Immune Cells, Serum Metabolites, and Ovarian Cancer: A Mediation Mendelian Randomization Study

Keywords
Ovarian cancer, Mendelian randomization, Serum metabolites, Mediation analysis, Immune cells phenotype

Abstract

Introduction

Recent studies have highlighted the potential functions of immune cells and serum metabolites in the progression of ovarian cancer (OC). Therefore, this study executed Mendelian randomization (MR) methodology to seek out causal links among serum metabolites, immune cells, and OC.

Material and methods

This study wielded data from multiple sources to obtain genetic data related to immune cell phenotypes, serum metabolites, and OC. The causal effects were estimated using the inverse variance weighted, MR-Egger, weighted median, simple mode, and weighted mode to assess potential causal effects. Finally, mediation analysis was conducted to ascertain the potential mediating functions of immune cell phenotypes and serum metabolites in OC.

Results

36 causal links between immune cell phenotypes and OC were recognized. "Resting CD4 regulatory T cell %CD4 regulatory T cell" (OR = 0.977, p = 0.018) was protective, while "IgD- CD38dim B cell %B cell" (OR = 1.027, p = 0.021) was risk factor. Additionally, 89 causal relationships were identified between serum metabolites and OC. "Gluconate levels" (OR = 0.925, p = 0.047) was protective, while "fructose levels" (OR = 1.097, p = 0.019) was risk factor for OC. Mediation analysis identified 3 serum metabolites that mediated the influence of immune cell phenotypes on OC, alongside 2 immune cell phenotypes acting as mediators between serum metabolites and OC. Notably, sensitivity analysis validated the robustness of these findings.

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Running head: Causal relationships in ovarian cancer

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1. Introduction

Ovarian cancer (OC) remains a lethal gynecological malignancy with a 5-year survival rate of approximately 47%. This is mostly due to the absence of early symptoms, resulting in the majority of patients being detected at an advanced stage[1]. Moreover, the etiology of OC remains unclear, further complicating early diagnosis[2]. While current therapies partially control progression, high recurrence rates persist, highlighting an urgent need for improved early diagnostic biomarkers and therapeutic targets[3].

Recent studies have linked the immune system to OC progression: macrophage homeostasis dysregulation drives tumor microenvironment immunosuppression[4,5]; and altered lymphocyte subsets (e.g., T/B cells) correlate with prognosis[6]. In particular, regulatory T cells (Tregs) and regulatory B cells (Bregs) inhibit the proliferation of other immune cells by expressing different immune checkpoint molecules and secreting

immunosuppressive substances, thereby promoting tumor growth and progression[7–10]. In parallel, the rapid advancement of metabolomics has offered a new lens to clarify OC pathogenesis while being widely utilized in OC studies over recent years[11,12]. Serum metabolites, as direct reflections of biochemical activities in the body, are considerable for risk assessment and prognosis prediction of female cancers[13]. Fatty acids (such as C16 and C22), amino acids, and various chemical substances have been recognized as possible serum indicators for OC[14,15]. These findings underscore the critical role of serum metabolites in OC, but their causal relationship with OC—and whether they mediate the crosstalk between immune cells and OC pathogenesis—has not been validated. Traditional observational studies, limited by confounding factors and reverse causality, cannot resolve these uncertainties.

Based on this, the present study aims to systematically evaluate the causal relationship between immune cells and serum metabolites with OC through Mendelian randomization (MR) analysis, which leverages genetic variants as instrumental variables (IVs) to infer causal relationships while minimizing bias[16,17]. We further integrated mediation analysis to dissect the potential pathways: specifically, whether serum metabolites mediate the effect of immune cells on OC, or vice versa. This approach aims to clarify the immunometabolic network in OC, providing a theoretical basis for developing novel diagnostic strategies and therapeutic interventions.

2. Materials and methods

2.1 Data collection

This study incorporated 731 immune phenotypes, all retrieved from the GWAS catalog (https://www.ebi.ac.uk/gwas/) (GCST90001391 to GCST90002121) and encompassing 3,757 individuals of European ancestry with no cohort overlap[18]. Additionally, 1,400 serum metabolites (GCST90199621 to GCST90201020) were retrieved from the GWAS catalog, originating from the Canadian Longitudinal Study on Aging (CLSA) cohort. This cohorts contained 8,299 randomly selected European-descent individuals with no blood relations[19]. Meanwhile, the GWAS data for OC (ieu-a-1120) were obtained from the IEU OpenGWAS project (https://gwas.mrcieu.ac.uk/), comprising 40,941 controls and 25,509 case samples of European origin, totaling 470,825 single nucleotide polymorphisms (SNPs).

2.3 Acquisition of IVs

MR analysis was conducted based on the following three fundamental assumptions: (1) IVs must demonstrate a consistent correlation with the exposure factors being examined; (2) IVs must be unaffected by any identifiable or unidentifiable confounding variables; (3) IVs must affect the outcome only via the exposure factor, rather than through alternative direct causal mechanisms. Based on these assumptions, we followed the initial step of selecting IVs by setting the SNP selection threshold at $p < 1 \times 10^{-5}$ when regarding immune cells and serum metabolites as exposure factors[20,21]. Second, the ieugwasr package (v 1.0.0) [22] was used to eliminate SNPs with linkage disequilibrium, (parameters: $r^2 = 0.001$, kb = 10000). Subsequently, we calculated the F-statistic for each genetic variant and retained only those with an F-statistic > 10. Based on GWAS catalog,

SNPs potentially associated with outcome GWAS traits were excluded at a threshold of $p < 1*10^{-5}$ to satisfy the independence assumption. Additionally, the Steiger method was employed for directionality testing to ensure the unidirectionality of causal relationships. To ensure that the effects of SNPs on exposure and outcome corresponded with those of the identical allele, palindromic SNPs were omitted.

2.4 MR analysis

This study followed the Mendelian reporting specifications for randomised studies (STROBE-MR)[23]. This study employed multiple MR analysis methods to assess causal relationships. The inverse variance weighted (IVW) [24] method, which typically has the highest statistical power[24,25], was chosen as the primary method, supplemented by other approaches including MR-Egger[26], weighted median[27], simple mode[28], and weighted mode[28]. Moreover, the mediating roles of immune cells and serum metabolites in OC was explored. First, the overall effect of the main exposure on OC was assessed (a), reflecting the combined direct and indirect effects of the exposure on OC without considering mediating variables. Subsequently, the effects of exposure on the mediator (c) and the mediator on the outcome (b) were analyzed. In quantifying mediating effects, c*b was used to represent the mediating effect. Additionally, the direct effect was computed using the formula a-c*b, while the proportion of mediation was quantified as c*b/a.

2.5 Statistical analysis

Sensitivity analyses were conducted to assess the robustness of causal inferences.

Heterogeneity was evaluated using the mr_heterogeneity function and pleiotropy was assessed through MR-Egger regression and MR-PRESSO methods-outliers with $p < 0.05$ were excluded. Additionally, leave-one-out analysis was conducted to test the reliance of results on individual SNPs: one SNP was removed sequentially to check if any single SNP influenced the causal estimates. All analyses were completed using the TwoSampleMR (v 0.6.0)[29] and MRPRESSO packages (v 1.0)[30], and the Delta method was utilized to compute standard errors for mediating effects, direct effects, and proportions mediated[31].

3. Result

3.1 Selection of IVs

SNPs were screened to meet the necessary assumptions for MR analysis. In the analysis of immune cells phenotype and OC, a total of 17,757 SNPs were included for further study, with F-statistics fluctuating from 19.537 to 3159.289 (Table S1). For the analysis of serum metabolites and OC, 34,513 SNPs were used, with F-statistics spanning from 19.503 to 2297.785 (Table S2). In the analysis from immune cells phenotype to serum metabolites, 17,836 SNPs were included, with F-statistics varying from 19.537 to 3159.289 (Table S3). Finally, in the analysis from serum metabolites to immune cells phenotype, 34,856 SNPs were included, with F-statistics fluctuating from 19.503 to 2297.785 (Table S4). All selected SNPs had F-statistics exceeding 10, thereby affirming their reliability.

3.2 Investigating the mediating role of serum metabolites in immune cells and OC

Given that immune cells are essential for ovarian development, pathogenic processes, and functional maintenance[32], we probed into the causal links between immune cells and OC. Among the 731 immune cells phenotype and OC were analyzed, 36 significant causal relationships were identified, with 15 being protective factors and 21 being risk factors. Specifically, "resting CD4 regulatory T cell %CD4 regulatory T cell" [odds ratio (OR) = 0.977, 95% confidence interval (CI) = 0.958-0.996, p = 0.018) and "CD25 on $CD39^{+}$ resting CD4 regulatory T cell" (OR = 0.946, 95% CI = 0.899-0.995, p = 0.032) were protective against OC. Conversely, "CD19 on IgD^{+} $CD38^{-}$ unswitched memory B cell" (OR = 1.037, 95% CI = 1.014-1.060, p = 0.002) and "IgD^{-} $CD38^{dim}$ B cell %B cell" (OR = 1.027, 95% CI = 1.004-1.050, p = 0.021) were risk factors for OC (Figure 1). Subsequently, reverse MR analysis was performed to exclude potential bidirectional effects, with no significant associations found, confirming the validity of the causal links identified in the forward MR analysis.

Considering the substantial effect of immune cells, we further explored their influence on OC through serum metabolites. This involved analyzing the causal links between 1,400 serum metabolites and OC, leading to the identification of 89 significant associations (Figure 2). On this basis, we checked into the causal relationships between immune cells phenotype and serum metabolites, finding 156 associations between 36 immune cells phenotype and 80 serum metabolites, including 78 protective and 78 risk factors (Table S5). In the mediation analysis, 4 significant associations were identified: "2R,3R−dihydroxybutyrate levels" increased the risk effect of "$CD11c^{+}$ $CD62L^{-}$

monocyte Absolute Count" on OC (11.300%). "N−lactoyl phenylalanine (Lac-Phe) levels" attenuated the risk effect of "CD27 on IgD^- $CD38^+$ B cell" on OC (21.230%), and "X−12221 levels" attenuated the risk effect of "CD19 on IgD^+ $CD38^-$ unswitched memory B cell" on OC (12.342%). "Pseudouridine levels" attenuated the protective effect of "CD4+CD8+ T cell Absolute Count" on OC (14.010%) (Figure 3). Due to the unclear levels of X−12221, 3 mediating associations were finally validated, uncovering the interactions between metabolites and immune cell phenotypes as well as their influence on OC risk.

3.3 Exploring the influence of serum metabolites on OC via immune cells phenotype

Metabolomics, an emerging branch of systems biology, has made significant progress in cancer research in recent years, substantially enhancing the understanding, diagnosis, and treatment of diverse cancers, including OC[33]. Metabolomics can provide detailed information on metabolic changes during disease onset and progression, offering new perspectives for early diagnosis. Therefore, MR analysis was conducted on 1,400 serum metabolites and OC. In this process, 89 significant associations were identified, including 49 protective factors and 40 risk factors for OC (Figure 2). "Mannose to mannitol to sorbitol ratio" (OR = 1.104, 95% CI = 1.008-1.208, p = 0.033) and "Oleoyl−linoleoyl−glycerol (18:1 to 18:2) to linoleoyl−arachidonoyl−glycerol (18:2 to 20:4) ratio" (OR = 1.058, 95% CI = 1.012-1.106, p = 0.013) exhibited risk effects on OC. Conversely, "Adenosine 5'−monophosphate (AMP) to acetoacetate ratio" (OR = 0.929, 95% CI = 0.866-0.997, p = 0.041) and "sulfate of piperine metabolite C18H21NO3 (1)

levels" (OR = 0.925, 95% CI = 0.860-0.996, p = 0.039) were protective factors. Similarly, to exclude the possibility of bidirectional effects, the causal impact of OC on these serum metabolites was assessed, with no significant reverse associations found.

Subsequently, the role of serum metabolites in influencing OC through immune cells phenotype was explored. By calculating the causal relationships between 731 immune cells phenotype and OC, 36 significant associations were identified (Figure 1). Based on these significant results, the causal links between serum metabolites and immune cells phenotype were evaluated, identifying 143 significant associations between 69 serum metabolites and 34 immune cells phenotype, including 68 protective and 75 risk factors (Table S6). Subsequently, mediation analysis was conducted to explore whether these serum metabolites influenced OC through specific immune cells. The results identified two significant associations: "IgD$^-$ CD24$^-$ AC" attenuated the protective effect of "arachidonate (20:4n6) to paraxanthine ratio" on OC (19.450%), and "CD4 Treg %CD4" attenuated the risk effect of "Gamma−CEHC glucuronide levels" on OC (17.580%) (Figure 4). These findings suggested that certain metabolites might alter the immune microenvironment of OC by adjusting immune cell activity or function, which in turn affects OC's onset and progression.

3.4 Sensitivity analysis

To verify the accuracy of causal inferences in this study, comprehensive sensitivity analyses were performed. The causal results were consistent with the MR-PRESSO method, with no outliers detected. The MR-Egger test results showed p-values exceeding

0.05, indicating no pleiotropic bias (Table S7). Subsequently, when facing heterogeneity in MR analysis, a random-effects IVW was employed (Table S7), still yielding robust causal inference results. Furthermore, leave-one-out analysis revealed that the causal inference conclusions remained largely unaffected despite the removal of one SNP in each iteration. These findings ensured the reliability of the analysis results.

4. Discussion

Given the unclear interrelationships among immune cells, serum metabolites, and OC, this study utilized MR analysis to systematically probe into the causal links between immune cells/serum metabolites and OC, further investigating the mediating roles of these factors in OC development. We ultimately identified 3 significant pairs of relationships mediated by serum metabolites and 2 associations mediated by immune cells. These findings not only established a novel theoretical framework for comprehending the etiology of OC but also presented new avenues for its early identification and therapy.

Through MR analysis, we identified "HLA DR^{++} monocyte %monocyte" as a protective factor for OC. Monocytes' HLA-DR surface expression denotes their activation state[34], which aids in determining their immunological status[35]. Some studies indicate that a certain monocyte subpopulation may possess immunosuppressive properties by suppressing T cell proliferation and differentiation, facilitating the development of regulatory T cells, and secreting anti-inflammatory mediators. This immune regulatory mechanism can safeguard the ovaries from autoimmune injury, therefore diminishing the

chance of premature ovarian failure (POF) [36], which seemed to be consistent with our findings. Besides, our research revealed that "$CD28^{-}$ $CD25^{++}$ $CD8^{+}$ T cell Absolute Count" is a risk factor for OC, corroborated by existing research results. Studies have shown that an increased relative count of $CD28^{-}$ CD25CD8 T cells correlates with an elevated risk of infertility in women[37]. Another MR analysis revealed that the greatest risk factor for infertility is $CD28^{-}CD25^{++}$ $CD8^{+}$ %T cells[38]. The results indicated that the onset and advancement of OC were intricately affected by the immune system.

Metabolites, as products and substrates of cellular metabolic processes, which was tightly tied to cancer[39]. Therefore, we marched investigation into the link between serum metabolites and OC and ultimately identified 89 associations with causal relationships. Among these associations, "palmitoylcarnitine levels" were considered a protective factor for OC. The primary job of palmitoylcarnitine is to ferry long-chain fatty acids into the mitochondria for β-oxidation, thereby providing cell energy[40]. Consequently, focusing on palmitoylcarnitine and leveraging its capacity to provoke oxidative stress in cancer cells may offer a possible supplementary approach for the treatment of OC[41,42]. In contrast, "Trimethylamine N−oxide (TMAO) levels" were recognized as a risk factor for OC. Studies have shown that TMAO can upregulate macrophage scavenger receptors, promotes cholesterol accumulation and foam cell formation, activates MAPK and nuclear factor-κB pathways, thereby promoting plaque formation and inflammatory responses[43]. Moreover, heightened plasma levels of TMAO may correlate with the pathophysiology of polycystic ovarian syndrome (PCOS) absent hyperandrogenism (HA)

and are significantly linked to augmented systemic inflammation[44]. This discovery indicates that TMAO may facilitate the onset and progression of OC via many pathways, which aligned with our results. In summary, the intricate connection between OC and serum metabolites has been made clear by this investigation.

To explore immune cells' mediating role in the serum metabolites-OC relationship, mediation analysis revealed that "CD4 Treg %CD4" attenuated OC risk associated with "Gamma−CEHC glucuronide levels"- a vitamin E metabolite and OC risk factor. Research has proven that the anticancer efficacy of tumor-infiltrating cytotoxic CD8+ T cells in OC seems to be impacted by the presence of CD4 Tregs[45], suggesting that CD4 Tregs may promote the progression of OC. However, studies have certified that depleting Tregs during OC can boost immunity and perhaps have therapeutic benefits[46,47]. Additionally, studies have maintained that and elevated number of CD4+ T cells is positively connected with the clinical features and tumor size of OC[48,49]. This suggested that modulating the ratio of CD4 Tregs to CD4+ T cells might provide a potential target for developing novel immunomodulatory therapeutic strategies. Additionally, we also addressed the mediating function of serum metabolites in the interaction between immune cells and OC. Mediation analysis showed that "Lac-Phe levels" attenuated the risk of OC associated with "CD27 on IgD^- $CD38^+$ B cell"- a confirmed risk factor for OC. Higher IgD^- $CD38^+$ B cells link to stronger inflammation[50], which may promote OC via tumor microenvironment formation. In both mice and humans, plasma Lac-Phe concentrations rise in response to stimuli that

enhance circulating lactate or phenylalanine levels or increase glycolytic flux[51]. Research indicates that elevated levels of Lac-Phe serve as a significant protective factor against OC[52]. These findings suggest Lac-Phe's mediated protective effect may stem from its role in modulating immune cell function and suppressing inflammation, offering a fresh perspective on OC's underlying immunometabolic mechanisms.

We have broken through the traditional single-variable research model in terms of perspective and are the first to use mediating MR to systematically evaluate the causal relationship among immune cell phenotypes, serum metabolites and OC. Specific targets such as "CD4 Treg %CD4" and "Lac-Phe levels" have been proposed and hold promise for advancing targeted regulatory therapies. For instance, strategies involving the selective depletion of CD4 Treg cells or the development of Lac-Phe or its analogs as metabolic intervention agents warrant further investigation and validation. Despite making significant progress in elucidating the immunometabolic mechanisms of OC, our research has encountered tons limitations. The principal constraint is the substantial dependence on data from European populations, potentially introducing specific biases. Furthermore, the outcomes of our mediation study have not been corroborated by further experimental trials, necessitating further research to validate these causal links. The stratification of the research subjects is not clear and only some immune cells and serum metabolites are focused on, which may limit the universality of the conclusion and miss key mediator pathways.

5. Conclusion

In summary, our MR study thoroughly investigated the causative connections among immune cell phenotypes, serum metabolites, and OC. The research discovered 36 notable causal relationships between immune cell phenotypes and OC, along with 89 connections between serum metabolites and and OC. Furthermore, by mediation analysis, we clarified the mediating role of serum metabolites and immune cells in OC. These findings elucidate the significant functions of immune cells and serum metabolites in OC and offer new perspectives for its early identification..

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Conflict of interest statement

The authors declare no conflicts of interest.

Ethics declarations

Not applicable.

Author contributions

Guansheng Chen: Writing – original draft, Software, Project administration, Methodology, Investigation, Data curation, Conceptualization.

YongjunWang: Validation, Software, Formal analysis.

Lingyu Liu: Validation, Software, Formal analysis.

Wenjing Li: Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization.

Data Sharing Statement

The genome-wide association study (GWAS) dataset for OC was obtained from the IEU OpenGWAS project (https://gwas.mrcieu.ac.uk/). Immune phenotypes were obtained from the GWAS catalog (https://www.ebi.ac.uk/gwas/).

Figure legends

Figure 1 Results of Mendelian randomization (MR) analysis of immune cells phenotype on ovarian cancer (OC). IVW, inverse variance weighted; nsnp, number of single nucleotide polymorphism; pval, pvalue; or, odds ratio; CI, confidence interval; pleio_P, pleiotropy pvalue.

Figure 2 Results of MR analysis between serum metabolites and OC. IVW, inverse variance weighted; nsnp, number of single nucleotide polymorphism; pval, pvalue; or, odds ratio; CI, confidence interval; pleio_P, pleiotropy pvalue.

Figure 3 The impact of immune cells phenotype on OC regulated by serum metabolites. nsnp, number of single nucleotide polymorphism; pval, pvalue; or, odds ratio; CI, confidence interval; pleio_P, pleiotropy pvalue; a, the total effect of immune cells phenotype on OC; b, the effect of serum metabolites on OC; c, the effect of immune cells phenotype on serum metabolites.

Figure 4 Results of mediation analysis of serum metabolites via immune cells phenotype for OC. nsnp, number of single nucleotide polymorphism; pval, pvalue; or, odds ratio; CI, confidence interval; pleio_P, pleiotropy pvalue; a, the total effect of serum metabolites on OC; b, the effect of immune cells phenotype on OC; c, the effect of serum metabolites on immune cells phenotype.

Supplementary information

Table S1. Screening results of single nucleotide polymorphisms (SNPs) with immune cells phenotype on ovarian cancer (OC).

Table S2. Screening results of SNPs with serum metabolites on OC.

Table S3. Screening results of SNPs with immune cells phenotype on serum metabolites.

Table S4. Screening results of SNPs with serum metabolites on immune cells phenotype.

Table S5. Mendelian randomization (MR) analysis between immune cells phenotype and serum metabolites.

Table S6. MR analysis between serum metabolites and immune cells phenotype.

Table S7. Heterogeneity and horizontal pleiotropy tests for MR analysis.

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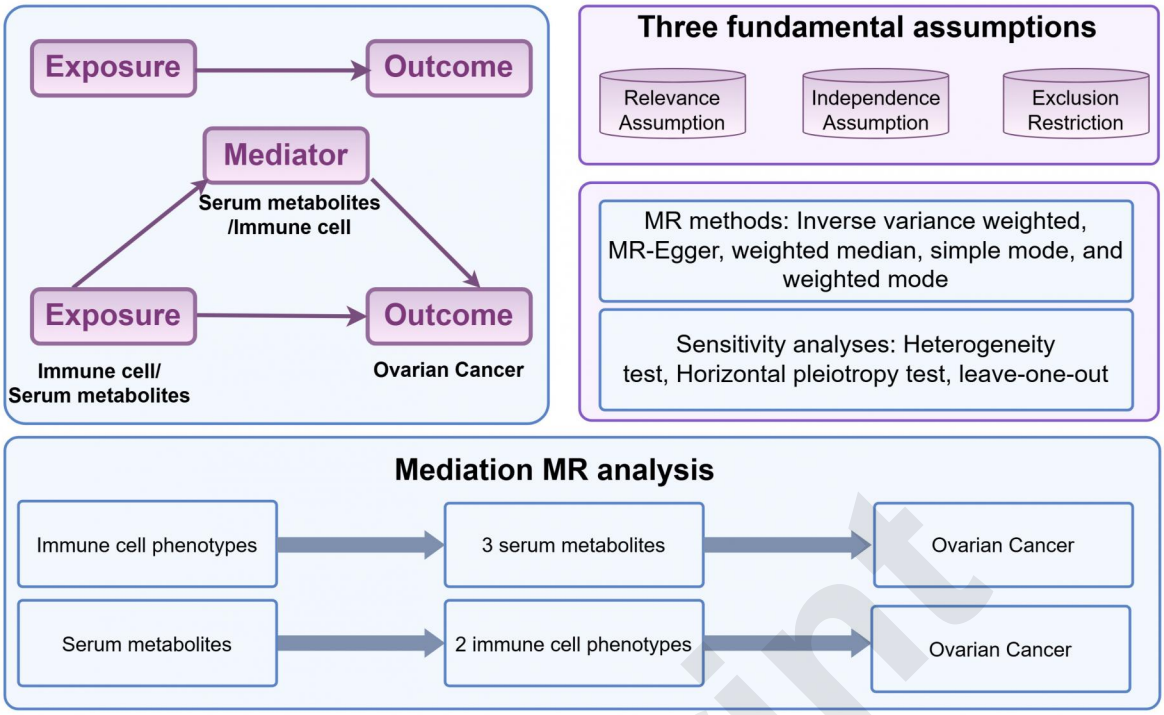
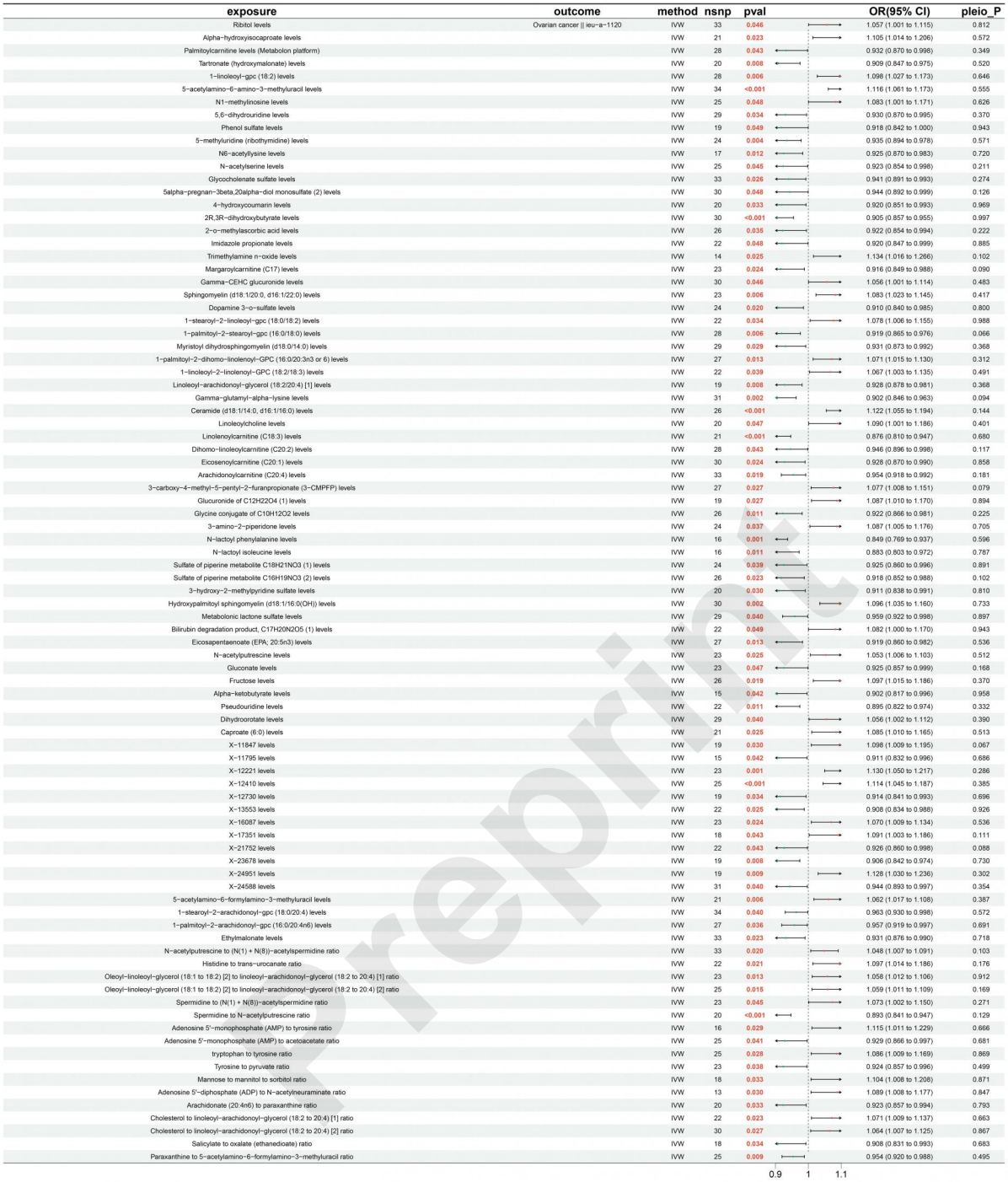
Immune Cells, Serum Metabolites, and Ovarian Cancer: A Mediation Mendelian Randomization Study



exposure	outcome	method	nsnp	pval	OR(95% CI)	pleio_P
IgD+ CD38− B cell Absolute Count	Ovarian cancer || ieu-a-1120	IVW	17	0.022	0.940 (0.892 to 0.991)	0.430
Plasma Blast−Plasma Cell %B cell		IVW	26	0.018	1.039 (1.007 to 1.072)	0.389
Plasma Blast−Plasma Cell Absolute Count		IVW	23	0.021	0.968 (0.942 to 0.995)	0.600
IgD− CD24− B cell Absolute Count		IVW	18	<0.001	1.081 (1.033 to 1.133)	0.633
IgD− CD38+ B cell %lymphocyte		IVW	27	0.036	0.976 (0.953 to 0.998)	0.835
IgD− CD38dim B cell %B cell		IVW	24	0.021	1.027 (1.004 to 1.050)	0.468
CD11c+ CD62L− monocyte Absolute Count		IVW	22	0.018	1.042 (1.007 to 1.079)	0.509
HLA DR++ monocyte %monocyte		IVW	20	0.008	0.957 (0.927 to 0.989)	0.657
CD4 regulatory T cell %CD4+ T cell		IVW	18	<0.001	1.077 (1.033 to 1.123)	0.352
Resting CD4 regulatory T cell %CD4 regulatory T cell		IVW	30	0.018	0.977 (0.958 to 0.996)	0.772
Resting CD4 regulatory T cell %CD4+ T cell		IVW	32	0.009	0.974 (0.954 to 0.993)	0.142
Central Memory CD4−CD8− T cell Absolute Count		IVW	4	0.003	1.079 (1.027 to 1.134)	0.340
CD4+CD8+ T cell Absolute Count		IVW	18	0.023	0.945 (0.900 to 0.992)	0.085
CD28− CD25++ CD8+ T cell Absolute Count		IVW	25	0.042	1.030 (1.001 to 1.061)	0.658
CD25++ CD8+ T cell %T cell		IVW	17	0.028	1.048 (1.005 to 1.093)	0.934
CD25++ CD8+ T cell %CD8+ T cell		IVW	22	0.006	1.059 (1.017 to 1.103)	0.171
CD28+ CD45RA+ CD8+ T cell Absolute Count		IVW	53	0.021	1.009 (1.001 to 1.016)	0.548
CD19 on IgD+ CD38− unswitched memory B cell		IVW	24	0.002	1.037 (1.014 to 1.060)	0.535
CD20 on IgD+ CD24− B cell		IVW	23	0.037	1.027 (1.002 to 1.053)	0.744
CD25 on CD20− CD38− B cell		IVW	14	0.028	1.064 (1.007 to 1.125)	0.481
CD27 on IgD− CD38+ B cell		IVW	11	0.037	1.079 (1.005 to 1.159)	0.793
CD38 on IgD+ CD24− B cell		IVW	17	0.044	1.047 (1.001 to 1.095)	0.867
CD62L on CD62L+ Dendritic Cell		IVW	24	0.042	1.040 (1.001 to 1.080)	0.136
CD3 on CD4 regulatory T cell		IVW	11	0.017	1.042 (1.007 to 1.078)	0.257
HVEM on Effector Memory CD8+ T cell		IVW	18	0.003	0.962 (0.937 to 0.987)	0.282
CD45 on HLA DR+ T cell		IVW	16	0.031	1.042 (1.004 to 1.082)	0.784
CD127 on CD45RA+ CD4+ T cell		IVW	21	0.035	1.039 (1.003 to 1.077)	0.389
CD25 on CD39+ resting CD4 regulatory T cell		IVW	15	0.032	0.946 (0.899 to 0.995)	0.941
CD25 on CD39+ activated CD4 regulatory T cell		IVW	12	0.044	1.061 (1.002 to 1.123)	0.457
CD33 on CD33+ HLA DR+ CD14dim		IVW	27	0.046	1.017 (1.000 to 1.034)	0.121
FSC−A on HLA DR+ CD4+ T cell		IVW	16	0.007	0.941 (0.901 to 0.984)	0.902
CCR2 on granulocyte		IVW	17	0.041	0.966 (0.934 to 0.998)	0.667
CD80 on CD62L+ myeloid Dendritic Cell		IVW	26	0.023	0.968 (0.941 to 0.996)	0.985
SSC−A on plasmacytoid Dendritic Cell		IVW	24	0.039	0.967 (0.936 to 0.998)	0.441
SSC−A on HLA DR+ T cell		IVW	22	0.036	0.958 (0.920 to 0.997)	0.217
CD45RA on naive CD4+ T cell		IVW	31	0.031	0.976 (0.954 to 0.998)	0.051

Figure 1 Results of Mendelian randomization (MR) analysis of immune cells phenotype on ovarian cancer (OC). IVW, inverse variance weighted; nsnp, number of single nucleotide polymorphism; pval, pvalue; or, odds ratio; CI, confidence interval; pleio_P, pleiotropy pvalue.



exposure	outcome	method	nsnp	pval	OR(95% CI)	pleio_P
Ribitol levels	Ovarian cancer || ieu-a-1120	IVW	33	0.046	1.057 (1.001 to 1.115)	0.812
Alpha-hydroxyisocaproate levels		IVW	21	0.023	1.105 (1.014 to 1.206)	0.572
Palmitoylcarnitine levels (Metabolon platform)		IVW	28	0.043	0.932 (0.870 to 0.998)	0.349
Tartronate (hydroxymalonate) levels		IVW	20	0.008	0.909 (0.847 to 0.975)	0.520
1-linoleoyl-gpc (18:2) levels		IVW	28	0.006	1.098 (1.027 to 1.173)	0.646
5-acetylamino-6-amino-3-methyluracil levels		IVW	34	<0.001	1.116 (1.061 to 1.173)	0.555
N1-methylinosine levels		IVW	25	0.048	1.083 (1.001 to 1.171)	0.626
5,6-dihydrouridine levels		IVW	29	0.034	0.930 (0.870 to 0.995)	0.370
Phenol sulfate levels		IVW	19	0.049	0.918 (0.842 to 1.000)	0.943
5-methyluridine (ribothymidine) levels		IVW	24	0.004	0.935 (0.894 to 0.978)	0.571
N6-acetyllysine levels		IVW	17	0.012	0.925 (0.870 to 0.983)	0.720
N-acetylserine levels		IVW	25	0.045	0.923 (0.854 to 0.998)	0.211
Glycocholenate sulfate levels		IVW	33	0.026	0.941 (0.891 to 0.993)	0.274
5alpha-pregnan-3beta,20alpha-diol monosulfate (2) levels		IVW	30	0.048	0.944 (0.892 to 0.999)	0.126
4-hydroxycoumarin levels		IVW	20	0.033	0.920 (0.851 to 0.993)	0.969
2R,3R-dihydroxybutyrate levels		IVW	30	<0.001	0.905 (0.857 to 0.955)	0.997
2-o-methylascorbic acid levels		IVW	26	0.035	0.922 (0.854 to 0.994)	0.222
Imidazole propionate levels		IVW	22	0.048	0.920 (0.847 to 0.999)	0.885
Trimethylamine n-oxide levels		IVW	14	0.025	1.134 (1.016 to 1.266)	0.102
Margaroylcarnitine (C17) levels		IVW	23	0.024	0.916 (0.849 to 0.988)	0.090
Gamma-CEHC glucuronide levels		IVW	30	0.046	1.056 (1.001 to 1.114)	0.483
Sphingomyelin (d18:1/20:0, d16:1/22:0) levels		IVW	23	0.006	1.083 (1.023 to 1.145)	0.417
Dopamine 3-o-sulfate levels		IVW	24	0.020	0.910 (0.840 to 0.985)	0.800
1-stearoyl-2-linoleoyl-gpc (18:0/18:2) levels		IVW	22	0.034	1.078 (1.006 to 1.155)	0.988
1-palmitoyl-2-stearoyl-gpc (16:0/18:0) levels		IVW	28	0.006	0.919 (0.865 to 0.976)	0.066
Myristoyl dihydrosphingomyelin (d18:0/14:0) levels		IVW	29	0.029	0.931 (0.873 to 0.992)	0.368
1-palmitoyl-2-dihomo-linolenoyl-GPC (16:0/20:3n3 or 6) levels		IVW	27	0.013	1.071 (1.015 to 1.130)	0.312
1-linoleoyl-2-linolenoyl-GPC (18:2/18:3) levels		IVW	22	0.039	1.067 (1.003 to 1.135)	0.491
Linoleoyl-arachidonoyl-glycerol (18:2/20:4) [1] levels		IVW	19	0.008	0.928 (0.878 to 0.981)	0.368
Gamma-glutamyl-alpha-lysine levels		IVW	31	0.002	0.902 (0.846 to 0.963)	0.094
Ceramide (d18:1/14:0, d16:1/16:0) levels		IVW	26	<0.001	1.122 (1.055 to 1.194)	0.144
Linoleoylcholine levels		IVW	20	0.047	1.090 (1.001 to 1.186)	0.401
Linolenoylcarnitine (C18:3) levels		IVW	21	<0.001	0.876 (0.810 to 0.947)	0.680
Dihomo-linoleoylcarnitine (C20:2) levels		IVW	28	0.043	0.946 (0.896 to 0.998)	0.117
Eicosenoylcarnitine (C20:1) levels		IVW	30	0.024	0.928 (0.870 to 0.990)	0.858
Arachidonoylcarnitine (C20:4) levels		IVW	33	0.019	0.954 (0.918 to 0.992)	0.181
3-carboxy-4-methyl-5-pentyl-2-furanpropionate (3-CMPFP) levels		IVW	27	0.027	1.077 (1.008 to 1.151)	0.079
Glucuronide of C12H22O4 (1) levels		IVW	19	0.027	1.087 (1.010 to 1.170)	0.894
Glycine conjugate of C10H12O2 levels		IVW	26	0.011	0.922 (0.866 to 0.981)	0.225
3-amino-2-piperidone levels		IVW	24	0.037	1.087 (1.005 to 1.176)	0.705
N-lactoyl phenylalanine levels		IVW	16	0.001	0.849 (0.769 to 0.937)	0.596
N-lactoyl isoleucine levels		IVW	16	0.011	0.883 (0.803 to 0.972)	0.787
Sulfate of piperine metabolite C18H21NO3 (1) levels		IVW	24	0.039	0.925 (0.860 to 0.996)	0.891
Sulfate of piperine metabolite C16H19NO3 (2) levels		IVW	26	0.023	0.918 (0.852 to 0.988)	0.102
3-hydroxy-2-methylpyridine sulfate levels		IVW	20	0.030	0.911 (0.838 to 0.991)	0.810
Hydroxypalmitoyl sphingomyelin (d18:1/16:0(OH)) levels		IVW	30	0.002	1.096 (1.035 to 1.160)	0.733
Metabolonic lactone sulfate levels		IVW	29	0.040	0.959 (0.922 to 0.998)	0.897
Bilirubin degradation product, C17H20N2O5 (1) levels		IVW	22	0.049	1.082 (1.000 to 1.170)	0.943
Eicosapentaenoate (EPA; 20:5n3) levels		IVW	27	0.013	0.919 (0.860 to 0.982)	0.536
N-acetylputrescine levels		IVW	23	0.025	1.053 (1.006 to 1.103)	0.512
Gluconate levels		IVW	23	0.047	0.925 (0.857 to 0.999)	0.168
Fructose levels		IVW	26	0.019	1.097 (1.015 to 1.186)	0.370
Alpha-ketobutyrate levels		IVW	15	0.042	0.902 (0.817 to 0.996)	0.958
Pseudouridine levels		IVW	22	0.011	0.895 (0.822 to 0.974)	0.332
Dihydroorotate levels		IVW	29	0.040	1.056 (1.002 to 1.112)	0.390
Caproate (6:0) levels		IVW	21	0.025	1.085 (1.010 to 1.165)	0.513
X-11847 levels		IVW	19	0.030	1.098 (1.009 to 1.195)	0.067
X-11795 levels		IVW	15	0.042	0.911 (0.832 to 0.996)	0.686
X-12221 levels		IVW	23	0.001	1.130 (1.050 to 1.217)	0.286
X-12410 levels		IVW	25	<0.001	1.114 (1.045 to 1.187)	0.385
X-12730 levels		IVW	19	0.034	0.914 (0.841 to 0.993)	0.696
X-13553 levels		IVW	22	0.025	0.908 (0.834 to 0.988)	0.926
X-16087 levels		IVW	23	0.024	1.070 (1.009 to 1.134)	0.536
X-17351 levels		IVW	18	0.043	1.091 (1.003 to 1.186)	0.111
X-21752 levels		IVW	22	0.043	0.926 (0.860 to 0.998)	0.088
X-23678 levels		IVW	19	0.008	0.906 (0.842 to 0.974)	0.730
X-24951 levels		IVW	19	0.009	1.128 (1.030 to 1.236)	0.302
X-24588 levels		IVW	31	0.040	0.944 (0.893 to 0.997)	0.354
5-acetylamino-6-formylamino-3-methyluracil levels		IVW	21	0.006	1.062 (1.017 to 1.108)	0.387
1-stearoyl-2-arachidonoyl-gpc (18:0/20:4) levels		IVW	34	0.040	0.963 (0.930 to 0.998)	0.572
1-palmitoyl-2-arachidonoyl-gpc (16:0/20:4n6) levels		IVW	27	0.036	0.957 (0.919 to 0.997)	0.691
Ethylmalonate levels		IVW	33	0.023	0.931 (0.876 to 0.990)	0.718
N-acetylputrescine to (N(1) + N(8))-acetylspermidine ratio		IVW	33	0.020	1.048 (1.007 to 1.091)	0.103
Histidine to trans-urocanate ratio		IVW	22	0.021	1.097 (1.014 to 1.186)	0.176
Oleoyl-linoleoyl-glycerol (18:1 to 18:2) [2] to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [1] ratio		IVW	23	0.013	1.058 (1.012 to 1.106)	0.912
Oleoyl-linoleoyl-glycerol (18:1 to 18:2) [2] to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio		IVW	25	0.015	1.059 (1.011 to 1.109)	0.169
Spermidine to (N(1) + N(8))-acetylspermidine ratio		IVW	23	0.045	1.073 (1.002 to 1.150)	0.271
Spermidine to N-acetylputrescine ratio		IVW	20	<0.001	0.893 (0.841 to 0.947)	0.129
Adenosine 5'-monophosphate (AMP) to tyrosine ratio		IVW	16	0.029	1.115 (1.011 to 1.229)	0.666
Adenosine 5'-monophosphate (AMP) to acetoacetate ratio		IVW	25	0.041	0.929 (0.866 to 0.997)	0.681
tryptophan to tyrosine ratio		IVW	25	0.028	1.086 (1.009 to 1.169)	0.869
Tyrosine to pyruvate ratio		IVW	23	0.038	0.924 (0.857 to 0.996)	0.499
Mannose to mannitol to sorbitol ratio		IVW	18	0.033	1.104 (1.008 to 1.208)	0.871
Adenosine 5'-diphosphate (ADP) to N-acetylneuraminate ratio		IVW	13	0.030	1.089 (1.008 to 1.177)	0.847
Arachidonate (20:4n6) to paraxanthine ratio		IVW	20	0.033	0.923 (0.857 to 0.994)	0.793
Cholesterol to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [1] ratio		IVW	22	0.023	1.071 (1.009 to 1.137)	0.663
Cholesterol to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio		IVW	30	0.027	1.064 (1.007 to 1.125)	0.867
Salicylate to oxalate (ethanedioate) ratio		IVW	18	0.034	0.908 (0.831 to 0.993)	0.683
Paraxanthine to 5-acetylamino-6-formylamino-3-methyluracil ratio		IVW	25	0.009	0.954 (0.920 to 0.988)	0.495

Figure 2 Results of MR analysis between serum metabolites and OC. IVW, inverse variance weighted; nsnp, number of single nucleotide polymorphism; pval, pvalue; or, odds ratio; CI, confidence interval; pleio_P, pleiotropy pvalue.

Outcome(Y)	Expouse(X)	Mediate(M)	beta	nsnp	pval	OR(95% CI)	pleio_P	Mediated effect	Direct effect	Mediated proportion(%)
Ovarian cancer	CD11c+ CD62L− monocyte Absolute Count	2R,3R−dihydroxybutyrate levels	a	22	0.018	1.042 (1.007 to 1.079)	0.509	c*b	a−c*b	c*b/a
			c	23	0.008	0.954 (0.921 to 0.988)	0.191	1.005 (1.000 to 1.009)	1.038 (1.002 to 1.074)	11.300 (0.656 to 21.944)
			b	30	<0.001	0.905 (0.857 to 0.955)	0.997	pval=0.0374	pval=0.0376	pval=0.0374
	CD19 on IgD+ CD38− unswitched memory B cell	X−12221 levels	a	24	0.002	1.037 (1.014 to 1.060)	0.535	c*b	a−c*b	c*b/a
			c	22	0.007	0.964 (0.939 to 0.990)	0.690	0.996 (0.991 to 1.000)	1.041 (1.018 to 1.065)	12.342 (0.380 to 24.303)
			b	23	0.001	1.130 (1.050 to 1.217)	0.286	pval=0.0431	pval=0.000463	pval=0.0431
	CD27 on IgD− CD38+ B cell	N−lactoyl phenylalanine levels	a	11	0.037	1.079 (1.005 to 1.159)	0.793	c*b	a−c*b	c*b/a
			c	15	0.003	1.104 (1.033 to 1.179)	0.776	0.984 (0.969 to 0.999)	1.097 (1.020 to 1.180)	21.230 (1.620 to 40.840)
			b	16	0.001	0.849 (0.769 to 0.937)	0.596	pval=0.0338	pval=0.0132	pval=0.0338
	CD4+CD8+ T cell Absolute Count	Pseudouridine levels	a	18	0.023	0.945 (0.900 to 0.992)	0.085	c*b	a−c*b	c*b/a
			c	19	0.001	0.931 (0.892 to 0.972)	0.442	1.008 (1.000 to 1.016)	0.938 (0.892 to 0.985)	14.010 (0.005 to 28.015)
			b	22	0.011	0.895 (0.822 to 0.974)	0.332	pval=0.0499	pval=0.0106	pval=0.0499

Figure 3 The impact of immune cells phenotype on OC regulated by serum metabolites. nsnp, number of single nucleotide polymorphism; pval, pvalue; or, odds ratio; CI, confidence interval; pleio_P, pleiotropy pvalue; a, the total effect of immune cells phenotype on OC; b, the effect of serum metabolites on OC; c, the effect of immune cells phenotype on serum metabolites.

Outcome(Y)	Expouse(X)	Mediate(M)	beta	nsnp	pval	OR(95% CI)	pleio_P	Mediated effect	Direct effect	Mediated proportion(%)
Ovarian cancer	Arachidonate (20:4n6) to paraxanthine ratio	IgD− CD24− AC	a	20	0.033	0.923 (0.857 to 0.994)	0.793	c*b	a−c*b	c*b/a
			c	21	0.002	1.220 (1.075 to 1.385)	0.829	1.016 (1.002 to 1.030)	0.909 (0.843 to 0.980)	19.450 (2.179 to 36.721)
			b	18	<0.001	1.081 (1.033 to 1.133)	0.633	pval=0.0273	pval=0.0125	pval=0.0273
	Gamma−CEHC glucuronide levels	CD4 Treg %CD4	a	30	0.046	1.056 (1.001 to 1.114)	0.483	c*b	a−c*b	c*b/a
			c	31	0.010	0.879 (0.797 to 0.970)	0.684	0.990 (0.981 to 1.000)	1.066 (1.010 to 1.126)	17.580 (0.495 to 34.666)
			b	18	<0.001	1.077 (1.033 to 1.123)	0.352	pval=0.0437	pval=0.021	pval=0.0437

Figure 4 Results of mediation analysis of serum metabolites via immune cells phenotype for OC. nsnp, number of single nucleotide polymorphism; pval, pvalue; or, odds ratio; CI, confidence interval; pleio_P, pleiotropy pvalue; a, the total effect of serum metabolites on OC; b, the effect of immune cells phenotype on OC; c, the effect of serum metabolites on immune cells phenotype.