Identification of key genes and infiltrating immune cells among acetaminophen-induced acute liver failure and HBV-associated acute liver failure

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Background: Acute liver failure (ALF) is a life-threatening complication that is relatively uncommon. ALF causes severe hepatocyte damage and necrosis, which can lead to liver dysfunction and even multi-organ failure. A growing body of evidence suggests that immune cell infiltration and some abnormally expressed genes are associated with ALF development. However, in ALF, they have yet to be thoroughly investigated.

Methods: The Gene Expression Omnibus (GEO) database was used to obtain microarray datasets such as GSE74000, GSE120652, GSE38941, and GSE14668, which were then examined via GEO2R to determine differentially expressed genes (DEGs) associated with ALF. Metascape was employed to annotate the underlined genes using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses. The mechanism of IGF1 in 2 different kinds of ALF including acetaminophen-induced ALF and hepatitis B virus (HBV)-induced ALF was studied using gene set enrichment analysis (GSEA). Next, immune cell infiltration was investigated and differentiated in ALF using CIBERSORT.

Results: Six genes (*HAO2*, *IGF1*, *PLA2G7*, *SC5D*, *GNE*, *SLC1A1*) were found to be abnormally expressed in the 2 distinct types of ALF i.e., acetaminophen-induced ALF and HBV-induced ALF. *IGF1* was identified as a hub gene in ALF and was found to be associated with several developmental cascades including immune responses, inflammatory responses, and intracellular calcium homeostasis. Additionally, the number of CD4 naive T cells, CD8 T cells, and follicular helper T cells was increased in acetaminophen-induced ALF, whereas the number of activated NK cells, resting NK cells, and plasma cells was increased in HBV-induced ALF.

Conclusions: The present study determined a potential molecular target, namely *IGF1*, in acetaminophen-induced ALF and HBV-induced ALF, which may provide novel insights into the pathophysiology and management of ALF. Concurrently, the putative immunological pathways have been found.

Keywords: IGF1; immune cell infiltration; HBV-associated acute liver failure; acetaminophen-induced acute liver failure

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Introduction

Acute liver failure (ALF) is a relatively uncommon and serious complication of hepatocyte injury that can progress to death within days or weeks. A significant correlation between rapid-onset aminotransferase elevation, altered mentation, and disrupted coagulation is observed after a range of injuries to liver cells (1). ALF has not been examined comprehensively due to its rarity. Furthermore, most treatment prescriptions are based on expert opinion. Although liver transplantation, which is performed in approximately 30% of patients with ALF, remains a life-saving alternative to conventional therapy, improvements in management have resulted in lower mortality (2). Liver transplantation is a significant therapeutic option for all types of ALF. The risk of serious postoperative complications is higher in patients with ALF who require an urgent liver transplant. Additionally, some patients are unable to obtain an appropriate liver donor, requiring them to continue waiting, thereby delaying therapy (3). Hence, investigating new therapeutic strategies that improve the survival of patients and gaining a better understanding of the molecular processes underlying the pathogenesis of ALF are required.

High doses of acetaminophen (APAP) and viral hepatitis are the 2 most common causes of ALF across the globe (4). However, Xie *et al.* revealed that dihydromyricetin (DHM) is a promising candidate for reversing liver injury (5). Another study demonstrated that the severity of the histopathology is directly linked to a specific gene signature in hepatitis B virus (HBV)-induced ALF. The degree of liver necrosis correlated favorably with HSPC (hepatic stem progenitor cells) activation and fibrogenesis (6). However, none of these approaches has reached a sufficient level of significance and success to be adopted in clinical practice.

According to several recent studies, many genes and cellular cascades are involved in the onset and development of liver cancer (7) and other liver illnesses (8). In addition, immunotherapy is a relatively new treatment approach that is gaining attention in the context of treatment, particularly in cancer. Research into immunotherapy's potential as a therapeutic target and a biomarker for prognosis and diagnosis in the microenvironment has demonstrated considerable promise (9,10). According to a study, anti-PD-1 immunotherapy may also be useful in the treatment of ALF (11). Herein, we aimed to evaluate viable targets for ALF treatment by constructing a possible network and analyzing the immune microenvironment in 2 different types of ALF. For the first time, we evaluated 4 datasets (GSE74000, GSE120652, GSE38941, and GSE14668) from the Gene Expression Omnibus (GEO) database to explore differentially expressed genes (DEGs) in APAP-induced ALF and HBV-induced ALF relative to healthy livers. We also generated a hub gene network and performed functional annotation and signaling cascade analysis. Moreover, CIBERSORT was employed to evaluate the state of immune cell infiltration in these 2 distinct types of ALF. As a result, we are the first to investigate the expression of key genes and their roles and immune infiltration in acetaminophen-induced ALF and HBV-induced ALF. We present the following article in accordance with the STREGA reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-2742/rc).

Table 1 Details of GEO data

Accession	Platform	Sample	Normal	Acute liver failure
GSE74000	GPL750	Liver	2	3
GSE120652	GPL6244	Liver	10	4
GSE38941	GPL570	Liver	8	4
GSE14668	GPL570	Liver	10	4

GEO, Gene Expression Omnibus.

Methods

Microarray datasets and data pre-processing

The GEO database (https://www.ncbi.nlm.nih.gov/geo/) was utilized to obtain the raw gene expression profiles i.e., GSE120652, GSE74000, GSE38941, and GSE14668. *Table 1* depicts the relevant information from the 4 databases. Different data could be obtained in various datasets using various experimental tools. A Perl script and the sva package from R software (R-project.org) were used to perform the merging and pre-processing of raw data to retrieve the crucial data of the 4 gene expression matrices (12).

Evaluation and functional enrichment analysis of DEGs

The DEGs between ALF tissue and healthy liver tissue were evaluated using the GEO2R tool (http://www.ncbi.nlm.nih.gov/geo/geo2r/) (13). The threshold for detecting DEGs was set at adjusted P value <0.05 and |log fold change (FC)| >1.5. The overlapping of DEGs in the 4 datasets

(GSE120652, GSE74000, GSE14668, and GSE38941) was evaluated using a Venn diagram, followed by generating candidate DEGs using Metascape by integrating more than 40 biological databases. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Gene set enrichment analysis (GSEA)

GSEA, a computer-based tool, has been employed to detect statistically significant variations in a group of genes among 2 biological conditions (14). After pre-processing of the data, the GSEA software was employed to analyze Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and to import merged gene matrices. The permutation type was set to "phenotype" and the number of permutations was set at 1,000. The Molecular Signatures Database (http://software.broadinstitute.org/gsea/msigdb/index.jsp) was also used to obtain gene set databases for this investigation.

CIBERSORT

The CIBERSORT algorithm was used to evaluate immune cell infiltration in samples. CIBERSORT is an *in silico* approach that utilizes gene expression data to predict the cellular composition of complicated tissues using pre-processed gene expression profiling (15). The CIBERSORT LM22 gene file was used to identify 22 immune cell subsets and analyze GEO data on healthy liver tissue and ALF tissue. P values and root mean squared errors for each expression file were counted in CIBERSORT, as detailed in prior work. This technique uses the default signature matrix, which had 100 permutations. Data with a CIBERSORT P value of less than 0.05 were retained for subsequent analysis. As a result of this direct integration, a whole matrix of immune cell subsets was generated. The findings of CIBERSORT were shown using the R packages corplot, vioplot, and ggplot2.

Principal component analysis (PCA) of infiltrated immune cells

PCA is a multivariate regression analysis approach that was utilized to determine the variations in immune cell infiltration between the 2 distinct ALF types in this study, namely APAP-induced ALF and HBV-induced ALF. The PCA graph was plotted via the ggplot2 package in R software. The PCA graph could be used to identify infiltrating immune cells as variables and to describe the variation between healthy liver tissue and ALF tissue.

Statistical analyses

All data visualization and statistical analysis were accomplished by R software (version 4.1.2). P value <0.05 was considered statistically significant.

Results

Evaluation of DEGs

Herein, we categorized the data into 2 groups i.e., APAP-induced ALF and HBV-induced ALF. GEO2R was used to reveal DEGs in ALF, as shown in *Figure 1A*,*1B*. After evaluation using the threshold of adjusted P value <0.05 and |logFC| >1.5, 224, 207, 206, and 213 DEGs were identified in the GSE74000, GSE120652, GSE38941, and GSE14668 datasets, respectively (*Figure 2A*). *Figure 2B* shows the overlap of DEGs in the 2 groups. Six genes (*HAO2*, *IGF1*, *PLA2G7*, *SC5D*, *GNE*, *SLC1A1*) were found to be abnormally expressed in the 2 groups. It has been demonstrated that *IGF1* is a hub gene in the protein-protein interaction (PPI) network and may serve as a potential marker gene for HBV-associated ALF (16,17). However, the role of *IGF1* in APAP-induced ALF is yet to be investigated.

GO and KEGG analysis of target genes

The online Metascape database (18) was used to perform GO and KEGG pathway enrichment analysis of upregulated and downregulated genes. *Figure 3A* shows that in APAP-induced ALF, *AKT1* (protein kinase B) was activated. Lipid oxidation has a key role in APAP-induced ALF. APAP increased lipid peroxides produced from n-6 fatty acids, indicating that auto-oxidation is the primary mechanism of lipid oxidation induced by APAP (19). Simultaneously, there is compelling evidence that immune-mediated inflammation plays a key role in ALF caused by HBV (20,21). Target genes were shown to be considerably enriched in lymphocyte-mediated immunity according to our findings. Moreover, allograft rejection was also triggered. The recognition of non-self donor alloantigens by recipient T-cells causes rejection. T-cells in lymphoid tissue proliferate and become activated in response to antigen recognition before migrating

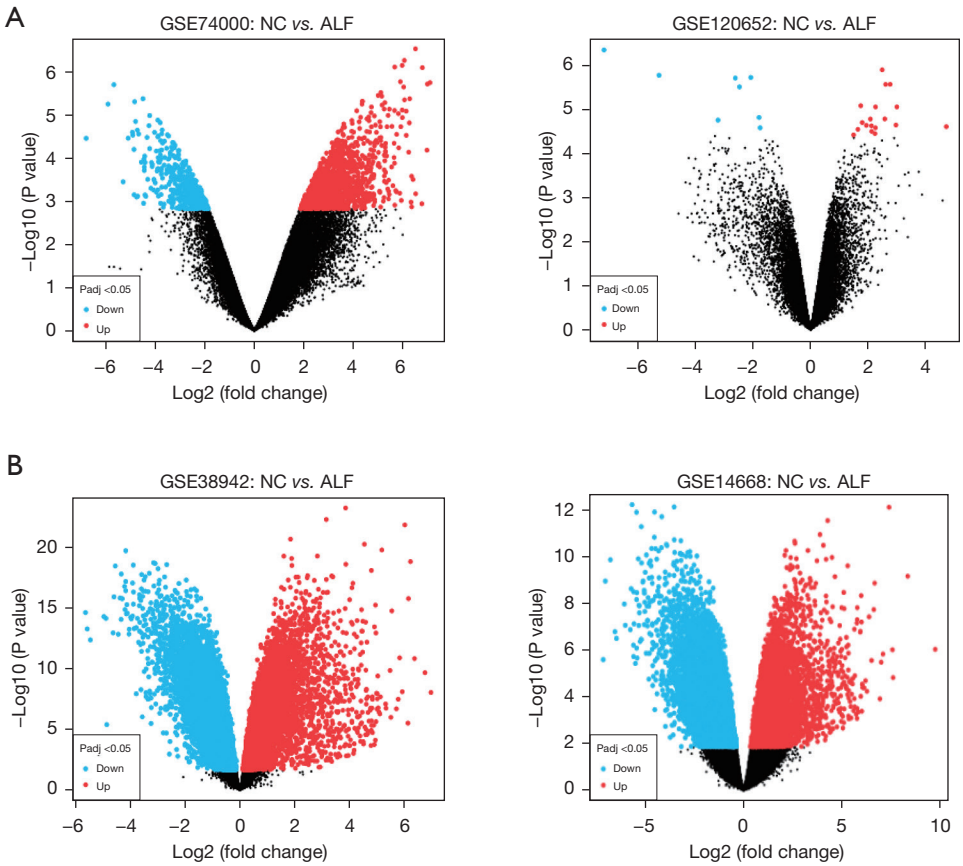

Figure 1 Volcano plot of DEGs (differentially expressed genes) in the mRNA expression profiling datasets. Volcano plots of DEGs in normal and acute liver failure samples in acetaminophen-induced ALF (A) and hepatitis B virus-induced ALF (B). Colors represent different genes: black nodes represent genes without significantly different expression, red nodes represent upregulated genes, blue nodes represent downregulated genes. ALF, acute liver failure; DEGs, differentially expressed genes.

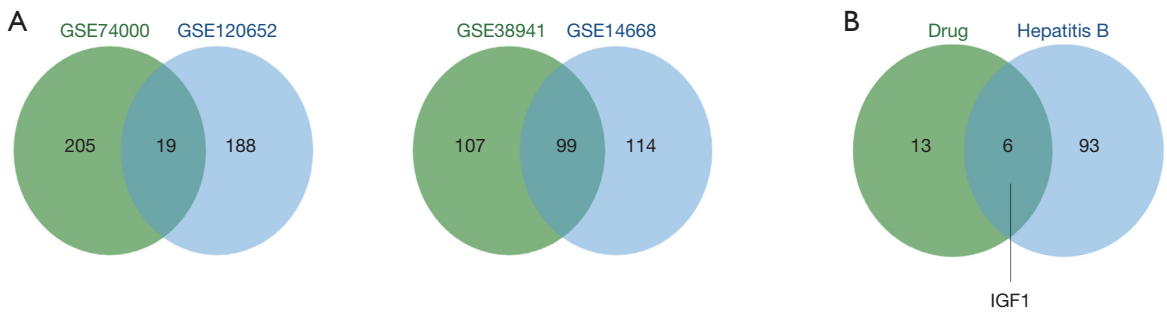

Figure 2 Venn diagram of DEGs. GEO2R was used to reveal DEGs in ALF by Venn diagrams. (A) Genes with significant changes in acetaminophen-induced ALF (left) and hepatitis B virus-induced ALF (right). (B) *IGF1* was identified as a hub gene in ALF and was to be further studied. DEGs, differentially expressed genes; ALF, acute liver failure.

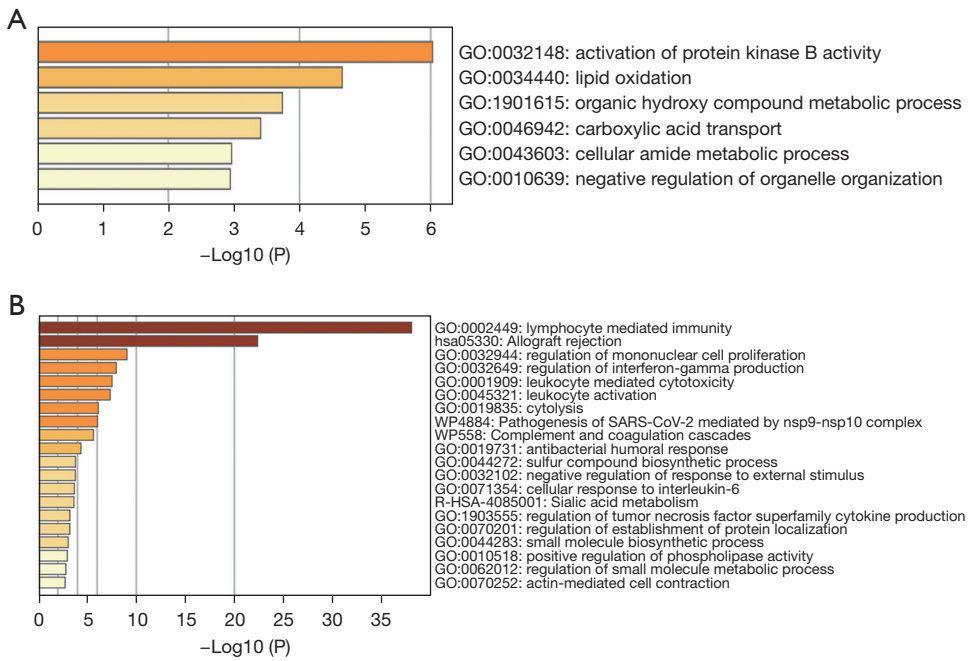

Figure 3 GO and KEGG annotation analysis for the target genes of DEGs in terms of biological process, cellular component, and molecular function. (A) Related pathways in acetaminophen-induced ALF. (B) Related pathways in hepatitis B virus-induced ALF. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes; ALF, acute liver failure.

to the allograft (*Figure 3B*) (22).

Evaluation of IGF1-associated cascades

According to previous studies (16,17), *IGF1* is a gene that plays a key role in ALF. Hence, GSEA was used to obtain *IGF1* expression data from GEO datasets. GSEA revealed that *IGF1* is implicated in a range of developmental cascades, which allowed us to better understand the biological implications of coregulated proteins. The immune response is stimulated (T cell and NK cell activation) and intracellular calcium homeostasis is destroyed in the 2 kinds of ALF in this study, as shown in *Figure 4A*. In APAP-induced ALF, inflammatory response cytochrome P450 was activated, and cysteine and methionine metabolism was inhibited (*Figure 4B*). MAPK and Ras signaling pathways were activated in HBV-associated ALF, as shown in *Figure 4C*.

Composition of infiltrated immune cells in 2 different kinds of ALF from the GEO expression array dataset

Using GEO expression array data, the infiltrated immune cell fraction was first reviewed between the 2 types of ALF i.e., APAP-induced ALF and HBV-induced ALF. The proportions of immune cells in the samples and groups were found to be significantly different. As illustrated in *Figure 5A*,*5B*, CD8 T cells, CD4 naive T cells, and follicular helper T cells were the top 3 highest in APAP-induced ALF. In contrast, active natural killer (NK) cells, resting NK cells, and plasma cells were found to be elevated in HBV-associated ALF (*Figure 6A*,*6B*). Furthermore, the results demonstrated that immune injury (mediated by T cells) plays a critical role in the pathogenesis of APAP-induced ALF, although the immune response caused by NK cells is the most common in HBV-associated ALF, as shown in *Figure* 7. Compared to HBV-associated ALF, APAP-induced ALF had a lower proportion of M1 macrophages, M2 macrophages, monocytes, and plasma cells, but a higher proportion of activated dendritic cells, activated mast cells, activated NK cells, resting NK cells, follicular helper T cells, and regulatory T cells (Tregs) (*Figure 8*). We performed a correlation analysis of infiltrating immune cells in the 2 types of ALF and evaluated many pairs of immune cells that were positively and negatively associated, as shown in *Figure 9A*. The correlation degree was indicated by the score. According to the underlined finding, plasma cells

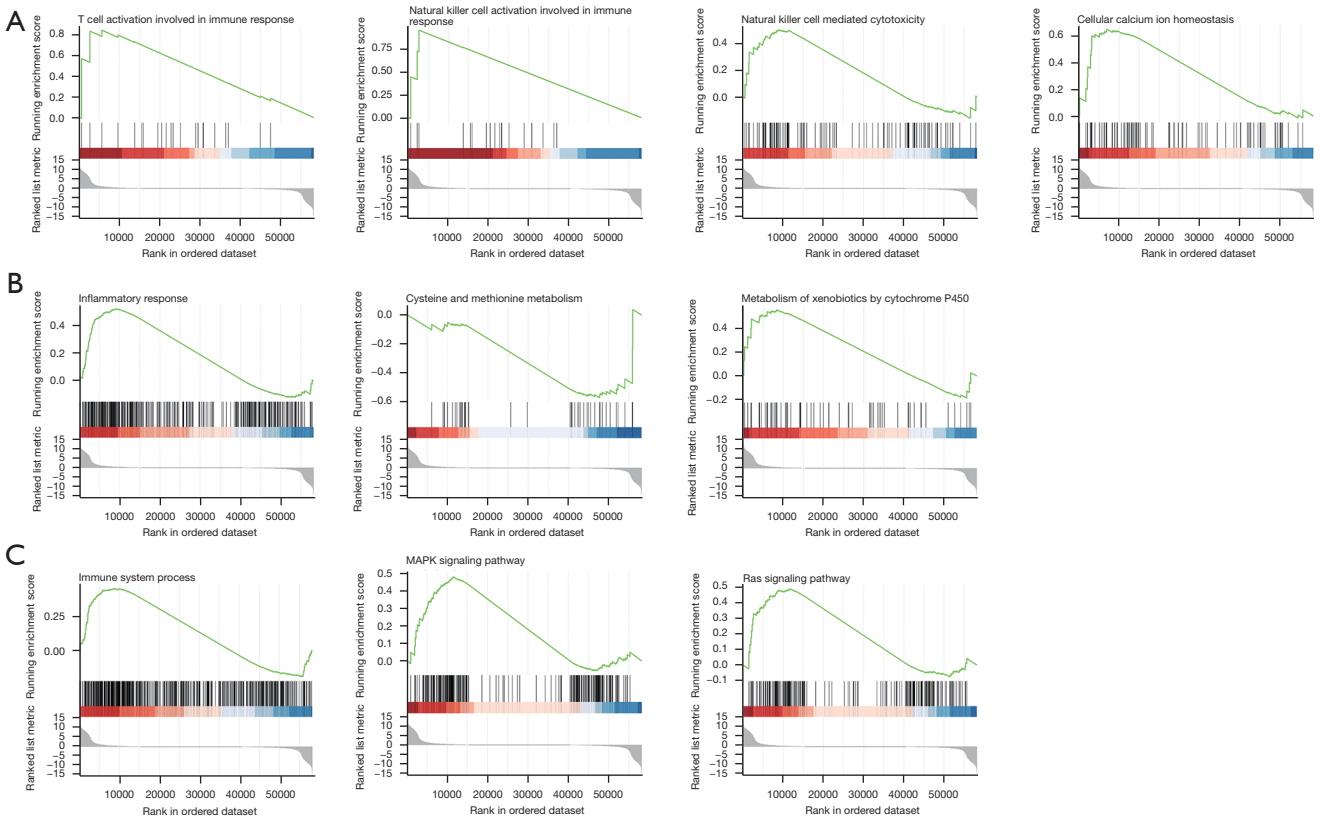

Figure 4 Significant pathways according to *IGF1* obtained by GSEA in GEO datasets. (A) For the 2 types of acute liver failure, in the course of the disease, the immune response is activated, and intracellular calcium homeostasis is destroyed. (B) In acetaminophen induced acute liver failure, inflammatory response cytochrome P450 was activated, while cysteine and methionine metabolism was inhibited. (C) The immune response, MAPK and Ras signalling pathways were activated in HBV-associated ALF. GEO, Gene Expression Omnibus; HBV, hepatitis B virus; ALF, acute liver failure.

and M2 macrophages had the strongest synergistic effect. Meanwhile, the most competitive effect was observed in eosinophils and resting dendritic cells, as well as activated memory T cells. The PCA results revealed that immune infiltration might be used to discriminate between the 2 types of ALF (*Figure 9B*).

Discussion

ALF is a condition that usually occurs in the intensive care unit and is unpredictable and potentially fatal. Although the majority of ALF deaths are caused by intracranial hypertension (ICH) and infections, patients may also present with varying degrees of hemodynamic abnormalities and renal failure (23). ALF is caused by a variety of reasons, including genetic predisposition, socioeconomic circumstances, and environmental exposures. One of the leading causes of ALF in Western countries is the excessive intake of APAP, and its prevalence appears to be rising (24). After APAP overdose, viral hepatitis is the most common cause of ALF, especially in underdeveloped nations (25). However, the precise mechanisms of these 2 types of ALF are unknown. Hence, further investigations are needed to explore the molecular mechanism of ALF in order to diagnose and treat liver diseases.

Microarray technology has recently made it easy to uncover thousands of genomic changes in the course of many diseases (26). In this study, DEGs in ALF were identified using data from 4 datasets: GSE120652, GSE74000, GSE14668, and GSE38941. The aberrantly expressed genes in ALF (induced by APAP) were found at the junction of GSE74000 and GSE120652, while the same data was also observed for GSE38941 and GSE14668 in HBV-associated ALF. The DEGs were then analyzed

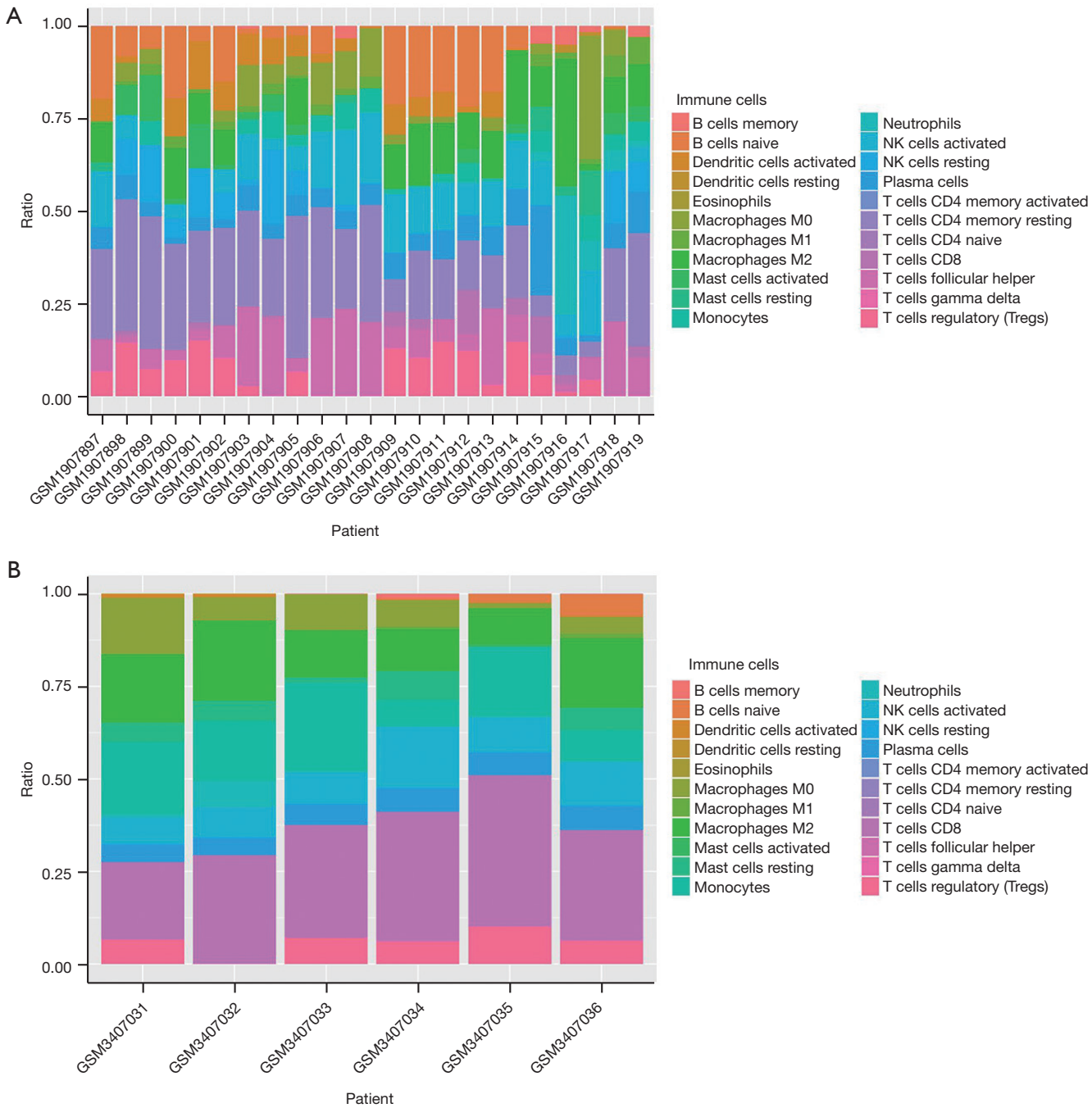

Figure 5 The fraction of 22 subsets of immune cells in acetaminophen-induced ALF. (A) GSE74000. (B) GSE120652. X axis: each GEO sample; Y axis: percentage of each kind of immune cell. GEO, Gene Expression Omnibus; ALF, acute liver failure.

for functional and pathway enrichment. *AKT1* was shown to be activated in APAP-induced ALF according to the findings. Patients with ALF indicated 'sepsis-like' immune paralysis, according to clustering algorithm analysis, and functional analysis revealed that endoplasmic reticulum (ER) stress or the *AKT1* gene (linked with downstream cascades) was differentially modified in ALF (27). The Akt cascade is known to have a protective function in death factor-mediated apoptosis, and researchers hypothesize that the triggering of Akt could be a therapeutic approach

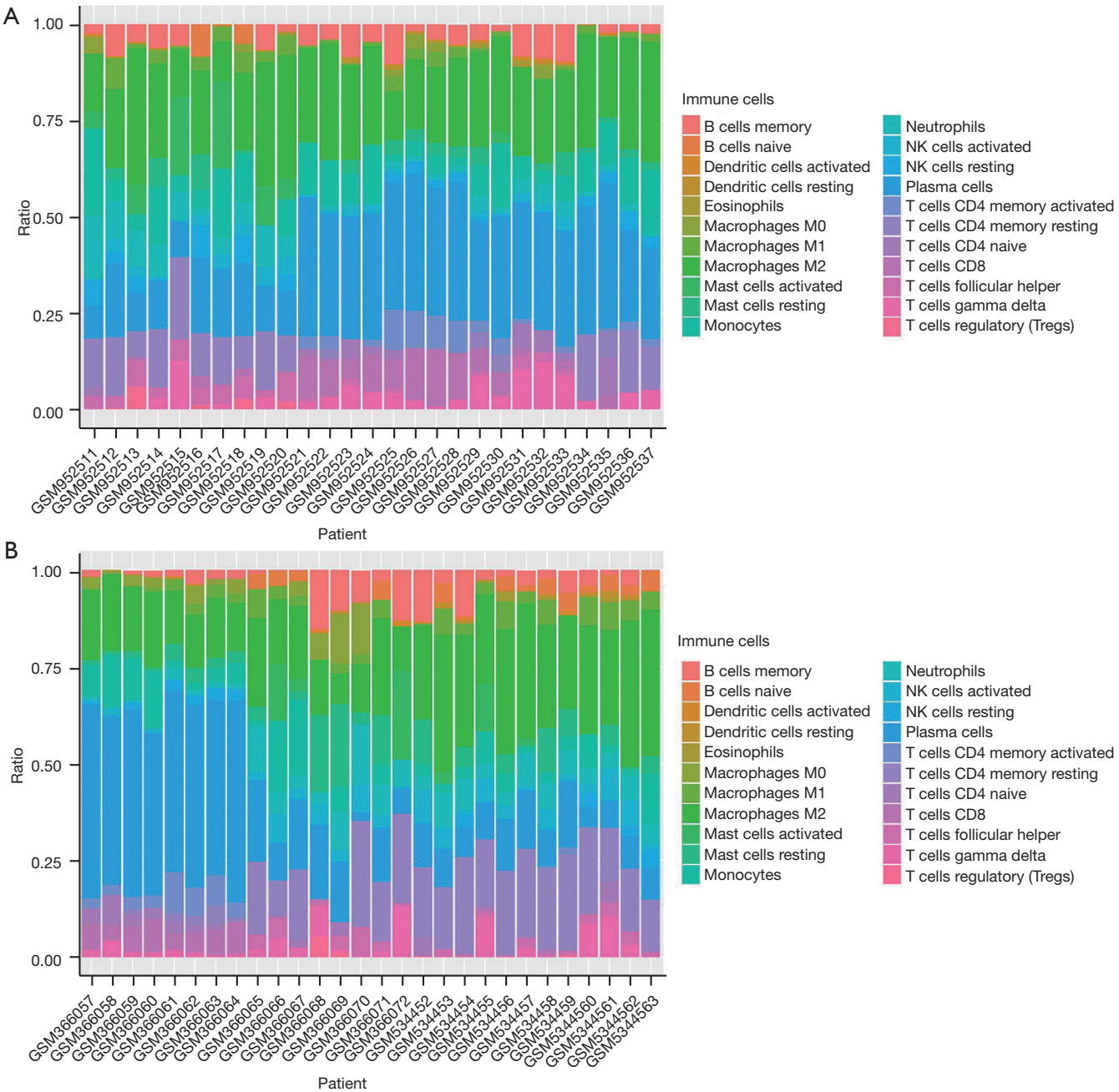

Figure 6 The fraction of 22 subsets of immune cells in hepatitis B virus-induced ALF. (A) GSE38941. (B) GSE14668 . X axis: each GEO sample; Y axis: percentage of each kind of immune cell. GEO, Gene Expression Omnibus; ALF, acute liver failure.

to decrease hepatocyte apoptosis and liver injury induced by TNF (28). From this aspect, we assumed that *AKT1* activation is the body's stress reaction when APAP induces liver failure. An elevated level of lipids may be the primary root cause of hepatocyte injury and inflammation (29). Hence, we believed that APAP-induced ALF may activate inflammation and the immune system by generating a high level of lipids. Immunity-mediated inflammation appears to play a key role in ALF induced by the HBV virus, according to findings.

Studies have revealed that *IGF1* has been implicated in HBV-associated ALF (16,17). GSEA revealed that *IGF1*

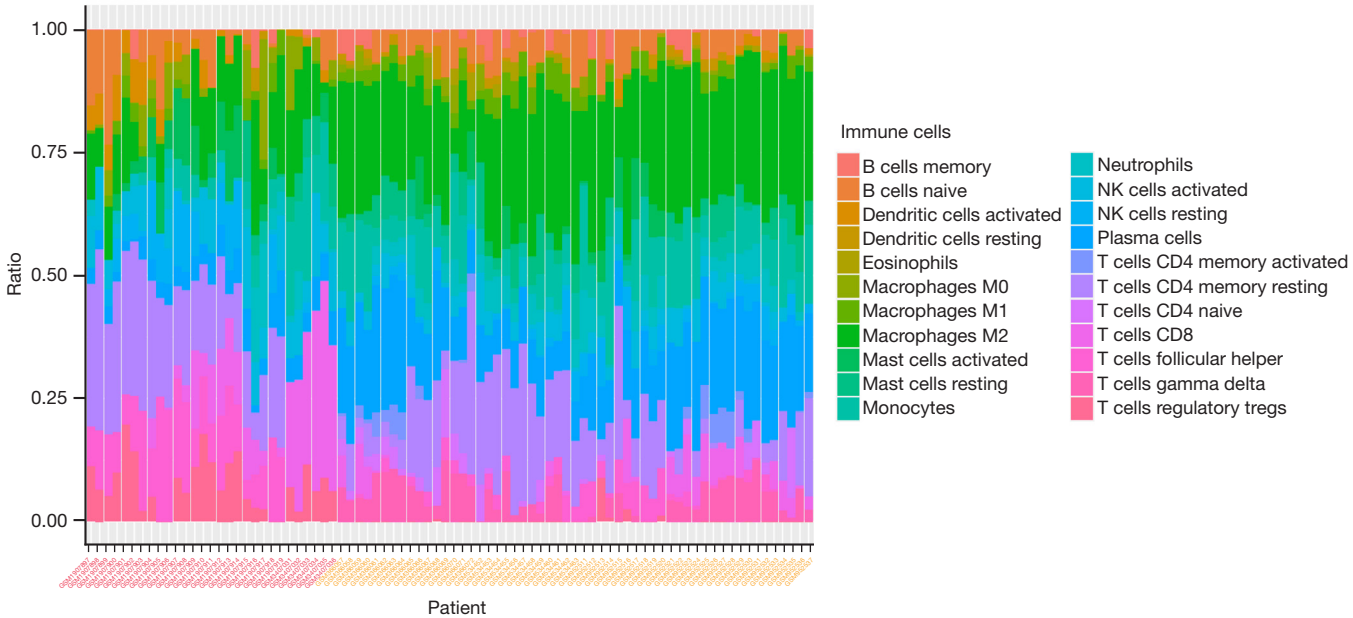

Figure 7 The fraction of 22 subsets of immune cells in 2 types of acute liver failure. X axis: each GEO sample, red represents acetaminophen-induced ALF, yellow represents hepatitis B virus-induced ALF; Y axis: percentage of each kind of immune cell. GEO, Gene Expression Omnibus; ALF, acute liver failure.

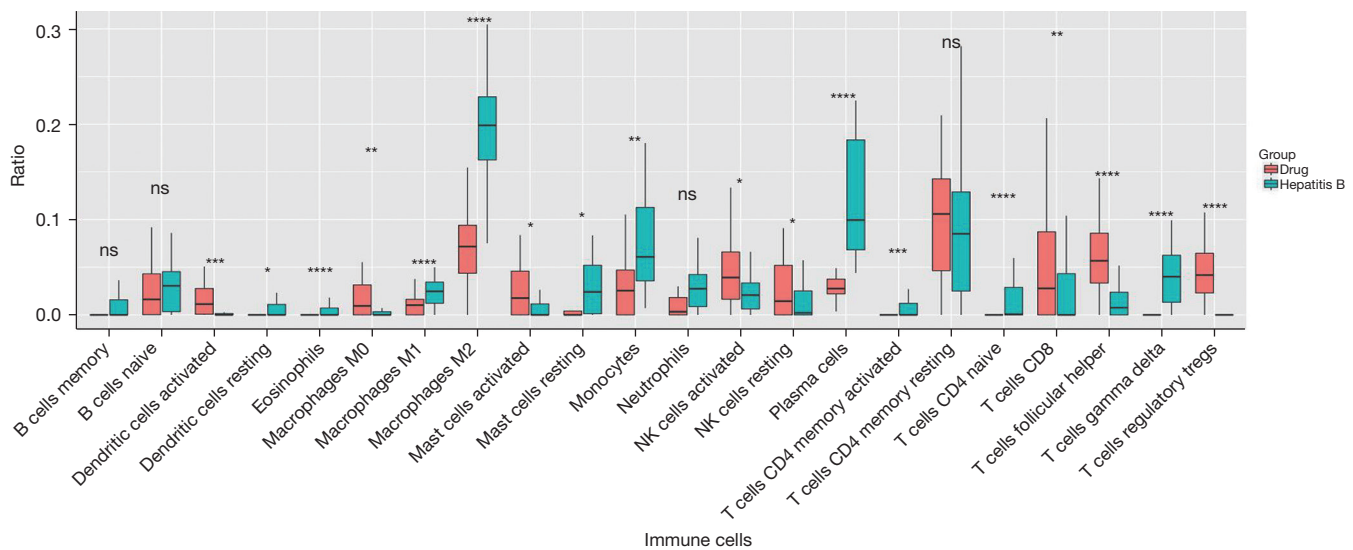

Figure 8 The violin graph shows the difference in immune infiltration between acetaminophen-induced ALF and hepatitis B virus-induced ALF. ns, no sense, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. ALF, acute liver failure.

aided the immune response and restricted intracellular calcium homeostasis in the 2 distinct types of ALF. In individuals with ALF, acute injury stimulates immune cells, resulting in cytokine and chemokine cascades that lead to liver damage, an aggressive systemic inflammatory response syndrome (SIRS), and an increased mortality rate. It has been suggested that the presence of immune soluble components is associated with both the course of the disease and the severity of symptoms (30). Intracellular calcium accumulation has been shown to cause cell death

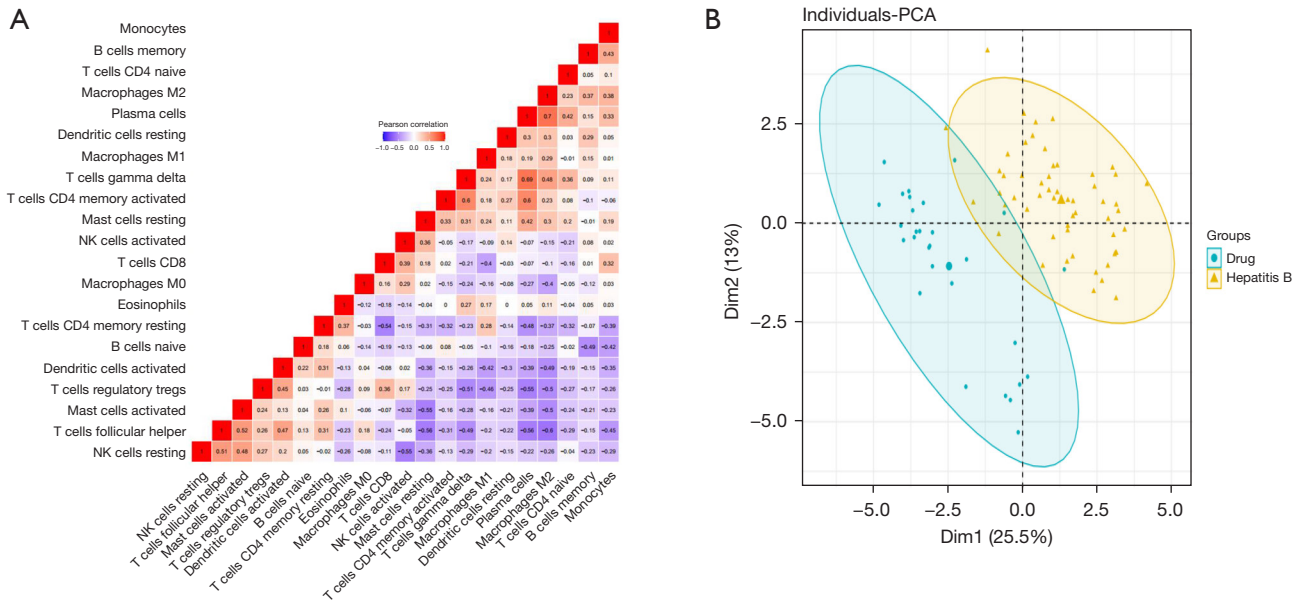

Figure 9 The co-expression patterns among fractions of immune cells. Red: positive correlation; purple: negative correlation (A). Principal component analyses performed on acute liver failure samples (B). PCA, principal components analysis.

via multiple signaling cascades in recent decades. A Study has shown that reducing intracellular calcium levels can prevent cellular damage and can be used to treat ALF characterized by severe liver necrosis and apoptosis (31). In addition, APAP is glucoronidated and sulfated by uridine 5-diphospho-glucuronosyltransferase and sulfotransferase into harmless chemicals that are eliminated via urine in nontoxic amounts (4 g/d). The leftover APAP is transformed into the hazardous metabolite N-acetyl-p-benzoquinone (NAPQI) by cytochrome P450 enzymes (CYPs), which is then conjugated to glutathione (GSH) to detoxify it. At an elevated level of APAP, such as an overdose, the excess NAPQI depletes GSH, causing covalent attachment to hepatic proteins, mitochondrial oxidative stress, and hepatocellular necrosis. Ethanol and other drugs, such as antibiotics and antiepileptics, activate CYPs, causing more NAPQI to be produced (4). As a result, an inflammatory response and cytochrome P450 activation in APAP-induced ALF and HBV-associated ALF may be seen. Furthermore, our findings suggest that in HBV-associated ALF, the MAPK and Ras signaling pathways are activated. Some compounds can prevent acute liver injury by directly decreasing the inflammatory response, thereby blocking the MAPK and Ras cascades (32,33).

ALF is an explosive disease, in which a large number of liver cells are damaged and liver function is significantly decreased in a short time. Current research suggests that the immune response is also involved in the development of this disease, researchers believe that dysfunction of innate and adaptive immune systems during ALF, resulting in paralysis of functional immune cells (34). However, the specific immune cells and the differences between immune cell infiltration in APAP-induced ALF and HBV-induced ALF are still unknown. According to the data obtained by CIBERSORT, the subsets of infiltrated immune cells were different in the 2 types of ALF. T cell-mediated immune damage is significantly involved in APAP-induced ALF, while the immune response caused by NK cells is the most common in HBV-associated ALF. T cell-mediated immune damage is present in many individuals with undiagnosed ALF (35), and Vα14iNKT cell loss protects against APAP-induced ALF by increasing hepatic GSH and altering APAP metabolism (36). The innate immune system relies heavily on NK cells. NK cells grow independently of the thymus in peripheral lymphoid tissues. Lipid antigens presented by the MHC-I-like molecule CD1d (37) can stimulate NK cell activity. Some findings, similar to our results, suggested that the inflammatory cytokines increased in HBV-ALF patients may boost NK cell-mediated cytotoxicity via the TRAIL cascade (38). In this view, the immune response mediated by NK cells is the most common in HBV-related ALF. In conclusion, immunotherapy may be a new treatment for

acute liver failure.

Conclusions

In this study, we evaluated the similarities and differences between 2 forms of ALF, namely APAP-induced ALF and HBV-induced ALF, using the GEO database and bioinformatics tools. The obtained results demonstrated that *IGF1* is a key molecular target in ALF mechanisms. Moreover, we explored the immunological landscape and demonstrated the underlying immune infiltration patterns in the 2 types of ALF. Our research contributes to a better understanding of the immune response and sheds light on the immunological mechanism underlying the progression of ALF for future studies.

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Footnote

Reporting Checklist: The authors have completed the STREGA reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-2742/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-2742/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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