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Effect of Different Blood Purification Methods on Residual Renal Function in Maintenance Hemodialysis Patients

Guangcai Zhang*

Weifang Fangzi District People's Hospital, Weifang 261200, Shandong Province, China

***Corresponding author:** Guangcai Zhang, 953479939@qq.com

Abstract: *Objective:* This study aims to evaluate the efficacy of different blood purification methods in protecting residual renal function (RRF) in patients with maintenance hemodialysis (MHD). *Methods:* The study selected 50 patients receiving MHD between September 2023 and September 2024. Using the random number table method, patients were divided into the control group and the study group, each with 25 participants. The control group received hemodiafiltration, while the study group was treated with hemodialysis plus hemoperfusion. The RRF measures of the two groups were compared. *Results:* There were significant differences in the RRF measures ($P < 0.05$). *Conclusion:* Combined hemodialysis and hemoperfusion therapy can effectively improve the RRF index of MHD patients with a significant curative effect.

Keywords: Blood purification; Maintenance hemodialysis; Residual renal function

1. Introduction

Chronic renal failure, also referred to as chronic renal insufficiency, is a condition characterized by gradual and progressive damage to the kidney's parenchymal tissue. Over time, this damage leads to the atrophy of the kidneys, rendering them unable to perform their essential functions. The failure to filter waste products results in the retention of metabolic byproducts, as well as imbalances in water, electrolytes, and acid-base regulation, affecting multiple organ systems. This clinical syndrome, often reaching its advanced stage in uremia, can take several years or even decades to develop from the initial disease to significant renal insufficiency. Chronic renal failure is a severe stage of renal insufficiency [1]. In multiple stages, uremia marks the terminal stage of chronic renal failure, at which time the patient's glomerular and tubular function is severely impaired, resulting in metabolic abnormalities in the body, which often leads to a series of clinical symptoms such as malnutrition and anemia. In the current treatment strategies, maintenance hemodialysis (MHD) has become an important tool in the treatment of uremia [2]. By removing toxins from the blood, hemodialysis not only delays the

progression of the disease but also helps to prolong the patient's life. However, the specific effects of MHD on residual renal function (RRF) are still insufficient [3]. Selecting a sample of 50 patients with chronic renal failure receiving different blood purification methods, this study observes and compares the specific effects of two different dialysis regimens on their RRF, and strives to provide a more sufficient reference for further clinical improvement and protection of RRF in the future.

2. Data and methods

2.1. General information

The selection period of this study was from September 2023 to September 2024, including 50 patients on MHD in our hospital. Using the random number table method, the patients were divided into the control group and the study group, with 50 patients in each group. In the control group, there were 16 men and 9 women with an age range from 30 to 70 years, with a mean age of 50.11 ± 3.97 years, dialysis duration from 10 to 50 months, and a mean dialysis duration of 30.19 ± 3.11 months. In the study group, there were 15 men and 10 women with an age range from 29 to 70 years, with a mean age of 49.67 ± 3.88 years and dialysis time from 12 to 50 months, with a mean dialysis time of 31.26 ± 3.55 months. The baseline data between the two groups showed no significant difference ($P > 0.05$), indicating that the data of the two groups were comparable.

Inclusion criteria included (1) patients had a plateau; (2) patients had an indication for blood purification; and (3) patients and their families had fully informed and given consent.

Exclusion criteria were (1) patients with immune dysfunction; (2) patients with abnormal blood coagulation function; and (3) patients who were participating in other research programs.

2.2. Methods

In the control group, hemodiafiltration treatment was given. The blood flow velocity was controlled at 250 ml per minute; the effective area of the acetate fiber membrane was 1.8 m^2; the dialysate speed was controlled at 800 ml per minute, and the posterior replacement and displacement amount was 50–70 ml per minute, once for four hours. Hemofiltration treatment was performed once a week, and hemodialysis was performed twice [4].

In the study group, combined treatment with hemoperfusion and hemodialysis was performed. Heparin (100 mg) was poured into the runner and reversed up and down approximately 10 times, then left to stand for 30 minutes. Saline (2000 ml) was used to flush the runner and line, and the dispenser and dialyzer were cleaned in series [5]. Irrigation was repeated with normal saline (1000 ml). The arteriovenous line was connected, and combined dialysis was performed for 0–2 hours, with the blood flow velocity controlled at 200 ml per minute. The perfusion device was removed after 2 hours, and hemodialysis continued for 2 more hours, with the blood flow controlled at 250 ml per minute. Combined dialysis was conducted once a week, with two additional sessions of hemodialysis [6]. All patients were treated for 6 months. During this period, drug treatment, such as blood pressure-lowering medications, was administered according to patients' actual conditions.

2.3. Observation indicators

For accurate assessment of residual renal function, fasting venous blood samples were taken for relevant biochemical tests, ensuring more reliable data and avoiding inaccuracy caused by food intake [7]. Patients fasted for at least 8 hours before blood drawing to eliminate the potential effects of food and fluid intake on renal

function determination values. Thereafter, professional medical staff drew venous blood samples under strict sterile conditions, and the collected blood was immediately sent to the laboratory for analysis.

2.4. Statistical methods

The calculation software used was SPSS25.0; measurement data were expressed as mean ± standard deviation (SD), count data were expressed as [n (%)], by t value and χ^2. $P < 0.05$ indicated a significant difference.

3. Results

3.1. Comparison of the clinical data

There was no significant difference between the age, disease duration, and primary disease type of each group, thus the groups are comparable, as shown in **Table 1**.

Table 1. Comparison of the clinical data

Groups	n	Mean age (years)	Mean course of disease (month)	Primary disease types			
				Chronic glomerulonephritis	Diabetic nephropathy	Hypertension kidney disease	Other
Control group	25	44.50 ± 3.27	15.36 ± 2.14	10	6	7	6
Observation group	25	45.68 ± 3.59	14.68 ± 1.62	9	5	7	7
χ^2/t	-	0.686	0.617	0.068	0.056	0.231	0.281
P	-	> 0.05	> 0.05	0.795	0.812	0.631	0.596

Table 1 presents the comparison of clinical data between the patients in the control and observation groups. Each group had 25 patients, the mean age of the control group was 44.50 years (SD 3.27), and the mean disease duration was 15.36 months (SD 2.14). The primary disease types included 10 cases of chronic glomerulonephritis, six diabetic nephropathy, seven hypertensive nephropathy, and six others. The mean age of the observation group was 45.68 years (SD 3.59), and the mean disease duration was 14.68 months (SD 1.62), including nine cases of chronic glomerulonephritis, five diabetic nephropathy, seven hypertensive nephropathy, and seven others. The statistical differences between the two groups in terms of age, duration of disease, and distribution of primary disease type were not significant ($P > 0.05$), indicating that the data of the two groups were comparable.

3.2. Comparison of RRF index

The data of the two groups were similar before dialysis, the mean values of the study group and the control group were 1.61 ± 0.26 and 1.62 ± 0.33, respectively, and the statistical differences were not significant ($P > 0.05$); after dialysis, the RRF index in the study group was significantly better than the control group ($P < 0.05$), as presented in **Table 2**.

Table 2. Comparison of RRF index (mean ± SD, ml/min)

Groups	n	Before dialysis	After dialysis
Study group	25	1.61 ± 0.26	1.09 ± 0.49
Control group	25	1.62 ± 0.33	0.63 ± 0.39
t	-	0.119	3.672
P	-	0.950	0.000

4. Discussion and conclusion

4.1. Factors affecting RRF and its mechanisms

Residual renal function refers to the portion of kidney function that remains intact despite significant damage during the progression of chronic kidney disease. This function includes, but is not limited to, the ability to remove toxins and regulate water and electrolyte balance. These functions are required for kidney health to maintain environmental stability in the body. Although the RRF may have declined substantially, even limited renal function is important for reducing the systemic toxin burden, maintaining the electrolyte and acid-base balance, and supporting other physiological processes such as hematopoiesis [8]. In fact, even a small RRF significantly reduces dialysis needs and improves patient nutritional status and overall prognosis. Therefore, it is crucial to closely monitor and protect patient RRF during the course of hemodialysis treatment.

The loss of RRF is influenced by a variety of factors, including individual physiological differences in the patient, the primary etiology of chronic kidney disease (e.g., diabetic nephropathy, hypertensive nephropathy, or autoimmune nephritis), and the chosen dialysis technique. Hemodialysis itself, although an important treatment to save the patient's life, may cause further damage to the kidney. The materials and methods used during the dialysis process may cause nephrotoxicity, such as certain dialysis membrane materials may trigger inflammatory reactions, and the composition of the dialysate may not be completely suitable for all patients, thus increasing the burden on the nephron. Mechanical pressure and chemical exposure during dialysis frequently trigger the release of inflammatory mediators such as cytokines and free radicals that can further damage kidney tissue, especially when renal function is already impaired. This dialysis-induced inflammation and kidney damage accelerates the loss of RRF, leading to an increased patient dependence on dialysis.

4.2. Protection of RRF by blood purification technology

4.2.1. Hemodiafiltration

Hemodiafiltration (HDF) is an advanced blood purification technology that combines traditional hemodialysis with an efficient filtration mechanism. In this process, special filter equipment is used to simultaneously remove dissolved toxins and excess water from the blood. This technique helps patients maintain a stable fluid state by effectively controlling water retention, thereby reducing complications caused by excessive water accumulation, such as increased heart burden and hypertension. HDF works with the use of a highly permeable filter membrane that allows the passage of larger molecules of toxins and excess water, while retaining essential components in the blood, such as red blood cells and plasma proteins [9].

During dialysis, HDF removes the solutes in a more stable manner, which is extremely important for maintaining the hemodynamic state of the patient. A more gradual solute clearance helps prevent potential blood pressure fluctuations and other hemodynamic instabilities during dialysis, which can otherwise impose additional strain on the heart or negatively impact residual renal function.

4.2.2. Hemoperfusion

Hemoperfusion uses a physical adsorption device to remove toxins from the blood, and its basic principle is adsorption. The filling column is composed of adsorbent and wrapping material. The adsorbent has resin and activated carbon, and has the ability to adsorb dissolved substances and colloidal substances in the liquid. According to the nature of the force between the surface of the adsorbent and the adsorbent, the adsorption can be divided into two basic types: physical adsorption and chemical adsorption. This method is an efficient blood purification technology, which is used through a physical adsorption device, such as activated carbon or special resin, to remove various toxins from the blood. This technique is particularly suited to remove harmful substances with large molecular weight and difficult to pass through conventional dialysis membranes. During hemoperfusion, blood is directed through filters that contain adsorbents that can capture and fix toxins in the blood [10]. By reducing the use of dialysate, the risk of complications related to dialysate quality issues, such as electrolyte imbalance and contamination, is correspondingly reduced. This lessens the burden on the kidneys, allowing the remaining nephrons to better perform their physiological functions, such as regulating body water and electrolyte balance, as well as processing other metabolic waste. Blood perfusion mainly removes harmful substances in the blood through adsorption, especially the aromatic amino acids adsorption capacity. However, it should be noted in the treatment that this technique can also adsorb the thyroid hormones T_3 and T_4, growth hormone, and hormones such as insulin. Therefore, in the long-term application of hemoperfusion, it is necessary to be alert to the possible decline of hormone levels, and timely supplement or adjust according to the need. Due to the adsorption materials, such as improper washing of the irrigation, excessive residual aldehydes or bubbles, may cause a series of adverse reactions, including hemolysis, headache, and even air embolism.

4.3. Comprehensive application and patient benefits

In exploring the effects of different blood purification techniques on RRF in patients with MHD, the comprehensive application of various blood purification methods, such as hemodialysis (HD) combined with hemoperfusion (HP), shows significant clinical advantages. With HD technology, small and medium molecule toxins can be effectively removed, while HP focuses on removing macromolecules and toxins that are hardly dialyzing. This dual clearance mechanism not only enhances the efficiency of toxin clearance but also alleviates the possible physiological burden of relying on a single purification technique. This comprehensive treatment modality promotes the overall health of patients, including improved nutritional status, by reducing toxin accumulation and improving water and electrolyte balance. The above improvements directly enhance the patient's quality of life and have the potential to indirectly prolong patient survival. Although a combination of both purification technologies may increase healthcare costs at early stages, it could in the long run reduce overall healthcare expenditure by reducing complications and prolonging patient survival. Furthermore, the maintenance and improvement of RRF reduces the financial burden and life disturbance associated with frequent dialysis. Future studies should further explore the effect of this combination treatment model in patients with different types of kidney disease to optimize the treatment plan and achieve the goal of individualized medicine.

Disclosure statement

The author declares no conflict of interest.

References

[1]   Pan H, Zhang Y, Gai Y, et al., 2024, Effect of Different Blood Purification Methods on the Levels of β2-MG, CRP, SCr, and UA in Maintenance Hemodialysis Patients. Journal of Molecular Diagnosis and Therapy, 16(07): 1216–1219 + 1224.

[2]   Xiao F, Zhou D, Xia Y, 2023, Analysis of the Clinical Effect of Vitamin D Combined with Different Blood Purification Methods in the Treatment of Secondary Hyperparathyroidism in Maintenance Hemodialysis Patients. Chinese and Foreign Medical Care, 42(35): 17–20.

[3]   He F, Liang M, Huang X, et al., 2023, Effect of Different Blood Purification Methods on Protein Energy Expenditure in Maintenance Hemodialysis Patients. Shenzhen Journal of Integrated Traditional Chinese and Western Medicine, 33(19): 80–83.

[4]   Xu J, Huang N, 2023, Effect of Different Blood Purification Methods on Mineral and Bone Metabolism in Maintenance Hemodialysis Patients. Medical Innovation in China, 20(18): 66–70.

[5]   Zhang J, Tu L, Yue Y, et al., 2023, Effect of Different Blood Purification Methods on Serum Levels of microRNA-144 and microRNA-155 in Maintenance Hemodialysis Uremic Atherosclerosis Patients and Their Clinical Significance. Progress in Modern Biomedical Sciences, 23(11): 2192–2196 + 2172.

[6]   Li M, 2023, Study on the Effect of LEARNS Mode in Self-Management of Maintenance Hemodialysis Patients, dissertation, Yunnan University of Traditional Chinese Medicine.

[7]   Ran Y, 2023, Meta-Analysis of TCM Treatment for Malnutrition in Maintenance Hemodialysis Patients, dissertation, Hubei University of Traditional Chinese Medicine.

[8]   Suda T, Fujii H, Asakura K, et al., 2023, Fat Embolism Syndrome After Humerus and Pelvis Fracture Complicated by Acute Kidney Injury Requiring Blood Purification: A Case Report and Literature Review. Renal Replacement Therapy, 9(1): 51.

[9]   Liu J, Zhang H, Diao Z, et al., 2023, Epidemiological Analysis of Death Among Patients on Maintenance Hemodialysis: Results from the Beijing Blood Purification Quality Control and Improvement Center. BMC Nephrology, 24(1): 236.

[10]   Solignac J, Lacroix R, Arnaud L, et al., 2022, Rheopheresis Performed in Hemodialysis Patients Targets Endothelium and Has an Acute Anti-Inflammatory Effect. Journal of Clinical Medicine, 12(1): 105.

A Pan-Cancer Analysis of GAPDH as a Common Biomarker for Various Cancers

Yasir Hameed*

Department of Biotechnology, Institute of Biochemistry, Biotechnology, and Bioinformatics, The Islamia University of Bahawalpur, Punjab 63100, Pakistan

*Corresponding author: Yasir Hameed, yasirhameed2011@gmail.com

Abstract: *Background:* The present study aimed to investigate the expression level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and explore its prognostic value across 24 different human cancers. This investigation was conducted using comprehensive bioinformatics and in vitro approaches that involved multiple layers of analysis. *Methods:* GAPDH expression and methylation levels were assessed via bioinformatics tools and validated using cell lines through RNA sequencing and targeted bisulfite sequencing analyses. The potential prognostic significance of GAPDH was evaluated through the use of a Kaplan–Meier plotter. Additionally, cBioPortal was employed to investigate genetic alterations associated with this gene. Pathway analysis was conducted using DAVID. Furthermore, a correlation analysis between GAPDH expression and CD8^{+} T immune cells was performed using TIMER and CDT. Lastly, a gene-drug interaction network analysis was conducted using Cytoscape to examine the relationship between GAPDH and drugs. *Results:* The GAPDH was found commonly up-regulated in 24 types of human cancers and its up-regulation was significantly correlated with the poor relapse-free survival (RFS) and overall survival (OS) of BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. This implies that GAPDH plays a significant role in the development of these cancers. The GAPDH up-regulation was also noticed to be associated with the different clinicopathological features of BLCA, CESC, HNSC, KIRP, LIHC, and LUAD patients. Pathway analysis has shown GAPDH involvement in different diverse pathways. Furthermore, notable correlations were observed between the expression of GAPDH and its promoter methylation level, genetic alterations, as well as the level of CD8^{+} T immune cells. Moreover, we identified significant regulatory drugs targeting GAPDH that have the potential to modulate its expression and potentially prevent conditions such as BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. *Conclusion:* Based on our findings, GAPDH emerged as a promising diagnostic and prognostic biomarker for BLCA, CESC, HNSC, KIRP, LIHC, and LUAD.
Keywords: Cancer; Expression; GAPDH; Biomarker

1. Introduction

Cancer is not a particular disease but a collection of various diseases [1]. It is characterized by the uncontrolled growth and spread of abnormal cells in the body. Cancer can arise in any part of the body and can manifest in numerous forms, such as breast, lung, prostate, and colon cancers [2]. Each type of cancer has its unique characteristics, including specific risk factors, symptoms, and treatment approaches. The impact of cancer extends beyond the physical realm, affecting individuals emotionally, socially, and economically [3]. It places a significant burden on patients, their families, and healthcare systems. The development of cancer can be influenced by various factors, including genetic mutations, environmental exposures, lifestyle

choices, and certain infections [4]. Therefore, cancer can also be described as a disorder of altered gene expression, as it primarily results from variations in the expressions of various DNA repair and tumor suppressor genes [5]. In line with 2019 disease prevalence and mortality statistics, cancer has been declared as the second leading cause of death worldwide with an estimated 9.6 million deaths, or one in six deaths after cardiovascular diseases [6].

Recently, an increasing body of evidence suggested that regulatory changes resulting in the alteration of gene expression play a critical role in complex traits and disorders, and such genomic changes with regulatory effects are also predicted to participate in the development of cancer [7]. Identification of the regulatory alterations and their effect on the gene expression level is an important aspect of understanding cancer biology. Mainly, the major cancer subtypes including breast cancer, colorectal cancer, and leukemia have usually been profiled for CpG methylation, post-transcriptional, post-translational changes, and mutational analysis of a few DNA repair and tumor suppressor genes to understand the molecular landscape of cancer development [8]. However, the effect of such regulatory alterations on the gene expression of several other essential genes is yet to be uncovered.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is an important enzyme in the human body that catalyzes the redox reaction via the glycolytic pathway [9]. GAPDH is a housekeeping enzyme and is thus frequently used as an internal control in various laboratory-based experiments including western blot and reverse transcription-polymerase chain reaction [10,11]. Earlier, the GAPDH up-regulation appears to be linked with cancer development, as the initial evidence was found in the Dunning R-3327 rat prostatic adenocarcinoma, where a higher expression of GAPDH was documented in cancer cells as compared to the ventral prostate tissue of normal rat [12]. In addition, another study by Tang et al. [13] has also reported higher GAPDH expression in human colon cancer (CC) tissue as compared to the controls, which was further found elevated in metastatic liver tissue, suggesting that GAPDH may contribute to colon cancer metastasis. In contrast, Seykora et al. have reported that GAPDH expression might be slightly diminished in melanoma metastases and nodular primary melanomas relative to melanocytic nevi [14], while other reports have shown a significant up-regulation of GAPDH in melanoma tissues as compared to normal tissues [15]. Furthermore, recent studies have revealed the identification of point mutations in the GAPDH gene as a novel melanoma tumor antigen. These mutations have been found to be recognized by tumor-infiltrating T-lymphocytes in a patient with metastatic melanoma [16].

However, the biomarker role of GAPDH in various other human cancers is less reported. In the current study, we analyzed the diagnostic and prognostic potential of GAPDH in 24 human cancer subtypes using a multi-layered bioinformatics and in vitro approach. The novelty of our study lies in the pan-cancer analysis of GAPDH expression across multiple cancer types. To our knowledge, such a comprehensive examination of GAPDH expression in a diverse range of cancers had not been conducted before. By performing this pan-cancer analysis, our study brings to light previously undiscovered trends and associations between GAPDH expression and different cancer types. This broader perspective allows us to identify commonalities and variations in GAPDH expression patterns across various cancers, shedding new light on its potential roles in different oncogenic processes. Therefore, our study adds a unique and valuable dimension to the existing body of research on GAPDH and its implications in cancer biology.

2. Methods

2.1. UALCAN

GAPDH pan-cancer analysis across distinct cancers was performed using the online resource UALCAN. This archive contains raw data from TCGA cancer projects, including the data of expression, methylation,

and clinicopathological parameters-based data [17]. UALCAN is based on data extracted from The Cancer Genome Atlas (TCGA) database, one of the largest and most comprehensive cancer genomics databases in the world. The UALCAN database offers access to processed and normalized transcriptome sequencing data for multiple cancer types, including lung, breast, colorectal, ovarian, and other types of cancer. One of the most significant benefits of the UALCAN database is that it aims to bridge the gap between cancer genomics data and easy-to-use visualization and statistical computation tools. The platform provides researchers with the option to explore cancer transcriptomic data easily and intuitively. The platform incorporates several analytical tools, including gene expression analysis, patient survival analysis, tumor mutation burden analysis, and more [17]. The gene expression module is one of the essential UALCAN features. It enables researchers to analyze the gene expression levels of their genes of interest. They can also compare the expression levels of their genes of interest with normal tissue samples and among tumor subgroups. Moreover, the gene expression tool provides charts, gene expression box plots, and heat maps. For statistics purposes, a student t-test was used by UALCAN. A P value of < 0.05 was considered statistically significant results.

2.2. Kaplan–Meier plotter

The GAPDH relapse-free survival (RFS) and overall survival (OS) in distinct cancer subtypes were evaluated through a user-friendly online tool, Kaplan–Meier (KM) Plotter [18]. KM Plotter is a data visualization tool designed to explore the survival rate of cancer patients based on their gene expression profile. This web-based application allows researchers to create KM survival plots, perform univariate and multivariate analyses, and identify genes or markers that correlate with disease prognosis. With KM Plotter, users can analyze large cohorts of cancer patients from several public databases, including TCGA and GEO, and investigate the impact of specific genes on patient outcomes. Moreover, this intuitive and user-friendly interface offers various customization options and statistical tools to facilitate data exploration and interpretation [18]. The outcomes of KM analysis include the following information: RFS and OS duration (in weeks) with best auto-selected cutoff criteria, P value, and hazard ratios (HR). A P value of < 0.05 was considered statistically significant results.

2.3. MEXPRESS

MEXPRESS [19] was assessed in our study to evaluate the Pearson correlation between GAPDH expression and its promoter methylation levels in different cancers. This database provides researchers with multiple analytical methods to interpret RNA sequencing data and identify relevant gene expression profiles. MEXPRESS allows users to explore gene expression data related to sample types, clinical parameters, and subtypes of cancer. Furthermore, users can access gene-level data, visualizations, and data exports. MEXPRESS is particularly helpful for identifying candidates for new treatments and biomarkers. This platform is free and open to the public, making it an accessible resource for cancer research. MEXPRESS offers a unique combination of user-friendliness, versatility, and trustworthiness that helps researchers gain insights into cancer biology in a straightforward manner [19]. A P value of < 0.05 was deemed significant.

2.4. cBioPortal

In our study, genetic alterations and copy number variation (CNVs) in GAPDH as well as their correlation with the expression level of GAPDH in distinct cancer subtypes were evaluated using the cBioPortal database [20]. cBioPortal is a comprehensive online platform for exploring multi-omics cancer data. It integrates genomic data from public cancer datasets with analysis tools to help researchers gain insights into the molecular mechanisms underlying cancer development and progression. This database provides access to multiple types of data such as gene expression, mutation, copy number variation, protein expression, and clinical information for thousands of cancer patients across numerous cancer types. Researchers can also find

and investigate potential cancer drivers, mutations, and clinical associations using a range of algorithms and visualizations [20].

2.5. Co-express genes, PPI network, and pathway analysis

GEPIA [21] database was used to identify co-express genes with GAPDH. STRING database [22] was used to construct a PPI network of the GAPDH genes via STRING using default settings. Subsequently, the PPI network was visualized using Cytoscape software 3.8.2 [23]. Furthermore, pathway analysis of the GAPDH-enriched genes was carried out using an online tool DAVID [24]. This tool is a bioinformatics software program used by researchers to identify the biological mechanisms and pathways involved in a set of genes or proteins. The tool offers a range of analytical methods, such as functional annotation, gene ontology analysis, pathway analysis, and clustering analysis. Users can upload their own gene lists or use pre-existing ones from publicly available datasets. The DAVID tool bridges the gap between the raw data and biological understanding by providing a comprehensive analysis of gene expression data. A *P* value of < 0.05 was considered significant.

2.6. GAPDH and infiltrating level of CD8$^+$ T cells

The Spearman correlation between GAPDH expression and CD8$^+$ T immune markers in BLCA (bladder urothelial carcinoma), CESC (cervical squamous cell carcinoma and endocervical adenocarcinoma), HNSC (head and neck squamous cell carcinoma), KIRP (kidney renal papillary cell carcinoma), LIHC (liver hepatocellular carcinoma), and LUAD (lung adenocarcinoma) patients was performed through a user-friendly resource, TIMER [25]. TIMER database includes 108 cancer types from the TCGA project. The TIMER2 offers several functionalities, including differential gene expression analysis, survival analysis, and gene correlations. The platform provides insights into the immune microenvironment of tumors and can aid in the development of immunotherapy strategies. The database uses a deconvolution algorithm that integrates multiple immune cell-specific markers to estimate the abundance of immune cells [25]. A *P* value of < 0.05 was considered significant.

2.7. Screening of GAPDH regulatory drugs

CTD database [26] is used in this study to find the GAPDH regulatory drug including both positive and negative regulated drugs. CTD is a valuable resource in the field of toxicology and genomics. It is a comprehensive and curated database that integrates information on gene-disease associations, chemical-gene interactions, and environmental factors related to toxicology. The CTD database plays a crucial role in understanding complex interactions among genes, chemicals, and diseases, ultimately contributing to the development of safer and more effective strategies for risk assessment and environmental health protection. Later, the identified drugs were visualized via Cytoscape 3.8.2.

3. Results

3.1. Expression level analysis of GAPDH

The UALCAN platform was used to analyze the TCGA expression profile of GAPDH in tumor samples and their corresponding normal tissues. This analysis aimed to identify any differences in GAPDH expression between tumor and normal tissues [27]. The results revealed a statistically significant (*P* < 0.05) overexpression of GAPDH in various human cancer samples, including BLCA, CESC, HNSC, KIRP, LIHC, and LUAD when compared to normal controls (**Figure 1**).
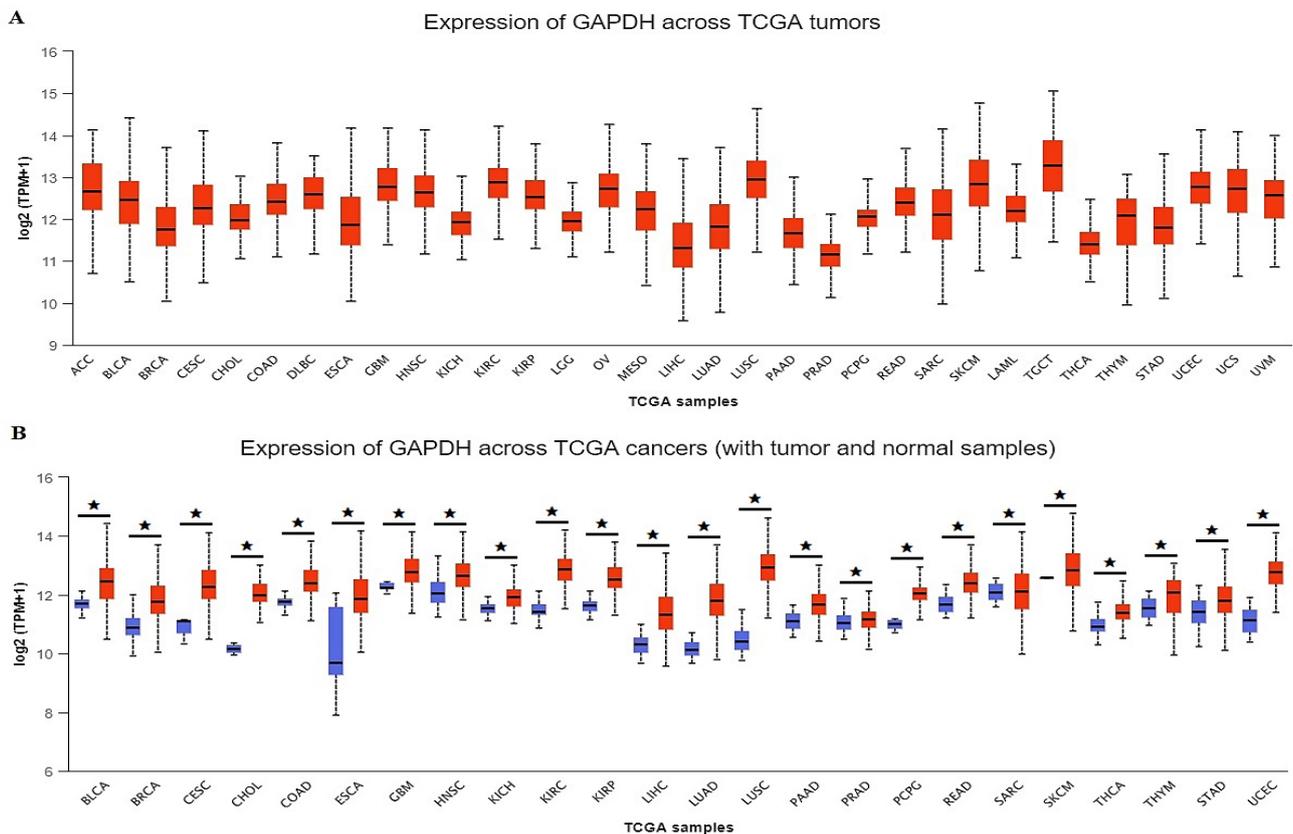
Figure 1. Profile of GAPDH expression across 24 different human cancers. (A) Expression profile of GAPDH in cancer samples exclusively. (B) Expression profile of GAPDH in both cancer samples and normal controls. *P* < 0.05 indicates significance.

3.2. GAPDH prognostic potential

Prognostic analysis of GAPDH in 24 cancer types was carried out using the KM plotter online tool. This analysis aimed to assess the relationship between GAPDH expression and the prognosis in terms of RFS and OS in 24 cancer types. It was observed that overexpressed GAPDH was significantly (*P* < 0.05) linked with the decreased RFS and OS duration of the BLCA (HR = 1.41, 95% CI: 1.05–1.9, *P* = 0.02; HR = 1.93, 95% CI: 1.16–3.19, *P* = 0.0095), CESC (HR = 1.97, 95% CI: 1.24–3.15, *P* = 0.0035; HR = 1.81, 95% CI: 0.83–3.95, *P* = 0.013), HNSC (HR = 1.63, 95% CI: 1.17–2.29, *P* = 0.0038; HR = 1.54, 95% CI: 0.73– 3.29, *P* = 0.026), KIRP (HR = 3.44, 95% CI: 2.01–6.6, *P* = 5.2e-06; HR = 1.84, 95% CI: 0.82–4.14, *P* = 0.013), LIHC (HR = 2.43, 95% CI: 1.69–3.51, *P* = 8.7e-07; HR = 1.75, 95% CI: 1.25–2.46, *P* = 0.001), and LUAD (HR = 1.92, 95% CI: 1.43–2.58, *P* = 1.2e-05; HR = 1.55, 95% CI: 0.96–2.49, *P* = 0.0069) patients (**Figure 2**). Nevertheless, in the context of other cancer types, elevated GPDH expression did not demonstrate a significant correlation with adverse outcomes in terms of RFS and OS. Collectively, these findings suggest that increased GAPDH expression is specifically linked to reduced RFS and OS in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD cancer types. Therefore, the next part of our study will mainly focus on the unique role of GAPDH in these six types of human cancers.
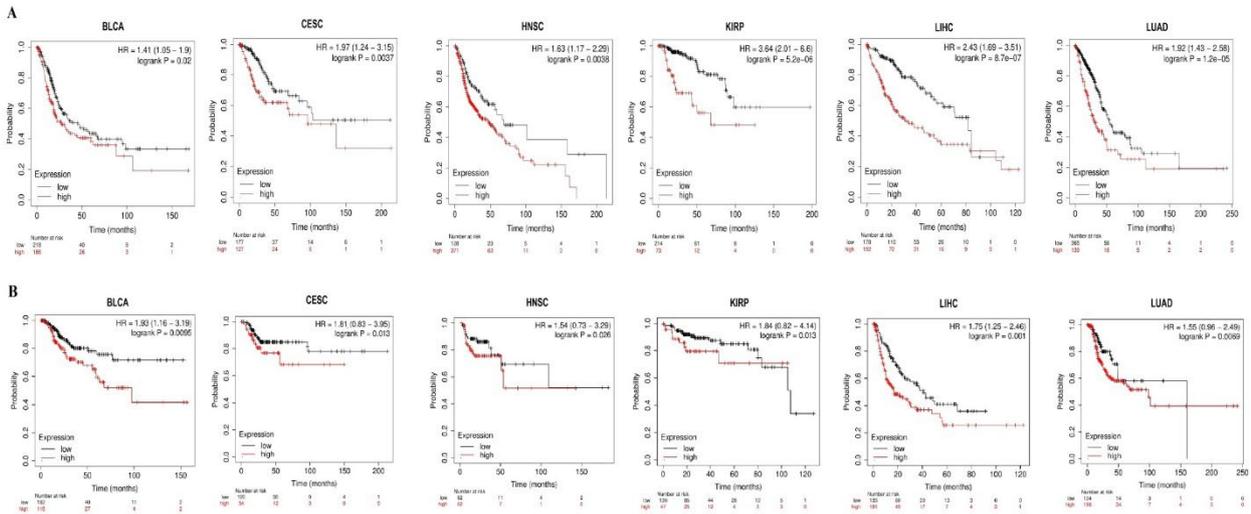
Figure 2. Elevated GAPDH linked to adverse RFS and OS in various cancer subtypes patients. (A) Association of GAPDH with RFS in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. (B) Association of GAPDH with OS in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. *P* < 0.05 indicates significance.

3.3. Association between GAPDH expression and clinicopathological characteristics

Expression analysis of GAPDH expression across different clinicopathological features including different cancer stages, patient's races, and nodal metastasis statuses of BLCA, CESC, HNSC, KIRP, LIHC, and LUAD patients has shown significant (*P* < 0.05) up-regulation of GAPDH relative to normal controls (**Figures 3** to **5**). The Student's *t*-test was employed for comparing the GAPDH expression. This test evaluated whether the means of the two groups were statistically different from each other, with *P* < 0.05 indicating the level of statistical significance.

Based on the observed trends in GAPDH expression across various cancer stages, it becomes evident that the expression of GAPDH is associated with distinct patterns in different types of cancer. In BLCA, for instance, GAPDH expression tends to rise significantly in stages 2 and 3, suggesting a potential role in disease progression during these stages. In CESC, GAPDH expression reaches its highest levels in stage 4, possibly indicating its involvement in advanced disease states. Conversely, in HNSC, GAPDH expression is more pronounced in stage 1, possibly reflecting its role in early-stage cancer. For KIRP, GAPDH expression appears to increase notably in stage 4, suggesting its potential significance in late-stage disease. In LIHC, GAPDH expression is more pronounced in stage 4, possibly indicating a role in advanced liver cancer. Finally, for LUAD, GAPDH expression is higher in stage 2, suggesting its involvement in this particular stage of lung cancer. These findings underscore the complexity of GAPDH expression regulation in different cancer stages. The variations in GAPDH expression patterns may reflect its multifaceted roles in cellular processes and highlight its potential as a marker for specific stages of cancer.

Regarding GAPDH expression in patients of different racial backgrounds, the following patterns emerged: In BLCA patients, African-American individuals exhibited higher GAPDH expression levels compared to their Caucasian and Asian counterparts. Among CESC patients, GAPDH expression was elevated in Caucasian patients relative to African-American and Asian patients. Similarly, in HNSC patients, GAPDH expression was also higher in Caucasian individuals compared to African-American and Asian patients. In KIRP patients, Asian individuals displayed higher GAPDH expression compared to African-American and Caucasian patients. In LIHC patients, African-American patients demonstrated higher

GAPDH expression levels than Asian and Caucasian patients. Lastly, in LUAD patients, GAPDH expression was higher in Caucasian patients compared to Asian and African-American patients (**Figure 4**).

Concerning GAPDH expression in cancer patients with varying nodal metastasis statuses, distinct trends emerged across different cancer types: In BLCA patients, those with N0 status exhibited higher GAPDH expression levels compared to those with N1–N3 status. In CESC patients, those with N1 status displayed elevated GAPDH expression in comparison to those with N0 status. Among HNSC patients, those with N3 status demonstrated higher GAPDH expression levels than those with N0–N2 status. In KIRP patients, individuals with N2 status showed increased GAPDH expression relative to those with N0 and N1 statuses. In LIHC patients, those with N1 status exhibited higher GAPDH expression compared to those with N0 status. Finally, in LUAD patients, those with N4 status displayed elevated GAPDH expression compared to those with N0–N3 statuses (**Figure 5**).

Elevated GAPDH expression points to a metabolic shift, known as the Warburg effect, commonly observed in cancer cells, enhancing glycolysis for rapid energy production and cell proliferation [28]. This phenomenon not only signifies a potential diagnostic tool for early cancer detection but also underscores the aggressive nature of these cancers. Furthermore, these findings may pave the way for innovative therapeutic strategies targeting the glycolytic pathway, including GAPDH, to disrupt cancer cell metabolism and improve treatment outcomes.
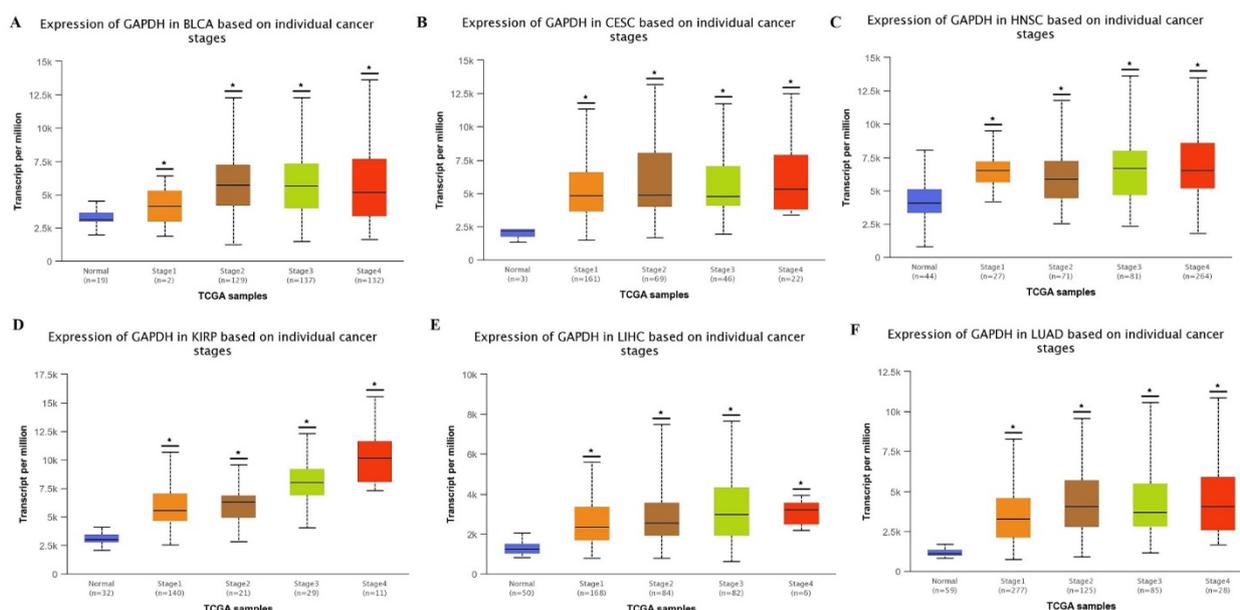

Figure 3. Cancer stage-specific expression patterns of GAPDH in various human cancers. (A) BLCA, (B) CESC, (C) HNSC, (D) KIRP, (E) LIHC, and (F) LUAD. *P* < 0.05 was deemed statistically significant.
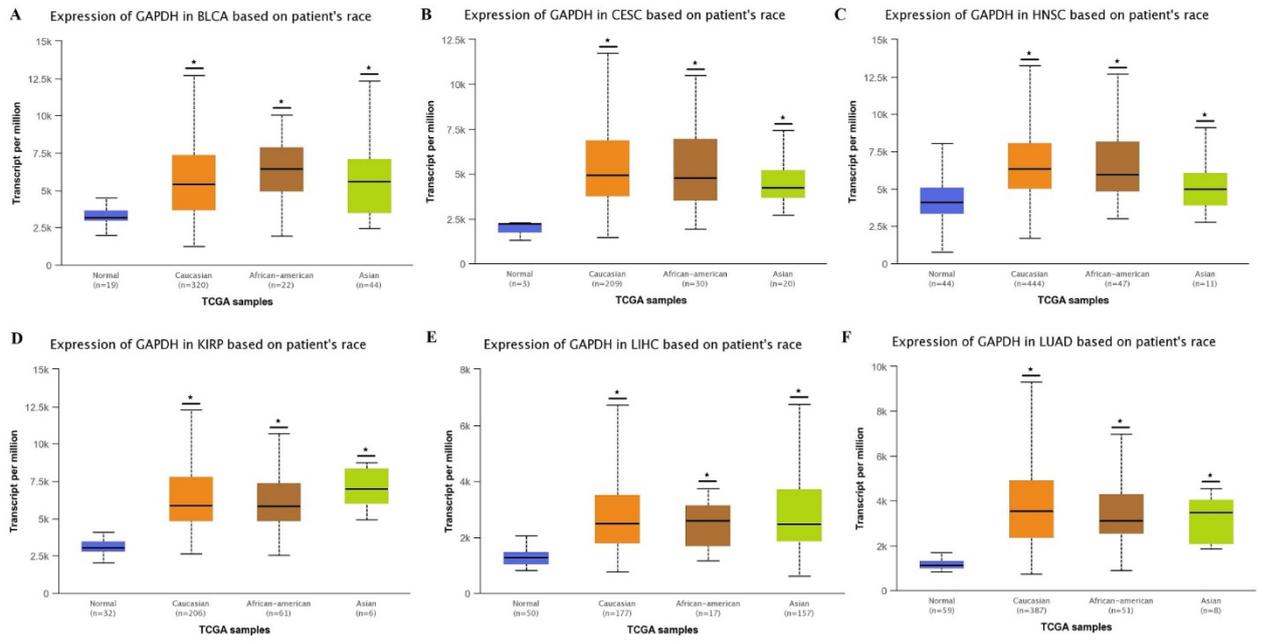
Figure 4. Race-specific expression patterns of GAPDH in various human cancers. (A) BLCA, (B) CESC, (C) HNSC, (D) KIRP, (E) LIHC, and (F) LUAD. *P* < 0.05 was considered indicative of statistically significant results.
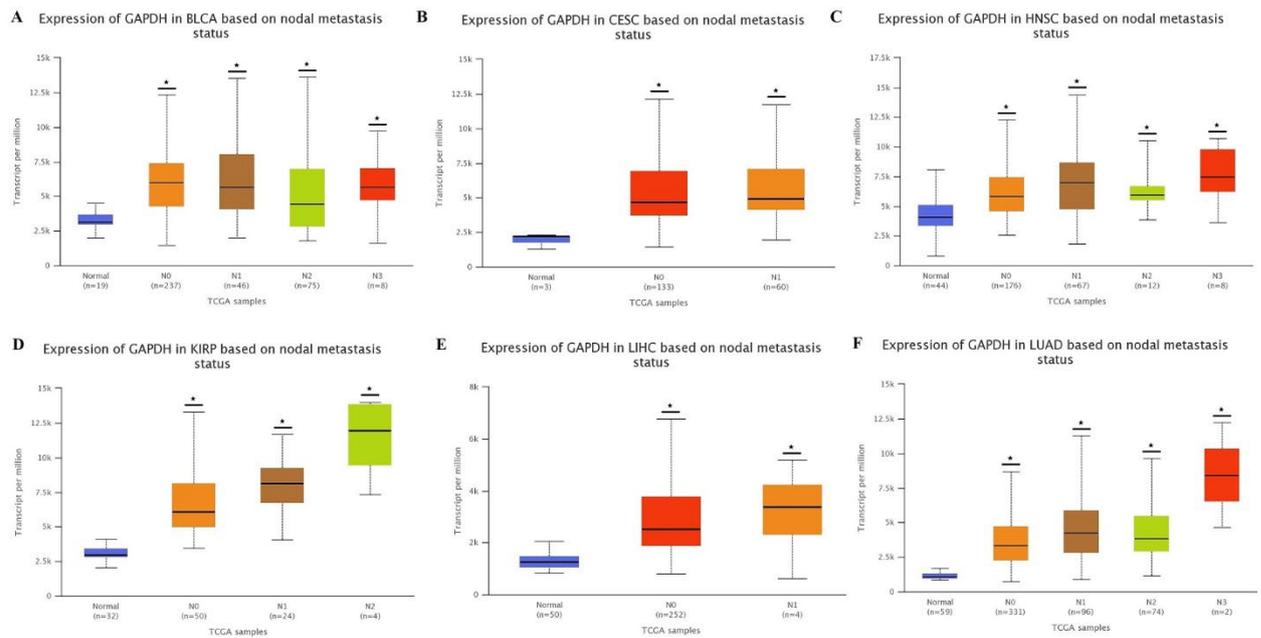
Figure 5. GAPDH expression profiles specific to nodal metastasis in various human cancers. (A) BLCA, (B) CESC, (C) HNSC, (D) KIRP, (E) LIHC, and (F) LUAD. *P* < 0.05 was considered statistically significant.

3.4. Promoter methylation

The MEXPRESS database was employed to examine the correlation between GAPDH promoter methylation and its expression in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. The analysis revealed a significant (*P* < 0.05) negative correlation between GAPDH promoter methylation level and its expression in these cancer types (**Figure 6**).
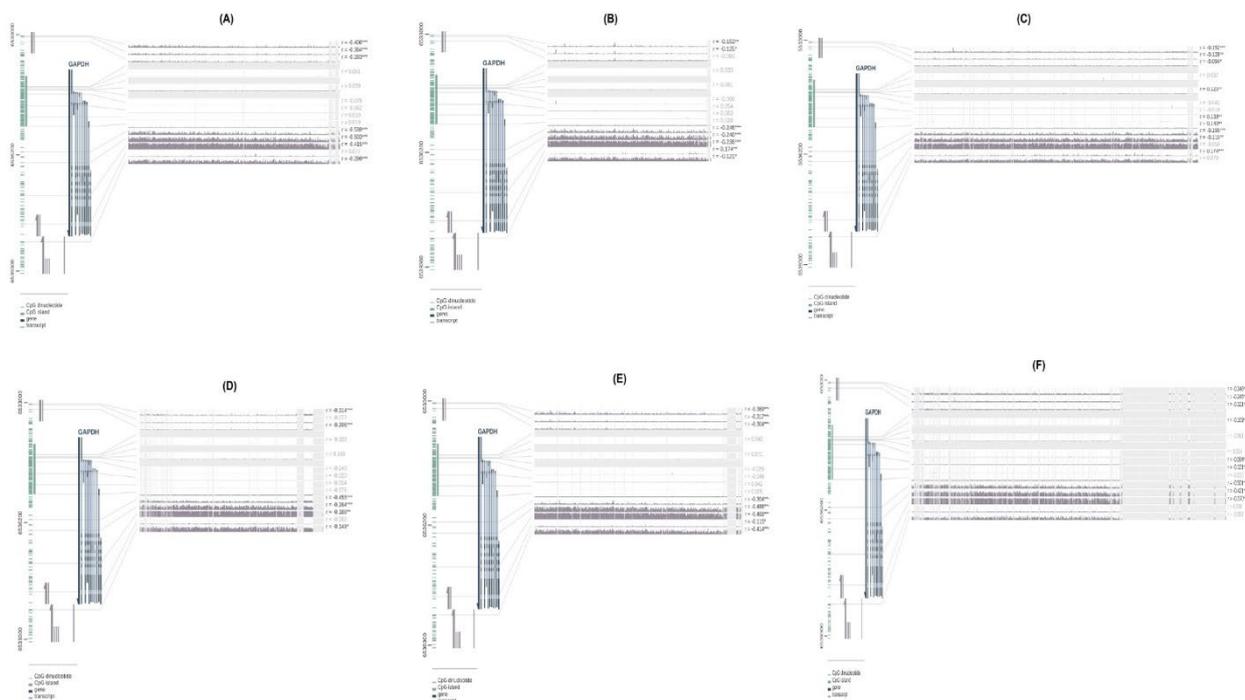
Figure 6. A correlation analysis between GAPDH expression and its promoter methylation level in different cancer subtypes via MEXPRESS. (A) In BLCA, (B) In CESC, (C) In HNSC, (D) In KIRP, (E) In LIHC, and (F) In LUAD. $P < 0.05$ was considered to indicate statistically significant results.

3.5. Genomic analysis

Details regarding genetic alterations in GAPDH within BLCA, CESC, HNSC, KIRP, LIHC, and LUAD were extracted from various TCGA datasets including, "Bladder Urothelial carcinoma (TCGA, Firehose Legacy consisting of 413 cancerous samples), Cervical Squamous Cell Carcinoma (TCGA, PanCancer Atlas consisting of 297 cancerous samples), Head and Neck Squamous Cell Carcinoma (TCGA, Firehose Legacy consisting of 530 cancerous samples), Kidney Renal Clear Cell Carcinoma (TCGA, Firehose Legacy, consisting of 538 cancerous samples), Liver Hepatocellular Carcinoma (TCGA, Firehose Legacy consisting of 379 cancerous samples), and Lung Adenocarcinoma (TCGA, Firehose Legacy consisting of 586 cancerous samples)." Results revealed that GAPDH harbors genetic alterations in 2.4% cases of the BLCA with maximum deep amplification, 1.4% cases of the CESC patients with maximum missense mutations, 2.4% cases of the HNSC patients with deep amplification, 0.5% cases of the KIRP with deep amplification, and 0.4% cases of the LIHC with missense mutations, and 4% cases of the LUAD patients with maximum deep deletion (**Figure 7A–F**). Moreover, GAPDH expression was assessed in two distinct groups: one comprising BLCA, CESC, HNSC, KIRP, LIHC, and LUAD samples without mutations in GAPDH, and the other consisting of samples from the same cancer types but with mutations in GAPDH. The results indicated that there was no significant disparity in overall gene expression, including the expression of GAPDH between these two sample groups (**Figure 7G**). These findings suggest that mutations in GAPDH have no involvement in the regulation of its expression across the studied cancers.
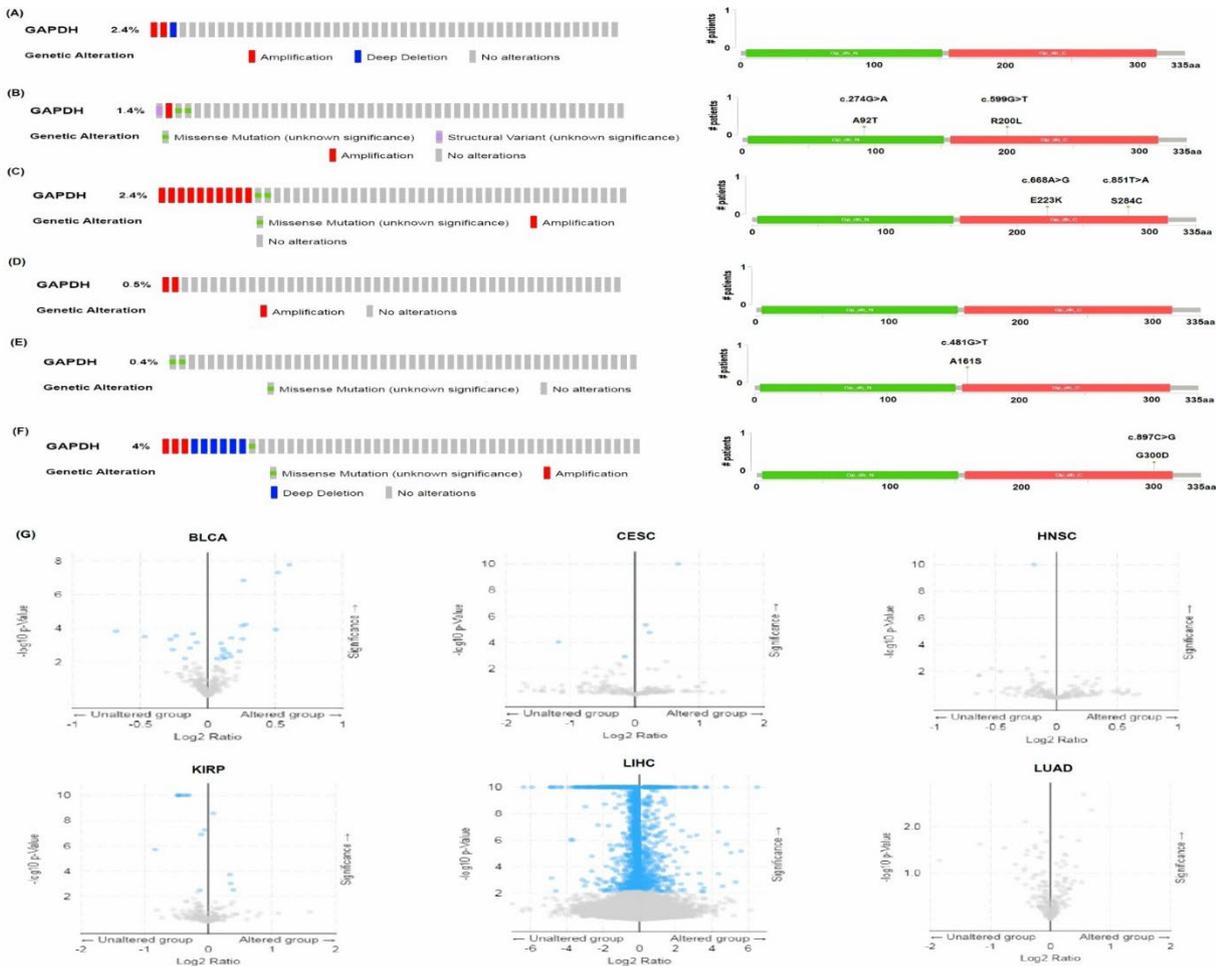
Figure 7. Analysis of genetic alterations in GAPDH across different TCGA datasets. (A) BLCA dataset, (B) CESC dataset, (C) HNSC dataset, (D) KIRP dataset, (E) LIHC dataset, and (F) LUAD dataset.

3.6. Co-express genes, PPI network, and pathway analysis

Firstly, GEPIA was used to identify GAPDH co-express genes in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. Analysis results showed that TPI1, GAPDHP1, PHB2, ALDOA, YBX3, ENO1, PKM, MRPL51, USP5, and NCAPD2 are the top nine co-express genes with GAPDH (**Figure 8A**). We further processed these GAPDH-enriched genes for pathway analysis using the DAVID tool. Results have shown that GAPDH-enriched genes were significantly involved in different pathways including "Fructose and mannose metabolism," "Glycolysis/Gluconeogenesis," "Biosynthesis of amino acids," "Carbon metabolism," etc. (**Figure 8B**).
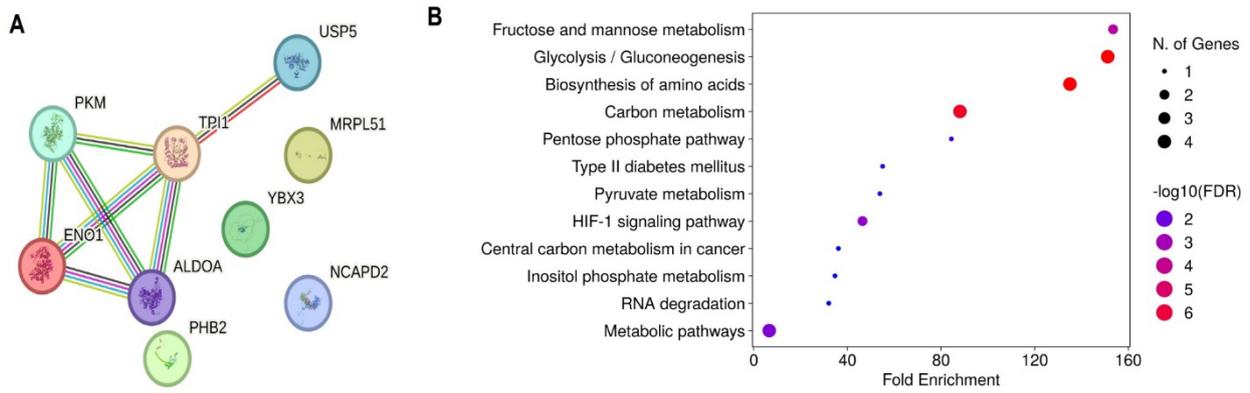
Figure 8. Construction of a protein-protein interaction (PPI) network and pathway analysis of genes enriched with GAPDH. (A) PPI network displaying the interactions among genes enriched with GAPDH, and (B) Pathway analysis illustrating the functional pathways associated with genes enriched with GAPDH.

3.7. GAPDH and infiltrating level of CD8$^+$ T cells

CD8$^+$ T immune cells play a crucial role in the success of current cancer immunotherapies [29]. Therefore, maintaining an appropriate level of CD8$^+$ T immune cells in cancer tissues is of utmost importance. In our study, TIMER was used to calculate the Spearman correlation between the expression of GAPDH and the level of CD8$^+$ T cells. The results demonstrated a significant ($P < 0.05$) positive correlation between the expression of GAPDH and CD8$^+$ T immune cell level in BLCA, while a significant ($P < 0.05$) negative correlation was observed in CESC, HNSC, KIRP, LIHC, and LUAD (**Figure 9**).
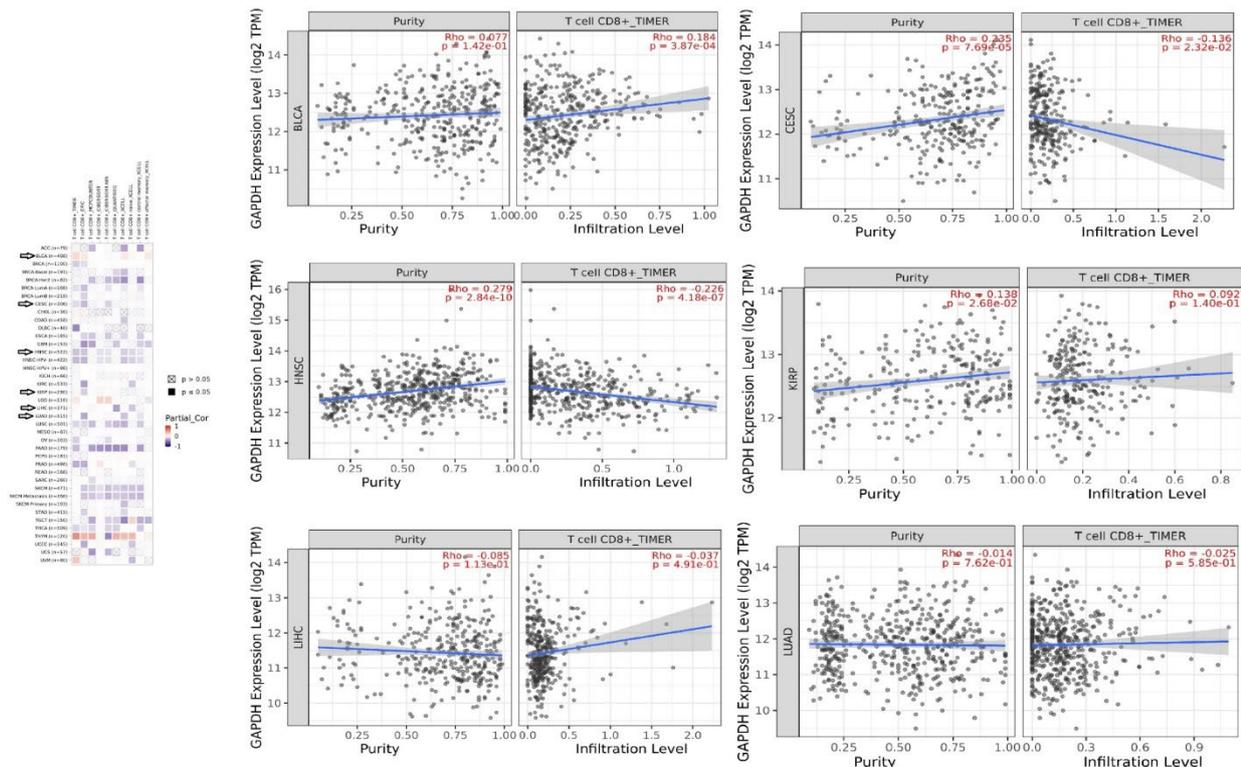
Figure 9. Correlational analysis of GAPDH expression and levels of CD8$^+$ T cells in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD using TIMER. $P < 0.05$ was considered indicative of statistically significant results.

3.8. GAPDH-associated drugs

We conducted a gene-drug interaction network analysis to identify drugs that are associated with GAPDH in the CTD database. The analysis revealed several drugs that have the potential to regulate the expression of GAPDH. For instance, bisphenol A and tretinoin were found to increase the expression level of GAPDH, while ethinyl estradiol and carbon tetrachloride were associated with a decrease in GAPDH expression level (**Figure 10**). These findings suggest that these drugs may have an impact on GAPDH expression and provide insights into potential therapeutic interventions.
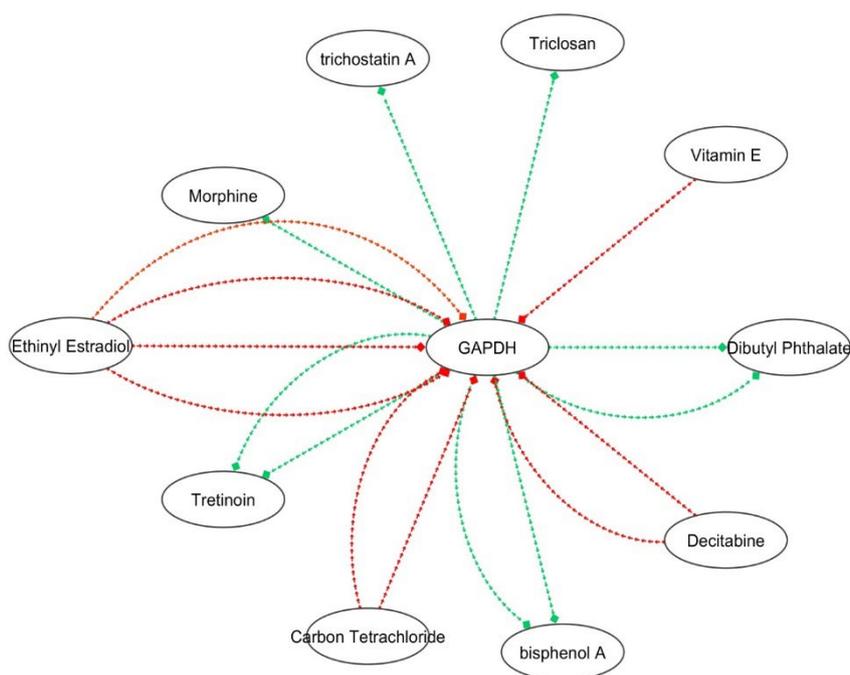

Figure 10. Network of drugs targeting GAPDH. Red color indicates drugs that can increase GAPDH expression, and green arrows represent drugs that can decrease GAPDH expression. The numbers of arrows denote the reference count of selected drugs.

4. Discussion

Under normal physiological conditions, healthy cells primarily rely on oxidative phosphorylation, a more energy-efficient process, to generate adenosine triphosphate (ATP) [30]. However, in stark contrast, cancer cells often exhibit a metabolic shift known as the Warburg effect. This effect refers to the active utilization of glycolysis, the breakdown of glucose, for ATP production, even in the presence of sufficient oxygen. Despite the availability of oxygen, cancer cells demonstrate a preference for glycolysis, leading to increased glucose uptake and lactate production [30]. The Warburg effect is a hallmark of many cancer types and provides cancer cells with metabolic advantages such as increased biosynthetic precursors and reduced dependence on oxygen for energy production. Understanding the mechanisms behind the Warburg effect is crucial for developing targeted therapeutic approaches that exploit the metabolic vulnerabilities of cancer cells while sparing normal cells [30]. GAPDH, a glycolytic enzyme explicitly catalyzed the conversion of glyceraldehyde-3-phosphate (G-3-P) to 1,3-diphosphoglycerate [31]. Besides, GAPDH also contributes to numerous other cellular functions, for instance, it participates in the export of nuclear tRNA, DNA repair, DNA replication, exocytosis, endocytosis, cytoskeletal organization carcinogenesis, and cell death [32,33].

Although GAPDH is commonly utilized as an internal control, its expression variations have also been documented in various human cell lines [34]. Remarkably, decreased expression of GAPDH has been observed in breast cancer, glioma, prostatic cancer, liver cancer, colorectal cancer, pancreatic cancer, gastric cancer, melanoma, and bladder cancer [35]. Increased levels of GAPDH have also been confirmed as a pro-apoptotic agent by Nakajima et al. [36]. Such variation in the expression of GAPDH in different cancer subtypes suggested its inconsistent role in the determination of cell fate [35].

To the best of our knowledge, no previous study has investigated the expression profile of GAPDH in different human cancer subtypes and its correlation with various clinicopathological features such as RFS, OS, promoter methylation status, genetic alterations, CNVs, and CD8$^+$ T immune cells level. Hence, this study aimed to examine the expression pattern of GAPDH across 24 types of human cancers and its association with diverse parameters including RFS, OS, promoter methylation status, genetic alterations, CNVs, and CD8$^+$ T immune cell level. By exploring these relationships, we aimed to enhance our understanding of the potential role of GAPDH in cancer development and its significance as a biomarker in different cancer types.

We observed that GAPDH was up-regulated in 24 major human cancers and its overexpression was significantly associated with decreased RFS and OS in patients with BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. These findings suggest a crucial role for GAPDH in the development of these specific cancer subtypes. Therefore, our study focused primarily on BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. Furthermore, we found that GAPDH was significantly overexpressed ($P < 0.05$) in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD patients across different clinicopathological features, including cancer stages, patient races, and nodal metastasis statuses, when compared to normal controls. The increased expression of GAPDH throughout all stages of these cancers suggests its involvement not only in glycolysis-related processes but also in non-glycolytic mechanisms during tumor development. Moreover, the observed elevated expression of GAPDH in patients of different races with BLCA, CESC, HNSC, KIRP, LIHC, and LUAD highlights the potential for race-independent treatment strategies in these patient populations. Moreover, an increased GAPDH expression in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD patients of different nodal metastasis statuses has implied that GAPDH may also affect the prognosis of these patients.

To investigate the factors contributing to the overexpression of GAPDH in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD, we performed a correlation analysis using the MEXPRESS database to examine the relationship between GAPDH and its expression levels. Results revealed an expected significantly negative correlation between its expression and promoter methylation level. Collectively, our findings suggest that GAPDH expression regulation in CESC, HNSC, KIRP, LIHC, and LUAD may be significantly influenced by GAPDH hypomethylation. However, further experimental studies on a larger scale are necessary to validate and expand upon these findings.

Several biomarkers have been identified so far for the diagnosis and prognosis of BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. For example, Recently, Brisuda et al. identified circulating tumor DNA (ctDNA) as a novel biomarker of BLCA [37]. Berger et al. [38] identified various mutated genes as novel biomarkers of CESC by analyzing Gene GEO datasets. Song et al. [39] developed a long noncoding RNA-microRNA-mRNA network in CESC using GEO datasets and provided novel insights into CESC Biology. Sawyers et al. published a review article in which they shed light on the role of novel HNSC molecular biomarkers including EGFR, CCND1, Bcl-2, Kip1, VEGF, and p53 [40]. Similarly, various KIRP-related diagnostic and prognostic biomarkers have been identified so far including VHL [41], VEGF [42], CAIX [43], and HIF1a/2a [44]. Furthermore, the diagnostic and prognostic potential of the different genes including TTF-1, p63, CK5/6, Napsin A, SPATS2, and ST6GALNAC1 have also been well identified in LUAD by a previous study [45]. However, there is a lack of generalization of any biomarkers in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD patients with diverse clinicopathological features. Herein, we observed a significant up-regulation of GAPDH expression in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD patients

with various clinicopathological features compared to the control group. Additionally, our analysis of GAPDH promoter methylation level, as well as the assessment of RFS and OS, supports its potential as a novel biomarker for BLCA, CESC, HNSC, KIRP, LIHC, and LUAD patients.

CD8^{+} T cells play a pivotal role in the immune response against cancer [46]. Our study revealed intriguing correlations between GAPDH expression and CD8^{+} T immune cells in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. These findings suggest that GAPDH might play a role in modulating the immune response and contribute to the development of these cancers.

In our study, pathway analysis of GAPDH enriched genes revealed their involvement in several KEGG pathways, including "Fructose and mannose metabolism," "Glycolysis/Gluconeogenesis," "Biosynthesis of amino acids," "Carbon metabolism," etc. In addition, we have also identified a few potential drugs that could help to prevent BLCA, CESC, HNSC, KIRP, LIHC, and LUAD by controlling GAPDH expression.

5. Conclusion

In summary, our study has identified the diagnostic significance of GAPDH in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD patients with diverse clinicopathological features. We have also assessed the prognostic values of GAPDH and established correlations with its expression, which could potentially aid in predicting the prognosis of BLCA, CESC, HNSC, KIRP, LIHC, and LUAD patients using GAPDH as a prognostic marker. However, further experimental investigations are warranted before translating these findings into clinical implications.

Disclosure statement

The author declares no conflict of interest.

References

[1]   Brucher BLDM, Lyman G, van Hillegersberg R, et al., 2014, Imagine a World Without Cancer. BMC Cancer, (14): 186.
[2]   Feng Y, Spezia M, Huang S, et al., 2018, Breast Cancer Development and Progression: Risk Factors, Cancer Stem Cells, Signaling Pathways, Genomics, and Molecular Pathogenesis. Genes Dis, (5): 77–106.
[3]   Stein KD, Syrjala KL, Andrykowski MA, 2008, Physical and Psychological Long-Term and Late Effects of Cancer. Cancer, (112): 2577–2592.
[4]   Anand P, Kunnumakkara AB, Sundaram C, et al., 2008, Cancer is a Preventable Disease that Requires Major Lifestyle Changes. Pharm Res, (25): 2097–2116.
[5]   Lahtz C, Pfeifer GP, 2011, Epigenetic Changes of DNA Repair Genes in Cancer. Journal of Molecular Cell Biology, (3): 51–58.
[6]   Siegel RL, Miller KD, 2020, Cancer Statistics, 2020. CA Cancer J Clin, (70): 7–30.
[7]   Khurana E, Fu Y, Chakravarty D, et al., 2016, Role of Non-Coding Sequence Variants in Cancer. Nat Rev Genet, (17): 93–108.
[8]   Sharma A, Jiang C, De S, 2018, Dissecting the Sources of Gene Expression Variation in a Pan-Cancer Analysis Identifies Novel Regulatory Mutations. Nucleic Acids Research, (46): 4370–4381.
[9]   Lazarev VF, Guzhova IV, Margulis BA, 2020, Glyceraldehyde-3-Phosphate Dehydrogenase is a Multifaceted Therapeutic Target. Pharmaceutics, (12): 416.
[10]   Mori R, Wang Q, Danenberg KD, et al., 2008, Both Beta-Actin and GAPDH Are Useful Reference Genes for Normalization of Quantitative RT-PCR in Human FFPE Tissue Samples of Prostate Cancer. Prostate, (68): 1555–1560.
[11]   Murthi P, Fitzpatrick E, Borg AJ, et al., 2008, GAPDH, 18S rRNA and YWHAZ Are Suitable Endogenous Reference Genes for Relative Gene Expression Studies in Placental Tissues from Human Idiopathic Fetal Growth Restriction. Placenta, (29): 798–801.

[12] Epner DE, Partin AW, Schalken JA, et al., 1993, Association of Glyceraldehyde-3-Phosphate Dehydrogenase Expression with Cell Motility and Metastatic Potential of Rat Prostatic Adenocarcinoma. Cancer Res, (53): 1995–1997.

[13] Tang Z, Yuan S, Hu Y, et al., 2012, Over-Expression of GAPDH in Human Colorectal Carcinoma as a Preferred Target of 3-Bromopyruvate Propyl Ester. J Bioenerg Biomembr, (44): 117–125.

[14] Seykora JT, Jih D, Elenitsas R, et al., 2003, Gene Expression Profiling of Melanocytic Lesions. The American Journal of Dermatopathology, (25): 6–11.

[15] Giricz O, Lauer-Fields JL, Fields GB, 2008, The Normalization of Gene Expression Data in Melanoma: Investigating the Use of Glyceraldehyde 3-Phosphate Dehydrogenase and 18S Ribosomal RNA as Internal Reference Genes for Quantitative Real-Time PCR. Analytical Biochemistry, (380): 137–139.

[16] Zhou J, Dudley ME, Rosenberg SA, et al., 2005, Persistence of Multiple Tumor-Specific T-Cell Clones is Associated with Complete Tumor Regression in a Melanoma Patient Receiving Adoptive Cell Transfer Therapy. Journal of Immunotherapy, (28): 53.

[17] Chandrashekar DS, Bashel B, Balasubramanya SAH, et al., 2017, UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia, (19): 649–658.

[18] Maciejczyk A, Szelachowska J, Czapiga B, et al., 2013, Elevated BUBR1 Expression is Associated with Poor Survival in Early Breast Cancer Patients: 15-Year Follow-Up Analysis. J Histochem Cytochem, (61): 330–339.

[19] Koch A, De Meyer T, Jeschke J, et al., 2015, MEXPRESS: Visualizing Expression, DNA Methylation and Clinical TCGA Data. BMC Genomics, (16): 636.

[20] Cerami E, Gao J, Dogrusoz U, et al., 2012, The cBio Cancer Genomics Portal: An Open Platform for Exploring Multidimensional Cancer Genomics Data. Cancer Discovery, (2): 401–404.

[21] Tang Z, Li C, Kang B, et al., 2017, GEPIA: A Web Server for Cancer and Normal Gene Expression Profiling and Interactive Analyses. Nucleic Acids Res, (45): W98–W102.

[22] von Mering C, Huynen M, Jaeggi D, et al., 2003, STRING: A Database of Predicted Functional Associations Between Proteins. Nucleic Acids Res, (31): 258–261.

[23] Shannon P, Markiel A, Ozier O, et al., 2003, Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. Genome Research, (13): 2498–2504.

[24] Huang DW, Sherman BT, Tan Q, et al., 2007, The DAVID Gene Functional Classification Tool: A Novel Biological Module-Centric Algorithm to Functionally Analyze Large Gene Lists. Genome Biology, (8): R183.

[25] Li T, Fu J, Zeng Z, et al., 2020, TIMER2.0 for Analysis of Tumor-Infiltrating Immune Cells. Nucleic Acids Research, (48): W509–W514.

[26] Mattingly CJ, Colby GT, Forrest JN, et al., 2003, The Comparative Toxicogenomics Database (CTD). Environmental Health Perspectives, (111): 793–795.

[27] Rio DC, Ares M Jr., Hannon GJ, et al., 2010, Purification of RNA Using TRIzol (TRI reagent). Cold Spring Harb Protoc, (6): 5439.

[28] Ghatak S, Muthukumaran RB, Nachimuthu SK, 2013, A Simple Method of Genomic DNA Extraction from Human Samples for PCR-RFLP Analysis. J Biomol Tech, (24): 224–231.

[29] Ziai J, Gilbert HN, Foreman O, et al., 2018, CD8^{+} T Cell Infiltration in Breast and Colon Cancer: A Histologic and Statistical Analysis. PLoS One, (13): e0190158.

[30] Warburg O, 1956, On the Origin of Cancer Cells. Science, (123): 309–314.

[31] Wang J, Li Y, Pan L, et al., 2021, Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) Moonlights as an Adhesin in Mycoplasma hyorhinis Adhesion to Epithelial Cells as Well as a Plasminogen Receptor Mediating Extracellular Matrix Degradation. Veterinary Research, (52): 80.

[32] Colell A, Green D, Ricci J, 2009, Novel Roles for GAPDH in Cell Death and Carcinogenesis. Cell Death & Differentiation, (16): 1573–1581.

[33] Ganapathy-Kanniappan S, Kunjithapatham R, Geschwind JF, 2012, Glyceraldehyde-3-Phosphate Dehydrogenase: A Promising Target for Molecular Therapy in Hepatocellular Carcinoma. Oncotarget, (3): 940–953.

[34] Caradec J, Sirab N, Revaud D, et al., 2010, Is GAPDH a Relevant Housekeeping Gene for Normalisation in Colorectal Cancer Experiments? British Journal of Cancer, (103): 1475–1476.

[35] Guo C, Liu S, Sun MZ, 2013, Novel Insight into the Role of GAPDH Playing in Tumor. Clinical and Translational Oncology, (15): 167–172.

[36] Nakajima H, Amano W, Fujita A, et al., 2007, The Active Site Cysteine of the Proapoptotic Protein Glyceraldehyde-3-Phosphate Dehydrogenase is Essential in Oxidative Stress-Induced Aggregation and Cell Death. Journal of Biological Chemistry, (282): 26562–26574.

[37] Brisuda A, Pazourkova E, Soukup V, et al., 2016, Urinary Cell-Free DNA Quantification as Non-Invasive Biomarker in Patients with Bladder Cancer. Urol Int, (96): 25–31.

[38] Berger AC, Korkut A, Kanchi RS, et al., 2018, A Comprehensive Pan-Cancer Molecular Study of Gynecologic and Breast Cancers. Cancer cell, (33): 690–705.

[39] Song J, Ye A, Jiang E, et al., 2018, Reconstruction and Analysis of the Aberrant lncRNA-miRNA-mRNA Network Based on Competitive Endogenous RNA in CESC. Journal of Cellular Biochemistry, (119): 6665–6673.

[40] Sawyers CL, 2008, The Cancer Biomarker Problem. Nature, (452): 548–552.

[41] Labrousse-Arias D, Martínez-Alonso E, Corral-Escariz M, et al., 2017, VHL Promotes Immune Response Against Renal Cell Carcinoma via NF-κB–Dependent Regulation of VCAM-1. Journal of Cell Biology, (216): 835–847.

[42] Hou Q, Li M-Y, Huang W-T, et al., 2017, Association Between Three VEGF Polymorphisms and Renal Cell Carcinoma Susceptibility: A Meta-Analysis. Oncotarget, (8): 50061.

[43] Yang X, Minn I, Rowe SP, et al., 2015, Imaging of Carbonic Anhydrase IX with an ^{111}In-Labeled Dual-Motif Inhibitor. Oncotarget, (6): 33733.

[44] Lai X-M, Liu S-Y, Tsai Y-T, et al., 2017, HAF Mediates the Evasive Resistance of Anti-Angiogenesis TKI Through Disrupting HIF-1α and HIF-2α Balance in Renal Cell Carcinoma. Oncotarget, (8): 49713.

[45] Alabiad M, Harb O, Abozaid M, et al., 2021, The Diagnostic and Prognostic Roles of Combined Expression of Novel Biomarkers in Lung Adenocarcinoma (LUAD) and Lung Squamous Cell Carcinoma (LUSC); An Immunohistochemical Study. Iranian Journal of Pathology, (16): 162–173.

[46] van der Leun AM, Thommen DS, Schumacher TN, 2020, CD8(+) T Cell States in Human Cancer: Insights from Single-Cell Analysis. Nature Reviews. Cancer, (20): 218–232.

Clinical Features and Risk Factors of COVID-19-Associated Fungal Infections in Kidney Transplant Recipients

Chonghao Sun[1,2†], Hao Dong[2†], Haotian Tan[1,2], Congrong Shen[2*], Zhenshan Ding[2], Ying Zhao[2], Jianfeng Wang[1,2*]

[1]Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing 100029, China
[2]Department of Urology, China-Japan Friendship Hospital, Beijing 100029, China

[†]These authors contributed equally to this work and shared first authorship.

*Corresponding authors: Jianfeng Wang, zryywjf@163.com; Congrong Shen, sdysscr@163.com

Abstract: *Objective:* To investigate the clinical features, outcomes, and risk factors of fungal infections in kidney transplant recipients (KTRs) as a result of coronavirus disease 2019 (COVID-19). *Methods:* We retrospectively analyzed 54 KTRs with COVID-19 who were hospitalized at the China-Japan Friendship Hospital from December 1, 2022, to April 1, 2023. With a mean age of 50 ± 12 years, there were 43 men and 11 women participated. For KTRs with COVID-19, we employed multivariate logistic regression analysis to identify the risk factors. *Results:* Twenty (37.0%) patients in this study had fungal infections as a result of COVID-19. Patients with fungal infections had significantly higher rates of mortality (50.0%, 10/20 vs. 2.9%, 1/34, $P < 0.001$), acute respiratory distress syndrome (ARDS) (65.0%, 13/20 vs. 26.5%, 9/34, $P = 0.005$), and acute kidney injury (AKI) (60%, 12/20 vs. 23.5%, 8/34, $P = 0.007$) than those without fungal infections. The result of the multivariate analysis showed that the incidence of fungal infections in KTRs with COVID-19 was independently correlated with age (increased by 10 years, OR = 2.221), history of diabetes mellitus (OR = 12.293), ARDS (OR = 12.849), and bacterial co-infections (OR = 30.461). *Conclusion:* Compared to KTRs without fungal infections, those with COVID-19-related fungal infections had worse clinical courses and less favorable results. The conditions including bacterial co-infections, ARDS, older age, and comorbidity of diabetes mellitus increased the incidence of secondary fungal infections.
Keywords: COVID-19; Kidney transplant; Fungal infections; Clinical features; Risk factors

1. Introduction

The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has ravaged the world in recent years [1]. Even after receiving vaccination against SARS-CoV-2, kidney transplant recipients (KTRs), who are particularly vulnerable to COVID-19, remain at high risk of COVID-19-related mortality [2]. Recently, it was reported that a subset of patients were involved with fungal infections owing to COVID-19. These infections were referred to as COVID-19-associated fungal infections. At the onset of the epidemic, China was the first country to report multiple cases

of *Aspergillus* co-infections in COVID-19 patients, all of whom had negative outcomes [3]. Since then, there has been a rise in the identification of COVID-19-associated fungal infections, particularly in immunosuppressed populations, raising widespread concerns about the serious medical burden [4-7]. However, the clinical features, outcomes, and risk factors of COVID-19-associated fungal infections in KTRs were unclear.

According to reports, epithelial barrier degradation, immune system dysregulation, immunosuppressive therapy, overuse of broad-spectrum antibiotics, invasive mechanical ventilation, and host-related comorbidities have all been linked to opportunistic fungal infections associated with COVID-19 infections [8,9]. The primary pathogens were *Aspergillus*, *Candida*, and *Mucorales*, and the diagnosis could be supported by the positive results of a laborious fungal culture [10]. Fungal infections, which were frequently in COVID-19 patients, were characterized by fever, cough, and dyspnea [11]. Furthermore, the diffuse lung damage induced by SARS-CoV-2 may conceal the imaging findings of fungal disease [8,9], and the atypical computed tomography (CT) features of SARS-CoV-2, including ground-glass opacities (GGO) and nodular lesions, were similar to those of fungal infections [12]. Therefore, it was difficult to diagnose these superinfections based on clinical, pathogenic, and radiological manifestations, and it was crucial to identify high-risk individuals as soon as possible.

The treatment of COVID-19-associated fungal infections mostly consists of immunosuppression induction, antifungal drug administration, respiratory support, and human immunoglobulin utilization. The survival and outcome of the target organ were greatly enhanced by prompt diagnosis and therapy. For COVID-19 high-risk patients, antifungal prophylactic techniques were suggested as a possible treatment [13]. Before beginning antifungal therapy in KTRs, drug-drug interactions and nephrotoxicity must be taken into account, particularly in patients with acute kidney injury (AKI) [14,15].

Compared to severe COVID-19 patients without secondary fungal infections, patients with COVID-19-associated fungal infections have been documented to have longer hospital stays, greater rates of ICU admission, longer durations of mechanical ventilation, and increased death [16-18]. Trujillo *et al.* reported that KTRs as an immunosuppressive population appear to have a worse prognosis than the general population [19].

Until now, few case reports and case series have been published to describe KTRs who had secondary fungal infections after COVID-19. It was unknown what the general clinical course, outcomes, and risk factors of fungal infections associated with COVID-19 in KTRs were. Herein, we conducted a retrospective cohort study to investigate the prevalence, clinical features, prognosis, and risk factors of COVID-19-associated fungal infections in KTRs.

2. Materials and methods
2.1. Subjects and data collection
Fifty-four KTRs with COVID-19 who were hospitalized at China-Japan Friendship Hospital were retrospectively enrolled in this study between December 1, 2022, and April 1, 2023. Following the COVID-19 diagnosis, all patients were categorized into two groups: those with fungal infections ($n = 20$) and those without fungal infections ($n = 34$), based on the positive results of fungi in body fluids. The clinical characteristics, laboratory indexes, imaging features, fungi species, therapies, and prognoses of the two groups were reviewed and compared. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

2.2. Definitions

The diagnostic criteria for COVID-19 were the positive results of real-time polymerase chain reaction (RT-PCR) assay of nasopharyngeal and oropharyngeal swab specimens. Fungal/bacterial infections were diagnosed when patients had clinical symptoms and presented infections with positive pathogenic findings of fungi/bacteria in any of the following tests, including bronchoalveolar lavage culture, sputum culture, blood culture, and metagenomics next-generation sequencing of bronchoalveolar lavage.

AKI was classified according to the Kidney Disease Improving Global Outcomes (KDIGO) guideline, which was defined as any of the following conditions (not graded): increase in serum creatinine (SCr) by ≥ 0.3 mg/dl (≥ 26.5 μmol/l) within 48 hours; or increase in SCr to ≥ 1.5 times baseline, which was known or presumed to have occurred within the prior seven days; or urine volume < 0.5 ml/kg/hour for six hours [20]. Acute respiratory distress syndrome (ARDS) was defined as arterial oxygen tension < 60 mmHg or oxygenation index < 300 [21].

2.3. Statistical analysis

Data analysis was conducted using the statistical software SPSS27.0. The χ^2 test was used to compare qualitative variables that were reported as numbers (n) and percentages (%). Quantitative variables were summarized with medians and interquartile ranges [Md (IQR)] and the Mann–Whitney U test was used for comparison between the two groups. Logistic regression was used to analyze the risk factors for COVID-19-associated fungal infections in KTRs, and ROC curves were employed to assess the diagnostic value of the risk factors that were promising predictors of secondary fungal infections in patients. The *P* value of less than 0.05 was considered statistically significant.

3. Results

3.1. Patient characteristics

This study comprised 54 hospitalized KTRs who had been diagnosed with COVID-19. There were 43 (79.6%) male patients. The median age and body mass index (BMI) of the enrolled patients were 50 ± 12 years and 23.2 ± 3.2 kg/m². The main comorbidities were hypertension (49/54, 90.7%), diabetes mellitus (25/54, 46.3%), cardiovascular disease (7/54, 11.6%), and cerebral disease (6/54, 11.1%). The median interval from kidney transplant to the COVID-19 episode was 227 (22, 565) days (**Table 1)**. Compared to KTRs without fungal co-infections, KTRs with fungal co-infections were predominantly male (100.0%, 20/20 vs. 67.7%, 23/34, *P* = 0.004) and significantly older [57 (47,60) vs. 47 (37,58) years, *P* = 0.029]. In addition, the group with fungal infections had a significantly higher rate of diabetes mellitus in the past (65.0%, 13/20 vs. 35.3%, 12/34, *P* = 0.035). There was no statistical difference between the two groups in terms of immunosuppressive therapy, the rate of acute rejection, and delayed graft function. In our study, the kidneys were obtained from deceased unrelated donors, with 75.92% coming from donation after circulatory death (DCD), 16.67% from donation after brain death (DBD), and 7.41% from donation after brain and cardiac death (DBCD).

Table 1. Clinical features of kidney transplantation recipients with COVID-19 ($N = 54$)

	Fungal infection group ($n = 20$)	Non-fungal infection group ($n = 34$)	*P* value
Demographics			
Male, *n* (%)	20 (100)	23 (67.7)	**0.004**
Age, years	57 (47–60)	47 (37–58)	**0.029**
Height, cm	170 (168–176)	170 (164–175)	0.264
Weight, kg	67.0 (60.8–74.0)	67.5 (60.0–75.3)	0.907
BMI, kg/m^2	22.6 (21.1–24.0)	23.2 (20.6–25.3)	0.629
Co-morbid conditions			
Hypertension, *n* (%)	19 (95.0)	30 (88.2)	0.732
Diabetes mellitus, *n* (%)	13 (65.0)	12 (35.3)	**0.035**
Cardiovascular disease, *n* (%)	4 (20.0)	3 (8.8)	0.446
Cerebral disease, *n* (%)	4 (20.0)	2 (5.9)	0.252
Transplantation characteristics			
Tacrolimus, *n* (%)	19 (95.0)	33 (97.1)	1.000
Cyclosporine, *n* (%)	1 (5.0)	1 (2.9)	1.000
Sirolimus, *n* (%)	2 (10.0)	6 (17.7)	0.713
Mycophenolate, *n* (%)	20 (100.0)	31 (91.2)	0.287
Mizoribine, *n* (%)	0 (0.0)	2 (5.9)	0.528
Steroids, *n* (%)	20 (100.0)	34 (100.0)	1.000
Delayed graft function, *n* (%)	6 (30.0)	4 (11.8)	0.193
Acute rejection, *n* (%)	9 (45.0)	8 (23.5)	0.101
Time after transplantation, days	227 (23–549)	224 (19–607)	0.778
Symptoms			
Fever, *n* (%)	18 (90.0)	32 (94.1)	0.984
Cough, *n* (%)	14 (70.0)	30 (88.2)	0.193
Dyspnea, *n* (%)	19 (95.0)	27 (79.4)	0.246
Hemoptysis, *n* (%)	3 (15.0)	1 (2.9)	0.273
Diarrhea, *n* (%)	5 (25.0)	7 (20.6)	0.706
Comorbidities and complications			
ARDS, *n* (%)	13 (65.0)	9 (26.5)	**0.005**
AKI, *n* (%)	12 (60.0)	8 (23.5)	**0.007**
Bacterial infection, *n* (%)	11 (55.0)	4 (11.8)	**< 0.001**

Abbreviations: BMI, body mass index; ARDS, acute respiratory distress syndrome; AKI, acute kidney injury.

3.2. COVID-19-associated fungal infections

A total of 20 (37%) KTRs developed COVID-19-associated fungal infections. The fungal pathogens comprised *Candida* (12/20, 60%), *Aspergillus* (11/20, 55%), and *Mucorales* (1/20, 5%). Candida mainly included *Candida albicans* (8/13, 61.5%), *Candida tropicalis* (2/13, 15.4%), *Candida paraphimosis* (2/13, 15.4%), and *Candida glabrata* (1/13, 7.7%). *Aspergillus* encompassed *Aspergillus fumigatus* (7/15, 46.7%), *Aspergillus flavus* (4/15, 26.7%), *Aspergillus niger* (3/15, 20.0%), and *Aspergillus nidulans* (1/15, 6.6%).

3.3. Clinical presentations

Fever (50/54, 92.6%), dyspnea (46/54, 85.2%), and cough (44/54, 81.5%) were the most common COVID-19-related symptoms and a proportion of patients presented diarrhea (12/54, 22.2%) and hemoptysis (4/54, 7.4%). There was no difference in clinical symptoms between the group with fungal infections and the group without fungal infections.

Twenty-two (40.7%) patients developed ARDS, 20 (37.0%) KTRs developed AKI, and 15 (27.8%) cases were diagnosed with bacterial infections. The fungal infection group had a significantly higher incidence of ARDS (65.0%, 13/20 vs. 26.5%, 9/34, $P = 0.005$), AKI (60.0%, 12/20 vs. 8/34, $P = 0.007$) and bacterial co-infections (55.0%, 11/20 vs. 11.8%, 4/34, $P < 0.001$).

3.4. Auxiliary examination

Compared with the others, KTRs with secondary fungal infections demonstrated lower lymphocyte count [0.29 (0.20–0.56) vs. 0.50 (0.36–0.99) x10^9/L, $P = 0.037$], lower albumin levels [30.1 (26.3, 32.6) vs. 33.1 (29.2, 37.9) g/L, $P = 0.008$], and higher blood urea levels [17.33 (13.65, 28.50) vs. 11.64 (8.07, 18.54) mmol/L, $P = 0.007$]. Quantitative analysis of T-lymphocytes was performed in 39 of the 54 KTRs. The CD4$^+$ T lymphocyte count [90 (51, 213) vs. 245 (90, 570)/μL, $P = 0.47$] and the CD8$^+$ T lymphocyte count [92 (69,207) vs. 266 (121, 457)/μL, $P = 0.19$] were significantly lower in the fungal infection group.

Following admission, all patients had a CT scan of the chest, and the most common radiographic findings were patch shadow (38/54, 70.4%), ground glass opacities (31/54, 57.4%), pleural effusion (22/54, 40.7%), air bronchogram (21/54, 38.9%), consolidation (15/54, 27.8%) and pleural lesions (13/54, 24.1%). The air bronchogram sign (60.0%, 12/20 vs. 44.1%, 9/34, $P = 0.015$) was more frequent in patients with secondary fungal infections (**Table 2**).

Table 2. Laboratory and imaging analysis of kidney transplantation recipients with COVID-19 ($N = 54$)

Clinical symptom	Fungal infection group ($n = 20$)	Non-fungal infection group ($n = 34$)	P value
Laboratory examination			
WBC, ×10^9/L	5.70 (3.68–8.40)	5.91 (4.10–8.19)	0.879
NEUT, ×10^9/L	4.58 (2.92–7.32)	4.49 (3.04–7.19)	0.914
LYMPH, ×10^9/L	0.29 (0.20–0.56)	0.50 (0.36–0.99)	**0.037**
NEUT %	87.9 (80.5–91.4)	83.7 (71.4–91.3)	0.452
LYMPH %	6.2 (4.1–11.7)	10.3 (4.4–17.6)	0.149
Hemoglobin, g/L	115 (97–127)	110 (96–122)	0.513
Platelet, ×10^9/L	188 (142–210)	149 (121–170)	**0.043**
CRP, mg/L	67.24 (43.37–87.01)	69.86 (22.73–120.19)	0.907
PCT, ng/ml	0.31 (0.11–0.97)	0.18 (0.10–0.68)	0.416
Albumin, g/L	30.1 (26.3–32.6)	33.1 (29.2–37.9)	**0.008**
Serum creatinine, μmol/L	232.2 (137.3–541.5)	142.5 (121.5–248.9)	0.062
eGFR, ml/min/1.73m^2	26.75 (9.72–50.10)	41.66 (23.94–57.43)	0.060
Urea, mmol/L	17.33 (13.65–28.50)	11.64 (8.07–18.54)	**0.007**
Imaging examinations, n (%)			
Ground glass opacity	12 (60.0)	19 (55.9)	0.768
Patch shadow	17 (85.0)	21 (61.8)	0.134
Consolidation	7 (35.0)	8 (23.5)	0.363
Pleural lesions	7 (35.0)	6 (17.6)	0.150
Air bronchogram	12 (60.0)	9 (44.1)	**0.015**
Pleural effusion	11 (55.0)	11 (32.4)	0.102

Abbreviations: WBC, white blood cell; NEUT, neutrophils; LYMPH, lymphocyte; CRP, C-reactive protein; PCT, procalcitonin; eGFR, estimated glomerular filtration rate.

3.5. Treatments

Forty (74.1%) cases received the antiviral treatment with Paxlovid, and 34 (62.9%) of the KTRs were treated with oral dexamethasone or intravenous methylprednisolone. Antibiotic therapy was given depending on the presence of confirmed or suspected bacterial infections.

Compared with patients without fungal infections, patients with secondary fungal infections were more likely to receive invasive mechanical ventilation (60.0%, 12/20 vs. 5.9%, 2/34, $P < 0.001$). We found the administration of meropenem (50.0%, 11/20 vs. 26.5%, 9/34, $P = 0.036$), piperacillin/tazobactam (50.0%, 11/20 vs. 20.6%, 7/34, $P = 0.010$), vancomycin (45.0%, 9/20 vs. 0.0%, 0/34, $P < 0.001$), and tigecycline (30.0%, 6/30 vs. 2.9%, 1/34, $P = 0.015$) were more common in KTRs with fungal co-infections than in those without fungal infections. There was no statistical difference between the two groups for intravenous corticoid therapy (**Table 3**).

Table 3. Treatment and outcomes of kidney transplantation recipients with COVID-19 ($N = 54$)

	Fungal infection group ($n = 20$)	Non-fungal infection group ($n = 34$)	P value
Treatments			
Invasive mechanical ventilation, n (%)	12 (60.0)	2 (5.9)	**< 0.001**
Paxlovid, n (%)	15 (75.0)	25 (73.5)	0.905
Intravenous steroid, n (%)	15 (75.0)	19 (55.9)	0.160
Meropenem, n (%)	11 (55.0)	9 (26.5)	**0.036**
Cefoperazone/sulbactam, n (%)	12 (60.0)	25 (73.5)	0.301
Piperacillin/tazobactam, n (%)	11 (55.0)	7 (20.6)	**0.010**
Fluoroquinolone, n (%)	9 (45.0)	12 (35.3)	0.480
Vancomycin, n (%)	9 (45.0)	0 (0.0)	**< 0.001**
Linezolid, n (%)	2 (10.0)	1 (2.9)	0.632
Tigecycline, n (%)	5 (25.0)	1 (2.9)	**0.015**
Sulfonamide, n (%)	12 (60.0)	13 (38.2)	0.121
Imipenem/cilastatin, n (%)	5 (25.0)	2 (5.9)	0.110
Outcomes			
ICU admission, n (%)	12 (60.0)	4 (11.8)	**< 0.001**
Death, n (%)	10 (50.0)	1 (2.9）	**< 0.001**
Length of hospital stay, days	23 (13–27)	13 (10–16)	**0.003**

Abbreviations: ICU, intensive care unit

3.6. Outcomes

The mortality rate for COVID-19-associated fungal infections was 50% (10/20), which was considerably higher when compared to those patients without fungal infections (50.0%, 10/20 vs. 2.9%, 1/34, $P < 0.001$). In addition, the group with the fungal infections had a higher rate of admission to the ICU (60.0%, 12/20 vs. 11.8%, 4/34, $P < 0.001$) and a longer hospital stay [23 (13, 27) vs. 13(10, 16) days, $P = 0.003$].

3.7. Analyses of risk factors

When COVID-19 KTRs with secondary fungal infections were compared to those without fungal infections, the results of the multivariate analysis showed that the following factors were independently related to the occurrence of COVID-19-associated fungal infections in KTRs: older age (increased by 10 years, OR = 2.221, 95% CI: 1.036–4.759), diabetes mellitus history (OR = 12.293, 95% CI: 1.485–101.758), ARDS (OR = 12.849, 95% CI: 1.487–111.012), and bacterial co-infections (OR = 30.461, 95% CI: 2.486–373.166) (**Table 4**).

Table 4. Risk factors of COVID-19-associated fungal infections in KTRs ($N = 54$)

	Univariate analysis			Multivariate analysis	
	Fungal infection group ($n = 20$)	Non-fungal infection group ($n = 34$)	P value	Odds ratio (95% CI)	P value
Age/10, years	5.7 (4.7–6.0)	4.7 (3.7–5.8)	0.029	2.221 (1.036–4.759）	**0.040**
LYMPH, $\times 10^9$/L	0.29 (0.20–0.56)	0.50 (0.36–0.99)	0.037	0.174 (0.017–1.780)	0.140
Diabetes mellitus, n (%)	13 (65.0)	12 (35.3)	0.020	12.293 (1.485–101.758)	**0.035**
ARDS, n (%)	13 (65.0)	9 (26.5)	0.005	12.849 (1.487–111.012)	**0.020**
Bacterial co-infection, n (%)	11 (55.0)	4 (11.8)	< 0.001	30.461 (2.486–373.166)	**0.008**
Air bronchogram, n (%)	12 (60.0)	9 (44.1)	0.015	0.222 (0.022–2.221)	0.200

Abbreviations: LYMPH, lymphocyte; ARDS, acute respiratory distress syndrome.

4. Discussion

In solid organ transplant (SOT) recipients, COVID-19-associated fungal infections have been reported to be especially deadly and incapacitating, resulting in longer hospital stays and increased medical expenses [2,10]. The features and risk factors of COVID-19-associated fungal co-infections have been well demonstrated in solid organ transplants, but the information available for KTRs is limited [22-25]. To the best of our knowledge, this study is the first to pinpoint the clinical traits and risk factors associated with COVID-19-associated fungal infections in KTRs in the Chinese population. We discovered that the patients had a severe clinical course, poorer kidney outcome, and worse survival, and that older age, history of diabetes mellitus, ARDS, and bacterial co-infections were independent risk factors.

This study found that COVID-19-associated fungal infections in KTRs are not rare, with an incidence of 37%. The prevalence rate was much higher than in the general population, which was between 1% and 33% [26]. In a large multicenter retrospective cohort study, the incidence of secondary fungal infections in SOT recipients with COVID-19 was reported to be 8%, which was lower than our data [22]. This may be due to the heterogeneity of patient groups and the diagnosis of fungal infections [27]. KTRs were more vulnerable to secondary fungal infections due to their long-term use of immunosuppressive drugs, especially at the second occurrence of specific infections. Additionally, we discovered that KTRs with a history of diabetes were more vulnerable to COVID-19-associated fungal infections, as the prolonged hyperglycemic state may impair neutrophil function and cause immune dysregulation [27]. Schwartz *et al.* found that a diabetic state could exacerbate the adverse effects of SARS-CoV-2 on T-lymphocytes and increase the risk of superinfections [28].

The fungal pathogens in our research were *Candida*, *Aspergillus*, and *Mucorales*, which was consistent with previous research [10]. We found that lymphocytes were significantly decreased in patients with secondary fungal infections, with CD4$^+$ and CD8$^+$ T cells being the most reduced. This phenomenon reflects the immunosuppressive state caused by the cytokine storm of COVID-19 [29]. Severe lymphocytopenia and lymphocyte dysfunction were closely associated with bacterial immune dysfunction and secondary fungal infections [10], and thrombocytopenia was a risk factor for mortality in SOT recipients with COVID-19 [30]. We discovered that patients with secondary fungal infections had a higher prevalence of hypoalbuminemia. Hypoalbuminemia was a non-specific indicator of illness severity and was associated with poor prognosis in patients with the novel coronavirus infections [31]. The synthesis of IL-10 by lymphocytes could be impaired by hypoalbuminemia and the lack of IL-10 increases the susceptibility to other infections [32]. Although the difference was not statistically significant, participants with fungal infections exhibited higher serum creatinine levels, pointing to a potential link between fungal infections and kidney damage. A larger sample size might produce more fruitful outcomes. Patients with fungal infections had platelet counts that were within normal range, but the counts were nevertheless much greater than those of uninfected patients. The activating effect of the glucosamine-glucan produced by *Aspergillus* could stimulate platelet production [33]. Imaging tests revealed that patients with secondary fungal infections have a higher frequency of bronchial symptoms. When the lung inflammation was severe, the lung parenchyma's opacity decreased, causing the bronchi to become more visible and dilated [34].

In our study, secondary fungal infections made the clinical course of COVID-19 more complicated. We found that the incidence of ARDS, AKI, and combined bacterial infections was significantly higher in patients with secondary fungal infections. The lungs of COVID-19 individuals who have experienced ARDS may be seriously damaged, necessitating the use of mechanical ventilation. However, it should be noted that mechanical ventilation may make the patients more vulnerable to fungal infections. This is especially dangerous for KTRs who are already at risk of infections [35,36]. In our study, 60% (12/20) of patients with secondary fungal infections developed AKI, significantly more than those without fungal infections. According to Yang et al., in their summary of 51 studies involving 21,531 participants, the incidence of AKI caused by COVID-19 was 12.3%, with a higher rate of 38.9% among recipients of transplants [37]. According to a meta-analysis study conducted by Duarsa et al., hospitalized KTRs with COVID-19 had a 3.78 times higher risk of AKI compared to the general population, often leading to worse outcomes [38]. In critically ill patients, the cytokine storms could occur in response to COVID-19 by impairing lymphocyte function through various mechanisms. This could lead to multiple organ dysfunction syndromes, including AKI [36]. Furthermore, some azole antifungal medications may inhibit cytochrome P450 3A4 (CYP3A4), and increase blood levels of tacrolimus and the risk of nephrotoxicity [39]. According to certain studies, COVID-19-induced cytokine storms may be made worse by secondary fungal infections, increasing the risk of AKI and fatality rates [36]. We discovered that individuals who developed secondary fungal infections often had mixed bacterial infections, with drug-resistant bacteria accounting for as much as 45.5% of these infections. Patients with KTRs were more susceptible to infections. When secondary infections were detected, the clinicians administered broad-spectrum antibiotics more frequently, which raised the possibility of secondary infections with drug-resistant bacteria and opportunistic pathogenic fungi [40]. Likewise, most COVID-19 patients with secondary bacterial infections were more likely to be transferred to ICU and receive invasive medical procedures, which greatly increased the risk of fungal infections [41].

We found that the air bronchogram sign was more frequent in secondary fungal infections, which could contribute to the diagnosis. Previous studies demonstrated that the diagnosis of fungal infections using biopsies and laboratory testing is often challenging and delayed. Fungal infections frequently exhibit non-specific symptoms and atypical radiographic findings, making early diagnosis difficult. Nevertheless, according to an autopsy report, 2.8% of ICU patients showed pathological signs of fungal infections. However, only 40% of these patients received a definitive diagnosis during their lifetime [42]. Therefore, it is possible that the actual incidence of these infections in KTRs is underestimated [43].

According to our research, COVID-19 KTRs with fungal infections had far greater rates of ICU admission, length of stay, and mortality than KTRs without infections. There were also significant differences between the two groups in the proportion of mechanical ventilation and antibiotic therapy. Consistent with our study findings, previous research has demonstrated that patients with COVID-19-associated fungal infections typically require more invasive ventilation and have longer hospital stays [16]. Superinfections need to be closely monitored since they have the potential to be the final cause of mortality [44]. According to a multicenter observational study by Prattes et al., ICU death was nearly twice as likely to occur in patients with fungal infections compared to those without superinfections [45]. KTRs were more vulnerable to fungal coinfections, and some case reports indicated unfavorable outcomes resulting from secondary fungal infections [7,46].

In the current study, the risk factors for secondary fungal infections in KTRs with COVID-19 in this cohort were advanced age (OR = 2.221), diabetes history (OR = 12.293), ARDS (OR = 12.849), and combined bacterial co-infections (OR = 30.461). It was a well-established fact that the elderly population was highly vulnerable to COVID-19 and fungal infections, due to their compromised immune system [1,47]. Several meta-analyses demonstrated that diabetes is an independent risk factor for fungal co-infections and poor prognosis in COVID-19 patients [10,48]. In addition, severely ill COVID-19 patients with multiple comorbidities were more susceptible to secondary fungal infections [35]. As with our research findings, we found that complications of ARDS and bacterial co-infections were independent risk factors for fungal infections in COVID-19 KTRs since these patients had higher rates of receiving mechanical ventilation and high doses of antibiotics. The overuse of broad-spectrum antibiotics was a major factor contributing to the emergence of drug-resistant bacterial infections and opportunistic pathogenic fungal infections [49]. By recognizing risk factors, healthcare professionals may be able to focus more on screening procedures and perhaps prescribe antifungal prophylaxis to high-risk individuals in an effort to prevent COVID-19-associated fungal infections.

Our study has several limitations. The data presented reflected a real-life scenario for which there are no predefined standards for inpatient administration. Well-designed prospective studies at multiple centers are necessary to further substantiate this entity, as the conclusions drawn from this retrospective study at a single center may not apply to all KTRs. The sample size is limited to demonstrate the intact risk factors of COVID-19-associated fungal infections in KTRs.

5. Conclusion

To summarize, KTRs with COVID-19-associated fungal infections have a high incidence and poor outcomes. Older age, history of diabetes mellitus, ARDS, and bacterial infections were the independent risk factors for COVID-19-associated fungal infections in KTRs. Long COVID, also known as post-acute sequelae of COVID-19, affects around 10% of infected individuals [50]. Thus, studies on COVID-related fungal infections in KTRs are still crucial. Clinicians should pay special attention to secondary fungal infections in KTRs with COVID-19, especially when treating patients with multiple risk factors.

Disclosure statement

The authors declare no conflict of interest.

Author contributions

Conceptualization: Hao Dong, Ying Zhao
Investigation: Congrong Shen, Chonghao Sun, Jianfeng Wang
Methodology: Haotian Tan
Writing – original draft: Chonghao Sun
Writing – review & editing: Jianfeng Wang, Zhenshan Ding

References

[1] Zhou F, Yu T, Du R, et al., 2020, Clinical Course and Risk Factors for Mortality of Adult Inpatients with COVID-19 in Wuhan, China: A Retrospective Cohort Study. Lancet, 395(10229): 1054–1062.

[2] Raja MA, Mendoza MA, Villavicencio A, et al., 2021, COVID-19 in Solid Organ Transplant Recipients: A Systematic Review and Meta-Analysis of Current Literature. Transplant Rev (Orlando), 35(1): 100588.

[3] Yang X, Yu Y, Xu J, et al., 2020, Clinical Course and Outcomes of Critically Ill Patients with SARS-CoV-2 Pneumonia in Wuhan, China: A Single-Centered, Retrospective, Observational Study. Lancet Respir Med 8(5): 475–481.

[4] Wang J, Yang Q, Zhang P, et al., 2020, Clinical Characteristics of Invasive Pulmonary Aspergillosis in Patients with COVID-19 in Zhejiang, China: A Retrospective Case Series. Crit Care, 24(1): 299.

[5] Raut A, Huy NT, 2021, Rising Incidence of Mucormycosis in Patients with COVID-19: Another Challenge for India Amidst the Second Wave? Lancet Respir Med, 9(8): e77.

[6] Naveen KV, Saravanakumar K, Sathiyaseelan A, et al., 2022, Human Fungal Infection, Immune Response, and Clinical Challenge-A Perspective During COVID-19 Pandemic. Appl Biochem Biotechnol, 194(9): 4244–4257.

[7] Cruzado Vega LL, Santos Garcia A, 2022, SARS-CoV-2 and *Aspergillus* Pneumonia in Kidney Transplantation: More Frequent Than We Think? Nefrologia (Engl Ed), 42(3): 359–360.

[8] Casalini G, Giacomelli A, Ridolfo A, et al., 2021, Invasive Fungal Infections Complicating COVID-19: A Narrative Review. J Fungi (Basel), 7(11): 921.

[9] Shishido AA, Mathew M, Baddley JW, 2022, Overview of COVID-19-Associated Invasive Fungal Infection. Curr Fungal Infect Rep, 16(3): 87–97.

[10] Hoenigl M, Seidel D, Sprute R, et al., 2022, COVID-19-Associated Fungal Infections. Nat Microbiol, 7(8): 1127–1140.

[11] Maartens G, Wood MJ, 1991, The Clinical Presentation and Diagnosis of Invasive Fungal Infections. J Antimicrob Chemother, 28(Suppl A): 13–22.

[12] Koehler P, Bassetti M, Chakrabarti A, et al., 2021, Defining and Managing COVID-19-Associated Pulmonary Aspergillosis: The 2020 ECMM/ISHAM Consensus Criteria for Research and Clinical Guidance. Lancet Infect Dis, 21(6): e149–e162.

[13] White PL, Dhillon R, Cordey A, et al., 2021, A National Strategy to Diagnose Coronavirus Disease 2019-Associated Invasive Fungal Disease in the Intensive Care Unit. Clin Infect Dis, 73(7): e1634–e1644.

[14] Wilmes D, Coche E, Rodriguez-Villalobos H, et al., 2021, Fungal Pneumonia in Kidney Transplant Recipients. Respir Med, (185): 106492.

[15] Tragiannidis A, Gkampeta A, Vousvouki M, et al., 2021, Antifungal Agents and the Kidney: Pharmacokinetics, Clinical Nephrotoxicity, and Interactions. Expert Opin Drug Saf, 20(9): 1061–1074.

[16] Gangneux JP, Dannaoui E, Fekkar A, et al., 2022, Fungal Infections in Mechanically Ventilated Patients with COVID-19 During the First Wave: The French Multicentre MYCOVID Study. Lancet Respir Med, 10(2): 180–190.

[17] Chong WH, Saha BK, Neu KP, 2022, Comparing the Clinical Characteristics and Outcomes of COVID-19-Associate Pulmonary Aspergillosis (CAPA): A Systematic Review and Meta-Analysis. Infection, 50(1): 43–56.

[18] Singh S, Verma N, Kanaujia R, et al., 2021, Mortality in Critically Ill Patients with Coronavirus Disease 2019-Associated Pulmonary Aspergillosis: A Systematic Review and Meta-Analysis. Mycoses, 64(9): 1015–1027.

[19] Trujillo H, Fernandez-Ruiz M, Gutierrez E, et al., 2021, Invasive Pulmonary Aspergillosis Associated with COVID-19 in a Kidney Transplant Recipient. Transpl Infect Dis, 23(2): e13501.

[20] Kellum JA, Lameire N, 2013, Diagnosis, Evaluation, and Management of Acute Kidney Injury: A KDIGO Summary (Part 1). Crit Care, 17(1): 204.

[21] Ranieri VM, Rubenfeld GD, Thompson BT, et al., 2012, Acute Respiratory Distress Syndrome: The Berlin Definition. JAMA, 307(23): 2526–2533.

[22] Permpalung N, Chiang TPY, Manothummetha K, et al., Invasive Fungal Infections in Inpatient Solid Organ Transplant Recipients With COVID-19: A Multicenter Retrospective Cohort. Transplantation, 108(7): 1613–1622.

[23] Avery RK, Chiang TPY, Marr KA, et al., 2021, Inpatient COVID-19 Outcomes in Solid Organ Transplant Recipients Compared to Non-Solid Organ Transplant Patients: A Retrospective Cohort. Am J Transplant, 21(7): 2498–2508.

[24] Pennington KM, Martin MJ, Murad MH, et al., Risk Factors for Early Fungal Disease in Solid Organ Transplant Recipients: A Systematic Review and Meta-Analysis. Transplantation, 108(4): 970–984.

[25] Fernandez-Ruiz M, Andres A, Loinaz C, et al., 2020, COVID-19 in Solid Organ Transplant Recipients: A Single-Center Case Series from Spain. Am J Transplant, 20(7): 1849–1858.

[26] Chong WH, Saha BK, Ananthakrishnan R, et al., 2021, State-of-the-Art Review of Secondary Pulmonary Infections in Patients with COVID-19 Pneumonia. Infection, 49(4): 591–605.

[27] Frydrych LM, Bian G, O'Lone DE, et al., 2018, Obesity and Type 2 Diabetes Mellitus Drive Immune Dysfunction, Infection Development, and Sepsis Mortality. J Leukoc Biol, 104(3): 525–534.

[28] Schwartz MD, Emerson SG, Punt J, et al., 2020, Decreased Naïve T-cell Production Leading to Cytokine Storm as Cause of Increased COVID-19 Severity with Comorbidities. Aging Dis, 11(4): 742–745.

[29] Li H, Liu L, Zhang D, et al., 2020, SARS-CoV-2 and Viral Sepsis: Observations and Hypotheses. Lancet, 395(10235): 1517–1520.

[30] Nimmo A, Gardiner D, Ushiro-Lumb I, et al., 2022, The Global Impact of COVID-19 on Solid Organ Transplantation: Two Years Into a Pandemic. Transplantation, 106(7): 1312–1329.

[31] Marjot T, Webb GJ, Barritt AS, et al., 2021, COVID-19 and Liver Disease: Mechanistic and Clinical Perspectives. Nat Rev Gastroenterol Hepatol, 18(5): 348–364.

[32] Austermeier S, Pekmezovic M, Porschitz P, et al., 2021, Albumin Neutralizes Hydrophobic Toxins and Modulates Candida albicans Pathogenicity. mBio, 12(3): e0053121.

[33] Deshmukh H, Speth C, Sheppard DC, et al., 2020, *Aspergillus*-Derived Galactosaminogalactan Triggers Complement Activation on Human Platelets. Front Immunol, (11): 550827.

[34] Algin O, Gokalp G, Topal U, 2011, Signs in Chest Imaging. Diagn Interv Radiol, 17(1): 18–29.

[35] Negm EM, Mohamed MS, Rabie RA, et al., 2023, Fungal Infection Profile in Critically Ill COVID-19 Patients: A Prospective Study at a Large Teaching Hospital in a Middle-Income Country. BMC Infect Dis, 23(1): 246.

[36] Tang L, Yin Z, Hu Y, et al., 2020, Controlling Cytokine Storm Is Vital in COVID-19. Front Immunol, (11): 570993.

[37] Yang X, Tian S, Guo H, 2021, Acute Kidney Injury and Renal Replacement Therapy in COVID-19 Patients: A Systematic Review and Meta-Analysis. Int Immunopharmacol, (90): 107159.

[38] Duarsa GWK, Sugianto R, Yusari IGAAA, et al., 2023, Predictor Factor for Worse Outcomes in Kidney Transplant Recipients Infected with Coronavirus Disease 2019: A Systematic Review and Meta-Analysis. Transpl Immunol, (76): 101739.

[39] Tolou-Ghamari Z, 2012, Nephro and Neurotoxicity of Calcineurin Inhibitors and Mechanisms of Rejections: A Review on Tacrolimus and Cyclosporin in Organ Transplantation. J Nephropathol, 1(1): 23–30.

[40] Fishman JA, 2017, Infection in Organ Transplantation. Am J Transplant, 17(4): 856–879.

[41] Zhang H, Zhang Y, Wu J, et al., 2020, Risks and Features of Secondary Infections in Severe and Critical Ill COVID-19 Patients. Emerg Microbes Infect, 9(1): 1958–1964.

[42] Tejerina EE, Abril E, Padilla R, et al., 2019, Invasive Aspergillosis in Critically Ill Patients: An Autopsy Study. Mycoses, 62(8): 673–679.

[43] Thompson GR, Miceli MH, Jiang J, et al., 2023, Secondary Invasive Fungal Infection in Hospitalised Patients with COVID-19 in the United States. Mycoses, 66(6): 527–539.

[44] Clancy CJ, Nguyen MH, 2020, Coronavirus Disease 2019, Superinfections, and Antimicrobial Development: What Can We Expect? Clin Infect Dis, 71(10): 2736–2743.

[45] Prattes J, Wauters J, Giacobbe DR, et al., 2022, Risk Factors and Outcome of Pulmonary Aspergillosis in Critically Ill Coronavirus Disease 2019 Patients-A Multinational Observational Study by the European Confederation of Medical Mycology. Clin Microbiol Infect, 28(4): 580–587.

[46] Stephens ML, Mathew A, Banerjee D, et al., 2022, COVID-Associated Pulmonary Aspergillosis and Herpes Simplex Virus Pneumonia in a Renal Transplant Recipient. Transpl Infect Dis, 24(6): e13978.

[47] Akbar AN, Gilroy DW, 2020, Aging Immunity May Exacerbate COVID-19. Science, 369(6501): 256–257.

[48] Villanego F, Mazuecos A, Perez-Flores IM, et al., 2021, Predictors of Severe COVID-19 in Kidney Transplant Recipients in the Different Epidemic Waves: Analysis of the Spanish Registry. Am J Transplant, 21(7): 2573–2582.

[49] Shafiekhani M, Shekari Z, Boorboor A, et al., 2022, Bacterial and Fungal Co-Infections with SARS-CoV-2 in Solid Organ Recipients: A Retrospective Study. Virol J, 19(1): 35.

[50] Ballering AV, van Zon SKR, Olde Hartman TC, et al., 2022, Persistence of Somatic Symptoms After COVID-19 in the Netherlands: An Observational Cohort Study. Lancet, 400(10350): 452–461.

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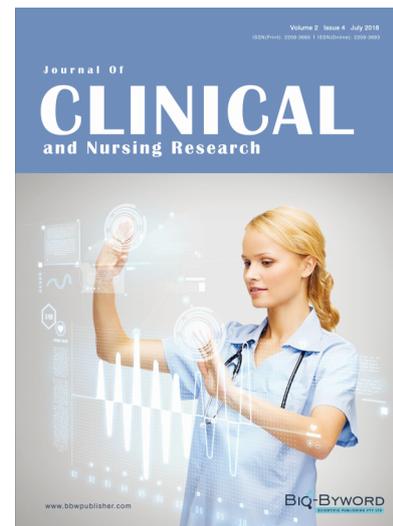
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