中文摘要

肾移植术后不同程度 BK 病毒感染与淋巴细胞亚群的相关性研究

研究背景：

肾脏移植是终末期肾病（End stage renal disease，ESRD）的最佳治疗手段。肾移植受者需要长期服用免疫抑制剂，在减少排斥反应发生率的同时也带来了更多的感染机会。BK 病毒在人群中普遍易感。初次感染 BK 病毒后，BK 病毒在肾小管上皮细胞和尿路移行上皮细胞中开始终身潜伏。当免疫力下降时逐渐依次进展为 BK 病毒尿症、BK 病毒血症，进而逐渐发展为 BK 病毒相关性肾病，对移植肾脏产生严重危害。由于特异性抗 BK 病毒药物的缺乏，BK 病毒感染逐渐受到移植管理团队的重视。免疫抑制剂主要作用于人体免疫系统中的淋巴细胞，因此，研究不同程度 BK 病毒与淋巴细胞亚群的相关性可能作为一个突破点，为临床上 BK 病毒的诊治提供参考。

研究目的：

研究肾移植术后不同程度 BK 病毒感染与淋巴细胞亚群之间的相关性，探究淋巴细胞亚群对不同程度 BK 病毒感染的预测能力。

研究方法：

回顾性分析 2018 年 1 月-2021 年 12 月于吉林大学第一医院行同种异体肾移植手术的 971 例患者的随访资料，随访截止日期至 2022 年 12 月 31 日。将尿液中 BK 病毒 DNA 载量阴性且血液也为阴性定义为 BK 病毒阴性组，尿液中 BK 病毒 DNA 载量＞10^3 拷贝/毫升且血液中 BK 病毒 DNA 载量阴性定义为尿 BK 病毒阳性组，将血液中 BK 病毒 DNA 载量＞10^3 拷贝/毫升且尿液中为任何值定义为血 BK 病毒阳性组。收集患者的尿 BK 病毒 DNA 载量、血 BK 病毒 DNA 载量、淋巴细胞亚群的检测结果，并同时收集患者的年龄、性别、体重指数（Kg/m^2）、移植后时长（月）、移植类型（DD 或 LD）、肾脏原发病因（肾炎性疾病、肾病性疾病、高血压肾病、糖尿病肾病及其他）信息。不同 BK 病毒感染程度的淋巴细胞亚群组间比较采用 Kruskal-Wallis 非参数检验，组间总体分布不同的进一步行两两比较，采用 Bonferroni 法进行 P 值校正，并采用校正后 P 值，相关性分析采用 Spearman 相关。选取有统计学意义的淋巴细胞亚群指标，绘制 ROC

曲线分析其对于预测 BK 病毒感染（尿 BK 病毒阳性或血 BK 病毒阳性）和 BK 病毒血症（血 BK 病毒阳性）的价值。

结果：

（1）971 例患者中共纳入规律随访且同时进行 BK 病毒定量检测及淋巴细胞亚群检测的 510 例患者为研究对象。510 例患者中男性 352 例（占比 69%），女性 158 例（占比 31%），平均年龄 42.03±11.83 岁，BMI 平均值为 22.97±2.86。510 例患者中共检出尿 BK 病毒阳性 172 例，尿阳性率 33.7%，血 BK 病毒阳性率 46 例，血阳性率 9%，三组在年龄、性别、体重指数、移植后时长、移植类型、肾脏原发病因等方面均无显著性差异。

（2）BK 病毒阴性组、尿 BK 病毒阳性组与血 BK 病毒阳性组三组之间在 $CD4^+$T 细胞比例（分别为 36%、29%和 19%，$P<0.001$）、$CD4^+$T 细胞数目（分别为 628、475 和 221，$P<0.001$）、CD4/CD8（分别为 1.0、0.8 和 0.5，$P<0.001$）方面两两比较差异均具有统计学意义。BK 病毒阴性组与血 BK 病毒阳性组在 $CD3^+$T 细胞数目（分别为 1309 和 824，$P=0.005$）、$CD8^+$T 细胞比例（分别为 33%和 42.5%，$P=0.005$）、$CD8^+$T 细胞数目（分别为 618 和 532，$P=0.041$）、NK 细胞数目（分别为 211 和 138.5，$P=0.004$）方面差异具有统计学意义。BK 病毒阴性组与尿 BK 病毒阳性组在 $CD8^+$T 细胞比例（分别为 33%和 37%，$P=0.047$）方面差异具有统计学意义。BK 病毒感染严重程度与 $CD3^+$T 细胞数目、$CD4^+$T 细胞比例及数目、$CD8^+$T 细胞数目、NK 细胞数目、CD4/CD8 呈负相关（$r<0$，$P<0.05$），与 $CD8^+$T 细胞比例呈正相关（$r>0$，$P<0.05$）。

（3）预测 BK 病毒感染和 BK 病毒血症的指标中具有最佳效果的为 CD4/CD8，曲线下面积（AUC）分别为 0.668 和 0.789，其次为 $CD4^+$T 细胞比例（0.647 和 0.761）、$CD4^+$T 细胞计数（0.642 和 0.746）、$CD3^+$T 细胞计数（0.568 和 0.628）、$CD8^+$T 细胞比例（0.584 和 0.620）、NK 细胞计数（0.580 和 0.605）、$CD8^+$T 细胞计数（0.545 和 0.603）。淋巴细胞亚群联合预测因子预测 BK 病毒感染和 BK 病毒血症的曲线下面积分别为 0.742 和 0.849。

结论：

（1）不同程度 BK 病毒感染与淋巴细胞亚群存在相关性，BK 病毒感染程度越高的患者 $CD3^+$T 细胞数目、$CD4^+$T 细胞比例及数目、$CD8^+$T 细胞数目、NK 细胞数目、CD4/CD8 越低，$CD8^+$T 细胞比例越高。

（2）淋巴细胞亚群对肾移植术后 BK 病毒感染和 BK 病毒血症具有良好的预测价值，其中 CD4/CD8 是预测 BK 病毒感染和 BK 病毒血症效果最佳的指标。提示当淋巴细胞亚群指标降低尤其是 CD4/CD8 降低时如果同时合并 BK 病毒感染要及时进行抗病毒治疗防止感染加重，当没有证据显示已发生 BK 病毒感染时要警惕存在近期发生 BK 病毒感染的可能性。

关键词：

肾移植，BK 病毒，淋巴细胞亚群，T 细胞，NK 细胞，受试者工作特征（ROC）曲线

Abstract

Correlation between different levels of BK virus infection and lymphocyte subsets after renal transplantation

Background：

Renal transplantation is the best treatment for end-stage renal disease (ESRD). The need for long-term immunosuppression in kidney transplant recipients reduces the incidence of rejection while also presenting an increased opportunity for infection. bk virus is universally susceptible in the population. After initial infection with BK virus, BK virus begins lifelong latency in renal tubular epithelial cells and urinary migratory epithelial cells. When immunity decreases it gradually progresses sequentially to BK viremia, BK viremia, and then gradually to BK virus-associated nephropathy, which poses a serious risk to the transplanted kidney. Due to the lack of specific anti-BK viral drugs, BK virus infection is gradually gaining attention from transplant management teams. Immunosuppressants mainly act on lymphocytes in the human immune system; therefore, studying the correlation between different levels of BK virus and lymphocyte subsets may serve as a breakthrough point for the clinical diagnosis and treatment of BK virus.

Objective:

To investigate the correlation between different levels of BK virus infection and lymphocyte subpopulations after renal transplantation and to investigate the predictive ability of lymphocyte subpopulations for different levels of BK virus infection.

Methods:

The follow-up data of 971 patients who underwent allogeneic kidney transplantation at the First Hospital of Jilin University from January 2018 to December 2021 were retrospectively analyzed until December 31, 2022. A negative BK virus DNA load in urine and also negative blood was defined as the BK virus negative group, a BK virus DNA load $>10^3$ copies/mL in urine and a negative BK virus DNA load in blood was defined as the urinary BK virus positive group, and a BK virus DNA load $>10^3$ copies/mL in blood and any

value in urine was defined as the blood BK virus positive group. The results of urinary BK virus DNA load, blood BK virus DNA load, and lymphocyte subpopulations were collected from patients, along with information on age, sex, body mass index (Kg/m^2), length of time since transplantation (months), type of transplantation (DD or LD), and primary cause of kidney disease (nephritic disease, nephrotic disease, hypertensive nephropathy, diabetic nephropathy, and others). Comparisons between groups of lymphocyte subpopulations with different degrees of BK virus infection were performed using the Kruskal-Wallis nonparametric test, further rows with different overall distributions between groups were corrected for P-values using the Bonferroni method, and corrected P-values were used for correlation analysis using Spearman correlation, and lymphocyte subpopulation indicators with statistical significance were selected and ROC curves were plotted separately Their value for predicting BK virus infection (urinary BK virus positive or blood BK virus positive) and BK viraemia (blood BK virus positive) was analyzed.

Result:

(1) A total of 510 patients were enrolled in the study among 971 patients who were regularly followed up with both quantitative BK virus testing and lymphocyte subset testing. 510 patients included 352 males (69%) and 158 females (31%), with a mean age of 42.03±11.83 years and a mean BMI of 22.97±2.86. A total of 510 patients were detected with urinary A total of 172 cases were detected positive for BK virus, with a urine positivity rate of 33.7%, and 46 cases were positive for blood BK virus, with a blood positivity rate of 9%. There were no significant differences between the three groups in terms of age, gender, body mass index, post-transplantation duration, type of transplantation, and primary cause of kidney disease.

(2) The differences between the BK virus negative, urine BK virus positive and blood BK virus positive groups were statistically significant in terms of $CD4^+$T-cell percentage (36%, 29% and 19%, respectively, $P<0.001$), $CD4^+$T-cell number (628, 475 and 221, respectively, $P<0.001$), CD4/CD8 (1.0, 0.8 and 0.5, respectively, $P<0.001$) The differences between the BK virus-negative group and the blood BK virus-positive group were statistically significant in terms of $CD3^+$T-cell number (1309 and 824, , $P=0.005$), $CD8^+$T-cell ratio (33% and 42.5%,

P=0.005), $CD8^+$T-cell number (618 and 532, *P*=0.041), The differences were statistically significant in terms of the number of NK cells (211 and 138.5,*P*=0.004). The differences between the BK virus negative group and the urinary BK virus positive group were statistically significant in terms of the proportion of $CD8^+$T-cell (33% and 37%, *P*=0.047). The differences between the severity of BK virus infection and the number of $CD3^+$T-cell， the proportion and number of $CD4^+$T-cell, the number of $CD8^+$T-cell, number of NK cells， and CD4/CD8 were negatively correlated ($r < 0$, $P < 0.05$) and positively correlated with the proportion of $CD8^+$T-cell ($r > 0$, $P < 0.05$).

(3) The best predictor of BK virus infection and BK viremia was CD4/CD8 with an area under the curve (AUC) of 0.668 and 0.789, followed by $CD4^+$T-cell ratio (0.647 and 0.761), $CD4^+$T-cell count (0.642 and 0.746), $CD3^+$T cell count (0.568 and 0.628), $CD8^+$T-cell ratio (0.584 and 0.620), NK cell count (0.580 and 0.605), and $CD8^+$T-cell count (0.545 and 0.603). The area under the curve of the combined predictors of lymphocyte subsets predicting BK virus infection and BK viremia was 0.742 and 0.849, respectively.

Conclusion:

(1) There was a correlation between different degrees of BK virus infection and lymphocyte subsets, with higher degrees of BK virus infection being associated with lower $CD3^+$T cell counts, $CD4^+$T cell ratios and counts, $CD8^+$T cell counts, NK cell counts, CD4/CD8, and higher $CD8^+$T cell ratios.

(2) Lymphocyte subsets have good predictive value for BK virus infection and BK viremia after renal transplantation, with CD4/CD8 being the best predictor of BK virus infection and BK viremia. It is suggested that when lymphocyte subsets are reduced, especially CD4/CD8, timely antiviral therapy should be administered to prevent worsening of the infection if it is combined with BK virus infection, and when there is no evidence of BK virus infection, the possibility of recent BK virus infection should be alerted.

Key words:

Kidney transplantation, BK virus, Lymphocyte subsets, T cells, NK cells, Receiver

operating characteristic (ROC) curve

关于学位论文使用授权的声明

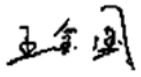
本人完全了解吉林大学有关保留、使用学位论文的规定，同意吉林大学保留或向国家有关部门或机构送交论文的复印件和电子版，允许论文被查阅和借阅；本人授权吉林大学可以将本学位论文的全部或部分内容编入有关数据库进行检索，可以采用影印、缩印或其他复制手段保存论文和汇编本学位论文。

（保密论文在解密后应遵守此规定）

论文级别：■硕士　□博士

学科专业：临床医学（外科学）

论文题目：肾移植术后不同程度 BK 病毒感染与淋巴细胞亚群的相关性研究

作者签名：　　指导教师签名： 

2023 年 5 月 31 日

目　　录

中英文缩略词对照表

英文缩写	英文全称	中文全称
ESRD	End stage renal disease	终末期肾脏病
DD	Donation after death	公民逝世后器官捐献
LD	Living donation	活体器官捐献
AUC	Area under the curve	受试者工作特征曲线
BKV	BK virus	BK 病毒
BKVN	BK virus associated nephropathy	BK 病毒性肾病
PCR	Polymerase chain reaction	聚合酶链式反应
Sag	Small tumor antigen	小 T 抗原
Tag	Large tumor antigen	大 T 抗原
NCCR	Noncoding control region	非编码控制区
TCR	Transcriptional control region	转录控制区
ER	Endoplasmic reticulum	内质网
mTOR	mammalian target of rapamycin	雷帕霉素
ATG	Antithymocyte globulin	抗胸腺细胞球蛋白
HLA	Human leukocyte antigen	人类白细胞抗原
DGF	Delayed graft function	移植肾功能延迟恢复
SOT	solid organ transplantation	实体器官移植
NK	Natural killer cell	自然杀伤细胞
KIRs	Killer cell immunoglobulin-like receptors	杀伤细胞免疫球蛋白样受体
TCMR	T cell-mediated rejection	T 细胞介导的排斥反应
IFN-γ	interferon-γ	γ 干扰素
qPCR	quantitative PCR	定量 PCR
dd-cfDNA	Donor derived-cell free DNA	供体来源的细胞游离 DNA
CNIs	Calcineurin inhibitors	钙调磷酸酶抑制剂
Tac	Tacrolimus	他克莫司
CsA	Cyclosporine	环孢素