REVIEW

Long Non Coding RNAs as Prognostic Factors or Diagnostic Biomarkers of Renal Transplant Rejection: A Systematic Review and Meta Analysis

Leila Zareian Baghdadabad[1], Razman Arabzadeh Bahri[1], Parnian Shobeiri[2], Iman Menbari Oskouie[1], Leonardo Oliveira Reis[3], Seyed Mohammad Kazem Aghamir[1]*

Purpose: Acute rejection (AR) of a kidney graft in renal transplant recipients is associated with microvascular injury leading to graft dysfunction and failure. Long noncoding RNAs (lncRNAs) may serve as markers for vascular injury and AR. We aimed to identify lncRNA biomarkers associated with graft loss after renal transplantation.

Materials and Methods: We searched PubMed, Scopus, Embase, and Web of Science. Odds ratios (ORs), hazard ratios (HRs), and their 95% confidence intervals (95% CIs) were calculated to assess effect sizes. All graphical designs and statistical analyses were performed using STATA version 17 (StataCorp LP, College Station, TX, USA) and the meta package.

Results: Of 291 initially identified articles, 10 met eligibility criteria and were included in the systematic review; 3 provided sufficient data for meta analysis. The pooled area under the curve (AUC) for lncRNA measurement in diagnosing acute kidney rejection was 0.79 in adults and 0.75 in pediatric populations, indicating good diagnostic accuracy. Leave one out sensitivity analyses confirmed the stability of these findings. However, the pooled HR for the prognostic value of lncRNAs was 0.81 (95% CI: 0.63–1.04), which was not statistically significant.

Conclusion: Assessment of lncRNA levels in plasma or urine appears promising as a diagnostic biomarker for acute kidney rejection. The prognostic value of lncRNAs in the course of acute kidney rejection requires further evaluation.

Keywords: biomarkers; graft rejection; kidney transplantation; prognosis; RNA, long noncoding

INTRODUCTION

Kidney transplantation is the best treatment available for patients with end stage kidney disease in terms of quality of life and mortality compared with dialysis[1]. The focus continues to be on reducing acute kidney rejection and other post transplant complications and improving long term outcomes. In addition, acute kidney rejection and subclinical rejection limit transplantation and patient outcomes after kidney transplantation[2-4]. Recent reports have shown that the incidence of acute kidney rejection affects 10% to 15% of patients within the first year of transplantation, depending on the immunosuppressive strategy[5,6], and has decreased by more than 50% since 2000 but remained stable over the past 5 years[7]. Acute cellular rejection (ACR) of any type according to the Banff classification can affect long term survival of allogeneic grafts, and vascular or late ACR may be poor predictors of graft survival[8,9]. Excessive immunosuppression should be avoided in clinical practice, as serious side effects may occur in low risk recipients[10].

Current strategies for monitoring renal transplantation include measurement of serum creatinine[11]. There are protocols for detection, clinical monitoring, and biopsy in some programs, but none is predictive of acute kidney rejection. Furthermore, as the gold standard for diagnosing acute kidney rejection, renal transplant biopsy has limitations such as risk of bleeding, damage to nearby organs, and misdiagnosis due to sampling errors[7-9]. Over the past three decades, clinicians have searched for noninvasive tools to rapidly detect acute kidney rejection and reduce the need for kidney biopsy[12]. Operationally defined lncRNAs are mRNA like transcripts ranging from 200 nucleotides to several kilobases in length that appear incapable of encoding proteins. Accumulating evidence suggests that circulating RNAs (cRNAs) are involved in biological, pathological, and developmental processes[13]. They act through mechanisms such as cis regulation in enhancers, chromatin reprogramming, and post transcriptional mRNA processing regulation. Like microRNAs, lncRNAs can also be used as biomarkers for diagnosis and prognosis. This suggests that lncRNAs can be used as novel biomarkers to identify patients with acute rejection and predict renal function loss. The aim of this systematic review and meta analysis was to establish an association between lncRNA expression profiles and acute kidney rejection or transplant risk.

[1]Urology Research Center, Tehran University of Medical Sciences, Tehran, Iran.
[2]School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
[3]UroScience and Department of Surgery (Urology), School of Medical Sciences, University of Campinas (Unicamp), and Pontifical Catholic University of Campinas (PUC Campinas), Campinas, São Paulo, Brazil.
*Correspondence: Urology Research Center, Sina Hospital, Hassan Abad Sq., Imam Khomeini Ave., Tehran, Iran.
Tel: (+9821) 6634 8560. Fax: (+9821) 6634 8561. E mail: mkaghamir@tums.ac.ir.
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Table 1. Characteristics of included studies

First author	Year	Sample type	lncRNA(s)	Main results
Chen	2014	Renal tissue biopsy	Upregulated: ASLNC04531; ASLNC09207; ASLNC14712; ASLNC08637; ASLNC13599; ASLNC07985; ASLNC19577; ASLNC03665; ASLNC12108; ASLNC21766; ASLNC18894; ASLNC11418; ASLNC03614; ASLNC11432; ASLNC04193; ASLNC12713; ASLNC00922; ASLNC00559; ASLNC17358; ASLNC14352. Downregulated: ASLNC06892; ASLNC02456; ASLNC08095; ASLNC01268; ASLNC10347; ASLNC19249; ASLNC07072; ASLNC01067; ASLNC04837; ASLNC22002; ASLNC08815; ASLNC17495; ASLNC01430; ASLNC21970; ASLNC12665; ASLNC12607; ASLNC00493; ASLNC10383; ASLNC13003; ASLNC22440. Differentially expressed (validated): AF113674; uc003wbj; uc010ftb; uc001fty; AK129917.	Five lncRNAs showed differential expression in acute kidney rejection.
Groeneweg	2020	Renal tissue biopsy	LNC EPHA6; LNC RPS24; MALAT1; LIPCAR	Higher LNC EPHA6 in rejection group than stable transplant group; lnc RPS24, LNC EPHA6, and LIPCAR correlated with vascular injury markers.
Qiu	2017	Renal tissue biopsy	lncRNA ATB	lncRNA ATB upregulated in acute kidney rejection versus controls.
Zou	2019	Renal tissue biopsy	RP11 25K19.1; ITGB2 AS1; MIR155HG; CARD8 AS1; RP6 159A1.4; TRG AS1	Six lncRNAs associated with acute kidney rejection; ITGB2 AS1, MIR155HG, CARD8 AS1 predicted future graft loss (AUC = 0.73).
Kölling	2019	Urine	hsa_circ_0071475; hsa_circ_0001334	Both increased in acute kidney rejection compared with control transplant patients.
Nafar	2019	Peripheral blood	OIP5 AS1; FAS AS1; TUG1; NEAT1; PANDAR	FAS AS1 significantly increased in males with acute rejection versus controls; not significant in females.
Lorenzen	2015	Urine	LNC MYH13 3:1; RP11 395P13.3 001; RP11 354P17.15 001	RP11 395P13.3 001 and RP11 354P17.15 001 upregulated in acute rejection and in cell culture supernatants under inflammatory conditions.
Ge	2017	Peripheral blood	AF264622; AK024956; AB209021; AK026078; BC036622; BX648207; CR606559; AK001279; AK021632; AK055670; AK090972; AK096729; AK098425; AL049951; AL157495; BC041913; BC043240; CR608275; CR611332; CR617316; CR617865; CR618720; CR622106	AF264622 and AB209021 had the most significant performance in adults and pediatrics.
Zhang	2020	Renal tissue biopsy	TRG AS1; LINC00645; LINC01187; TCL6; DANCR; LINC00982; CTD 3080P12.3; EMX2OS; TRAM2 AS1; LINC00671; WAC AS1; ATP1A1 AS1; AC112198.1; WDFY3 AS2; LINC00886; AL022344.5; RPARP AS1; SMIM2 AS1; RUSCI AS1; C12orf77; ITGB2 AS1; PCED1B AS1; LINC00592; ADIRF AS1; AC005523.2; ZNF213 AS1; TRHDE AS1; LINC00911; AC092192.1; ELOVL2 AS1; GS1 124K5; EPB41L4A AS1; APTR; LINC01410; HMMR AS1; LINC00472; LINC01222; FLJ37453; SEPSES AS1	ATP1A1 AS1, CTD 3080P12.3, EMX2OS, and LINC00645 were significantly downregulated in acute rejection.
Xu	2020	Renal tissue biopsy	RP11 280K24.1; AC126763.1; LINC01137; WASIR2; RP1 276N6.2; AD000684.2	Six lncRNAs associated with allograft rejection (AUC = 0.94).

MATERIALS AND METHODS

The present study was conducted based on the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA 2020) guidelines. The protocol was registered in PROSPERO (CRD42022343992).

Search strategy

A comprehensive search of PubMed, Scopus, Embase, and Web of Science was conducted by two authors from database inception to January 24, 2023, to identify articles evaluating the prognostic or diagnostic accuracy of lncRNAs in patients with acute kidney rejection. No language restrictions were applied. An updated search was performed one week prior to manuscript submission to include the most recent literature.

Search terms were grouped into kidney transplantation, transplant rejection, and lncRNA. The kidney transplantation group included keywords such as renal transplantation, kidney grafting, and renal grafting. The transplant rejection group included graft rejection and transplant rejection. The lncRNA group included long non coding RNA, long non protein coding RNA, long

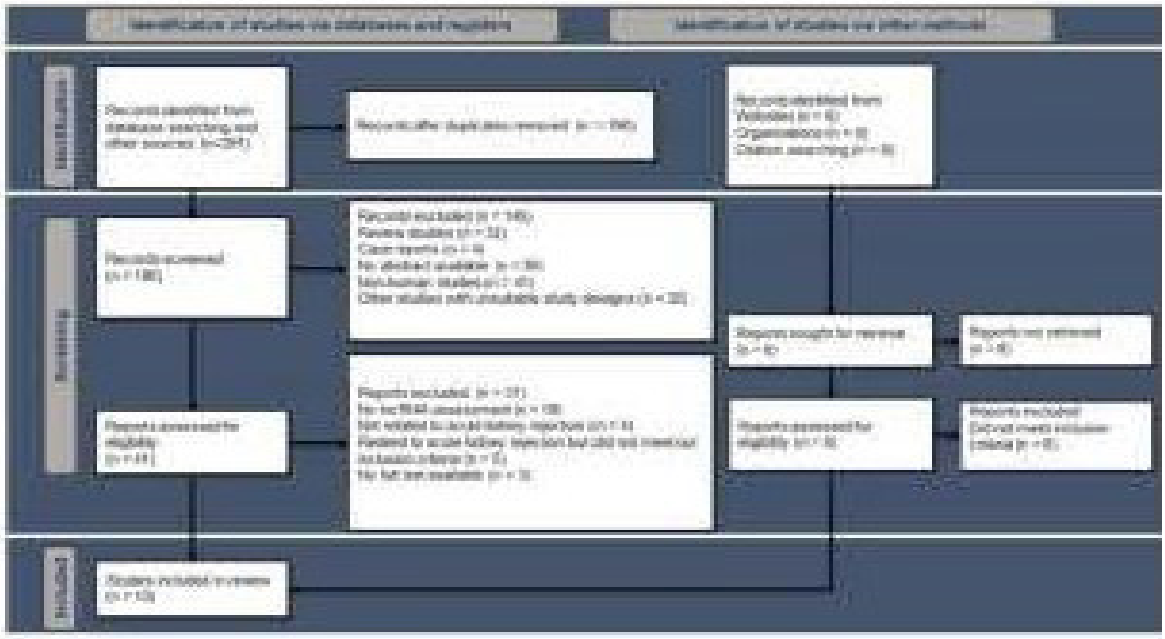

Figure 1. PRISMA flowchart of the literature search and selection of the articles.

ncRNA, and lnc RNA. "OR" was used within groups and "AND" between groups.

Eligibility criteria

Inclusion criteria: (a) patients with acute kidney rejection confirmed by clinical examinations or histopathological findings; (b) reported expression levels of lncRNAs in patients and controls; (c) reported correlation between lncRNA expression and sufficient data regarding HR, survival outcomes, sensitivity, specificity, or sample sizes; and (d) reported expression of lncRNAs detected in tissue, urine, or serum.

Exclusion criteria: (a) review articles, congress abstracts, commentaries, case reports, or letters to the editor; (b) non human studies; and (c) articles unrelated to lncRNAs and acute kidney rejection.

This review did not differentiate between cellular rejection and antibody mediated rejection. Studies assessing lncRNA expression across urine, peripheral blood, and kidney biopsy samples were included, depending on each study's methodology.

Data extraction and quality assessments

Data extraction was performed independently by two authors using predefined Excel sheets. Discrepancies were resolved through discussion and consensus; if disagreements persisted, a third author adjudicated. Extracted data included first author's name, year of pub-

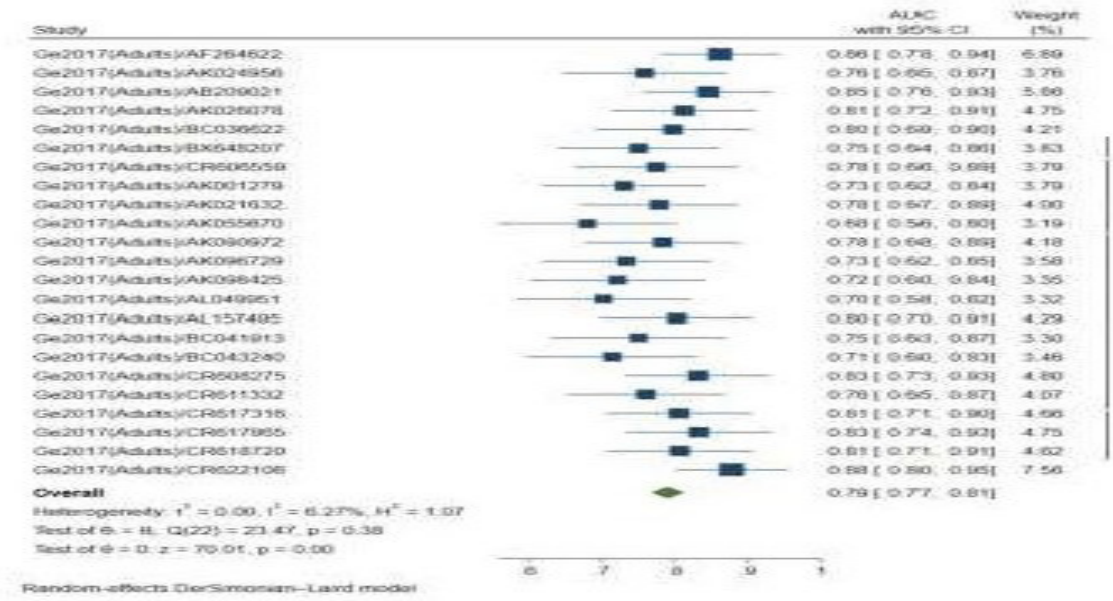

Figure 2. Forest plot of diagnostic accuracy of lncRNAs in acute kidney rejection in adults.

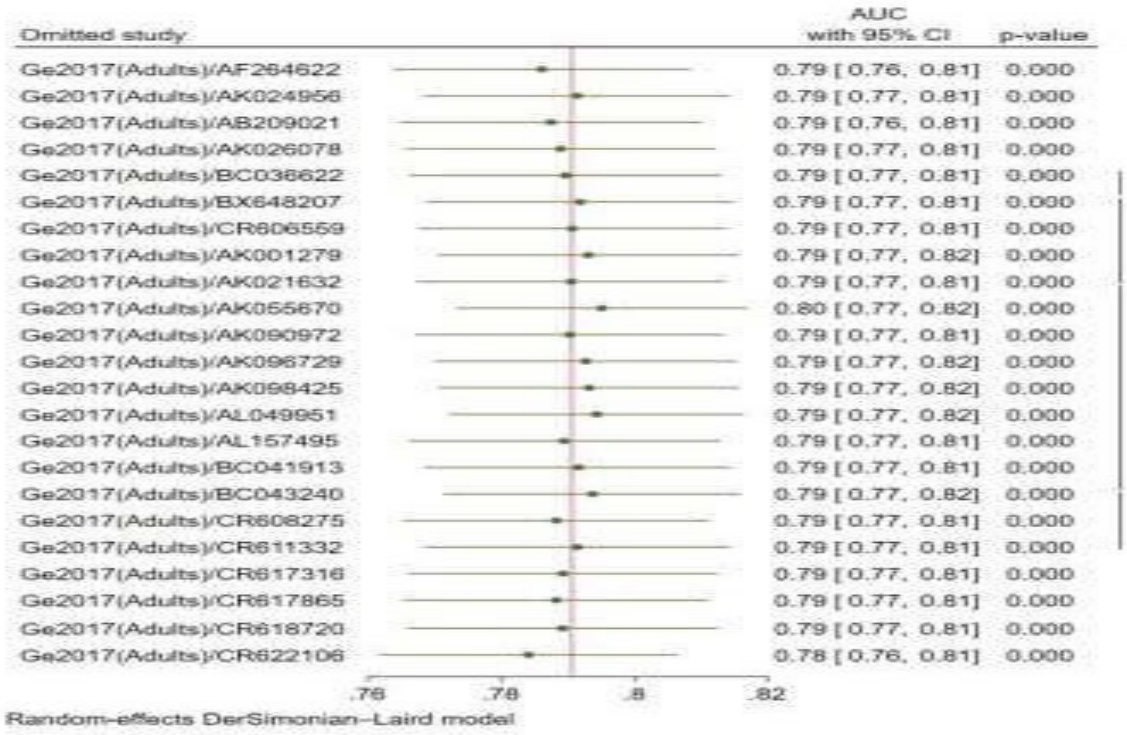

Figure 3. Leave one out analysis of diagnostic accuracy of lncRNAs in acute kidney rejection in adults.

lication, lncRNA type, sample sizes, sensitivity, specificity, or area under the curve (AUC) for diagnostic studies, HRs, *P* values, and 95% CIs for survival analysis. Based on Tierney et al.[14], Kaplan–Meier curves were used to estimate HRs when patient level data were unavailable. Study quality was assessed using QUIPS for prognostic studies and QUADAS 2 for diagnostic accuracy studies.

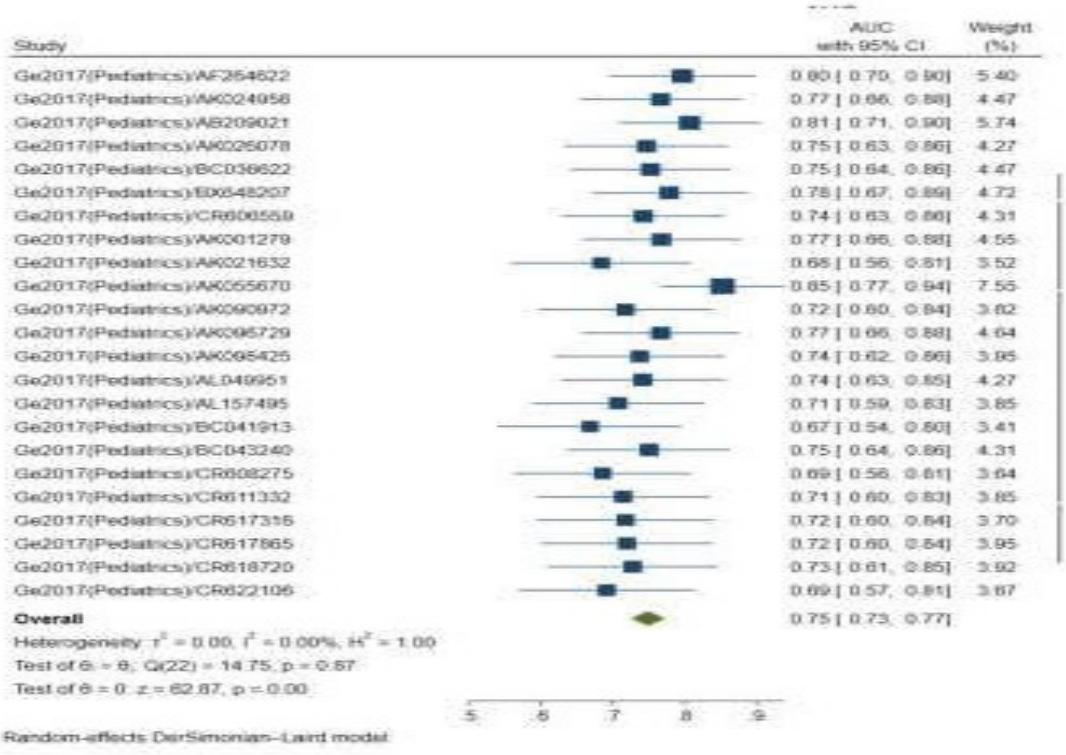

Figure 4. Forest plot of diagnostic accuracy of lncRNAs in acute kidney rejection in pediatrics.

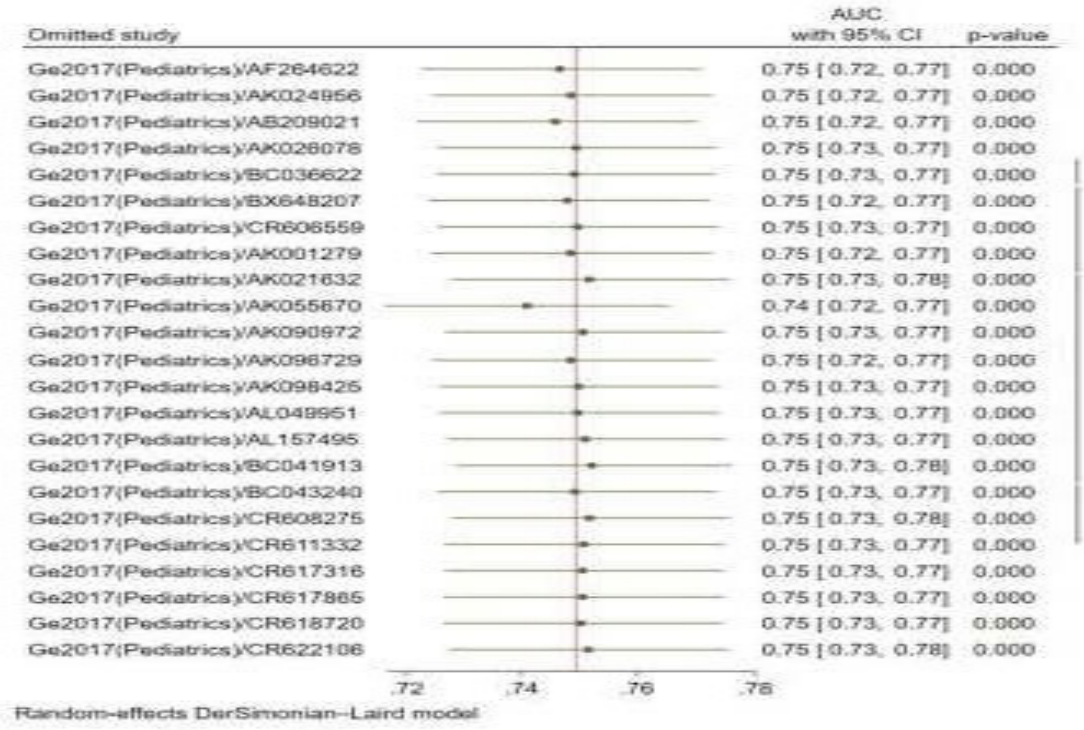

Figure 5. Leave one out analysis of diagnostic accuracy of lncRNAs in acute kidney rejection in pediatrics.

Statistical analysis

Heterogeneity was assessed using I^2 statistics and the chi square test. Significant heterogeneity was defined as $I^2 > 50\%$. A random effects model was used in the presence of high heterogeneity; otherwise, a fixed effect model was applied. Effect sizes included AUC, sensitivity, and specificity for diagnostic meta analysis; HRs with 95% CIs for prognostic studies; and ORs with 95% CIs for clinicopathological features. HR > 1 indicated an unfavorable survival outcome. Analyses were performed using STATA version 17 and the meta package. A two sided *P* value < 0.05 was considered statistically significant.

RESULTS

Identification of studies

A total of 291 articles were retrieved. After removing duplicates, 186 articles remained for title/abstract screening. Subsequently, 145 studies were excluded based on eligibility criteria. Full texts of 41 articles were reviewed, and 10 studies were included in the systematic review and meta analysis[15-24](**Figure 1**).

Study characteristics

Table 1 presents study characteristics. The included studies were published between 2014 and 2020, with most from 2019–2020. Most were conducted in China (n = 6)[15,16,20-23]; others were from Germany (n = 2)[18,24], the Netherlands (n = 1)[17], and Iran (n = 1)[19]. For meta analyses, diagnostic accuracy of lncRNA measurement for acute kidney rejection was evaluated in one study with sufficient data on 23 different lncRNAs in pediatric and adult cohorts.[16] Prognostic value was assessed in two studies with data on 45 different lncRNAs[21,22]. Seven studies were systematically reviewed but were not eligible for meta analysis.[15,17-19,23-24]

Meta-analysis

The pooled AUC of lncRNA measurement for evaluating acute kidney rejection in adults was 0.79 (95% CI: 0.77–0.81; test of heterogeneity: $I^2 = 6.27\%$, $P < 0.001$) (**Figure 2**). Leave one out analysis showed that removing any single lncRNA did not materially affect the total AUC (**Figure 3**). The pooled AUC in pediatrics was 0.75 (95% CI: 0.73–0.77; test of heterogeneity: $I^2 = 0.00\%$, $P < 0.001$) (**Figure 4**), with similar stability on leave one out analysis (**Figure 5**).

The pooled HR from prognostic studies was 0.81 (95% CI: 0.63–1.04; $I^2 = 80.4\%$, $P < 0.001$), which was not statistically significant for evaluating prognosis in acute kidney rejection ($P = 0.094$) (**Figure 6**).

DISCUSSION

This systematic review and meta analysis identified 10 studies evaluating lncRNA levels in acute kidney rejection. The analyses indicate that measurement of various lncRNAs plays an important role in diagnosing acute kidney rejection. The pooled AUCs were 0.79 in adults and 0.75 in pediatrics. The pooled HR of 0.81 suggests a potentially favorable association but did not reach statistical significance.

Notably, the pooled HR of 0.81 (95% CI: 0.63–1.04) for lncRNA expression in prognostic studies, although not statistically significant ($P = 0.094$), suggests a potential protective association of certain lncRNAs against progression of acute renal transplant rejection. This inverse association may indicate regulatory or anti inflammatory effects within the transplant microenvironment. For instance, Qiu et al.[20] showed lncRNA ATB upregulation during rejection episodes with implications for kidney cell phenotypes and nephrotoxic effects of immunosuppressive agents, suggesting functional roles beyond passive biomarker expression. These findings highlight

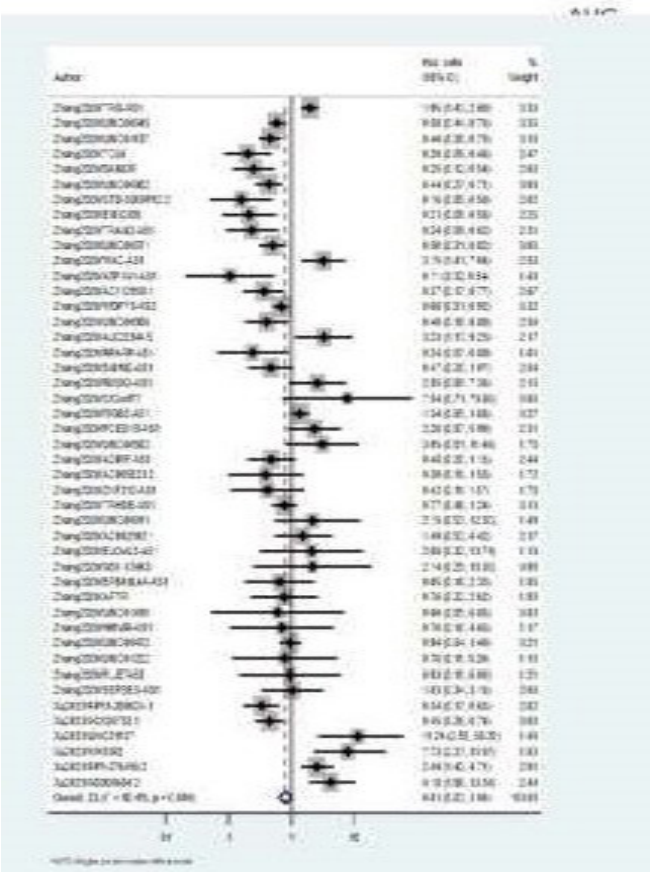

Figure 6. Forest plot of prognostic value of lncRNAs in acute kidney rejection.

the complexity of lncRNA mediated pathways, some of which may be compensatory or protective responses to immune activation and warrant mechanistic validation. Acute kidney rejection is a major cause of graft failure within the first year after kidney transplantation,[25,26] although its long term course remains under study. Acute rejection can cause microvascular endothelial injury. Growth factors and cytokines produced in the alloimmune response can lead to endothelial activation and microvascular destabilization[27-32], promoting chronic ischemia, loss of the microvascular system, and cell death.[33,34] lncRNAs are RNAs of ≥200 nucleotides without protein coding capacity. More than 90% of the human genome is transcribed into non protein coding RNA[35]. Multiple lncRNA classes influence human disease by regulating neighboring genes and chromatin topology, scaffolding, and protein decoying.[36-37] lncRNAs have roles in renal ischemia, transplantation, injury, fibrosis, glomerular diseases, inflammation, and renal cell carcinoma.[38] They may help predict chronic damage after kidney transplantation.[21] Beyond transplantation, circulating lncRNAs show biomarker potential in other diseases, such as Alzheimer's disease.[39] Animal studies also support lncRNA involvement in renal allograft rejection.[40] Groeneweg et al.[17] first evaluated LNC EPHA6 in acute kidney rejection, showing higher levels during rejection that decreased after resolution while glomerular filtration rate remained stable, and LNC EPHA6 has been linked to diabetic nephropathy.[41]

Limitations include: (1) limited available data for meta analysis—only one study with sufficient diagnostic accuracy data and two with prognostic data; (2) overall scarcity of research on this topic, highlighting the need for more comprehensive evaluations; (3) substantial heterogeneity in prognostic analyses (elevated I2), likely reflecting differences in populations, lncRNA targets, sample sources, and methods; and (4) potential sparse data bias, with large HR estimates and wide CIs in several included studies due to small event counts or sample sizes, necessitating cautious interpretation of prognostic significance.

CONCLUSIONS

Assessment of lncRNA levels in plasma or urine is a promising noninvasive approach for diagnosing acute kidney rejection. The prognostic value of lncRNAs requires further validation in larger, standardized studies.

SUMMARY

A pooled analysis shows lncRNAs can help diagnose acute kidney rejection (AUC 0.79 adults; 0.75 pediatrics), while prognostic value (HR 0.81) is not yet definitive; larger standardized studies are needed.

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CONFLICT OF INTEREST

The authors declare no conflict of interest for this article.

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