Criteria Grid
Hepatitis C Research Studies, Tools, and Surveillance Systems

Best Practice/Intervention:	Fabrizi F. et al. (2014) Meta-analysis of observational studies: hepatitis C and survival after renal transplant. *Journal of Viral Hepatitis*, 21(5):314-324
Date of Review:	March 11, 2015
Reviewer(s):	Christine Hu

Part A

Category:	**Basic Science** ☐ **Clinical Science** ☐ **Public Health/Epidemiology** ☐ **Social Science** ☐ **Programmatic Review** ☒
Best Practice/Intervention:	**Focus:** **Hepatitis C** ☒ **Hepatitis C/HIV** ☐ **Other:** **renal transplant** **Level:** **Group** ☒ **Individual** ☐ **Other:** **Target Population:** **HCV patients with renal transplant** **Setting:** **Health care setting/Clinic** ☒ **Home** ☐ **Other:** **Country of Origin:** **Italy** **Language:** **English** ☒ **French** ☐ **Other:**

Part B

	YES	NO	N/A	COMMENTS
Is the best practice/intervention a meta-analysis or primary research?	☒	☐	☐	Meta-analysis; to determine the impact of HCV on the relative risks of all-cause mortality and graft loss after renal transplant
Has the data/information been used for decision-making (e.g. program funding developments, policies, treatment guidelines, defining research priorities and funding)?	☐	☒	☐	Article suggested that healthcare providers should be aware of the risk of increased mortality and graft loss among renal transplant patients with HCV.
Do the methodology/results described allow the reviewer(s) to assess the generalizability of the results?	☒	☐	☐	
Are the best practices/methodology/results described applicable in developed countries?	☒	☐	☐	

	YES	**NO**	**N/A**	**COMMENTS**
Are the best practices/methodology/results described applicable in developing countries?	☒	☐	☐	Methodology and finding can be extended to similar studies in various countries.
The research study/tool/data dictionary is easily accessed/available electronically	☒	☐	☐	Available to download from http://onlinelibrary.wiley.com
Is there evidence of cost effective analysis with regard to interventions, diagnosis, treatment, or surveillance methodologies? If so, what does the evidence say? ***Please go to Comments section***	☐	☒	☐	
Are there increased costs (infrastructure, manpower, skills/training, analysis of data) to using the research study/tool/data dictionary?	☐	☒	☐	
How is the research study/tool funded? ***Please got to Comments section***	☒	☐	☐	This work has been supported in part by the grant 'Project Glomerulonephritis'
Is the best practice/intervention dependent on external funds?	☐	☒	☐	
Other relevant criteria: _____	☐	☐	☐	- Significant relationship between HCV and increase mortality and graft loss in renal transplant patients
WITHIN THE SURVEILLANCE SYSTEM FOR REVIEW				
Are these data regularly collected?	☐	☐	☒	Unclear
Are these data regularly collected at and/or below a national level?	☐	☐	☒	Unclear
Are these data collected manually or electronically?	☐	☐	☐	Electronically: National Library of Medicine's MEDLINE database
RESEARCH REPORTS				
Has this research been published in a juried journal?	☒	☐	☐	*Journal of Viral Hepatitis*
Does the evidence utilize the existing data/surveillance information or has it generated new data and/or information?	☐	☐	☐	utilize the existing data/surveillance information

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Meta-analysis of observational studies: hepatitis C and survival after renal transplant

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SUMMARY. Recent evidence has shown that anti-HCV-positive serologic status is significantly linked to lower patient and graft survival after renal transplant, but conflicting results have been given on this point. The aim of this study was to conduct a systematic review of the published medical literature concerning the impact of HCV infection on all-cause mortality and graft loss after RT. The relative risk of all-cause mortality and graft loss was regarded as the most reliable outcome end-point. Study-specific relative risks were weighted by the inverse of their variance to obtain fixed- and random-effect pooled estimates for mortality and graft loss with HCV across the published studies. We identified eighteen observational studies involving 133 530 unique renal transplant recipients. The summary estimate for adjusted relative risk (aRR) of all-cause mortality was 1.85 with a 95% confidence interval (CI) of 1.49; 2.31 ($P < 0.0001$); heterogeneity statistics, Ri = 0.87 (P-value by Q-test = 0.001). The overall estimate for adjusted RR of all-cause graft loss was 1.76 (95% CI, 1.46; 2.11) ($P < 0.0001$), heterogeneity statistics, Ri = 0.65 (P-value by Q-test = 0.001). Stratified analysis did not change meaningfully these results. Meta-regression showed that living donor rate had a favourable influence on patient ($P = 0.031$) and graft survival ($P = 0.01$), whilst diabetes mellitus having a detrimental role on patient survival ($P = 0.001$). This meta-analysis of observational studies supports the notion that HCV-positive patients after RT have an increased risk of mortality and graft loss. Further studies are in progress to understand better the mechanisms underlying the relationship between HCV and mortality or graft dysfunction after renal transplant.

Keywords: graft loss, Hepatitis C renal transplant, liver disease, survival.

INTRODUCTION

Hepatitis C virus (HCV) infection is a common complication after renal transplantation in both developed and less-developed countries. The natural history of HCV infection remains unclear [1] even if HCV is a well-known cause of liver disease after RT, and chronic liver disease represents the fourth most common cause of death in many series of RT recipients [2].

Post-transplant immuno-suppression has a permissive effect on viral replication, and this has the potential to accelerate pre-existing liver disease or to reactivate HCV infection after renal transplant. Defining the natural history of HCV is difficult even in patients with normal kidney function: the disease has a very long duration [3], determining its onset may be difficult, and various factors can modify the course including co-infection with hepatitis B virus (HBV) or human immunodeficiency virus (HIV), and alcohol use. Antiviral therapy is widely used – thus, natural history studies of chronic HCV will not be possible in the near future; finally, post-transfusion HCV that forms of HCV infection where the onset of infection is easily assessed no longer occurs [3].

The evaluation of the natural history of HCV after RT is even more problematic because of additional characteristics of this population. Clinicians have been reluctant to perform liver biopsy due to concern about abnormalities in platelet function in uraemia. Amino-transferase levels are lower in patients with kidney insufficiency than the nonuraemic population, and this may hamper recognition of HCV-related liver disease on the grounds of biochemical tests [4]. Third-generation anti-HCV testing is specific and sensitive in patients with end-stage renal disease; however, a small proportion of ESRD patients have HCV viraemia in serum, but lacked detectable anti-HCV antibody in serum because of the blunted humoral immune response that occurs with renal disease [5].

Abbreviations: aRR, adjusted relative risk; ELISA, enzyme-linked immunosorbent assay; HCV, hepatitis C virus; PCR, polymerase chain reaction; RIBA, recombinant immunoblot assay; TMA, thrombotic microangiopathy.

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Mortality is a reliable end-point in the natural history of HCV-related liver disease after RT. Recent information has been accumulating showing that HCV plays a role in lowering patient and graft survival among kidney transplant recipients, but controversial evidence is still present [6]. As an example, a retrospective study (44 renal transplant patients with more than one liver biopsy) recently concluded that kidney transplant does not seem to accelerate liver injury; 77% (24/31) of kidney recipients who underwent follow-up biopsies showed stable or improved liver histology [7].

The primary aim of this study was to analyse the available evidence on the relationship between anti-HCV seropositive status and the relative risks of all-cause death and graft loss after RT. A systematic review of the medical literature was carried out on this issue with a meta-analysis of clinical observational studies.

PATIENTS AND METHODS

Search strategy and data extraction

Electronic searches of the National Library of Medicine's MEDLINE database, Current Contents and manual searches of selected specialty journals were performed to identify all pertinent literature. Various MEDLINE database engines (Ovid, PubMed and GratefulMed), and Embase were used. The keywords 'Hepatitis C virus', 'Renal Transplantation', 'Graft loss' and 'Mortality' were used. Reference lists from qualitative topic reviews and published clinical trials were also searched. Our search was limited to human studies that were published in the English literature. Data extraction was conducted independently by two investigators (F.F., V.D.) and consensus was achieved for all data. Studies were compared to eliminate duplicate reports for the same patients, which included contact with investigators when necessary. Eligibility and exclusion criteria were prespecified.

Criteria for inclusion

We included studies evaluating patients with end-stage renal disease who underwent RT. Both case–control and cohort studies were considered eligible for inclusion in the analysis. To be considered for inclusion, studies had to define HCV infection by testing for anti-HCV in serum. Information on anti-HCV status had to be registered at the time of enrolment. Patient outcomes collected included death, cause of death and loss to follow-up.

Ineligible studies

Studies were excluded if they reported inadequate data on survival. Studies that were only published as abstracts or as interim reports were excluded; letters and review articles were not considered for this analysis.

End-points of interest

The primary end-point was the adjusted relative risk (aRR) and 95% confidence interval (CI) of all-cause mortality among RT recipients who were anti-HCV-positive relative to those not infected. The aRR of all-cause mortality (and graft loss) was specified by Cox proportional hazard analysis in each study. The Cox proportional hazard analysis was used to estimate the independent effect of anti-HCV serologic status on survival after adjustment for different follow-up time and distribution of potential confounders (e.g. age, gender, race, time on dialysis, diabetes mellitus, HBsAg sero-positive status, history of previous transplants). The relative risk and 95% CI of death rate due to liver disease among anti-HCV-positive RT recipients relative to those who were anti-HCV negative were also calculated.

The secondary end-point was the adjusted RR and 95% CI of graft loss among RT recipients who were anti-HCV positive relative to those not infected. The aRR of graft loss was specified by Cox proportional hazard analysis in each study. Cox proportional hazards regression was carried out to assess the effect of HCV serology status *per se* on graft loss after adjustment for differential follow-up time and distribution of potential confounders.

Statistical methods

A summary estimate of the aRR of all-cause mortality in anti-HCV-positive to anti-HCV-negative patients was generated by weighting the study-specific RRs by the inverse of the variance. We computed fixed and random-effect estimates [8]. R*i* (the proportion of total variance due to between studies variance) was used to assess heterogeneity. Heterogeneity was also analysed by a parametric version (1000 replication) of the DerSimonian and Laird *Q*-test, when the number of studies to be meta-analysed was not large [9]. To further explore the origin of heterogeneity, we restricted the analysis to subgroups of studies defined by study characteristics such as size (population-based/single-centre), country of origin (Europe/USA) or reference year. Meta-regression was carried out to look at the effect of potential and continuous covariates on the outcome of interest. We performed random-effects meta-regression using the method of moments or maximum likelihood approaches where appropriate; a single predictor is allowed in each model (simple meta-regression) [10]. A funnel plot was performed in order to detect a publication bias in the relation exposure-disease at hand. The publication bias was analysed by the Egger test. Statistical analysis was made by the software HEpiMA, version 2.1.3 [11], and Comprehensive Meta-analysis (CMA), version 2.0 (Biostat Inc., USA, 2005) [12]. The 5% significance was used for alpha risk. Every estimate was given with its 95% CIs.

RESULTS

Literature review

Our electronic and manual searches identified 1022 studies, of which 171 were considered potentially relevant and were selected for full text review. Nineteen papers fulfilled the inclusion criteria [13–31] and 152 were excluded. The trials by Pereira *et al.* (study 1 and 2) were addressed in three reports [13–15]. A total of eighteen clinical studies (with 133 530 unique renal transplant patients) were included in our meta-analysis. There were two controlled clinical trials [13–15], whilst the others had retrospective design. Five studies investigated the relationship between HCV infection and death after RT from a population perspective [17,24,26,29,30]. The list of the 171 references is available from the authors on request. There was a 100% concordance between reviewers with respect to final inclusion and exclusion of studies reviewed based on the predefined inclusion and exclusion criteria.

Study design of clinical trials

The report by Pereira *et al.* included 29 recipients who had received organs (kidneys [$n = 19$], hearts [$n = 6$] and livers [$n = 4$]) from 13 anti-HCV-positive cadaver donors (study group) and 74 recipients of organs (kidneys [$n = 57$], hearts [$n = 6$] and livers [$n = 11$]) from 37 randomly selected anti-HCV-negative cadaver donors (control group) [11,13]. Another study by Pereira *et al.* described 103 randomly selected recipients of kidneys from anti-HCV-negative donors; testing of pretransplant stored sera revealed positive results by anti-HCV ELISA in 23 (22%) and negative results in 80 (78%) recipients who constituted the control group [12,13].

The diagnosis of HCV was based on the presence (or absence) of serum anti-HCV by ELISA in most clinical studies included in the review. Confirmation of all anti-HCV-positive patients by immunoblot techniques was performed in some reports (Table 4). In Mahmoud's study, diagnosis of HCV was made by HCV RNA testing with polymerase chain reaction (PCR) (Table 4) [21].

The rate of living donors ranged between 0% [11–13] and 100% [21,24] in the reports included in our systematic review (Table 4).

Information on the use of induction immunosuppression (lymphocyte-depleting agents, interleukin-2 receptor blockers or both) was given in 9 (50%) reports. The percentage of patients receiving induction immunosuppressive therapy ranged between 5.3% (7/133) [21] and 82% [26] (Table 4); it seems that induction immunosuppressive therapy was given irrespective of anti-HCV serologic status.

Patient characteristics

Shown in Tables 1–4 are some salient demographic characteristics of subjects enrolled in the included studies. Six were from centres in North America, six from Western Europe and three from Asia. The mean age of subject cohort was between 40 ± 22 and 51 ± 21 years of age. The gender distribution varied from 57% to 77% male. The rate of patients with diabetes mellitus ranged from 5.5% to 30%.

Table 1 Baseline characteristics of studies included in the analysis

Authors	Reference year	Country	Patients, *n*	Anti-HCV positive, *n*
Pereira B., *et al.* (study 1)	1997	USA	103	21 (22.8%)
Pereira B., *et al.* (study 2)	1997	USA	103	23 (22.3%)
Legendre C., *et al.*	1998	France	499	112 (22.4%)
Batty D., *et al.*	2001	USA	28 692	1624 (5.7%)
Breitenfeldt M., *et al.*	2002	Germany	927	160 (17.2%)
Forman J., *et al.*	2004	USA	354	26 (7.3%)
Mahmoud I., *et al.*	2004	Egypt	133	80 (60.1%)
Bruchfeld A., *et al.*	2004	Sweden	571	51 (8.9%)
Aroldi A., *et al.*	2005	Italy	541	244 (45.1%)
Mitwalli A., *et al.*	2006	Saudi Arabia	448	286 (63.8%)
Einollahi B., *et al.*	2007	Iran	3028	NA
Ingsathit A., *et al.*	2007	Thailand	346	22 (6.3%)
Luan F., *et al.*	2008	USA	79 337	3708 (4.7%)
Gentil M., *et al.*	2009	Spain	3861	232 (6.7%)
Ridruejo E., *et al.*	2010	Argentina	542	180 (33.2%)
Morales J., *et al.*	2010	Spain	4304	587 (13.6%)
Scott D., *et al.*	2010	Australia, NZ	7572	140 (1.8%)
Singh N., *et al.*	2012	USA	2169	154 (7.1%)

Table 2 Baseline characteristics of studies included in the analysis

Authors	Age, yrs	Gender, male	AA patients, *n*	HBsAg positive, *n*
Pereira B., *et al.* (study 1)	40 ± 22/48 ± 23	20 (69%)/46 (62%)	NA	1 (4%)/1 (2%)
Pereira B., *et al.* (study 2)	51 ± 21/43 ± 19	13 (57%)/50 (63%)	NA	0/0
Legendre C., *et al.*	38 ± 1	69 (61.6%)/240 (62%)	NA	0
Batty D., *et al.*	45.2 ± 10/42.8 ± 15	1129 (69%)/16 219 (59.9%)	273 (54.8%)/16 219 (59.9%)	NA
Breitenfeldt M., *et al.*	40 ± 12/42 ± 13	93 (71%)/484 (63%)	1 (0.001%)	37 (3.9%)
Forman J., *et al.*	45.5 ± 13/44 ± 13	20 (77%)/195 (59.5%)	NA	NA
Mahmoud I., *et al.*	32 ± 10/30 ± 10	59 (74%)/25 (54%)	NA	0
Bruchfeld A., *et al.*	45.4 ± 13/45.5 ± 13	27 (52.9%)/340 (65.4%)	NA	0
Aroldi A., *et al.*	32 ± 12/34 ± 11	118 (56%)/152 (60%)	NA	77 (14%)
Mitwalli A., *et al.*	40 ± 12/36.8 ± 11	204 (71%)/124 (76%)	NA	8 (1.8%)
Einollahi B., *et al.*	36.4 ± 0.3	1919 (63.4%)	NA	NA
Ingsathit A., *et al.*	NA	16 (73%)/199 (61%)	NA	23 (6.7%)
Luan F., *et al.*	49/48	2706 (73%)/45 377 (60%)	1965 (53%)/18 151 (24%)	NA
Gentil M., *et al.*	NA	NA	NA	NA
Ridruejo E., *et al.*	42.03 ± 13.03	116 (64%)/210 (58%)	0	23 (4.2%)
Morales J., *et al.*	46.6 ± 13.2	2668 (62%)	NA	0
Scott D., *et al.*	NA	101 (72.1%)/4519 (60.8)	20 (14.3%)/677 (9.1%)	NA
Singh N., *et al.*	NA	1295 (59.7%)	228 (10.5%)	NA

Figures are given for anti-HCV-positive/anti-HCV-negative patients when appropriate. NA, not available; AA, African-American.

Table 3 Baseline characteristics of studies included in the analysis

Authors	Diabetes, *n*	Mean follow-up after RT (months)	Prior renal transplant	Time on dialysis prior RT
Pereira B., *et al.* (study 1)	NA	68 (2–107)/70 (0–112)	7 (24%)/5 (6.7%)	34 ± 54/14 ± 18
Pereira B., *et al.* (study 2)	NA	68 (1–104)/83 (1–112)	5 (22%)/8 (10%)	27 ± 81/16 ± 18
Legendre C., *et al.*	NA	79 ± 2/81 ± 5	0	72 ± 5/40 ± 2
Batty D., *et al.*	6307 (21.9%)	NA	51 (10.4%)/1311 (5.9%)	24.4 ± 20/18.4 ± 17
Breitenfeldt M., *et al.*	NA	110.4 ± 52.8	0	64.8 ± 50/45 ± 41
Forman J., *et al.*	93 (26%)	28 ± 21.8/28 ± 21.3	11 (42.3%)/29 (8.8%)	41.2 ± 52/23 ± 29
Mahmoud I., *et al.*	NA	94 ± 29/98 ± 28	6 (4.5%)	20 ± 16/9 ± 18
Bruchfeld A., *et al.*	7 (13.7%)/98 (18.8%)	130	12 (23%)/53 (10%)	68.4 ± 77/27 ± 43
Aroldi A., *et al.*	NA	172.8 ± 67/168 ± 60	24 (9.8%)/6 (2%)	42 ± 31/26.4 ± 23
Mitwalli A., *et al.*	NA	70.2 ± 33.6	0	NA
Einollahi B., *et al.*	NA	NA	157 (5.2%)	NA
Ingsathit A., *et al.*	3 (14%)/37 (11.4%)	44.4 (6–81)	NA	44.2 ± 29/22.2 ± 22
Luan F., *et al.*	1112 (30%)/21 932 (29%)	NA	0	40.9/29.9
Gentil M., *et al.*	191 (4.9%)	NA	360 (9.3%)	48.0 ± 53
Ridruejo E., *et al.*	NA	76.8 ± 59.5	40 (22%)/22 (6%)	NA
Morales J., *et al.*	237 (5.5%)	NA	525 (12%)	82.3 ± 64/31.7 ± 35
Scott D., *et al.*	16 (11.4%)/803 (10.8%)	NA	718 (9.5%)	NA
Singh N., *et al.*	565 (26%)	72.2 ± 51.1	441 (20.3%)	NA

Figures are given for anti-HCV-positive/anti-HCV-negative patients when appropriate. NA, not available.

Table 4 Baseline characteristics of studies included in the analysis

Authors	Kidney source, cadaveric	Induction immunosuppression	HCV diagnosis
Pereira B., *et al.* (study 1)	103 (100%)	NA	ELISA
Pereira B., *et al.* (study 2)	103 (100%)	9 (39%)/43 (54%)	ELISA
Legendre C., *et al.*	499 (100%)	0	ELISA
Batty D., *et al.*	1319 (81.2%)/18 934 (69.9%)	645 (39.7%)/10 123 (37.4%)	ELISA
Breitenfeldt M., *et al.*	119 (97%)/789 (98%)	NA	ELISA + Western Blot
Forman J., *et al.*	20 (77%)/154 (47%)	9 (37%)/85 (27%)	ELISA
Mahmoud I., *et al.*	0	7 (5.3%)	PCR
Bruchfeld A., *et al.*	39 (76%)/331 (64%)	0	ELISA + PCR
Aroldi A., *et al.*	219 (89%)/260 (87%)	31 (13%)/46 (15%)	ELISA + RIBA
Mitwalli A., *et al.*	42 (14.7%)/19 (11.7%)	NA	ELISA
Einollahi B., *et al.*	0	NA	ELISA + RIBA
Ingsathit A., *et al.*	15 (68%)/113 (35%)	NA	ELISA
Luan F., *et al.*	3077 (83%)/52 940 (70%)	3114 (84%)/62 016 (82%)	ELISA
Gentil M., *et al.*	3861 (100%)	NA	ELISA
Ridruejo E., *et al.*	135 (75%)/232 (64%)	110 (61%)/257 (71%)	ELISA
Morales J., *et al.*	NA	NA	ELISA
Scott D., *et al.*	NA	NA	ELISA
Singh N., *et al.*	2169 (100%)	648 (29.8%)	ELISA

Figures are given for anti-HCV-positive/anti-HCV-negative patients when appropriate. NA, not available; Induction therapy, Use of biological agents (depleting or nondepleting antibody) at RT; ELISA, Enzyme-linked immunosorbent assay; RIBA, recombinant immunoblot assay; PCR, polymerase chain reaction.

The average follow-up was between 44.4 months and 172.8 months. The frequency of anti-HCV-positive patients varied from 1.8% to 63.8%.

Summary estimates of outcome: All-cause mortality

Detailed information on the all-cause mortality rate of the patients was reported in thirteen studies. All included studies used Cox proportional hazard models to adjust for differential follow-up times and distribution of potential confounders in isolating the effect of anti-HCV sero-positive status on all-cause mortality. Figure 1 reports the Forest plot on the impact of HCV on all-cause mortality after renal transplant, and the summary estimate for aRR of all-cause mortality with anti-HCV across the identified studies was 1.85 with a 95% CI of 1.49; 2.31. The association was significant ($P < 0.0001$). Tests for homogeneity of the aRR across the thirteen studies gave an R*i* value of 0.87, that is, the homogeneity assumption was rejected. As shown in Table 5, there was no substantial difference in

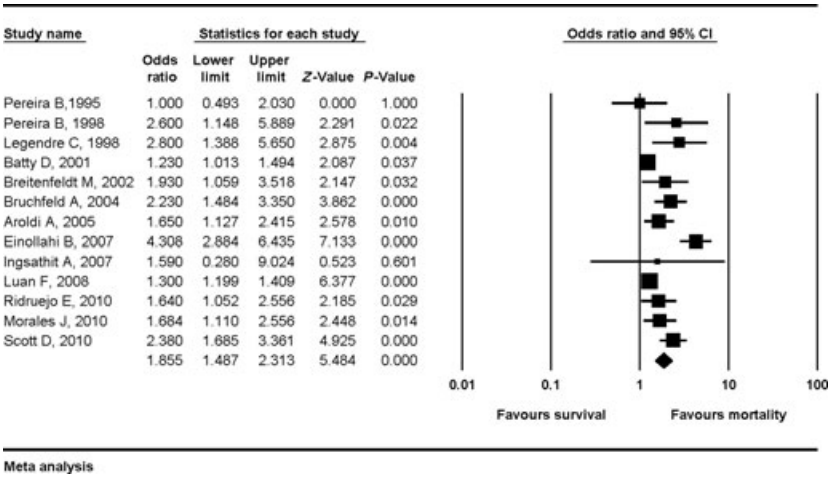

Fig. 1 Forest plot: Impact of HCV infection on all-cause mortality after renal transplant.

Table 5 Summary estimates for adjusted relative risks (aRR = adjusted relative risk by Cox proportional hazard model) of all-cause mortality and hepatitis C virus (HCV) after renal transplant

	Study, *n*	Fixed- effects aRR (95% CI)	Random-effects aRR (95% CI)	R*i*	*P*-value (by *Q*-test)
All studies	13	1.43 (1.34; 1.53)	1.85 (1.49; 2.31)	0.87	0.00
Population-based studies	5	1.38 (1.29; 1.48)	1.87 (1.32; 2.66)	0.95	0.000
Recent studies (since 2000)	10	1.42 (1.33; 1.52)	1.84 (1.45; 2.34)	0.90	0.00
Studies from USA	4	1.29 (1.20; 1.39)	1.29 (1.15; 1.44)	0.34	0.31
Studies from Europe	5	1.91 (1.55; 2.35)	1.91 (1.55; 2.35)	0.0	0.62
ELISA-based studies	9	1.35 (1.26; 1.45)	1.58 (1.30; 1.92)	0.77	0.00

Pereira [1] = aRR adjusted for time on dialysis, prior transplant, age, type of organ; Pereira [2] = aRR adjusted for age, time on dialysis, prior transplant; Legendre = aRR adjusted age, time on dialysis, gender, and transplantation year; Batty = aRR adjusted for age, race, gender, end-stage renal disease due to diabetes mellitus, weight, year of transplant, duration of pretransplant dialysis, prior transplant, donor and recipient age, donor and recipients race, donor and recipients gender, delayed graft function, allograft rejection, induction therapy; Breitenfeldt = aRR adjusted for time on dialysis, HBsAg status, age, acute rejection, HBV/HCV co-infection and HCV at RT; Bruchfeld = aRR adjusted for age, gender, diabetes mellitus, prior transplant, type of transplant and time on dialysis; Aroldi = aRR adjusted for age, gender, immunosuppression, HBV/HCV co-infection, type of donor (live/deceased), number of prior transplants; Einollahi = aRR adjusted for donor (age, gender, source) and recipient characteristic (age, gender, aetiology of ESRD, diabetes mellitus, blood group); Ingsathit = aRR adjusted for recipient age, gender, delayed graft function, time on dialysis, diabetes mellitus, acute rejection, HBV/HCV co-infection; Luan = aRR adjusted for recipient characteristics (age, gender, race, diabetes mellitus, aetiology of end-stage renal disease, time on dialysis, panel reactive antibody level, availability of private insurance) and donor characteristics (age, living donor, extended criteria donor, cold ischaemia time, arterial hypertension, creatinine level and cause of death); Ridruejo = aRR adjusted for gender, age, time on dialysis, acute rejection, graft type, number of transplants, induction therapy, type of maintenance immunosuppression; Morales = aRR adjusted for age, serum creatinine at 1 year, arterial blood pressure, donor age, recipient age, acute rejection, proteinuria, steroid treatment; Scott = aRR adjusted for year of transplant, gender, age, ethnicity, country, primary renal disease, co-morbid diabetes mellitus, cardiovascular disease, smoking status, body mass index, CMV antibody, peak PRA, current PRA, time since ESRD onset, graft number, live or deceased donor, ischaemia time, number of HLA mismatches, donor age, donor gender, donor ethnicity, and whether multiple organ transplant.

pooled aRR across designs (i.e. USA, European, population-based studies) even if the homogeneity assumption was rejected in some subgroups only.

The funnel plot concerning the publication bias is reported in Fig. 2. The Egger test demonstrated significant publication bias ($P = 0.022$). Meta-regression showed a negative impact of diabetes mellitus ($P = 0.001$) on the outcome of interest (the adjusted RR of all-cause mortality); living donor status was associated with higher survival ($P = 0.031$) (Table 6).

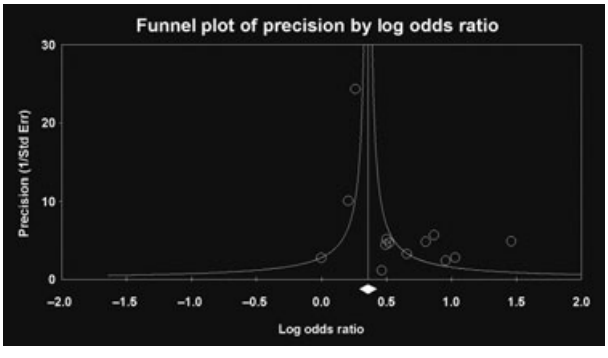

Fig. 2 HCV and survival in dialysis: Funnel plot.

Summary estimates of outcome: Disease-specific mortality among RT recipients

Nine studies gave detailed information on liver-related mortality in HCV-positive compared with HCV-negative recipients after renal transplant [13–16,18,20–22,25,31]. As shown in Table 7, the risk of liver-related death rate was strongly increased in HCV-positive recipients. Seven reports [13–16,18,20,21,31] provided data on the death rate due to infections after RT; the risk of infection-related mortality was not significantly enlarged. Four studies [18,20,21,31] gave information on cardiovascular mortality after RT; this was greater in HCV-positive than HCV-negative recipients (Table 7).

Summary estimates of outcome: Graft survival

Detailed information on the all-cause graft loss was reported in fourteen studies (Fig. 3). All studies used Cox proportional hazard models to adjust for confounders and follow-up time. The Forest plot shown in Fig. 3 gives an adjusted relative risk of graft loss in HCV-positive compared with HCV-negative RT recipients of 1.76 (95% CI, 1.46;

Table 6 Meta-regression: impact of continuous covariates on adjusted RR of all-cause mortality

Variable	Regression coefficient	Standard error	95% CI	*P*-value
Study size	−0.000	0.000	−0.00; 0.00	0.110
Male	2.355	3.141	−3.80; 8.51	0.45
Reference year	0.0124	0.027	−0.040; 0.06	0.646
HBsAg status	−1.413	1.658	−4.66; 1.83	0.39
First transplant	0.121	0.28	−0.43; 0.67	0.66
Diabetes mellitus	−1.97	0.62	−3.20; −0.74	0.001
Living donors	0.81	0.37	0.07; 1.55	0.031
Follow-up time	0.00	0.00	−0.00; 0.00	0.94

Table 7 Summary estimates for unadjusted relative risks (aRR) of disease-specific mortality and hepatitis C virus (HCV) among RT recipients

	Study, *n*	Fixed-effects unadjusted OR (95% CI)	Random-effects unadjusted OR (95% CI)	*P*-value (by *Q*-test)	Z-value
Liver disease–related mortality	9	11.6 (5.54; 24.4)	11.6 (5.54; 24.4)	0.0001	6.48
Cardiovascular mortality	4	2.15 (1.58; 2.91)	2.15 (1.58; 2.91)	0.0001	4.91
Infectious disease–related mortality	7	1.62 (1.13; 2.33)	1.64 (0.77; 3.49)	0.19	1.29

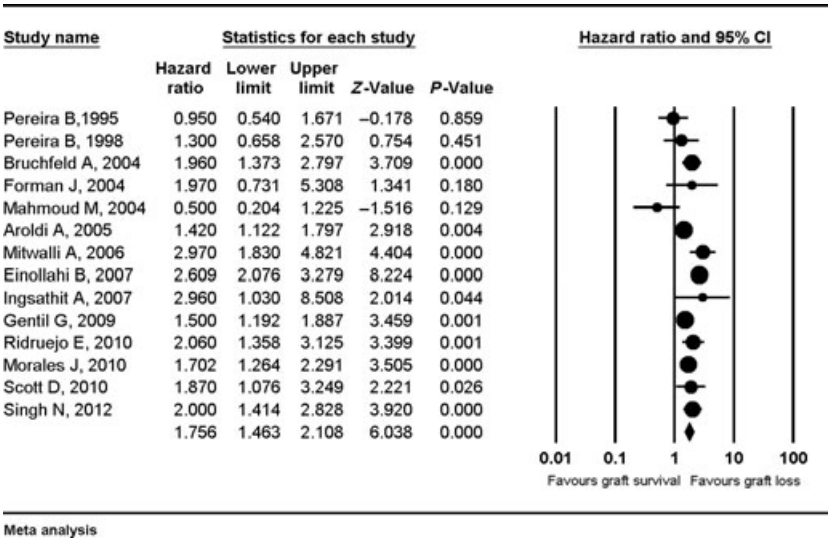

Fig. 3 Forest plot: Impact of HCV infection on all-cause graft loss after renal transplant.

2.11); the relationship was significant ($P < 0.0001$). Tests for homogeneity of the aRR across the fourteen studies gave an R*i* value of 0.65, that is, the homogeneity assumption was rejected.

As listed in Table 8, no substantial difference in pooled aRR occurred across designs (i.e. USA, European, population-based studies); the homogeneity assumption was rejected in some patient subsets. No publication bias was detected by the Egger test ($P = 0.61$).

Meta-regression revealed that male gender ($P = 0.0007$) and living donor status ($P = 0.01$) had favourable influence on all-cause graft survival (Table 9).

DISCUSSION

Controversy exists about the natural history of HCV infection both in individuals with intact kidney function and in renal transplant recipients. We have previously published a meta-analysis of clinical and observational studies ($n = 7$), and an independent and significant impact of HCV infection on lower patient and graft survival was found; the summary estimate for RR was 1.79 (95% CI, 1.57; 2.03) and 1.56 (95% CI, 1.35; 1.80), respectively [32]. Recent information, based on population-based surveys, has been accumulating on the link between HCV and lower survival;

Table 8 Summary estimates for adjusted relative risks (aRR = adjusted relative risk by Cox proportional hazard model) of all-cause graft loss and hepatitis C virus (HCV) after renal transplant

	Study, *n*	Fixed-effects aRR (95% CI)	Random-effects aRR (95% CI)	R*i*	*P*-value (by *Q*-test)
All studies	14	1.79 (1.63; 198)	1.76 (1.46; 2.11)	0.65	0.000
Population-based studies	4	1.91 (1.66; 2.19)	1.89 (1.40; 2.53)	0.77	0.000
Recent studies (since 2000)	12	1.84 (1.67; 2.03)	1.86 (1.54; 2.23)	0.65	0.001
Studies from USA	4	1.60 (1.24; 2.08)	1.50 (1.01; 2.22)	0.50	0.14
Studies from Europe	4	1.57 (1.37; 1.79)	1.57 (1.37; 1.79)	0.0	0.45
ELISA-based studies	10	3.05 (2.85; 3.27)	2.01 (1.34; 3.02)	0.97	0.00

Pereira [1] = aRR adjusted for time on dialysis, prior transplants, age, type of organ; Pereira [2] = aRR adjusted for age, time on dialysis, prior transplants; Bruchfeld = aRR adjusted for age, gender, diabetes mellitus, prior transplant, type of transplant and time on dialysis; Forman = aRR adjusted for acute humoral rejection, delayed graft function, HLA mismatches, PRA, prior transplant, donor type (cadaveric or living), recipients characteristics (age, gender, time on dialysis, diabetes mellitus, arterial hypertension), induction immuno-suppression; Mahmoud = aRR adjusted for donor and recipient age and gender, aetiology of end-stage renal disease, HLA mismatch, number of transplants, time on dialysis, proteinuria, transplant year, number of acute rejection episodes; Aroldi = aRR adjusted for age, gender, immunosuppression, HBV/HCV co-infection, type of donor (deceased/live), number of prior transplants; Mitwalli = aRR adjusted for age, gender, type of donor, hepatitis status, blood pressure; Einollahi = aRR adjusted for donor (age, gender, source) and recipient characteristic (age, gender, aetiology of ESRD, diabetes mellitus, blood group); Ingsathit = aRR adjusted for recipient age, gender, delayed graft function, time on dialysis, diabetes mellitus, acute rejection, HBV/HCV co-infection; Gentil = aRR adjusted for gender, recipient age, diabetes mellitus, re-transplant status, duration of prior RRT, transplant year; Ridruejo = aRR adjusted for gender, age, time on dialysis, acute rejection, graft type, number of transplants, induction therapy, type of maintenance immunosuppression; Morales = aRR adjusted for age, serum creatinine at 1 year, arterial blood pressure, donor age, recipient age, acute rejection, proteinuria, steroid treatment; Scott = aRR adjusted for year of transplant, gender, age, ethnicity, country, primary renal disease, co-morbid diabetes mellitus, cardiovascular disease, smoking status, body mass index, CMV antibody, peak PRA, current PRA, time since ESRD onset, graft number, live or deceased donor, ischaemia time, number of HLA mismatches, donor age, donor gender, donor ethnicity and whether multiple organ transplant; Singh = aRR adjusted for recipient age, race, gender, diabetes mellitus, arterial hypertension, prior transplant, HBV/HCV co-infection, donor age, gender, race, CMV status, maintenance immunosuppressive therapy.

Table 9 Meta-regression: impact of continuous covariates on adjusted RR of all-cause graft loss

Variable	Regression coefficient	Standard error	95% CI	*P*-value
Study size	0.000	0.000	−0.000; 0.000	0.86
Male	4.797	1.418	2.016; 7.578	0.0007
Reference year	0.034	0.026	−0.016; 0.086	0.184
HBsAg status	0.004	0.007	−3.5; 1.1	0.44
First transplant	3.294	1.985	−0.596; 7.185	0.097
Diabetes mellitus	0.873	0.831	−0.755; 2.50	0.293
Living donors	0.741	0.296	0.161; 1.322	0.01
Follow-up time	0.003	0.004	−0.004; 0.007	0.74

this prompted us to review this issue. The current meta-analysis of observational studies aimed to clarify the impact of HCV on all-cause death and graft loss after renal transplant; our results confirmed the prior evidence [32] even if the number of studies included ($n = 18$) or the size of the current meta-analysis ($n = 133{,}530$ unique patients) makes our estimates more reliable.

The natural history of HCV infection after renal transplant is controversial as several authors claimed that the detrimental impact of HCV upon patient/graft survival that has been found in various series was related to retrospective studies, dating back to the 1990s when immunosuppressive regimens were different from those used today. They relied on azathioprine, cyclosporine, high doses of steroids and sometimes an induction therapy with lymphocyte-depleting agents; all of which increased HCV replication in a consistent manner. In addition, it has been observed that many of these studies examined

outcomes in patients in whom a diagnosis of HCV infection was made only after transplant. This could have resulted in under-recognition of more advanced cases of liver disease at the time of transplantation, accounting for increased rates of decompensated liver disease in the reported cohorts. Further support for these views has come from studies based on liver histology, although results are conflicting. Some single-centre surveys, published in the last decade and which provided sequential post-transplant liver biopsies, concluded that in many patients, hepatic injury does not progress after kidney transplant [7,33]. Kamar *et al.* enrolled 51 anti-HCV-positive patients with detectable HCV RNA in serum who underwent a mean of three post-transplant serial liver biopsies over a follow-up of >6 years. They identified three patient groups: those in whom liver fibrosis remained stable ($n = 21$), those with progressing liver fibrosis ($n = 21$) and those with a regression in liver fibrosis ($n = 10$) [33]. In contrast, Zylberberg *et al.* have shown that liver disease progressed more rapidly in RT recipients compared with patients with intact kidney function or those on regular dialysis [34].

This current systematic review included a stratified analysis which did not modify meaningfully our findings; the link between HCV and lower survival after RT was demonstrated irrespective of reference year, country of origin or size of the study group. In contrast, meta-regression revealed that patient and graft survival are dependent on living donor rate; also, the frequency of diabetic transplant recipients had a detrimental influence upon all-cause mortality. These data confer robustness to our conclusions even if we found significant heterogeneity in many of our comparisons – this clearly hampers definitive conclusions. It is clear that our subgroup analysis with meta-regression was not able to capture all the sources of heterogeneity we have observed.

The mechanisms explaining the link between HCV and lower survival after RT remain largely unknown and are currently an area of avid research. According to our univariate analysis on disease-specific mortality after RT, the excess risk of death in HCV-positive renal transplant recipients may be at least attributed to chronic liver disease with its attendant complications (hepato-cellular carcinoma and liver cirrhosis) [32]. We found that the unadjusted OR for cardiovascular mortality in HCV-positive RT recipients was significantly increased, and this is in keeping with the multivariate analysis by Scott *et al.* [30]; they demonstrated a greater cardiovascular mortality in HCV-positive patients after RT. Anti-HCV status emerged by logistic regression as an independent factor for bloodstream infection (OR, 3.14; 95% CI, 1.19–8.24) in the RESITRA/REIPI cohort [35]. The development of new-onset diabetes after transplant [36], recurrence of HCV-associated glomerulonephritis [37] and chronic rejection/transplant glomerulopathy have been cited to explain the reduced graft survival seen in HCV-positive RT recipients. Baid-Agrawal *et al.* studied 209 consecutive renal allograft indication biopsies for chronic allograft dysfunction and found that the majority of patients with confirmed thrombotic microangiopathy (TMA) were also hepatitis C positive, and the majority of hepatitis C-positive patients had TMA [38]. This meta-analysis is potentially biased by a number of issues. First, all the clinical studies included in the current meta-analysis had an observational design. Although much has been learned about the course of HCV in patients on long-term dialysis, the available data are of limited use due to the lack of comparative studies with baseline data and sequential follow-up. The cross-sectional design of many studies does not allow firm conclusions on causality. Second, the studies of this meta-analysis might give incomplete information on additional unmeasured confounders that could introduce bias into the analysis. A peculiar feature of clinical databases as opposed to research databases is the great number of missing data or insensitive codes for co-morbidity diagnoses; our review gives incomplete information on race, HCV RNA or HBsAg status, and others. Third, individual findings from each study (e.g. 'patient-level data') were not available; thus, it was impossible to perform our own adjustments. Based on the RR reported in each study, we have calculated our summary estimate for RR of mortality with anti-HCV across the studies. However, we used adjusted RR obtained by the Cox model in each longitudinal study – this approach takes into account both differential follow-up time and differential distribution of covariates to isolate the effect of anti-HCV sero-positive status *per se*. Finally, as with all meta-analyses, this study has the potential limitation of publication bias; negative trials are less likely to be published. To limit the possible effect of publication bias, we used several strategies for identifying studies to include published and unpublished studies. We have not enrolled trials reported as abstracts; information presented in abstract format is often of poor quality and can give higher treatment effects [39]. Inclusion criteria, established *a priori*, were chosen to increase the likelihood that high-quality studies would be considered.

In conclusion, this meta-analysis of observational studies demonstrates a significant relationship between HCV and increased mortality and graft loss among RT patients. Healthcare providers should be aware of this risk, and more research at basic or clinical level is needed to deepen the link between HCV serologic status and survival among renal transplant recipients.

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