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ORIGINAL ARTICLE

Corticosteroid-free immunosuppression in liver transplantation: a meta-analysis and meta-regression of outcomes

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Abstract

To examine the impact of steroid withdrawal from the immunosuppression protocols in liver transplantation. The electronic databases Medline, Embase, Pubmed and the Cochrane Library were searched. Meta-analysis pooled the effects of outcomes of a total of 2590 patients enrolled into 21 randomized controlled trials (RCTs), using classic and modern meta-analytic methods. Meta-analysis of RCTs addressing patients transplanted for any indication showed no differences between corticosteroid-free immunosuppression and steroid-based protocols in most of the analyzed outcomes. More importantly, steroid-free cohorts appeared to benefit in terms of *de novo* diabetes mellitus development [R.R = 1.86 (1.43, 2.41)], Cytomegalovirus (CMV) infection [R.R = 1.47 (0.99, 2.17)], cholesterol levels [WMD = 19.71 (13.7, 25.7)], the number of patients that received the allocated treatment [O.R = 1.55 (1.17, 2.05)], severe acute rejection [R.R = 1.71 (1.14, 2.54)] and overall acute rejection [R.R = 1.31 (1.09, 1.58)] (when steroids were replaced in the steroid-free arm). Taking RCTs into account independently when steroids were not replaced, overall acute rejection was favoring the steroid-based arm [R.R = 0.75 (0.58, 0.98)]. Studies addressing exclusively transplanted HCV patients demonstrated a significant advantage of steroid-free protocols considering HCV recurrence [R.R = 1.15 (1.01, 1.13)], acute graft hepatitis [O.R = 3.15 (1.18, 8.40)], and treatment failure [O.R = 1.87 (1.33, 2.63)]. No unfavorable effects were observed after steroid withdrawal during short-term follow-up. On the contrary, significant advantages were documented.

Introduction

The utilization of corticosteroids has been the basis of lessening rejection risk following liver transplantation. Steroids, conversely, are associated with a large number of side-effects including the potential recurrence of hepatitis C virus (HCV). Chronic hepatitis C virus infection is the most prevalent indication for liver transplantation, accounting for at least 40% of all transplants performed in the United States [1]. With considerably improved short-term outcomes over the last two decades [2], the contemporary preoccupations in liver transplantation have moved away from avoiding rejection *per se* to precluding toxicity from anti-rejection regimens. Many authors believe that steroids can be withdrawn early without jeopardizing safety [3,4], but the duration of steroid administration after liver transplantation and the likely role of total steroid avoidance remain contentious.

A number of randomized clinical trials (RCT) have been published to study outcomes with steroid avoidance in liver transplantation. A recently published good meta-analysis addressing the subject has to be seen with skepticism because of some flaws in methodology [5].

The purpose of this study was to draw conclusions from the pooled analysis of these RCT of steroid avoidance in liver transplantation, via classic and modern meta-analytic and meta-regression methodology.

Methods

Literature search

All randomized control trials concerning steroid withdrawal in liver transplantation were identified [6–26]. In order to identify appropriate studies, the electronic databases Medline, Embase, Pubmed and the Cochrane Library were used to search for articles from 1990 to 2008 in the English language literature that included the following terms and/or combinations in their titles, abstracts or keyword lists: Randomized controlled trials, double-blind, liver transplantation, steroids, withdrawal, glucocorticoids, prednisone, methylprednisone, orthotopic liver transplantation and allograft. Where it was applicable the above mentioned terms were used in '[MESH]' (Pubmed and the Cochrane Library) otherwise the terms were combined with 'AND/OR' and asterisks. In addition, the abstracts from national and international conferences were searched using online search engines corresponding to the particular conference.

The scheme for this repetitive search is shown in Fig. 1. After the initial screening, additional criteria were imposed. These were as follows: (i) at least one treatment arm had early withdrawal or not at all steroid administration and a second treatment arm in which the patients received at least 3 months of steroids (ii) the analysis to be by intention to treat and (iii) studies of pediatric patients or both pediatric and adult patients were excluded.

Data extraction

Two authors (G.S., C.K) independently selected studies for inclusion and exclusion and reached consensus when they did not agree in the initial assignment. The following variables concerning studies addressing either collectively patients with any indication or exclusively patients with HCV undergoing orthotopic liver transplantation were recorded: authors, journal and year of publication, country of origin, trial duration, participant demographics and data concerning rejection, adverse events, complications, follow up and survival. Where it was felt necessary, the corresponding authors were contacted to obtain supplementary information.

Interventions and outcome definition

Rejection episodes

The diagnosis of graft rejection was biopsy-proven in the first 3 months. Acute rejection was also graded histologically as mild, moderate, or severe (high grade or refractory to treatment). The histologic features of chronic rejection were ductopenia and cholestasis. Corticosteroid-

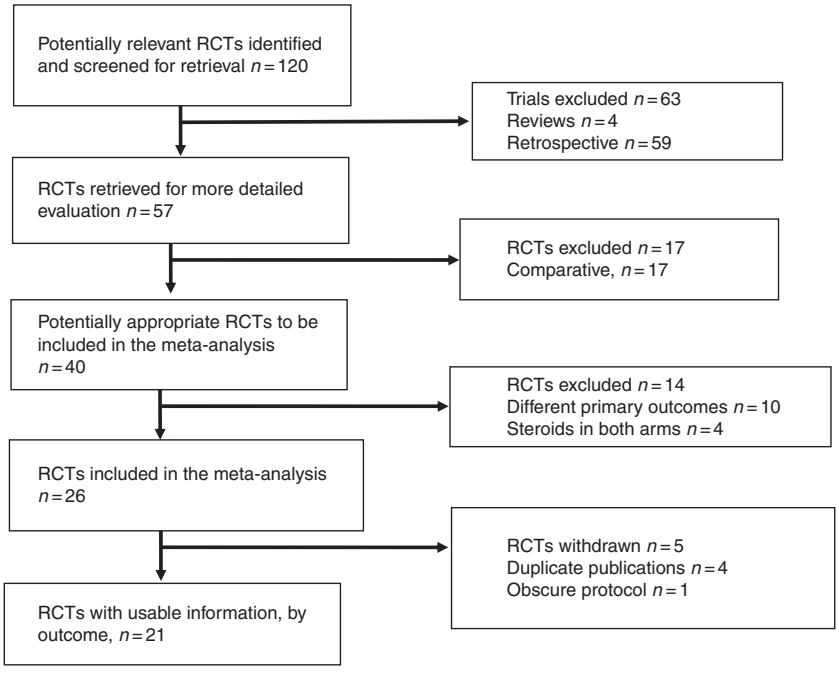


Figure 1 Progress through the stages of the meta-analysis of Randomized Controlled Trials.

resistant rejection was defined as a biopsy-proven rejection episode treated with corticosteroids that led to repeat biopsy showing unchanged or worsening rejection.

Recurrent hepatitis C
Hepatitis C virus infection after grafting associated with histologic signs of hepatitis.

Graft loss
Measured by provided actual survival not censored for death.

CMV infection
Symptomatic cytomegalovirus antigenemia.

Infectious complications
Bacterial and fungal infectious complications requiring hospitalization or intensive care unit management.

Treatment failure
Considered collectively various failure factors, i.e. the numbers of death, graft loss and withdrawal.

Data analysis

A formal meta-analysis (according to the guidelines of the QUOROM statement) [27,28] was made for all RCTs concerning early withdrawal or not at all steroid administration and a second treatment arm in which the patients received at least 3 months of steroids. The primary outcomes used for this study were: i) rejection ii) adverse events iii) graft and patient survival and iv) HCV recurrence.

In order to protect the analysis against false positive conclusions, we prespecified the following covariates to be investigated by subgroup analyses or meta-regression:

Administration of tacrolimus (Tacro) versus cyclosporine (CyA); use of perioperative steroids in the corticosteroid-free immunosuppression arm; replacement of steroids with another immunosuppression agent (versus omitting steroids without replacement) and study quality. Studies with three treatment arms [6,11] were treated as being two separate studies for outcome measures.

In order to quantify the level of agreement between reviewers the Maxwell test statistic and the generalized McNemar statistic were calculated. Pooled estimates of outcomes were calculated using a fixed-effects model but a randomized-effects model was used according to heterogeneity. For dichotomous data, results for each trial were expressed as an odds ratio (OR), relative risk (RR), incidence rate difference (IRD) or risk difference (RD), with 95% confidence intervals (CIs). For continuous outcomes, the effect size was measured as the weighted mean difference (WMD) with 95% CIs. For each total or subtotal the test for heterogeneity and the test for overall effect were given.

In order to explore sources of heterogeneity, the Moses-Shapiro-Littenberg method was applied by adding covariates to the model. If there was a threshold effect, the summary of study results was done by a ROC (Receiver Operating Characteristics) curve [symmetrical or asymmetrical curves around the 'Sensitivity–Specificity' line, depending on whether the diagnostic odds ratio (DOR) was constant or not]. Random effects between studies were taken into account using the restricted maximum likelihood method. The model parameters were obtained by the weighted least squares method, and weights were the inverse of variance of the log of the DOR.

Individual studies were categorized into more than one group where the overall cumulative effect size and group cumulative effect sizes were calculated and tested for significance and the total heterogeneity in effect sizes was partitioned into variance explained by the model and residual error variance not explained by the model. Bootstrap confidence intervals were used to estimate the range of uncertainty for a given test-statistic and to generate the lower and upper 95% confidence limits. Randomization tests were used to generate a statistical distribution of test-statistics from the given data to determine the significance of a test-statistic. Cumulative Meta-analysis was applied to the summary statistics of the successively added studies and compared one another to determine when a given result could have been discovered.

Bias was studied using sensitivity analysis by removing individual studies from the data set and analyzing the overall effect size and the weighted regression tests described by Egger *et al.* [29]. Fail-Safe tests were performed for estimation of the magnitude of the publication bias. In normal quantile plot, the standardized effect size was plotted against the normal quantile values for visual inspection of potential publication bias.

Meta-regression was used to assess the effect of covariates on treatment outcomes.

Results were significant if $P < 0.5$. The RevMan Version 4.2 [30,31], the Statsdirect version 2.6.5, the Meta Disk version 1.4 [32] and Meta-Win version 2.1 [33] was used for the data analysis.

Study quality assessment

Quality assessment of the methodology of the studies integrated in the meta-analysis was scored using the Jadad composite scale [34]. According to that five-point scale (0 point for 'No', 1 point for 'Yes' for the following parameters: randomized study; randomization described;

double-blind study; double-blinding described; description of withdrawals and dropouts) low-quality studies have a score of ≤2 and high-quality studies a score of ≥3 [35].

Results

Twenty-one of 120 screened RCTs were finally included [6–26] with a total of 2590 participants. The main reasons for RCT exclusion were inclusion of pediatric population, duplicates, different primary outcomes, randomization after a time period of steroid administration and use of corticosteroids in both arms.

The median follow-up varied among studies from 0 to 67 months. Fifteen studies reported median follow-up of more than 1 year, 4 studies from 0 to 36 months, while the remaining two studies report 3 and 6 months respectively.

Maxwell test statistic was not significant ($P = 0.852$) indicating that the raters did not disagree significantly. The generalized McNemar statistic ($P = 0.56$) indicated that the agreement was spread evenly.

The mean Jadad score of the studies included was 2.76 points. Three studies had a score of 5 points [9,17,19], one study of 4 points [13], nine studies of 3 [7,8,10,11,14,16,21,22,24], four studies of 2 points [6,20,25,26] and four studies of 1 point [12,15,18,23] respectively. The baseline characteristics of the patients in the included trials are summarized in Table 1.

Of the 21 studies, nine studies reported replacement of steroids with another immunosuppression agent [7,8,10–12,15,18,25,26] (in four studies by daclizumab, in one study by thymoglobulin, in two studies by mycophenolate mofetil, in one study by basiliximab and in one study by a combination of daclizumab and mycophenolate mofetil) and 13 studies included complementary agents in both arms (in one study thymoglobulin, in two studies basiliximab, in five studies mycophenolate mofetil, in one study azathioprine, in two studies a combination of basiliximab and mycophenolate mofetil, in yet another study a combination of daclizumab and mycophenolate mofetil and in one more a combination of basiliximab and azathioprine). All studies included a calcineurin inhibitor as part of the immunosuppression regimen (seven studies reported cyclosporine, and 14 tacrolimus). Nine studies administered perioperative steroids (1 or 2 doses – two studies: 7 and 14 days respectively) to the steroid-free arm, 11 studies did not administer perioperative steroids to the steroid-free arm, and one study did not specify.

By encompassing all the 21 RCTs, three comparisons were conducted with consideration to studies addressing collectively patients transplanted for any indication:

(I) Results concerning rejection (Table 2),

(II) Results of adverse events (Table 3)

(III) Results of graft and patient survival (Table 4) and one comparison with consideration to the:

(IV) Results of studies addressing exclusively transplanted HCV patients (Table 5).

Considering the first (I) comparison: corticosteroid-free immunosuppression group was equivalent to the steroid group in comparisons related to the following outcomes: acute rejection (mild, moderate) chronic rejection and steroid-resistant rejection. Considering overall acute rejection, contrast to the results of meta-analysis (comparable results between treatment arms), meta-regression showed that taking in account independently RCT that replaced steroids the outcome was favoring the corticosteroid-free immunosuppression arm (Fig. 2), while the reverse was true when steroids were not replaced.

Heterogeneity among studies in terms of acute rejection was observed ($P < 0.001/I^2 = 61\%$) and the threshold effect was documented by calculating the Spearman correlation coefficient [inverse correlation was observed (-0.188, P-value = 0.403)]. The summary of the study results are depicted in (Fig. 3). Sensitivity analysis showed that the significant heterogeneity among reported trials could be attributed principally to the trials of Belli *et al.* [6] and Reggiani *et al.* [21]. The aforementioned RCT do not have the sample size required in power analysis. By omitting these studies heterogeneity was no longer observed ($P = 0.27/I^2 = 20\%$).

Normal quantile plots did not detect any obvious publication bias concerning all outcomes (Fig. 4). Funnel plots and Egger's regression analysis showed significant publication bias for 'acute rejection' (Table 2).

We used the fail-safe method (Rosenthal's) to estimate the number of additional studies with a mean effect size of zero required in order to reduce the combined significance to a level (0.05). This analysis showed that 20 studies to be necessary (in the cases wherein steroids were not replaced in the steroid-free arm) and 23 studies (in the cases wherein steroids were replaced) for such effect. In view of the fact that there have been no more than 21 studies published over the past 18 years, it is highly improbable that such a large number of similar studies would have gone unpublished or have been missed by our search strategy.

Concerning the second (II) comparison: corticosteroid-free immunosuppression group was equivalent to the steroid group in comparisons pertaining to the following outcomes: renal insufficiency and severe renal insufficiency requiring hemofiltration, *de novo* hypertension development, neurologic disorders and infectious complications. In favor of the corticosteroid-free immunosuppression group (Fig. 5) was the development of post-transplant diabetes mellitus, CMV infection and cholesterol levels at 6 months.

Table 1. Baseline study characteristics.

Study ID	Inclusion	Participants	Interventions	Outcomes	Study duration/rejection treatments protocols
Belli [6]	HCV positive	Group A = 13 Group B = 11 Group C = 13	RATG + AZA + CyA + ST (3 m)/ RATG + AZA + CyA/ RATG + AZA + CyA + ribavirin	Acute rejection, chronic rejection, HCV recurrence	November 1997–November 1999 Not specified
Boillot [7]	Adult patients undergoing first OLT	Group A = 351 (103) Group B = 347 (106)	TACRO + Daclizumab/ TACRO + ST (3 m)	Acute rejection, corticosteroid resistant acute rejection, graft survival	July 2000– February 2002 Increasing TACRO dose and/or steroids
Eason [8]	Adult patients undergoing first OLT	Group A = 59 (34) Group B = 60 (31)	RATG + TACRO + MMF/ST (3 m) + TACRO + MMF	Patient survival, graft survival, rejection, Adverse events, HCV recurrence	December 1999–August 2002 Increasing TACRO or adding MMF or sirolimus; steroids if no improvement after 48 h
Filliponi [9]	HCV positive	Group A = 74 Group B = 66	Basiliximab + ST(3 m) + CyA + AZA/ Basiliximab + CyA + AZA	HCV recurrence, patient survival, graft survival, treatment failure	October 1998–March 2001 Methylprednisolone bolus for 3 days
Kato [10]	HCV positive	*1st Period* GroupA=15 GroupB=16/ *2nd Period* Group A = 16 Group B = 23	*1st Period* TACRO + Daclizumab/ TACRO + ST(3m)/ *2nd Period* TACRO + Daclizumab + MMF/ TACRO + ST(3 m) + MMF	Fibrosis stage, acute rejection, adverse events, predictors	November 1999–2001 Methylprednisolone bolus ± taper; OKT3 for severe or treatment -resistant rejection
Klintmalm [11]	HCV positive	Group A = 80 Group B = 79 Group C = 153	TACRO + ST (3 m)/TACRO + ST (3 m) + MMF/ Daclizumab + TACRO + MMF	Risk factors, rejection, HCV recurrence, treatment failure	Methylprednisolone bolus ± taper; mild rejection increasing tacrolimus ± antimetabolite (MMF or azathioprine) Antilymphocyte antibody for corticosteroid-resistant rejection
Langrehr [12]	HCV positive	Group A = 27 Group B = 26	TACRO + ST (3 m)/TACRO + MMF	Rejection, HCV recurrence	Not specified
Lerut [13]	Adult patients undergoing first OLT	Group A = 50 Group B = 50	TACRO + ST (3 m)/TACRO	Acute rejection, Graft survival, adverse events	Not specified
Llado [14]	Adult patients undergoing first OLT	Group A = 102 (45) Group B = 96 (43)	Basiliximab + CyA + ST(3 m)/ Basiliximab + CyA	Acute rejection, patient survival, Graft survival, infection	April 2001–September 2004 Methylprednisolone bolus for 3 days ± taper ± increase in TACRO
Lupo [15]	Adult patients undergoing first OLT	Group A = 20(9) Group B = 21(11)	CyA + ST (3 m)/CyA + Basiliximab	Acute rejection	Methylprednisolone bolus for 3 days
Margarit [16]	Adult patients undergoing first OLT	Group A = 28 (20) Group B = 32 (15)	TACRO/TACRO + ST (3 m)	Acute rejection, severe acute rejection, HCV recurrence, 3 years-graft survival	October 1998–September 2000 Increasing tacrolimus dose; methylprednisolone bolus for 3 days ± taper for severe rejection

Table 1. *continued*.

Study ID	Inclusion	Participants	Interventions	Outcomes	Study duration/rejection treatments protocols
Moench [17]	Adult patients undergoing first OLT	Group A = 56 (15) Group B = 54 (16)	TACRO/TACRO + ST(6 m)	Patient survival, graft survival, acute rejection, chronic rejection, adverse events	February 2000–August 2004 Methylprednisolon; tacrolimus adjusted higher level
Nashan [18]	Adult patients undergoing first OLT	Group A = 25(15) Group B = 26 (15)	Basiliximab + CyA + ST(3 m)/ Basiliximab + CyA + MMF	Rejection, HCV recurrence	January 1999–December 2000 Not specified
Pageaux [19]	Adult patients undergoing first OLT	Group A = 90 Group B = 84	Basiliximab + CyA + ST (6 m)/ Basiliximab + CyA + placebo	Acute rejection, 6-month graft and patient survival, treatment failure, recurrent HCV, adverse events	December 1999–August 2001 Not specified
Pelletier [20]	Adult patients undergoing first OLT	Group A = 36 Group B = 36	TACRO + MMF + ST (3–6 m)/ TACRO + MMF	Rejection, HCV recurrence, Graft survival Patient survival	June 2002–May 2004 Pulse steroids
Reggiani [21]	Adult patients undergoing first OLT	Group A = 18 Group B = 12	TACRO + MMF + ST(3 m)/ TACRO + MMF	Acute rejection, Adverse events, pharmacokinetics of MPA	Not specified/Increasing tacrolimus for mild rejection; methylprednisolone bolus 3 days ± taper for moderate rejection; OKT3 for steroid-resistant rejection
Samonakis [22]	HCV positive	Group A = 27 Group B = 29	TACRO/TACRO + ST (3–4 m) + AZA	Acute rejection, Survival, Re-transplantation, adverse events	
Studenik [23]	Adult patients undergoing first OLT	Group A = 19 Group B = 20	TACRO + Daclizumab + ST(3 m) + MMF/TACRO + Daclizumab + MMF	Acute rejection	February 2003–November 2004 Not specified
Tisone [24]	Adult patients undergoing first OLT	Group A = 22 Group B = 23	CyA + AZA + ST (3 m)/ CYA + AZA	Graft survival, adverse events, HCV recurrence	Not specified/methylprednisolone bolus for 3 days only for severe rejection duct damage
Varo [25]	Adult patients undergoing first OLT	Group A = 79 Group B = 78	TACRO + ST(3 m)/TACRO + Daclizumab + MMF	Acute rejection	Not specified/Up to 3 full courses of high dose steroids
Washburn [26]	Adult patients undergoing first OLT	Group A = 15 Group B = 15	TACRO + MMF + ST (15 m)/ Daclizumab + TACRO + MMF	Adverse events, rejection	April 1999–October 1999 Increasing tacrolimus dose; steroid bolus for moderate rejection

Numbers within brackets in the third column show the number of HCV transplanted patients.
CyA, cyclosporine; TACRO, tacrolimus; ST, steroids; RATG, rabbit antithymocyte globulin; AZA, azathioprine; MMF, mycophenolate mofetil; m, months; OKT3, murine monoclonal IgG2a antibody.

Funnel plots did not detect any obvious publication bias concerning all outcomes. As heterogeneity among studies was observed ($P < 0.05/I^2 = 50\%$) in terms of 'CMV infection' we examined the threshold effect by calculating the Spearman correlation coefficient. No inverse correlation was observed (0.450, P-value = 0.22), thus putting the presence of the threshold effect in question (Fig. 3).

Heterogeneity was not confirmed and re-sampling tests derived from 999 iterations and bootstrapping were used to generate confidence intervals around the overall cumulative mean effect size of 'CMV infection' (RR: −0.2370, Bootstrap CI: −0.6446 to −0.0181) showing a nonsignificant probability (Qtotal = 10.5329, $P = 0.160$).

By considering the third (III) comparison corticosteroid-free immunosuppression group was equivalent to the

Table 2. Rejection in studies addressing patients transplanted for any indication.

Outcome or subgroup	Studies	*n*	Effect Estimate (95% CI)	Heterogeneity	Test for overall effect	Publication Bias (indicator/ *P* value)	Favors group
Overall acute rejection	23	2590	R.E, R.R = 1.0 [0.83, 1.22]	***P* < 0.001** I^2 = 52%	χ^2 = 1.49E-03 *P* = 0.969	Egger: bias = −0.11 *P* = 0.838	None
MR ST not Replaced	13		R.E. R.R = 0.75 [0.58, 0.98]	***P* < 0.001** I^2 = 61%	χ^2 = 4.46 ***P* < 0.05**	Egger: bias = −1.71 ***P* < 0.001**	Steroid
ST Replaced	10		F.E. R.R = 1.31 [1.09, 1.58]	*P* = 0.139 I^2 = 34%	χ^2 = 7.59 ***P* < 0.01**	Egger: bias = 1.45 ***P* < 0.01**	Not steroid
Acute rejection (severe)	12	1490	F.E, R.R = 1.71 [1.14, 2.54]	*P* = 0.40 I^2 = 3.8%	χ^2 = 6.87 ***P* < 0.01**	Egger: bias = 0.78 *P* = 0.155	Not steroid
Chronic rejection	11	1106	F.E, R.R = 1.52 [0.71, 3.23]	*P* = 0.2 I^2 = 31%	χ^2 = 1.17 *P* = 0.28	Horbold-Egger: bias = −0.11 *P* = 0.952	None
Steroid resistant rejection	9	1540	F.E, R.R = 1.34 [0.87, 2.08]	*P* = 0.08 I^2 = 44%	χ^2 = 1.77 *P* = 0.283	Egger: bias = −0.78 *P* = 0.52	None
Acute rejection (mild)	6	1363	F.E, R.R = 0.94 [0.69, 1.29]	*P* = 0.25 I^2 = 24%	χ^2 = 0.14 *P* = 0.711	Egger: bias = 0.9 *P* = 0.428	None
Acute rejection (moderate)	6	1363	F.E, R.R = 1.02 [0.83, 1.27]	*P* = 0.61 I^2 = 0%	χ^2 = 0.05 *P* = 0.822	Egger: bias = −0.37 *P* = 0.649	None

MR, meta-regression; RR, relative risk; CI, confidence interval; F.E., Fixed Effects model; R.E., Random Effects model.

Table 3. Adverse events in studies addressing patients transplanted for any indication.

Outcome or subgroup	Studies	*n*	Effect estimate (95%CI)	Heterogeneity	Test for overall effect	Publication bias (indicator/*P* value)	Favors group
De Novo diabetes Mellitus	11	1727	F.E, R.R = 1.86 [1.43, 2.41]	*P* = 0.071 I^2 = 42%	χ^2 = 21.37 ***P* < 0.001**	Egger: bias = 0.87 *P* = 0.78	Not-steroid
Infectious complications	11	1692	F.E, R.R = 1.07 [0.96, 1.2]	*P* = 0.23 I^2 = 23%	χ^2 = 1.58 *P* = 0.208	Egger: bias = −0.17 *P* = 0.794	None
De novo hypertension	9	1578	F.E, R.R = 1.07 [0.9, 1.27]	*P* = 0.76 I^2 = 0%	χ^2 = 0.63 *P* = 0.426	Egger: bias = −0.05 *P* = 0.367	None
CMV infection	9	1401	R.E, R.R = 1.47 [0.99, 2.17]	***P* < 0.05** I^2 = 50%	χ^2 = 3.67 ***P* < 0.05**	Egger: bias = 0.87 *P* = 0.273	Not-steroid
Abnormal kidney function	7	1241	F.E, R.R = 0.93 [0.78, 1.11]	*P* = 0.54 I^2 = 0%	χ^2 = 0.63 *P* = 0.437	Egger: bias = 0.28 *P* = 0.665	None
Neurologic disorders	5	572	F.E, O.R = 0.76 [0.51, 1.13]	*P* = 0.56 I^2 = 0%	χ^2 = 1.61 *P* = 0.204	Egger: bias = 0.43 *P* = 0.717	None
Severe renal insufficiency	5	1087	F.E, O.R = 0.98 [0.52, 1.81]	*P* = 0.9 I^2 = 0%	χ^2 = 6.32E-03 *P* = 0.936	Egger: bias = 0.97 *P* = 0.1276	None
Cholesterol levels	5	1080	F.E, WMD= 19.71 [13.7, 25.7]	*P* = 0.07 I^2 = 53%	*Z* = 6.44 ***P* < 0.001**	Egger: bias = −1.1 *P* = 0.42	Not-steroid

OR, odds ratio; CI, confidence interval; WMD, weighted mean difference; RR, relative risk; F.E., Fixed Effects model; R.E., Random Effects model.

steroid group in comparisons relevant to the following outcomes: overall number of deaths during follow up, 1-year patient and graft survival (Fig. 6), re-transplantations, deaths within 6 months, death incidence rate difference and 3-month graft survival. Corticosteroid-free immunosuppression group was superior with impact to the number of patients which received the allocated intervention (Table 4).

Funnel plots did not detect publication bias concerning any of the outcomes.

By considering the fourth (IV) comparison corticosteroid-free immunosuppression group was equivalent to the steroid group in comparisons pertaining to the following outcomes: overall deaths in HCV patients, deaths in HCV-recurrence patients, and 1-year patient and graft survival (Table 5). Corticosteroid-free immunosuppression group was superior with impact to relative risk of HCV recurrence (Fig. 7), acute graft hepatitis and the number of patients sustaining treatment failure (collectively patients with graft loss/deaths/withdrawal).

Table 4. Follow-up in studies addressing patients transplanted for any indication.

Outcome or Subgroup	Studies	n	Effect Estimate (95%CI)	Hetero geneity	Test for overall effect	Publication Bias (indicator/*P* value)	Favors group
Total deaths at follow up	21	2257	F.E, R.R = 0.9 [0.72, 1.13]	$P = 0.11$ $I^2 = 28\%$	$\chi^2 = 0.84$ $P = 0.36$	Egger: bias = −0.48 $P = 0.441$	None
One-year patient survival	10	1014	F.E, O.R = 0.1 [0.69, 1.45]	$P = 0.59$ $I^2 = 0\%$	$\chi^2 = 4.94E\text{-}03$ $\|P = 0.944$	Egger: bias = 0.39 $P = 0.64$	None
One-year graft survival	8	832	F.E, O.R = 0.8 [0.56, 1.15]	$P = 0.34$ $I^2 = 11\%$	$\chi^2 = 1.27$ $P = 0.26$	Egger: bias = 0.29 $P = 0.837$	None
Re-transplantation	7	1189	F.E, O.R = 0.82 [0.45, 1.52]	$P = 0.18$ $I^2 = 33\%$	$\chi^2 = 0.22$ $P = 0.639$	Egger: bias = 0.29 $P = 0.7773$	None
Deaths up to 6 months	5	1175	F.E, R.D = −0.01 [−0.04, 0.02]	$P = 0.25$ $I^2 = 25\%$	$\chi^2 = 0.28$ $P = 0.593$	Egger: bias = 0.23 $P = 0.829$	None
Death incidence rate difference	4	407	F.E, I.R.D = 2.81E-04 [−2.47E-03, 3.03E-03]	$P = 0.33$ $I^2 = 12\%$	$Z = 0.2$ $P = 0.841$	Egger: bias = 2.03 $P = 0.262$	None
Three-months graft survival	4	1170	F.E, O.R = 1.24 [0.79, 1.25]	$P = 0.56$ $I^2 = 0\%$	$\chi^2 = 0.68$ $P = 0.409$	Egger: bias = 0.41 $P = 0.668$	None
Received the allocated intervention	4	1101	F.E, O.R = 1.55 [1.17, 2.05]	$P = 0.28$ $I^2 = 21\%$	$\chi^2 = 8.78$ **$P = 0.003$**	Egger: bias = −0.06 $P = 0.9773$	Not-steroid

OR, odds ratio; RR, relative risk; RD, risk difference; I.R.D., incidence rate difference; CI, confidence interval; F.E., Fixed Effects model; R.E., Random Effects model.

Table 5. Outcomes of HCV transplanted patients.

Outcome or subgroup	Studies	n	Effect estimate (95% CI)	Hetero geneity	Test for overall effect	Publication bias (indicator/ *P* value)	Favors group
HCV recurrence	14	1418	F.E, R.R = 1.15 [1.01, 1.13]	$P = 0.91$ $I^2 = 0\%$	$\chi^2 = 4.21$ **$P < 0.05$**	Egger: bias = 0.46 $P = 0.134$	Not-steroid
Overall deaths in HCV patients	7	757	F.E, R.R = 0.92 [0.52, 1.65]	$P = 0.72$ $I^2 = 0\%$	$\chi^2 = 0.06$ $P = 0.8$	Egger: bias = 0.64 $P = 0.374$	None
Deaths in HCV –recurrence patients	5	290	F.E, R.D = 0.01[−0.05, 0.07]	$P = 0.44$ $I^2 = 0\%$	$\chi^2 = 0.08$ $P = 0.775$	Egger: bias = 1.57 $P = 0.251$	None
Treatment failure (death, graft loss, withdrawal)	5	745	F.E, O.R = 1.87 [1.33, 2.63]	$P = 0.19$ $I^2 = 30\%$	$\chi^2 = 12.54$ **$P = 0.001$**	Egger: bias = −2.72 $P = 0.255$	Not-steroid
One-year graft survival	4	622	F.E, O.R = 0.68 [0.42, 1.08]	$P = 0.67$ $I^2 = 0\%$	$\chi^2 = 2.97$ $P = 0.084$	Horbold-Egger: bias = 11.83 $P = 0.0862$	None
One-year patient survival	4	622	F.E, O.R = 0.63 [0.37, 1.08]	$P = 0.95$ $I^2 = 0\%$	$\chi^2 = 2.37$ $P = 0.123$	Egger: bias = −0.83 $P = 0.281$	None
Acute graft hepatitis	3	72	F.E, O.R = 3.15 [1.18, 8.40]	$P = 0.13$ $I^2 = 50\%$	$\chi^2 = 4.53$ **$P = 0.03$**	Horbold-Egger: bias = 1.93 $P = 0.892$	Not-steroid

OR, odds ratio; RR, relative risk; RD, risk difference; CI, confidence interval; F.E., Fixed Effects model; R.E., Random Effects model.

Funnel plots did not detect significant publication bias concerning HCV recurrence (Fig. 4).

Meta-regression analysis showed that the type of calcineurin inhibitor as part of the immunosuppression regimen, the addition of perioperative steroids in the corticosteroid-free immunosuppression group, the administration of complementary agents in both arms or in the corticosteroid free arm and the high Jadad score of RCT (≥3 vs. ≤2) had no impact on any of the outcomes.

Meta-regression analysis also disclosed that there was no difference between studies that reported either replacement or nonreplacement of steroids in the corticosteroid-free immunosuppression group in terms of HCV recurrence ($P = 0.610$) and *de novo* diabetes mellitus development ($P = 0.087$), infectious complications ($P = 0.698$), severe acute rejection ($P = 0.967$).

On the contrary, there was statistical difference between studies that reported either replacement or

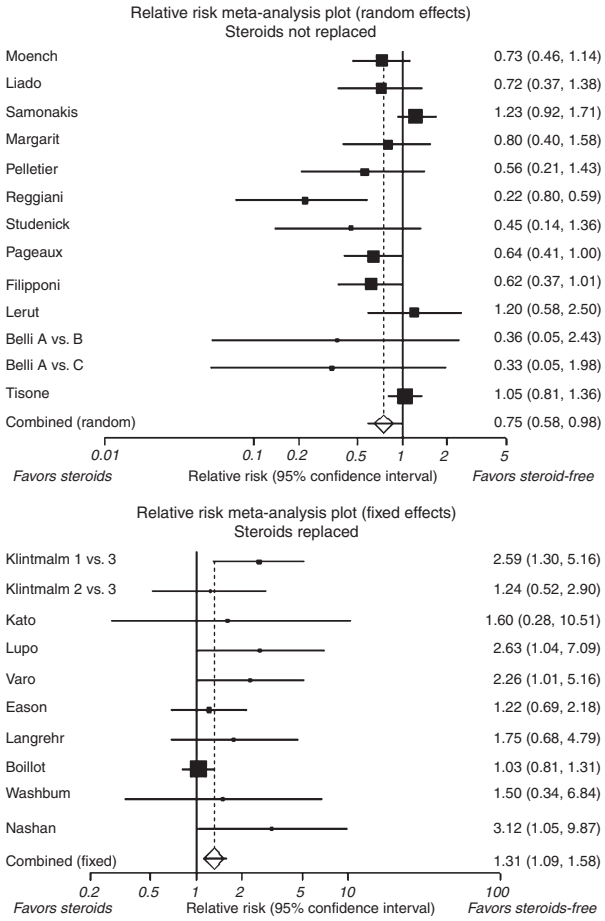


Figure 2 Meta-analysis of outcomes of 'acute rejection' in studies addressing patients transplanted for any indication. Steroids not replaced (upper panel); Steroids replaced (lower panel).

nonreplacement of steroids in the corticosteroid-free immunosuppression group concerning acute rejection ($P = 0.036$).

Randomization tests were used to test (re-sampling tests generated from 999 iterations) the significance of our model structure in terms of acute rejection [pooling the effects of different regimens (different immunosuppressive agents substituting steroids) in the corticosteroid-free immunosuppression group]. Mean Effect Size was equal to −0.0802 and Bootstrap 95% CI = −0.1278 to −0.0341and probability $P = 0.998$).

Cumulative meta-analysis showed a relatively consistent evidence of no statistical difference in the incidence of HCV recurrence between steroid and corticosteroid-free immunosuppression group over the years 2005–2008 (Fig. 8). Based on this evidence, the addition of any future study would contribute little to the cumulative body of evidence (Mean effect size: −0.0802, Bootstrapping CI: −0.1231 to −0.0345).

As far as it concerns cumulative meta-analysis for acute rejection, the point estimates and their confidence intervals stabilized from year 2005 and on and remained unchanged when steroids were not replaced (Mean effect size: 0.1912 Bootstrapping CI: 0.0528 to 0.3542) but when steroids were replaced this became evident only from year 2007 (Mean effect size: −0.2078 Bootstrapping CI: −0.6771 to −0.0786) (Fig. 9).

Discussion

Meta-analysis of RCT addressing collectively patients transplanted for any indication showed no differences

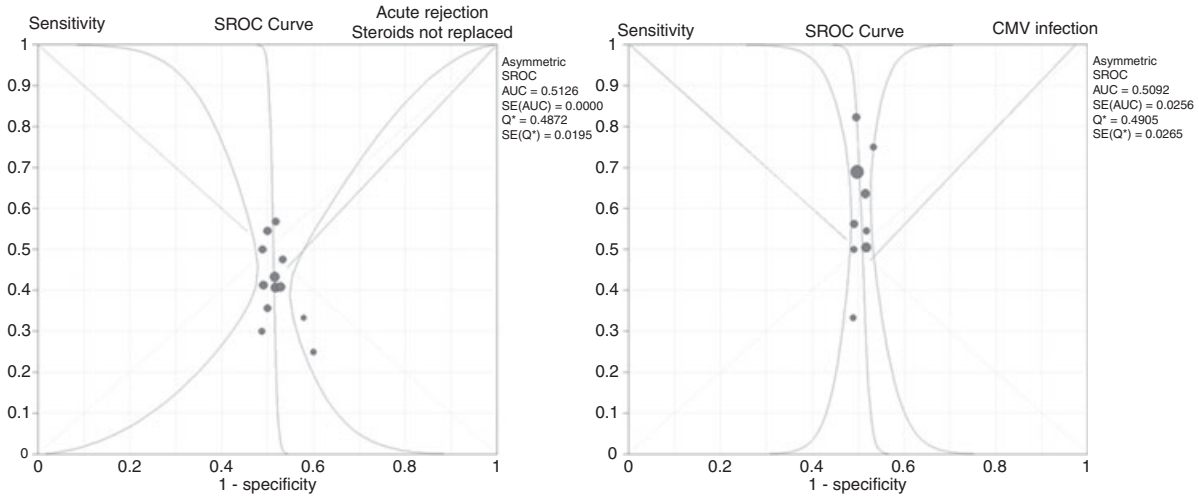


Figure 3 Summary of study results concerning 'acute rejection (steroids not replaced)' (left panel) and 'CMV infection' (right panel) on a ROC curve: the fitting of the ROC curve is presented by asymmetrical curves around the 'Sensitivity–Specificity' line as the Diagnostic Odds Ratio was not constant. All but two of the studies (concerning only acute rejection) depicted by the filled circles are within the curvilinear lines.

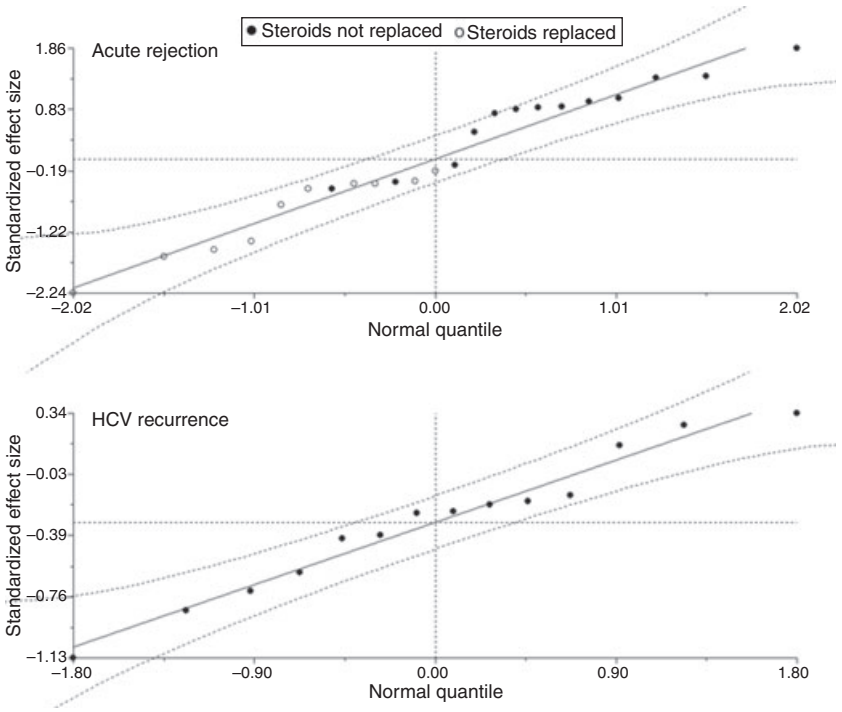


Figure 4 Normal quantile plot for inspection of publication bias concerning 'acute rejection' and 'HCV recurrence'. The standardized effect size (Odds Ratio) is plotted against the normal quantile values. The distribution of effect sizes is similar to the distribution of normal quantiles, as data points fall close to the line X = Y.

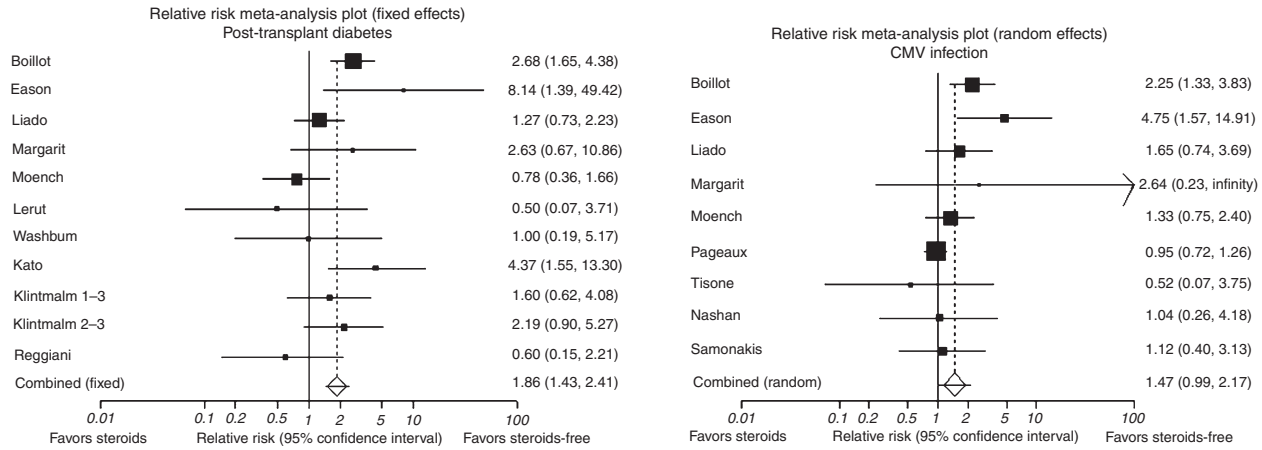


Figure 5 Meta-analysis of outcomes: 'post-transplant diabetes development' (fixed effects) and 'CMV infection' (random effects).

between corticosteroid-free immunosuppression and steroid-based protocols in most of the analyzed outcomes. More importantly, steroid-free cohorts appeared to benefit in terms of *de novo* diabetes mellitus development, CMV infection, cholesterol levels, the number of patients that received the allocated treatment, severe acute rejection and in overall acute rejection (when steroids were replaced in the steroid-free arm). Taking RCTs into account independently when steroids were not replaced, overall acute rejection was favoring the steroid-based arm.

Studies addressing exclusively transplanted HCV patients demonstrated a significant advantage of steroid-free protocols considering HCV recurrence, acute graft hepatitis, and treatment failure.

We recognize the potential heterogeneity of evaluating studies with different immunosuppression protocols. Actually, meta-regression established that differences in the use of main and complementary immunosuppression agents did not influence our conclusions: the type of calcineurin inhibitor as part of the immunosuppression protocol, the addition of perioperative steroids in the

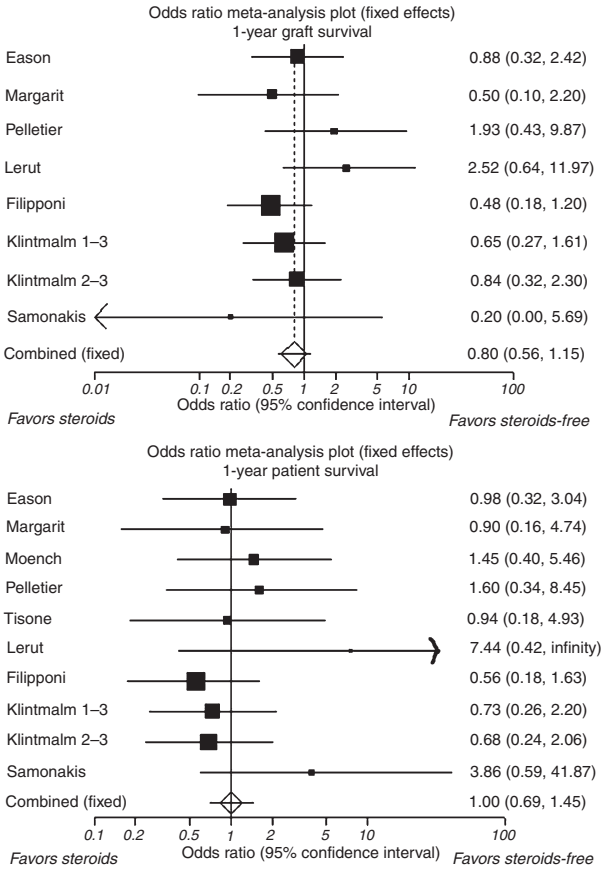


Figure 6 Meta-analysis of outcomes: 'one-year graft survival' (fixed effects) and 'one-year patient survival' (fixed effects).

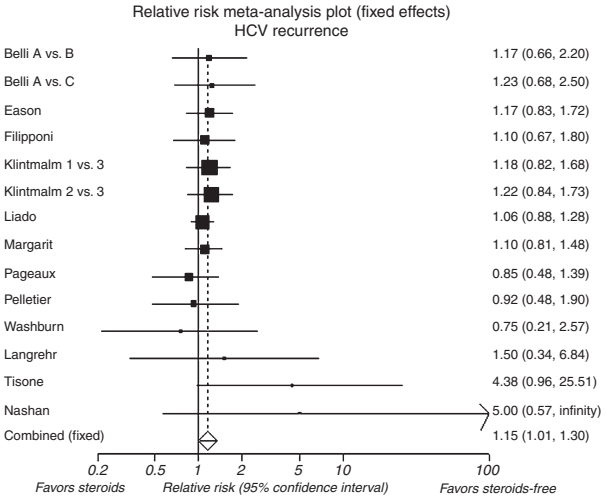


Figure 7 Meta-analysis of outcome: 'HCV recurrence' (fixed effects).

corticosteroid-free immunosuppression group, the administration of complementary agents in both arms and study quality had no impact on any of the outcomes.

Significant heterogeneity was noted between studies that dropped steroids or replaced steroids with different immunosuppression agents in the steroid-free arm concerning acute rejection. Sensitivity analysis disclosed the two studies contributing to heterogeneity. Besides randomization tests were used to test the significance of this model structure (pooling the effects of different regimens) showing nonsignificant probability, justifying this way of our comparisons.

There was one important potential extra source of heterogeneity among the analyzed studies including the different threshold to define positive and negative test results. This was relevant for the policy of defining HCV recurrence (biopsy-proven rejection, combined with each of the following: clinical recurrence, Ishak score, fibrosis, high liver function tests and HCV RNA) and the timing of protocol biopsies. This assumption was not confirmed by the results.

When considering the *completeness and applicability of evidence*, the included studies provided with satisfactory data and properly addressed the issues including rejection, adverse events, graft and patient survival and HCV recurrence. Additionally, their inclusion criteria (adult-liver-only recipients undergoing first orthotopic transplantation) and approaches (open-label studies/eligible patients randomized 1:1) emerged to almost be identical and thus ensuring comparable types of analyzed interventions and outcomes.

Making an allowance for the *quality of the evidence*, the 21 included studies had a total of 2590 participants, with good methodological quality (mean Jadad score = 2.76 points). Only nine out of the 21 studies that we analyzed were sufficiently powered to document their findings. Despite this fact, the application of Cumulative meta-analysis as a means of identifying the benefit in acute rejection and HCV recurrence of corticosteroid-free immunosuppression resolved the matter. Significant but explicable heterogeneity was revealed in only two out of 30 comparisons and a publication bias in only two instances. Modern meta-analytic techniques declined heterogeneity of studies in one of the two occasions.

The search only of the English language literature, could represent a potential publication bias during the review process, however, going through the abstracts, there were no suitable studies found in the non English language literature. The results of the fail-safe tests also provide a relative certainty against missing studies. The Harbold–Egger test was used to maintain the power of the Egger test in reducing the false positive rate, which was a problem in cases of large treatment effects and few events per trial.

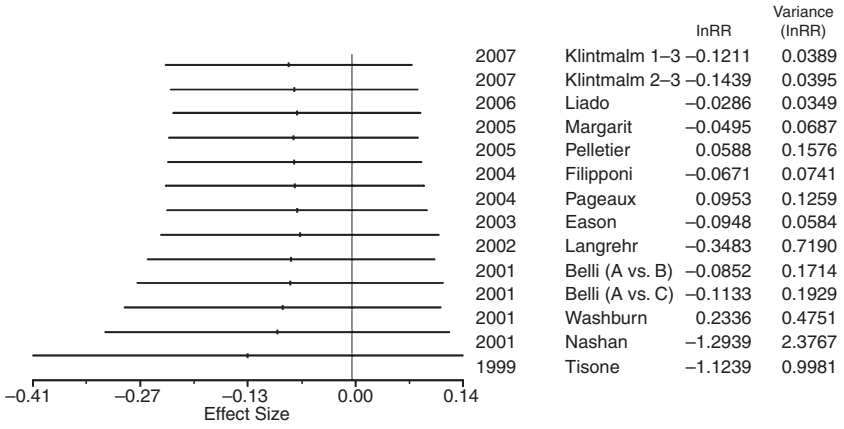
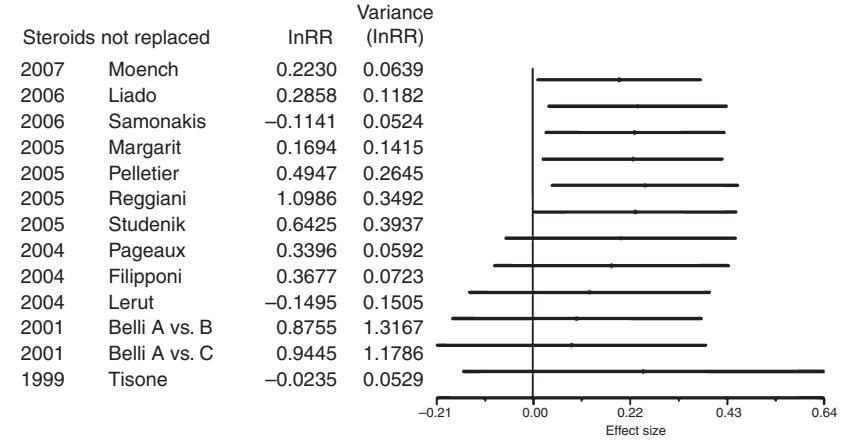


Figure 8 Cumulative Meta-Analysis for 'HCV recurrence': studies were successively added to the analysis based on chronological order according to the year of publication. The point estimates and their confidence intervals stabilized over the years 2005–2008.



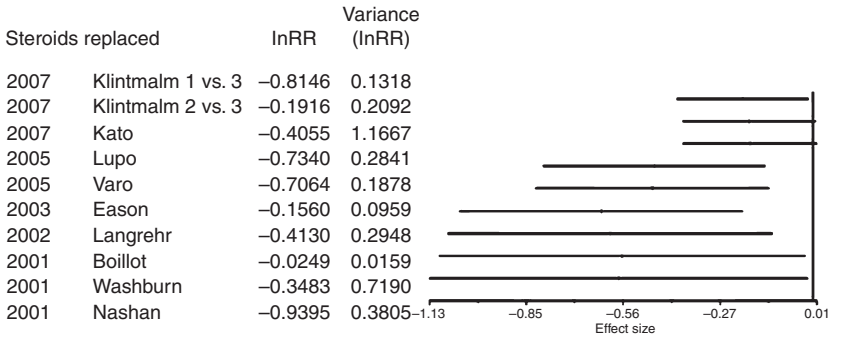


Figure 9 Cumulative Meta-Analysis for 'overall acute rejection–steroid not replaced' and 'overall acute rejection–steroid replaced': studies were successively added to the analysis based on chronological order according to the year of publication. The point estimates and their confidence intervals stabilized over the years 2005–2008 in the first instance, but the fact became evident only from year 2007 in the second.

Trying to make an *overall judgment of the external validity* of this meta-analysis, we balanced its outcomes with those of a well-structured recent meta-analysis [5]. We additionally included two sufficiently powered RCTs [17,19] in our meta-analysis and analyzed separately studies addressing exclusively transplanted patients with HCV.

Similar to our evidence, there were no differences in analyzed outcomes in exception that our meta-regression analysis did not differentiate between studies reporting either replacement or nonreplacement of steroids in the steroid-free arm in terms of post-transplant diabetes mellitus and severe acute rejection. This is attributed to the fact that this preceding meta-analysis: i) included erroneously the study of Samonakis *et al.* [22] (reporting reduction of post-transplant diabetes mellitus: there is not *de novo* development) in the first case and ii) included erroneously the study of Varo *et al.* [25] (it is clearly stated that there were no cases of severe acute rejection) in the second case.

There is also much skepticism about the methodology: when estimating the relative risk of death and graft loss at 1 year, authors do not extrapolate data to 1 year in

some of the included trials because this kind of information is not reported in the respective studies. Quite the reverse, our meta-analysis addresses precisely this subject. Besides the death incidence rate difference is provided.

Moreover, the most striking difference of the present meta-analysis is the analysis of heterogeneity and the validation of our results by cumulative meta-analysis, randomization tests and bootstrapping.

Similar to our results a published review confirms that steroid avoidance and steroid withdrawal strategies in kidney transplantation were not associated with increased mortality or graft loss despite an increase in acute rejection. These immunosuppression strategies allowed for safe steroid avoidance or elimination a few days after kidney transplantation if antibody induction treatment was prescribed or after 3 to 6 months if such induction was not used [36].

Considering early steroid withdrawal in liver transplant recipients with autoimmune hepatitis a prospective randomized study suggests that it should be attempted in OLT recipients, most will benefit from MMF without jeopardizing their allografts but only after 1 year of steroid administration [37].

Cumulative meta-analysis showed relatively consistent evidence over the years 2005–2008. Based on this, the addition of any future study would contribute little to the cumulative body of evidence.

In summary, steroid-free cohorts appeared to benefit in terms of *de novo* diabetes mellitus development, CMV infection, cholesterol levels, in terms of the number of patients that received the allocated treatment, severe acute rejection and overall acute rejection- only when steroids were replaced in the steroid-free arm. Studies addressing exclusively transplanted HCV patients demonstrated a significant advantage of steroid-free protocols considering HCV recurrence, acute graft hepatitis, and treatment failure. Regardless of the fact that no unfavorable effects after steroid withdrawal were observed during short-term, reports of the long term follow up of the existing studies or new sufficiently powered randomized controlled trials must be most welcomed.

Authorship

All authors fulfill three authorship criteria. GS: designed study, analyzed data, acquisition of data. AR: designed study, acquisition of data. IF: drafted paper, acquisition of data; SM: substantial review, drafted paper. KG: critical review, drafted paper. IG: critical review, drafted paper. HL: FACS designed study, critical revision; CK: designed study, critical revision. All authors finally approved the version to be published.

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