrent multifocal osteomyelitis, inflammatory infiltration sites were found to be rich in CD8+ T cells [30]. These studies collectively reveal that $CD8^+$ T cells play a significant regulatory role in OM pathogenesis, with CD127 potentially serving as a key target for modulating the immune response to OM. Additionally, B cells may regulate osteoclastogenesis within the immune microenvironment of OM [31]. Wagner *et al*. [32] demonstrated alterations in cytokines and increased B cell activity in post-traumatic OM patients, further identifying RANKL-dependent osteoclastogenesis that speeds up bone resorption. Another study found that mesenchymal stem cells derived from adipose tissue can restore bone regeneration in post-traumatic OM models and downregulate B cell expression, which is associated with osteoclast formation [33]. Consistent with these findings, our study suggested that CD27 on switched memory B cells might act as a risk factor for OM, showing a positive correlation with the risk of OM occurrence.

In the absence of effective biomarkers for early diagnosis and treatment targets for OM, metabolomic analysis offers a promising new approach to identify potential biomarkers [34, 35]. Omega-3 polyunsaturated fatty acids, such as docosahexaenoic acid, have demonstrated strong anti-inflammatory properties [36, 37]. During the initial inflammatory response, docosahexaenoic acid is converted into resolvins, which regulate neutrophil infiltration and suppress the inflammatory response [38]. Our study also found that docosapentaenoic acid (22:5n-3) levels are positively correlated with the risk of OM and play a negative mediating role in the causal relationship between CD127 on $CD8^+$ T cells and OM. Additionally, carnitine palmitoyltransferase-II, which is linked to immunity and infectious diseases, facilitates the release of acyl-coenzyme A (CoA) in mitochondria to accelerate β-oxidation, thus participating in the circulating metabolic processes associated with OM [39, 40]. In this study, we found that levels of ximenoylcarnitine (C26:1) serve as a protective factor in OM by influencing the circulating metabolic changes in the disease. Interestingly, Isogai *et al*. [16] conducted a metabolomic analysis of potential biomarkers in the plasma of mice with OM and found a significant decrease in acylcarnitine and fatty acids, particularly ω-3 and ω-6 polyunsaturated fatty acids, in the OM group. Although there is currently no clinically relevant metabolomic evidence, these results suggest that acylcarnitine may become a potential diagnostic and therapeutic target for clinical OM.

This study is the first to integrate and explore the causal relationships between immunophenotypes, plasma metabolites, and OM, investigating potential mechanisms of action through mediation analysis and enhancing the robustness of the results with heterogeneity and sensitivity analyses However, several limitations should be noted. Firstly, due to the limited availability of database samples and cohorts, future studies should use independent validation sets to confirm the accuracy of the MR results. Secondly, more demographic and clinical information on OM patients is needed to facilitate subgroup analyses, which would help guide stratified treatment strategies. Lastly, the lack of stratified cohorts for different OM pathogens in the GWAS data may affect the heterogeneity of the results. In the future, it will be crucial to validate immune-metabolic features in cohorts that categorize osteomyelitis by specific pathogens. Additionally, more in-depth mechanistic experiments are needed to explore the value of key biomarkers.

In conclusion, through MR analysis, we identified three immunophenotypes and nine plasma metabolites with potential causal relationships

to OM. Further mediation analysis revealed that three plasma metabolites may mediate the causal connections between two immunophenotypes and OM, offering new ideas and insights for exploring biomarkers related to the diagnosis and treatment of OM.

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Availability of data and materials

The data used to support the findings of this study are available from the previous studies and open GWAS datasets (https://gwas.mrcieu.ac.uk/). The processed data are available from the corresponding author upon request.

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Ethical approval

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

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