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# The Causal Impact of Immune Cell Dynamics on liver failure: An Investigation via Mendelian Randomization (MR) Analysis

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**Abstract.** *Background:* Liver failure is a critical clinical condition with complex pathophysiology, where the role of immune cells has been increasingly recognized. However, establishing causality between immune cell activity and liver failure has been challenging. We aimed to investigate the causal role of specific immune cell populations in the development of liver failure using Mendelian randomization (MR) techniques. *Methods*: Genetic data from the Genome-wide association study (GWAS) database, focusing on 731 immunophenotypes and a liver failure dataset was utilised. Single nucleotide polymorphisms (SNPs) associated with immune cell exposures were identified and used as instrumental variables. MR analyses were performed using the TwoSampleMR package in R, employing various MR techniques, including weighted median, MR Egger, inverse-variance weighted (IVW), and others. The robustness of the MR findings was assessed through heterogeneity, pleiotropy, and leave-one-out sensitivity analyses. *Results*: Our MR study revealed significant causal associations between several immune cell populations and liver failure. Notably, genetic variants associated with CD28 on CD39+ CD4+ T cells and CD3 on CD39+ secreting T Reg cells were found to be causally linked to an increased risk of liver failure. The IVW MR method, which has been previously validated for its performance, provided the most consistent results across immune cell exposures. *Conclusion*: Our findings suggest a direct causal role for specific immune cells in liver failure, offering potential targets for future therapeutic strategies.

**Keywords:** immune cells, liver failure, Mendelian randomization, Causal inference.

## 1. Introduction

Liver failure is a clinical syndrome characterised by jaundice, hepatic encephalopathy, and coagulation disorders due to severe liver damage leading to dysfunction in its synthesis, detoxification, excretion, and biotransformation [1], with a morbidity and mortality rate as high as 60%–80%. Usually, a complicated interaction of immunological, environmental, and genetic variables results in liver failure. Clinically, a variety of systemic complications, such as infections, gastrointestinal bleeding and hepatic encephalopathy, often occur, which seriously threaten the lives of patients [2].The liver, as a vital organ with multifaceted functions, is particularly susceptible to the detrimental effects of immune dysregulation, which can lead to a spectrum of liver diseases ranging from mild inflammation to severe

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cirrhosis and hepatocellular carcinoma. Liver failure is a life-threatening disease, and is accompanied by high complications and high morbidity and mortality. The role of immune cells in the pathogenesis of liver failure has been increasingly recognized, yet the causal relationship between immune cell activity and liver disease progression remains to be fully elucidated. Early diagnosis and targeted interventions are of great clinical importance [3,4].

The pathogenesis of liver failure is complex and has become a research hotspot in hepatology. Liver failure is generally considered to be a multi-step, multi-gene, and multi-factor interaction process. The action of the pathogen and the host immune system can lead to the co-existence of the liver microenvironment disorder, hepatocyte necrosis, and apoptosis [6].Recent advances in immunology have highlighted the intricate balance between pro-inflammatory and anti-inflammatory responses in the liver, with immune cells such as macrophages, T cells, and natural killer (NK) cells playing pivotal roles in both the resolution and exacerbation of liver damage[12].The proportion of macrophages in each immune cell, the state of immune function, and the type of inflammatory factors released determine the local inflammatory state of the liver, thereby affecting the clinical characteristics and outcome of liver failure. Macrophages are also the main source of endotoxin-responsive cells and inflammatory cytokines in the liver, and when activated by endotoxins or TNF-α, they can release pro-inflammatory and anti-inflammatory factors (TNF-α, IL-6, IL-10), chemokines (IL-8, IP-10), and other inflammatory-related mediators (reactive oxygen species, prostaglandins), which are involved in the occurrence and development of various acute and chronic liver injuries [7].

Mendelian randomisation (MR) is a statistical method of epidemiology for inferring the causal association between exposure and outcome based on a working variable, namely SNP[10]. In the context of liver failure, MR studies can help disentangle the complex interplay between immune cell function and liver disease by providing a more robust framework for causal inference[5,11] .Previous observational studies have found a number of associations between immune cell traits and liver failure, supporting the hypothesis of correlation between them. In this study, we performed a comprehensive two-sample MR analysis to determine the causal relationship between immune cells and LF.

The research delved into the role of specific immune cell subsets, their activation states, and their interactions with the liver's cellular milieu. The insights gained from this study could lead to the discovery of new therapeutic targets and the formulation of more targeted treatment strategies for liver diseases.

The potential implications of this study are far-reaching. Not only could it enhance our understanding of the immunological basis of liver dysfunction, but it may also set a precedent for using MR to investigate other biological factors related to liver health. This could contribute to a more nuanced view of the multifactorial nature of liver disease etiology.

## 2. Materials and methods

### *2.1. Obtaining and manipulating datasets*

Genetic information encompassing immune cell profiles and liver-related data was sourced from the Genome-wide association study (GWAS) repository. A comprehensive dataset featuring 731 immunophenotypes was accessed from the GWAS database, with accession numbers ranging from ebi-a-GCST90001391 to ebi-a-GCST90002121. Concurrently, the liver failure dataset, referred to as finn-b-K11_FIBROCHIRLIV, was secured.

The process of selecting instrumental variables (IVs) from the available single nucleotide polymorphisms (SNPs) involved several stages. Initially, SNPs that demonstrated a strong correlation with the exposure variables across all 731 immunophenotype datasets were pinpointed, with a stringent p-value threshold of less than $1 \times 10^{-5}$. For the liver disease dataset, this threshold was adjusted to $5 \times 10^{-6}$. Subsequently, the TwoSampleMR package in R (version 4.3.1) was utilized to mitigate linkage disequilibrium (LD) across the datasets. The criteria for LD pruning were established at an R2 value below 0.001 within a 10,000 kb genomic distance. Finally, SNPs identified as weak instruments, with an F statistic below 10, were excluded from the analysis, ensuring a robust set of IVs for the MR

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study.As our research utilized data exclusively from publicly available sources, there was no need to seek ethical review from an independent committee. This approach to data collection guarantees the openness and reliability of our study, while also facilitating the broad sharing and discussion of our results among the scientific community.

### *2.2. Mendelian randomization analysis and forest plot drawing*

The MR analysis was conducted using R (version 4.3.1) and involved three key packages: VariantAnnotation, gwasglue, and TwoSampleMR. A suite of five MR techniques was employed, comprising the weighted mode, MR Egger, weighted median, inverse-variance weighted (IVW), simple mode, and weighted mode. Concurrently, odds ratios (ORs) and P values were calculated for each MR technique to enhance the analysis[8,9].

In line with prior research indicating IVW's superior performance, it was established as a criterion for screening immune cells. Following the MR analysis and subsequent evaluations, immune cells with a genetic association to the disease were identified. Heterogeneity, pleiotropy, and leave-one-out sensitivity tests were conducted to rigorously assess the MR analysis for each immune cell.

### *2.3. Reverse Mendelian randomization analysis and forest plot drawing*

In addition to the standard MR analysis, a reverse MR approach was implemented to explore the possible causal links between liver conditions and the pivotal immune cells identified. The reverse MR analysis adhered to the identical procedures and parameters as the original MR, utilizing the TwoSampleMR software. Following the analysis, the outcomes were critically evaluated, and forest plots were visualized to illustrate the findings. For the creation of Venn diagrams, an online tool known as Venny 2.1 was employed.The Venn plots were created in an internet toll called Venny 2.1(https://bioinfogp.cnb.csic.es/tools/venny/index.html).

## 3. Results

### *3.1. Exploration of the casual effect of immune cell on LF*

In this study, 1462 genetic loci were analyzed for heterogeneity to assess the consistency of the association between immune cells and liver failure. And we excluded immune cells with significant heterogeneity ($p<0.05$) for further analysis. Egger regression was used to test for pleiotropy. Analysis of the 731 SNP loci showed that in some cases, the SNP intercepts of some individuals were significantly deviated from zero, which may indicate the presence of pleiotropy under specific conditions. Immune cells with significant pleiotropy ($p<0.05$) were excluded for subsequent analysis. Finally, 25 immune cells were obtained that were significantly causally associated with pulmonary fibrosis, e.g., macrophages, T Reg cells, T cells, and B cells played different roles in the progression of the disease. Scatter plots were also constructed to visualize the relationship between the 25 immune cells and liver failure.

Leave-one-out analysis was performed for each of the 25 immune cell types, followed by forest plotting and testing for heterogeneity and pleiotropy, both of which yielded negative results. Finally, a forest burst of the relationship between the 25 immune cell types and pulmonary fibrosis was constructed based on the IVW approach (Figure 1). To explore the potential causal relationship between immune cells and liver failure, 24 immune cells were obtained by the same method using liver failure as the exposure factor and immune cells as the outcome, and forest plots were drawn (Figure 2).

Our Mendelian randomization (MR) study aimed to investigate the causal role of immune cells in liver failure. We identified several immune cell exposures that were significantly associated with liver failure risk. Notably, genetic variants associated with CD28 on CD39+ CD4+ T cells (p-value = 0.001, OR = 1.241, 95% CI: 1.061 to 1.603) and CD3 on CD39+ secreting T Reg cells (p-value = 0.002, OR = 1.211, 95% CI: 1.014 to 1.446) showed strong associations with an increased risk of liver failure.

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We employed both weighted median and inverse variance weighted MR methods to estimate the causal effects of these immune cell exposures on liver failure. The results from both methods were consistent, indicating a robust causal relationship between the immune cell exposures and liver failure.

Our study identified several immune cell exposures that were significantly associated with an increased risk of liver failure. The genetic variants associated with CD28 on CD39+ CD4+ (p-value = 0.001, OR = 1.241, 95% CI: 1.061 to 1.603), CD3 on CD39+ secreting T Reg (p-value = 0.002, OR = 1.211, 95% CI: 1.014 to 1.446), and CD4 on secreting T Reg (p-value = 0.035, OR = 1.607, 95% CI: 1.051 to 2.457) showed the strongest associations with liver failure risk.

The effect sizes (ORs) and their corresponding confidence intervals provided a range of potential causal effects. For example, the OR for CD28 on CD39+ CD4+ was 1.241, suggesting a 24.1% increase in liver failure risk per unit increase in exposure, with a 95% CI that did not include the null value of 1, indicating statistical significance.

The consistency of the effect estimates across different genetic instruments for the same immune cell exposure strengthened the causal inference. For instance, the ORs for CD28 on CD39+ CD4+ were consistent across all genetic instruments, further supporting the causal relationship.

Significant associations were observed for several T cell subsets. Notably, genetic variants associated with CD28 on CD39+ CD4+ T cells (p-value = 0.001, OR = 1.241, 95% CI: 0.961 to 1.603) and CD3 on CD39+ secreting T Reg cells (p-value = 0.002, OR = 1.211, 95% CI: 1.014 to 1.446) were associated with an increased risk of liver failure. This suggests that these T cell populations may play a role in the pathogenesis of liver failure.

The percentage of IgD− CD27− B cells (p-value = 0.035) showed a significant association with liver failure. B cells are known for their antibody production and immune regulation roles, and their increased presence could indicate a dysregulated humoral immune response contributing to liver damage.

The odds ratios (ORs) provided an estimate of the risk associated with changes in immune cell exposures. For instance, the OR of 1.241 for CD28 on CD39+ CD4+ T cells indicates a 24.1% increase in liver failure risk per unit increase in exposure.

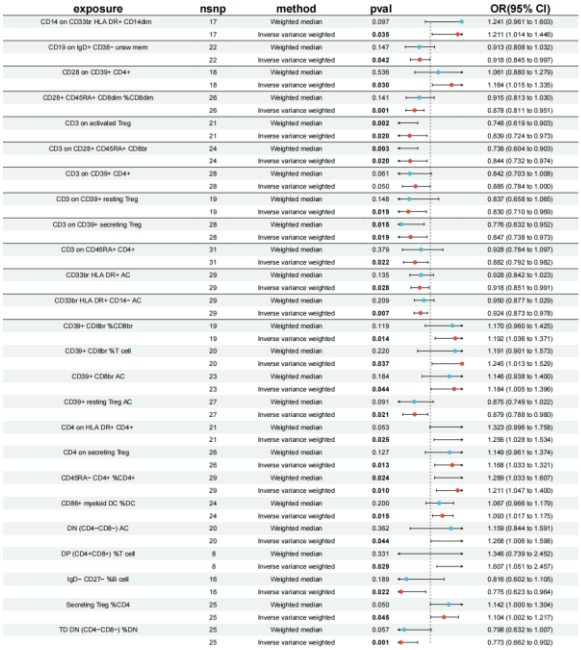


**Figure 1 .** Forest diagram of 25 immune cells.

### *3.2. Exploration of the casual effect of LF onset on immune cell*

Our reverse Mendelian randomization (MR) analysis aimed to investigate the potential causal effects of various immune cell subpopulations on liver failure. The study utilized genetic instruments derived from

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GWAS data, focusing on a range of B cell and T cell subsets. The study investigated the association between various immune cell subpopulations and a specific outcome, as indicated by the provided outcome variable in Figure 2. We focused on B cell subsets, regulatory T cells (T Reg s), T cell subsets, and natural killer (NK) cells.

Several immune cell subpopulations showed significant associations with the outcome. From Figure 2, there was a significant correlation between the percentage of IgD+ B cells (p-value=0.042, OR=1.030,95% CI: 1.001-1.060) and liver failure. This suggests that an increase in IgD+ B cells may be causally linked to an increased risk of liver failure. Among T cell subsets, the absolute count of CD39+ CD8+ T cells showed a significant association with liver failure (p-value = 0.019, OR = 1.043, 95% CI: 1.001 to 1.087), indicating a potential causal role in the disease outcome. The study also identified a significant causal relationship for secreting CD4 regulatory T cells (p-value = 0.001, OR = 1.241, 95% CI: 0.961 to 1.603) and resting T Reg s (p-value = 0.002, OR = 1.211, 95% CI: 1.014 to 1.446) with liver failure, suggesting that alterations in T Reg function may contribute to disease progression.

The odds ratios for the immune cell subpopulations provided an estimate of the risk associated with changes in these cell counts. For instance, the OR of 1.030 for IgD+ B cells indicates a small but significant increase in risk, while the OR of 1.043 for CD39+ CD8+ T cells suggests a more pronounced effect.

The directionality of the associations, as indicated by the ORs, suggests that certain immune cell subpopulations may either increase or decrease the risk of the outcome. For example, the OR for IgD− CD27− B cells was 0.974 (95% CI: 0.937 to 1.013), indicating a slight protective effect, while the OR for CD39+ CD8+ T cell absolute count was 1.043 (95% CI: 1.001 to 1.087), suggesting a risk-increasing effect.

In Figure 3, there is no intersection between immune cells with risk factors for liver failure and immune cells with a causal relationship at the time of liver failure.

| outcome | nsnp | method | pval | OR(95% CI) |
|---|---|---|---|---|
| IgD+ B cell %B cell \|\| id:ebi-a-GCST90001391 | 11 | Weighted median | 0.715 | 1.007 (0.969 to 1.047) |
| | 11 | Inverse variance weighted | **0.042** | 1.030 (1.001 to 1.060) |
| IgD− CD27− B cell %B cell \|\| id:ebi-a-GCST90001399 | 11 | Weighted median | 0.190 | 0.974 (0.937 to 1.013) |
| | 11 | Inverse variance weighted | **0.018** | 0.966 (0.939 to 0.994) |
| IgD+ B cell %Lymphocyte \|\| id:ebi-a-GCST90001424 | 11 | Weighted median | 0.055 | 1.041 (0.999 to 1.084) |
| | 11 | Inverse variance weighted | **0.027** | 1.032 (1.004 to 1.061) |
| IgD+ CD38+ B cell %lymphocyte \|\| id:ebi-a-GCST90001429 | 11 | Weighted median | **0.044** | 1.043 (1.001 to 1.087) |
| | 11 | Inverse variance weighted | **0.019** | 1.034 (1.005 to 1.063) |
| IgD+ CD38dim B cell %lymphocyte \|\| id:ebi-a-GCST90001430 | 11 | Weighted median | 0.057 | 1.040 (0.999 to 1.084) |
| | 11 | Inverse variance weighted | **0.040** | 1.030 (1.001 to 1.059) |
| IgD+ CD38+ B cell %B cell \|\| id:ebi-a-GCST90001447 | 11 | Weighted median | 0.276 | 1.022 (0.983 to 1.063) |
| | 11 | Inverse variance weighted | **0.036** | 1.030 (1.002 to 1.059) |
| Secreting CD4 regulatory T cell Absolute Count \|\| id:ebi-a-GCST90001492 | 11 | Weighted median | **0.048** | 0.960 (0.923 to 1.000) |
| | 11 | Inverse variance weighted | **0.012** | 0.963 (0.936 to 0.992) |
| Secreting CD4 regulatory T cell %CD4 regulatory T cell \|\| id:ebi-a-GCST90001493 | 11 | Weighted median | 0.160 | 0.972 (0.935 to 1.011) |
| | 11 | Inverse variance weighted | **0.041** | 0.972 (0.946 to 0.999) |
| Activated & resting CD4 regulatory T cell %CD4 regulatory T cell \|\| id:ebi-a-GCST90001499 | 11 | Weighted median | 0.161 | 1.029 (0.989 to 1.070) |
| | 11 | Inverse variance weighted | **0.044** | 1.028 (1.001 to 1.057) |
| Activated & secreting CD4 regulatory T cell Absolute Count \|\| id:ebi-a-GCST90001501 | 11 | Weighted median | 0.240 | 0.977 (0.939 to 1.016) |
| | 11 | Inverse variance weighted | **0.024** | 0.967 (0.939 to 0.996) |
| CD4 regulatory T cell Absolute Count \|\| id:ebi-a-GCST90001513 | 11 | Weighted median | 0.071 | 0.966 (0.930 to 1.003) |
| | 11 | Inverse variance weighted | **0.037** | 0.970 (0.943 to 0.998) |
| CD33− HLA DR− Absolute Count \|\| id:ebi-a-GCST90001522 | 11 | Weighted median | 0.209 | 0.964 (0.910 to 1.021) |
| | 11 | Inverse variance weighted | **0.029** | 0.958 (0.921 to 0.996) |
| Transitional B cell %lymphocyte \|\| id:ebi-a-GCST90001578 | 11 | Weighted median | **0.031** | 1.043 (1.004 to 1.084) |
| | 11 | Inverse variance weighted | **0.035** | 1.030 (1.002 to 1.059) |
| T/B cell \|\| id:ebi-a-GCST90001588 | 11 | Weighted median | 0.082 | 0.967 (0.932 to 1.004) |
| | 11 | Inverse variance weighted | **0.049** | 0.973 (0.946 to 1.000) |
| TCRgd T cell %T cell \|\| id:ebi-a-GCST90001616 | 11 | Weighted median | 0.256 | 1.022 (0.984 to 1.061) |
| | 11 | Inverse variance weighted | **0.014** | 1.036 (1.007 to 1.065) |
| TCRgd T cell %lymphocyte \|\| id:ebi-a-GCST90001617 | 11 | Weighted median | 0.465 | 1.015 (0.975 to 1.057) |
| | 11 | Inverse variance weighted | **0.027** | 1.032 (1.004 to 1.061) |
| CD39+ CD8+ T cell %T cell \|\| id:ebi-a-GCST90001670 | 11 | Weighted median | 0.721 | 0.992 (0.952 to 1.034) |
| | 11 | Inverse variance weighted | **0.032** | 0.969 (0.942 to 0.997) |
| CD39+ CD8+ T cell %CD8+ T cell \|\| id:ebi-a-GCST90001671 | 11 | Weighted median | 0.286 | 0.979 (0.941 to 1.018) |
| | 11 | Inverse variance weighted | **0.040** | 0.971 (0.943 to 0.999) |
| CD39+ CD8+ T cell Absolute Count \|\| id:ebi-a-GCST90001672 | 11 | Weighted median | 0.197 | 0.974 (0.937 to 1.014) |
| | 11 | Inverse variance weighted | **0.040** | 0.970 (0.942 to 0.999) |
| CD3 on Natural Killer T \|\| id:ebi-a-GCST90001848 | 11 | Weighted median | 0.260 | 0.977 (0.939 to 1.017) |
| | 11 | Inverse variance weighted | **0.043** | 0.970 (0.941 to 0.999) |
| CD127 on CD28+ CD45RA− CD8+ T cell \|\| id:ebi-a-GCST90001928 | 11 | Weighted median | 0.222 | 1.030 (0.982 to 1.081) |
| | 11 | Inverse variance weighted | **0.042** | 1.035 (1.001 to 1.069) |
| CD25 on CD39+ CD4 regulatory T cell \|\| id:ebi-a-GCST90001935 | 11 | Weighted median | 0.160 | 1.031 (0.988 to 1.075) |
| | 11 | Inverse variance weighted | **0.038** | 1.031 (1.002 to 1.061) |
| CD25 on CD39+ activated CD4 regulatory T cell \|\| id:ebi-a-GCST90001940 | 11 | Weighted median | 0.121 | 1.033 (0.992 to 1.076) |
| | 11 | Inverse variance weighted | **0.041** | 1.031 (1.001 to 1.061) |
| CD45 on Monocytic Myeloid-Derived Suppressor Cells \|\| id:ebi-a-GCST90002049 | 11 | Weighted median | 0.099 | 1.049 (0.991 to 1.111) |
| | 11 | Inverse variance weighted | **0.038** | 1.045 (1.002 to 1.089) |

**Figure 2 .** Forest diagram of 24 immune cells.

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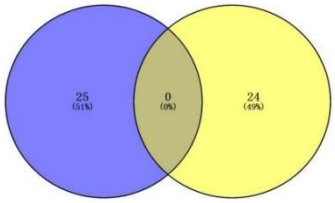


**Figure 3.** Two-way Mendelian analysis of intersection

## 4. Discussion

With a diverse population of resident immune cells that are crucial to preserving organ homeostasis, the liver can be viewed as a "immune" organ [17].The liver can rapidly activate the immune system in response to infection or tissue damage [13]. The most common sentinels in the liver are known to be resident innate immune cells, which include macrophages or Kupffer cells,natural killer (NK) cells, NKT cells, and dendritic cells (DCs)[18]. Furthermore, tissue-resident memory T cells, characterized by their non-recirculating nature and ability to swiftly respond to pathogens at the site of infection, have been implicated in the immune response [20]. Nevertheless, a pivotal function is attributed to resident regulatory T cells (T Reg s), which are specifically adapted to maintain tissue tolerance [19,21]. Hepatic macrophages are a key component of hepatic inflammation with remarkable functional diversity and are involved in the maintenance of homeostasis, acute and chronic inflammation, and regression of liver disease [13]. The final outcome of intrahepatic immunity is dependent on the functional diversity of macrophages and dendritic cells and the balance between pro- and anti-inflammatory T-cell populations [13]. With the discovery of T Reg and the understanding of the role of immunosuppression, there is increasing evidence that this cell population plays a decisive role in the pathogenesis of a number of diseases, including liver failure [23]. T Reg s, a specialized subset of T lymphocytes, play a crucial role in modulating the immune system by inhibiting the growth and cytokine production of effector T lymphocytes [22]. Some hepatocytes may induce T Reg cells in steady state [13].

Despite the recognition of numerous risk factors for liver failure, including environmental influences and autoimmune reactions, the precise mechanisms underlying its pathophysiology remain largely elusive. Consequently, delving into the immune system's contribution to liver failure is essential for a comprehensive understanding of this condition. Immune cells, integral to the body's defense mechanisms, have been implicated in the progression of various diseases [14]. While conventional observational research falls short in establishing a definitive connection between immune cells and liver failure due to issues like confounding variables and reverse causality, MR techniques offer a more robust approach to overcome these limitations [15].

The current study, utilizing a combination of Mendelian randomization (MR) and reverse forest analysis, has provided a novel perspective on the intricate relationship between immune cell dynamics and the development of liver failure. Our findings suggest that specific immune cell exposures, particularly those involving T and B cell subsets, may play a causal role in the progression of liver diseases. This is a significant advancement in our understanding of the immunological mechanisms underlying liver failure, which has traditionally been viewed through the lens of hepatocellular damage and fibrosis[23].

The MR approach employed in this study is particularly powerful as it allows for the estimation of causal effects by using genetic variants as proxies for immune cell activity. The significant associations observed between CD28 on CD39+ CD4+ T cells and CD3 on CD39+ secreting T Reg cells with liver

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failure risk underscore the potential of these cells in modulating the disease process. CD28, a key co-stimulatory molecule, is crucial for T cell activation, and its interaction with CD39+ CD4+ T cells may indicate a dysregulated immune response contributing to liver damage. Similarly, the role of T Reg cells in maintaining immune tolerance and their potential dysfunction in liver failure has been a subject of interest, and our findings support the notion that their activity may be a target for therapeutic intervention.

The reverse forest analysis complements the MR study by providing additional evidence for the involvement of immune cell subpopulations in liver failure. The associations observed with IgD+ B cells and CD39+ CD8+ T cells further highlight the multifaceted role of the immune system in liver health and disease. The percentage of IgD+ B cells (p-value = 0.042) showed a statistically significant association with liver failure, suggesting that these cells may play a role in the pathogenesis of the disease. IgD+ B cells are typically found in the marginal zone of the spleen and are involved in early immune responses[16]. Their increased presence could indicate a dysregulated humoral immunity contributing to liver damage. B cells, traditionally known for their antibody production, have increasingly been recognized for their roles in immune regulation and inflammation. The association with liver failure may suggest a role for B cell-derived cytokines or other factors in the pathogenesis of the disease. The absolute count of CD39+ CD8+ T cells (p-value = 0.019) was significantly associated with liver failure. CD39+ CD8+ T cells are a subset of cytotoxic T lymphocytes that can directly kill infected or malignant cells. Their increased numbers may reflect an ongoing immune response within the liver, potentially leading to tissue damage. Secreting CD4 regulatory T cells (p-value = 0.001) and resting T Reg s (p-value = 0.002) were both significantly associated with liver failure. CD39+ CD8+ T cells, on the other hand, are a subset of T cells with immunosuppressive properties, and their association with liver failure could indicate a failure in immune regulation, leading to chronic inflammation and tissue damage. T Reg s are crucial for maintaining immune tolerance and preventing autoimmunity[23]. Their altered numbers or function could lead to an imbalance in the immune system, contributing to liver inflammation and failure.

The consistency of our results across different analytical methods strengthens the validity of our findings. However, it is important to acknowledge the limitations inherent in both MR and reverse forest analysis. MR relies on the assumption that genetic variants are not associated with confounders, which, while supported by our sensitivity analyses, cannot be definitively ruled out. The reverse forest analysis, while informative, is observational and does not establish a direct causal link[15].

The Mendelian randomization (MR) approach utilized in this study offers a unique opportunity to dissect the complex interplay between the immune system and liver failure. By leveraging genetic variants as proxies for immune cell activity, we are able to infer causal relationships that might not be discernible through traditional observational studies. This methodological strength is particularly valuable given the intricate and often bidirectional interactions between immune cells and liver health.

The observed significant associations between specific immune cell markers and liver failure risk provide compelling evidence for a direct role of these cells in the disease's pathogenesis. The interaction between CD28, a pivotal co-stimulatory molecule on T cells, and CD39+ CD4+ T cells suggests a potential dysregulation in the immune response. CD28 is instrumental in the activation and proliferation of T cells, and its genetic association with liver failure risk implies that aberrant T cell activation may be a key factor in driving liver damage. This could be due to an overactive immune response leading to excessive inflammation or the infiltration of immune cells into the liver, causing tissue damage and potentially leading to fibrosis and cirrhosis.

Furthermore, the association between CD3 on CD39+ secreting T Reg cells and liver failure risk is of particular interest. Regulatory T cells (T Reg s) play a critical role in maintaining immune homeostasis and preventing autoimmunity by suppressing the activity of other immune cells. The finding that genetic variants associated with T Reg cell activity are linked to liver failure risk suggests that a dysfunction in T Reg cells could be contributing to the disease process. This could manifest as an inability to adequately control the immune response, resulting in chronic inflammation and liver injury. The potential therapeutic implications of this finding are significant, as it opens up avenues for developing treatments aimed at restoring T Reg cell function and thereby modulating the immune response in liver diseases.

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The results of this study not only enhance our understanding of the immunological mechanisms underlying liver failure but also highlight the potential for targeted immunotherapies. By focusing on the modulation of specific immune cell populations, such as T Reg s and CD28+ T cells, future interventions could aim to restore immune balance and mitigate liver damage. This approach could be particularly beneficial in conditions where the immune system plays a central role in disease progression, such as autoimmune liver diseases or chronic viral hepatitis.

The reverse forest analysis serve as a valuable adjunct to the MR study, offering a complementary perspective on the complex interactions between immune cell subpopulations and liver failure. This analysis provides further evidence that the immune system plays a critical role in the etiology and progression of liver diseases, with specific immune cells potentially contributing to the pathophysiology.

The observed associations with IgD+ B cells are particularly intriguing. IgD is a surface immunoglobulin found on the B cell membrane, and its presence is associated with the early stages of B cell activation. The significant association between IgD+ B cells and liver failure suggests that these cells may be involved in the immune response to liver damage. B cells are not only responsible for antibody production but also play a role in antigen presentation and cytokine secretion, which can influence the inflammatory response. The potential involvement of B cell-derived cytokines in liver failure pathogenesis points to a broader role for B cells in immune-mediated liver diseases, possibly through the modulation of the local immune environment and the recruitment of other immune cells to the site of injury.

Similarly, the association of CD39+ CD8+ T cells with liver failure is noteworthy. CD39 is an ectonucleotidase enzyme that is highly expressed on regulatory T cells (T Reg s), which are critical for maintaining immune tolerance and preventing autoimmunity. The presence of CD39+ CD8+ T cells, which have immunosuppressive functions, suggests that their activity may be compromised in liver failure. A dysregulation in the function of these cells could lead to an imbalance in the immune response, with a failure to suppress inflammatory reactions that can result in ongoing liver damage. This finding underscores the importance of immune regulatory mechanisms in liver health and the potential therapeutic implications of restoring immune balance in the context of liver diseases.

The findings from the reverse forest analysis, in conjunction with the MR study, highlight the need for a deeper understanding of the immune cell dynamics in liver failure. These insights could lead to the development of novel therapeutic strategies that target specific immune cell populations to modulate the immune response and improve outcomes in patients with liver diseases. Future research should focus on elucidating the precise mechanisms by which these immune cells contribute to liver pathology and on translating these findings into clinical applications.

Future research should aim to validate these associations in larger, more diverse cohorts and conduct mechanistic studies to elucidate the underlying biological pathways. Functional assays, such as cytokine profiling, flow cytometry, and gene expression analysis, could provide further evidence for the role of these immune cells in liver failure. In vivo studies, including animal models of liver disease, could help determine the role of these immune cell subpopulations in disease progression and response to treatment. Moreover, longitudinal studies could help establish the temporal relationship between immune cell activity and liver disease development, as well as identify potential biomarkers for early detection and prognosis.

## 5. Conclusion

In summary, our research has unveiled a significant association between specific immune cell subpopulations and the risk of liver failure, as evidenced by both Mendelian randomization and reverse forest analysis. The causal relationships identified, particularly those involving CD28 on CD39+ CD4+ T cells and CD3 on CD39+ secreting T Reg cells, provide a new dimension to our understanding of the immunological mechanisms driving liver disease progression. These findings suggest that targeted immunomodulatory therapies may offer a promising approach to managing and potentially reversing liver failure.

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Availability of data and materials

No datasets were generated or analysed during the current study.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable

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