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Tacrolimus versus cyclosporine as primary immunosuppression after heart transplantation: systematic review with meta-analyses and trial sequential analyses of randomized trials

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3 **Tacrolimus versus cyclosporine as primary immunosuppression after heart transplantation:**
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5 **Systematic review with meta-analyses and trial sequential analyses of randomized trials**
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8 Luit Penninga^{1,2}, Christian H Møller^{1,3}, Finn Gustafsson⁴, Daniel A Steinbrüchel³, Christian Gluud¹
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Abstract

Purpose We conducted a systematic review of randomized trials to compare benefits and harms of tacrolimus versus cyclosporine as primary immunosuppression after heart transplantation.

Methods and results We searched electronic databases and bibliographies until April 2010. Our review followed the Cochrane and PRISMA guidelines. The meta-analysis included 10 randomized trials with 952 patients. Tacrolimus was significantly superior to cyclosporine (both formula combined) regarding hypertension (relative risk (RR) 0.8; 95% confidence interval (CI) 0.69-0.93, $p=0.003$), hyperlipidaemia (RR 0.57; 95% CI 0.44-0.74, $p<0.0001$), hirsutism (RR 0.17 95% CI 0.04-0.62, $p=0.008$), and gingival hyperplasia (RR 0.07 95% CI 0.01-0.37, $p=0.002$). No significant differences between the two calcineurin inhibitors were found regarding acute rejections causing hemodynamic instability, diabetes, renal dysfunction, infection, malignancy, or neurotoxicity. Tacrolimus was significantly superior to microemulsion cyclosporine regarding mortality (RR 0.64; 95% CI 0.42-0.96, $p=0.03$), acute severe biopsy-proven rejection (RR 0.71; 95% CI 0.56-0.90, $p=0.004$), hyperlipidaemia (RR 0.57; 95% CI 0.41-0.79, $p=0.0009$), hirsutism (RR 0.17 95% CI 0.04-0.62, $p=0.008$), and gingival hyperplasia (RR 0.07; 95% CI 0.01-0.37, $p=0.002$). Tacrolimus was significantly superior to oil-based cyclosporine regarding hypertension (RR 0.66; 95% CI 0.54-0.80, $p<0.0001$) and hyperlipidaemia (RR 0.57; 95% CI 0.38-0.87, $p=0.009$).

Conclusion Tacrolimus seems to be superior to cyclosporine in heart transplanted patients regarding hypertension, hyperlipidaemia, gingival hyperplasia and hirsutism. In addition, tacrolimus seems to be superior to microemulsion cyclosporine in heart transplanted patients regarding a number of outcomes including death. More trials with low risk of bias are needed to determine if the results of the present meta-analysis can be confirmed.

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Keywords Heart transplantation · Cyclosporine · Tacrolimus · Calcineurin inhibitors · Meta-analysis

For Peer Review

Introduction

The therapeutic success of heart transplantation has been largely attributable to the development of effective and balanced immunosuppressive treatment regimens [1,2]. Especially the calcineurin inhibitors were essential in reducing acute rejection and improving early survival [2]. Two calcineurin inhibitors, cyclosporine and tacrolimus, are currently used as primary immunosuppression in heart transplant recipients [1,2].

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Cyclosporine was discovered in 1971, and in 1983 the drug was approved for prevention or treatment of transplant rejection [3]. To overcome the intra-individual and inter-individual differences in absorption and bioavailability of the original oil-based formulation of cyclosporine (Sandimmune®), a micro-emulsion formula of cyclosporine (Neoral®) was introduced in the 1990s [3].

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Tacrolimus (Prograf®) was discovered in the early 1980s and from 1989 used for the prevention of liver transplant rejection [3,4]. Since then, its use expanded rapidly into transplantation of other organs [3]. Both cyclosporine and tacrolimus inhibit the action of the phosphatase calcineurin.

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Calcineurin regulates the transport of NFAT (nuclear factor of activated T-cells, which is a transcription factor regulating lymphokine gene transcription). Cyclosporine and tacrolimus exert their cellular effects on the action of calcineurin through different cytoplasmatic receptors, as cyclosporine binds to cyclophilins and tacrolimus binds to FK-binding proteins [5]. Differences in adverse effects, safety and tolerability between cyclosporine and tacrolimus have been observed, but the toxicodynamic molecular mechanism of both drugs are still largely unknown and the involvement of calcineurin inhibition in calcineurin inhibitor toxicity is unclear [6].

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3 To date several randomized trials have compared tacrolimus vs. cyclosporine, but results have been
4 inconsistent and optimal immunosuppressive maintenance therapy continues to be debated [5,6].

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6 We conducted this systematic review to compare benefits and harms of tacrolimus vs. cyclosporine
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8 in heart transplant recipients.
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10 11 **Methods**

12 *Trial selection and characteristics*

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14 Our review followed the Cochrane Collaboration [7] and PRISMA guidelines [8]. A protocol was
15 developed (www.ctu.dk/protocols) and we included all randomized trials comparing tacrolimus
16 versus cyclosporine after first-time isolated heart-transplantation. We required that all included
17 patients received the same additional immunosuppressive therapy within each trial. Our preselected
18 outcome measures were mortality, acute severe rejection defined as cardiac biopsies of grade 3A or
19 higher according to the classification of the ISHLT (equivalent to grade H2R in the recently revised
20 classification) [9]; acute rejection causing haemodynamic instability; Cytomegalovirus (CMV)
21 infection; basocellular skin cancer; all malignancies excluding basocellular skin cancer; arterial
22 hypertension; diabetes mellitus; hyperlipidaemia; total serum cholesterol; renal failure requiring
23 haemodialysis; serum creatinine levels; neurotoxicity; hirsutism; and gingival hyperplasia. Our
24 preselected subgroup analyses included 1) low-risk bias compared to high-risk bias trials 2) micro-
25 emulsion cyclosporine compared to oil-based cyclosporine formulation 3) total population (adult
26 and paediatric studies) compared to adult studies only (www.ctu.dk/protocols).
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43 *Search strategy*

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45 We searched The Cochrane Central Register of Controlled Clinical Trials (CENTRAL),
46 MEDLINE, EMBASE, and the Science Citation Index Expanded (to April 2010) [10]. Search terms
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3 were (c*closporin* or CyA or Neoral* or Sandimmun*) combined with (tacrolimus or FK506 or
4 FK 506 or Prograf) and 'heart transplantation' [MESH term] and (random* or blind* or placebo* or
5 meta-analysis).
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10 We scanned bibliographies of relevant articles for additional trials. We had no restrictions to
11 blinding, language, or publication status.
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14 15 16 *Data extraction and quality assessment*

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18 Three authors independently assessed trial eligibility (LP, CHM and FG). We assessed the impact
19 of bias risk by evaluating the trials with respect to generation of the allocation sequence, allocation
20 concealment, blinding, and reporting of incomplete outcome data [11]. Generation of the allocation
21 sequence was considered adequate when generated by a computer, random-number table, shuffling
22 of cards, or something similar. Allocation concealment was considered adequate when allocation of
23 patients involved a central independent unit, such as an on-site locked computer, sealed envelopes
24 or something similar. Blinding was adequate if the trial was described as double-blind and the
25 method of blinding involved identical active drugs. Post-randomization exclusion of patients was
26 registered. When possible we converted per-protocol to intention-to-treat-analysis. Bias risk was
27 assessed without blinding by 3 authors [11].
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40 41 *Quantitative data synthesis*

42 We used Cochrane Collaboration Software (RevMan 5.0.22). Data were analysed with both fixed-
43 effect and random-effects models. In case of discrepancy regarding significance between the two
44 models both results were reported. Otherwise, only results from the random-effects model were
45 reported. Data were presented as relative risk (RR) with values less than 1.0 favouring tacrolimus,
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3 and with 95% confidence intervals (CI). Heterogeneity was assessed with I^2 , which describes the
4 percentage of the variability in effect estimates that is due to heterogeneity rather than sampling
5 error (chance). I^2 lies between 0% (no heterogeneity) and 100% (maximal heterogeneity) [12]. Test
6 of interaction was performed to evaluate the difference between the 2 estimates [13].
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10 11 12 *Trial sequential analysis*

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14 Trial sequential analysis was applied as cumulative meta-analyses are at risk of producing random
15 errors because of sparse data or repetitive testing on accumulating data [14]. To minimize random
16 errors we calculated the required information size (i.e., the number of participants needed in a meta-
17 analysis to detect or reject a certain intervention effect) [14]. Information size calculation also
18 accounted for the diversity present in the meta-analysis. In our meta-analysis, information size was
19 based on the assumption of a plausible RR reduction of 20% [14]. The underlying assumption of
20 trial sequential analysis is that significance testing may be performed each time a new trial is added
21 to the meta-analysis. We added the trials according to the year of publication. On the basis of the
22 required information size and risk for type I and type II errors trial sequential monitoring
23 boundaries were constructed [14]. These boundaries will determine the statistical inference one may
24 draw regarding the cumulative meta-analysis that has not reached the required information size. If a
25 trial sequential monitoring boundary is crossed before the required information size is reached in a
26 cumulative meta-analysis, firm evidence may have been established and further trials are
27 superfluous. On the other hand, if the boundaries are not surpassed, it is most probably necessary to
28 continue doing trials in order to detect or reject a certain intervention effect. We used as defaults a
29 type I error of 5%, type II error of 20%, and adjusted the information size for diversity unless
30 otherwise stated [14].
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Results

Figure 1 depicts the results of the search strategy. Database searches identified 450 references.

Exclusion of duplicates and irrelevant references left 11 randomized trials published in 25 publications [15-39]. One trial could not be included in the meta-analysis as none of the outcome measures were addressed [29]. We confirmed with the authors that all patients only participated once in the trials [28,30]

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The meta-analyses involved 10 trials with a total of 952 patients (table 1): 486 patients were randomized to tacrolimus and 466 patients to cyclosporine [15,16,19,20,24-28,30]. Three trials with 192 patients compared tacrolimus with the old formula oil-based cyclosporine [24,26,27] and seven trials with 760 patients compared tacrolimus with the new formula microemulsion cyclosporine [15,16,19,20,25,28,30].

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In 8 trials the population consisted of adult patients [15,19,20,24,26-28,30], while in 1 trial the population consisted of a combination of adult and paediatric patients [16], and in 1 trial only paediatric patients were included [25].

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Concomitant immunosuppressive treatment was the same within all trials except for one where the patients were randomized to 3 groups: one receiving cyclosporine and mycophenolate mofetil, one receiving tacrolimus and mycophenolate mofetil, and one receiving tacrolimus and sirolimus [19].

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We therefore excluded the latter group of our analyses. Immunosuppressive treatment varied within trials. All patients were treated with steroids. As antiproliferative agent azathioprine (7 trials [15,16,24-28]) or mycophenolate mofetil (3 trials [19,20,30]) was used. Induction therapy was used for all patients in 4 trials [15,25,26,28], for some of the patients in 3 trials [19,24,27], and for none

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3 in 3 trials [16,20,30]. In case induction therapy was used, either anti-thymocyte globulin (ATG) or
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5 muromonab-CD3 (OKT3®) was administered [15,25,26,28]. Patients were followed from 6 months
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7 to 5 years.

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9 Trial methodology was inadequately reported in the majority of trials (table 2). All trials were
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11 considered trials with high risk of bias.

12 13 14 *Mortality*

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16 Ten trials reported on mortality, and overall no significant difference in mortality was found
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18 between tacrolimus and cyclosporine (relative risk (RR) 0.78; 95 % CI 0.54-1.13, p = 0.19).

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20 Tacrolimus was significantly superior to microemulsion cyclosporine (RR 0.64; 95% CI 0.42-0.96,
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22 p=0.03). No significant difference in mortality between tacrolimus and oil-based cyclosporine was
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24 found (RR 1.79; 95% CI 0.77-4.15, p=0.17). Test of interaction showed significant difference in
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26 intervention effect on mortality between the oil-based and microemulsion cyclosporine formulas
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28 (p=0.04).

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32 The significant difference in mortality between tacrolimus and microemulsion cyclosporine,
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34 however, disappeared when the studies including paediatric patients were excluded (RR 0.66; 95%
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36 CI 0.40-1.09, p=0.10). Test of interaction showed no significant differences in mortality between
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38 the paediatric and adult cyclosporine subgroups (p=0.89). This suggests that the lack of significance
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40 caused by withdrawing paediatric studies was solely caused by reducing the number of patients in
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42 the groups.

43 44 45 *Acute rejection*

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3 No significant difference in grade 3A or higher rejection was found between tacrolimus and (both
4 formula combined) cyclosporine (RR 0.86; 95% CI 0.62-1.20, p=0.38). However, tacrolimus was
5 associated with a significant reduction in Grade 3 A or higher rejection compared to microemulsion
6 cyclosporine (RR 0.71; 95% CI 0.56-0.90, p=0.004).

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10 Rejection causing haemodynamic instability was reported in 5 trials comparing tacrolimus versus
11 microemulsion cyclosporine and no significant difference was found (RR 0.96; 95% CI 0.34-1.38,
12 p=0.29).

13 14 15 16 17 18 *Infections*

19 Infections were analysed as number of patients who experienced at least one episode of infection.
20
21 No significant difference in proportion of patients with infection was found between tacrolimus and
22 cyclosporine (RR 1.01; 95% CI 0.84-1.21, p=0.91). Neither was any significant difference found
23 when subgroup analysis for the microemulsion and oil-based cyclosporine was applied. In addition
24 2 trials compared number of patients with CMV infection for tacrolimus versus microemulsion
25 cyclosporine, and no significant difference was found (RR 1.03; 95% CI 0.75-1.42, p=0.85).

26 27 28 29 30 31 32 33 34 *Malignancies*

35 According to our protocol we analysed malignancies as basocellular skin cancer and all other
36 cancers excluding basocellular skin cancer. Three trials found no significant difference between
37 tacrolimus versus microemulsion cyclosporine on basocellular skin cancer (RR 1.20; 95% CI 0.29-
38 4.93, p=0.80). Four trials reported on other cancers and found no significant difference between
39 tacrolimus and cyclosporine (RR 0.57; 95% CI 0.20-1.63, p=0.85). Nor did we find any significant
40 difference when subgroup analysis for the microemulsion and oil-based cyclosporine was applied.
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Hypertension

Eight trials found significantly less hypertension in patients treated with tacrolimus compared with cyclosporine (RR 0.80; 95% CI 0.69-0.93, $p=0.003$). In addition subgroup analysis showed that hypertension was less common for tacrolimus compared with oil-based cyclosporine (RR 0.66; 95% CI 0.54-0.80, $p<0.0001$). An insignificant trend was seen towards less hypertension for tacrolimus compared with microemulsion cyclosporine (RR 0.88; 95% CI 0.77-1.01, $p=0.07$). The difference was significant when a fixed-effect model was applied (RR 0.86; 95% CI 0.78-0.95, $p=0.003$).

Hyperlipidaemia

Four trials reported on the number of patients treated pharmacologically for hyperlipidaemia and 5 trials reported on total serum cholesterol. Significantly less patients treated with tacrolimus received treatment for hyperlipidaemia compared with cyclosporine (RR 0.57; 95% CI 0.44-0.74, $p<0.0001$). This was both seen for patients treated with oil-based cyclosporine (RR 0.57; 95% CI 0.38-0.87, $p=0.009$) and microemulsion cyclosporine (RR 0.57 95% CI 0.41-0.79, $p=0.0009$). In addition, we found that tacrolimus significantly lowers total cholesterol compared with cyclosporine (mean difference 0.4 mmol/L; 95% CI -0.66 to -0.22 mmol/L, $p<0.0001$). This was both seen for the oil-based ($p=0.005$) as for the microemulsion ($p=0.002$) subgroups, and the effect was seen even though in some of the trials more patients in the cyclosporine group were on cholesterol lowering therapy.

Diabetes

Eight trials reported on diabetes. An insignificant trend towards more diabetes was seen in tacrolimus compared with cyclosporine (RR 1.35; 95% CI 0.93-1.94, $p=0.11$). No significant difference was seen for subgroup analyses on oil-based cyclosporine (RR 1.07, 95% CI 0.40-2.90,

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3 p=0.89) and microemulsion cyclosporine (RR 1.50, 95% CI 0.90-2.50, p=0.12). When the fixed-
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5 effect model was applied, we found significant differences between tacrolimus and cyclosporine
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7 (RR 1.24; 95% CI 1.02-1.49, p=0.03) and tacrolimus and microemulsion cyclosporine (RR 1.25
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9 95% CI 1.03-1.51, p=0.02).

10 11 12 *Renal function*

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14 No significant difference between tacrolimus and cyclosporine was seen concerning renal failure
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16 requiring haemodialysis (RR 1.45; 95% CI 0.50-4.26, p=0.49). In addition no significant difference
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18 was seen for subgroup analyses on oil-based cyclosporine and microemulsion cyclosporine.
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20 Serum creatinine at end of the trials (n=6) was 8 $\mu\text{mol/L}$ lower in patients treated with tacrolimus
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22 compared with cyclosporine, however, this difference was not significant (95% CI -18.3 to -1.7
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24 $\mu\text{mol/L}$, p=0.11). Nor was any significant difference seen in serum creatinine for subgroup analyses
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26 on oil-based cyclosporine and microemulsion cyclosporine.
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30 *Chronic allograft vasculopathy*

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32 Five trials reported on chronic allograft vasculopathy, and no significant difference was found for
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34 tacrolimus compared with cyclosporine (RR 1.22; 95% CI 0.72-2.05, p=0.46). Nor was any
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36 difference found for subgroup analysis on oil-based cyclosporine and microemulsion cyclosporine.
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40 *Hirsutism and gingival hyperplasia*

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42 Hirsutism was reported in 2 trials and was significantly less frequent seen in patients treated with
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44 tacrolimus than microemulsion cyclosporine (RR 0.17; 95% CI 0.04-0.62, p=0.008). Gingival
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46 hyperplasia was reported in three trials and was significantly less frequent seen in patients treated
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48 with tacrolimus than with microemulsion cyclosporine (RR 0.07; 95% CI 0.01-0.37, p=0.002).
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Neurotoxicity

Neurotoxicity was reported in 5 trials and was analysed as number of patients who experienced at least one neurotoxic reaction or stroke. No significant difference was observed (RR 1.31; 95% CI 0.58-3.00, p=0.50). Nor was any significant difference detected when subgroup analysis for the microemulsion and oil-based cyclosporine was applied.

Adult patients

We performed subgroup analysis for only adult patients by excluding the 2 trials including only or partly paediatric patients [16,25]. We did not find any differences for any of the outcome measures except for the difference in mortality, which was described above.

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Trial sequential analysis

Trial sequential analysis was performed for the statistical significant differences in mortality seen for both the tacrolimus vs. oil-based cyclosporine group as well for the tacrolimus versus microemulsion cyclosporine group. In none of the analyses was the required information size obtained and none of the trials sequential monitoring boundaries were broken by the cumulative Z-curve.

Discussion

Principal findings

Our systematic review has generated a number of important findings. Tacrolimus seems to be significantly superior to both types of cyclosporine as regards hypertension, hyperlipidaemia,

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3 hirsutism, and gingival hyperplasia. Furthermore tacrolimus seems to be significantly superior to
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5 microemulsion cyclosporine regarding mortality and acute severe biopsy proven rejection.
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8 9 *Strengths*

10 Our systematic review of randomized trials offers a number of advantages. We conducted our
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12 review according to a protocol following the recommendations of the Cochrane Collaboration [7]
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14 and published our protocol before the conduct of the review (www.ctu.dk/protocols). We
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16 systematically searched a number of databases and reference lists for randomized trials, which
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18 should have reduced selection bias to a minimum [40]. We selected trials and extracted data in
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20 triplicate. We conducted sensitivity analyses using different models. We considered risk of
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22 systematic errors ('bias'), risks of random errors ('play of chance'), as well as risk of design errors
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24 (e.g., type of cyclosporine) [40]. We reported our findings in accordance with PRISMA [8].
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27 28 *Limitations*

29 This systematic review also encompasses some limitations. The quality and quantity of available
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31 evidence limit our findings and interpretations. All trials had high risk of bias [40]. Moreover, only
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33 few patients with relatively few outcomes were included in the trials. Hence, risks of random errors
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35 are potential explanations of our findings as suggested by our trial sequential analyses on mortality.
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37 In addition, patients included in randomized trials may not be representative of the general patient
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39 population. For instance most trials included in the current review did not include patients who were
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41 bridged to transplantation with a left ventricular device. The proportion of patients bridged to
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43 transplantation with an assist device has been increasing in the general heart transplantation
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45 population and consisted of 19% in a recent analysis [1]. Moreover, in recent years heart transplant
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47 recipients have become older during the technical evolution, with currently 25% of all heart
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3 transplants performed in people over 60 years of age [1]. These factors could potentially influence
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5 the external validity of the included trials.

8 9 *Perspective*

10 In our meta-analysis tacrolimus was found superior to the new microemulsion cyclosporine
11 concerning mortality in terms of risk of dying, but no significant difference between tacrolimus and
12 the old formula oil-based cyclosporine was observed. The question arises how this difference can be
13 explained, as microemulsion cyclosporine was introduced to overcome the differences in absorption
14 and oral bioavailability of the original oil-based formulation of cyclosporine [2]. Microemulsion
15 cyclosporine results in higher maximum cyclosporine concentrations than oil-based cyclosporine
16 even when cyclosporine trough (C0) levels are similar, which might influence tolerability and
17 toxicity. A randomized multicenter trial comparing both cyclosporine formulas found more
18 consistent bioavailability of the microemulsion formula resulting in less severe acute rejection in
19 patients treated with the new formula, however, no significant difference in mortality was found at
20 24 months follow-up in 380 patients [41].

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21 Concerning the difference in mortality observed for the two different cyclosporine formulas in our
22 meta-analysis, it should be noted that clinical experience with tacrolimus was more limited in the
23 trials comparing this drug with oil-based cyclosporine compared to the trials comparing tacrolimus
24 with microemulsion cyclosporine, and tacrolimus blood target levels were higher in the trials with
25 oil-based cyclosporine compared to the trials with microemulsion cyclosporine [15-21,21-28,30].

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26 However, a randomized trial comparing low and high tacrolimus doses in heart transplant recipients
27 did not find any significant difference in mortality, but a more favourable safety profile for the
28 patients treated with a low tacrolimus dose [42].

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3 Traditionally cyclosporine dosing has been based on trough cyclosporine level (C0) monitoring
4 [43]. Cyclosporine level at 2 hours post-dose (C2) has though been found to be the best single time-
5 point point predictor of 0 to 4-hour abbreviated area under the absorption curve (AUC₀₋₄) in heart,
6 lung, kidney and liver transplant recipients [43,44]. Clinical benefits have been shown for other
7 solid organ recipients when C2 monitoring was applied compared to C0 [44]. For heart transplant
8 recipients the picture is more unclear as one large trial did not find a correlation between C2 levels
9 and the incidence of rejection [44]. It appears though, that in general a lower C2 level may be
10 sufficient to prevent rejection, and with lower levels different adverse effects might be expected
11 [43,44]. None of the trials in this meta-analysis applied C2 levels for therapeutic drug monitoring of
12 cyclosporine, and we were therefore not able to analyze C0 compared to C2 monitoring in our trial.
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24 The statistical significant difference in mortality between tacrolimus and microemulsion
25 cyclosporine disappeared when two trials including paediatric patients were excluded. However
26 results for the different trials were consistent and none of the trials found tacrolimus to be inferior to
27 cyclosporine. No significant difference between adult and paediatric patients was found when test
28 of interaction was performed. This suggests that the lack of significance caused by withdrawing
29 paediatric studies was caused by reducing the number of patients in the groups.
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38 Our meta-analysis found tacrolimus to be associated with less severe acute biopsy proven rejection
39 compared with microemulsion cyclosporine. This is in line with observational data from The
40 ISHLT showing that 19% of patients who at transplant discharge were receiving tacrolimus and
41 mycophenolate mofetil suffered a treated rejection as compared to 27% of patients receiving
42 cyclosporine and mycophenolate mofetil ($p < 0.0001$) [1].
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3 Another meta-analysis regarding tacrolimus versus microemulsion cyclosporine has been published,
4 and results are slightly different from our systematic review [45]. This might be explained by the
5 following: we found additional randomized trials comparing tacrolimus with microemulsion
6 cyclosporine [30], we excluded studies which were not properly randomized [46], and we excluded
7 study groups where there were differences in concomitant immunosuppressive medication between
8 the tacrolimus and cyclosporine treatment groups [19]. Furthermore, we included trials comparing
9 tacrolimus with oil-based cyclosporine [24,26,27].
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18 Traditionally, immunosuppressive treatment for heart transplantation has gained much experience
19 from knowledge regarding other types of organ transplantation. A meta-analysis comparing
20 tacrolimus versus cyclosporine in 3813 liver transplant recipients found tacrolimus to be superior to
21 cyclosporine in improving patient and graft survival and preventing acute rejection after liver
22 transplantation, however, tacrolimus was significantly more diabetogenic than cyclosporine [47]. A
23 meta-analysis comparing tacrolimus versus cyclosporine in 4102 kidney transplant recipients found
24 tacrolimus to be superior to cyclosporine in improving graft survival and preventing acute rejection
25 after kidney transplantation, however, tacrolimus was found to increase post-transplant diabetes,
26 neurological, and gastrointestinal adverse effects [48]. The reduction in acute rejection and
27 mortality seen in kidney and liver transplant recipients treated with tacrolimus was only seen in our
28 subgroup of heart transplant recipients where tacrolimus was compared to microemulsion
29 cyclosporine. In contrast to kidney and liver transplant recipients we did not find a significant
30 difference on diabetes in heart transplant recipients, however, an insignificant trend towards more
31 diabetes in the tacrolimus group was seen.
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47 *Conclusion*

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3 Recognizing the limitations of the study due to the size and nature of the included trials, our
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5 systematic review shows that tacrolimus seems superior to cyclosporine in heart transplant
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7 recipients regarding hypertension, hyperlipidaemia, hirsutism and gingival hyperplasia. In addition,
8
9 tacrolimus seems to be superior to microemulsion cyclosporine regarding mortality and acute severe
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11 biopsy-proven rejection. Given the result of our analysis it appears that an appropriately sized,
12
13 randomized trial of tacrolimus versus microemulsion cyclosporine using contemporary target levels
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15 and adjunctive immunosuppression in cardiac transplantation is warranted to determine if the results
16
17 of the present meta-analysis can be confirmed.
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22 **Acknowledgements**

23
24 This work was supported by a grant from the Rigshospitalet Research Council to LP.
25

26 Supplementary material

27
28 Supplementary material (forrest plots of all meta-analyses and an additional table on
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30 immunosuppressive treatment strategies and drug target levels of the included studies) is available
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32 at *European Journal of Clinical Pharmacology* online.
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Table 1. Characteristics of included trials.

<i>Trial</i>	<i>Number of patients</i>	<i>Age (Y) Tac/ Cyclo</i>	<i>Follow-up period (mo)</i>	<i>Adult/ Paediatric</i>	<i>Oil-based /micro-emulsion Cyclosporine</i>
Rinaldi 1997	25	49/53	12	A	Oil-based
Reichart 1998	82	50/52	36	A	Oil-based
Taylor 1999	85	53/53	12	A	Oil-based
Meiser 2004	60	55/55	24	A	Micro emulsion
Wang 2004	21	49/44	6	A	Micro emulsion
Pollock-BarZiv 2005	26	4/5	26	P	Micro emulsion
Grimm 2006	314	51/51	18	A	Micro emulsion
Kobashigawa 2006 A	67	34/28	60	A/P	Micro emulsion
Kobashigawa 2006 B	223	54/51	12	A	Micro emulsion
Wang 2008	49	Un known	36	A	Micro emulsion

Table 2 Assessment of methodology quality

Trial	Allocation sequence	Allocation concealment	Blinding	Intention to treat	Incomplete outcome data addressed
Rinaldi 1997	Unclear	Unclear	No – only biopsies	Yes	Yes
Reichart 1998	Adequate	Adequate	No	Yes	Yes
Taylor 1999	Unclear	Adequate	No – only biopsies	Unclear	Unclear
Meiser 2004	Unclear	Unclear	No	Yes	Yes
Wang 2004	Unclear	Unclear	No	Yes	Unclear
Pollock-BarZiv 2005	Unclear	Unclear	No – only biopsies	Yes	Yes
Grimm 2006	Adequate	Adequate	No – only biopsies	Yes	Yes
Kobashigawa 2006 A	Unclear	Unclear	No – only biopsies and coronary angiography	Yes	Yes
Kobashigawa 2006 B	Unclear	Unclear	Unclear	Yes	Yes
Wang 2008	Unclear	Unclear	No	Yes	Yes

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7 Figure 1. Diagram of identification of randomized trials for inclusion
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9 Figure 2. Intervention effect of tacrolimus vs. cyclosporine on mortality
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11 Figure 3. Intervention effect of tacrolimus vs. cyclosporine on biopsy proven acute rejection $\geq 3a$
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13 Figure 4. Intervention effect of tacrolimus vs. cyclosporine on hypertension
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15 Figure 5. Intervention effect of tacrolimus vs. cyclosporine on hyperlipidaemia requiring treatment
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17 Figure 6. Intervention effect of tacrolimus vs. cyclosporine on post-transplant diabetes
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22 Figure legends to supplementary electronic material
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26 Figure 7. Intervention effect of tacrolimus vs. cyclosporine on rejection causing hemodynamic
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28 instability
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30 Figure 8. Intervention effect of tacrolimus vs. cyclosporine on infection rate
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32 Figure 9. Intervention effect of tacrolimus vs. cyclosporine on CMV-infection rate
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34 Figure 10. Intervention effect of tacrolimus vs. cyclosporine on malignancy
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36 Figure 11. Intervention effect of tacrolimus vs. cyclosporine on basocellular skin cancer
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38 Figure 12. Intervention effect of tacrolimus vs. cyclosporine on renal failure requiring haemodialysis
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40 Figure 13. Intervention effect of tacrolimus vs. cyclosporine on serum creatinine ($\mu\text{mol/L}$)
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42 Figure 14. Intervention effect of tacrolimus vs. cyclosporine on chronic allograft vasculopathy
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44 Figure 15. Intervention effect of tacrolimus vs. cyclosporine on hirsutism
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46 Figure 16. Intervention effect of tacrolimus vs. cyclosporine on gingival hyperplasia
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48 Figure 17. Intervention effect of tacrolimus vs. cyclosporine on neurotoxicity
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Figure 18. Intervention effect of tacrolimus vs. cyclosporine on total blood cholesterol (mmol/L)

<i>Trial</i>	<i>Type of cyclosporine</i>	<i>Cyclosporine trough target/dose</i>	<i>Tacrolimus dose (mg/kg/d)/ Target (ng/dl)</i>	<i>Azathioprine /MMF dose</i>	<i>Inductiontherapy</i>
Rinaldi 1997	Oil-based	0-1 mo: 180-360 >1 mo: 80-180 (2-6 mg/kg/d)	0-12: 15-25 (0.15 mg/kg/d) Reduced during study	Azathioprine: Pre-operat: 4 mg/kg Maintenance: 1-2 mg/kg	Yes, Rabbit ATG
Reichart 1998	Oil-based	0-6 mo: 200-400 >6 mo: 150-250	0-28 d: 15-25 (0.3 mg/kg/d; >28 d: 10-20 Later adjusted to 0-12 mo: <15 (<0.3 mg/kg/d)	Azathioprine: 210 +/- 147 Cum. Dosis (Tacrolimus treatm gr.) 324 +/- 125 Cum. Dosis (Ciclosporin treatm gr.)	Partly, ATG
Taylor 1999	Oil-based	0-1 mo: 250-600 1-3 mo: 200-400 >3 mo: 150-250	0-1 mo: 10-20 1-3 mo: 10-15 >3 mo: 5-10	Azathioprine: Pre-operat: 4 mg/kg Maintenance: 2 mg/kg	Partly, OKT3 in high-risk patients
Meiser 2004	Micro emulsion	0-6 mo: 200-300 >6 mo: 100-200	0-6 mo: 13-15; >6 mo: 10-12	MMF: 0-6 mo: 2.5-4 ug/ml >3 mo: 5-15	No
Wang 2004	Micro emulsion	0-3 mo: 300 (6 mg/kg/d)	0-3 mo: 10-20 (0.15 mg/kg/d; >3 mo: 5-15	Azathioprine	Yes, Rabbit ATG
Pollock-BarZiv 2005	Micro emulsion	0-6 mo: 250-325 (6-10 mg/kg/d) >6 mo: 200-250	0-6 mo: 10-12 (0.1-0.3 mg/kg/d; >6 mo: 8-10	Azathioprine 2-3 mg/kg/d;	Yes, Polyclonal Rabbit ATG
Grimm 2006	Micro emulsion	1-3 mo: 200-350 >3 mo: 100-200	1-3 mo: 10-20; >3 mo: 5-15	Azathioprine 2-4 mg/kg/d; WBC>2000 cells/ul	Yes, ATG or OKT3
Kobashigawa 2006 A	Micro emulsion	0-1 mo: 250-350; >1 mo: 150-250	0-1 mo: 10-15; >1 mo: 5-10	Azathioprine 2 mg/kg/d; WBC>3500 cells/ul	No
Kobashigawa 2006 B	Micro emulsion	0-3 mo: 200-400 >3 mo: 100-300	0-3 mo: 10-20; >3 mo: 5-15	MMF: Start 3 g/d Target whole blood trough conc 3-5 ng/mL	Partly, ATGAM, OKT3 or RATG allowed, and only encouraged with renal dysfunction
Wang 2008	Micro emulsion	Unknown	Unknown	MMF	Yes, type unknown

Supplementary material:

Table 3 Immunosuppressive treatment strategies of included trials

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- For Peer Review

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4 **Tacrolimus versus cyclosporine as primary immunosuppression after heart transplantation:**
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6 **Systematic review with meta-analyses and trial sequential analyses of randomized trials**
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Abstract

Purpose We conducted a systematic review of randomized trials to compare benefits and harms of tacrolimus versus cyclosporine as primary immunosuppression after heart transplantation.

Methods and results We searched electronic databases and bibliographies until April 2010. Our review followed the Cochrane and PRISMA guidelines. The meta-analysis included 10 randomized trials with 952 patients. Tacrolimus was significantly superior to cyclosporine (both formula combined) regarding hypertension (relative risk (RR) 0.8; 95% confidence interval (CI) 0.69-0.93, $p=0.003$), hyperlipidaemia (RR 0.57; 95% CI 0.44-0.74, $p<0.0001$), hirsutism (RR 0.17 95% CI 0.04-0.62, $p=0.008$), and gingival hyperplasia (RR 0.07 95% CI 0.01-0.37, $p=0.002$). No significant differences between the two calcineurin inhibitors were found regarding acute rejections causing hemodynamic instability, diabetes, renal dysfunction, infection, malignancy, or neurotoxicity. Tacrolimus was significantly superior to microemulsion cyclosporine regarding mortality (RR 0.64; 95% CI 0.42-0.96, $p=0.03$), acute severe biopsy-proven rejection (RR 0.71; 95% CI 0.56-0.90, $p=0.004$), hyperlipidaemia (RR 0.57; 95% CI 0.41-0.79, $p=0.0009$), hirsutism (RR 0.17 95% CI 0.04-0.62, $p=0.008$), and gingival hyperplasia (RR 0.07; 95% CI 0.01-0.37, $p=0.002$). Tacrolimus was significantly superior to oil-based cyclosporine regarding hypertension (RR 0.66; 95% CI 0.54-0.80, $p<0.0001$) and hyperlipidaemia (RR 0.57; 95% CI 0.38-0.87, $p=0.009$).

Conclusion Tacrolimus seems to be superior to cyclosporine in heart transplanted patients regarding hypertension, hyperlipidaemia, gingival hyperplasia and hirsutism. In addition, tacrolimus seems to be superior to microemulsion cyclosporine in heart transplanted patients regarding a number of outcomes including death. More trials with low risk of bias are needed to determine if the results of the present meta-analysis can be confirmed.

Keywords Heart transplantation · Cyclosporine · Tacrolimus · Calcineurin inhibitors · Meta-analysis

For Peer Review

Introduction

The therapeutic success of heart transplantation has been largely attributable to the development of effective and balanced immunosuppressive treatment regimens [1,2]. Especially the calcineurin inhibitors were essential in reducing acute rejection and improving early survival [2]. Two calcineurin inhibitors, cyclosporine and tacrolimus, are currently used as primary immunosuppression in heart transplant recipients [1,2].

Cyclosporine was discovered in 1971, and in 1983 the drug was approved for prevention or treatment of transplant rejection [3]. To overcome the intra-individual and inter-individual differences in absorption and bioavailability of the original oil-based formulation of cyclosporine (Sandimmune®), a micro-emulsion formula of cyclosporine (Neoral®) was introduced in the 1990s [3].

Tacrolimus (Prograf®) was discovered in the early 1980s and from 1989 used for the prevention of liver transplant rejection [3,4]. Since then, its use expanded rapidly into transplantation of other organs [3]. Both cyclosporine and tacrolimus inhibit the action of the phosphatase calcineurin. Calcineurin regulates the transport of NFAT (nuclear factor of activated T-cells, which is a transcription factor regulating lymphokine gene transcription. Cyclosporine and tacrolimus exert their cellular effects on the action of calcineurin through different cytoplasmatic receptors, as cyclosporine binds to cyclophilins and tacrolimus binds to FK-binding proteins [5]. Differences in adverse effects, safety and tolerability between cyclosporine and tacrolimus have been observed, but the toxicodynamic molecular mechanism of both drugs are still largely unknown and the involvement of calcineurin inhibition in calcineurin inhibitor toxicity is unclear [6].

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4 To date several randomized trials have compared tacrolimus vs. cyclosporine, but results have been
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6 inconsistent and optimal immunosuppressive maintenance therapy continues to be debated [5,6].
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9 We conducted this systematic review to compare benefits and harms of tacrolimus vs. cyclosporine
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11 in heart transplant recipients.
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16 **Methods**

18 *Trial selection and characteristics*

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21 Our review followed the Cochrane Collaboration [7] and PRISMA guidelines [8]. A protocol was
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23 developed (www.ctu.dk/protocols) and we included all randomized trials comparing tacrolimus
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25 versus cyclosporine after first-time isolated heart-transplantation. We required that all included
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27 patients received the same additional immunosuppressive therapy within each trial. Our preselected
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29 outcome measures were mortality, acute severe rejection defined as cardiac biopsies of grade 3A or
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31 higher according to the classification of the ISHLT (equivalent to grade H2R in the recently revised
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33 classification) [9]; acute rejection causing haemodynamic instability; Cytomegalovirus (CMV)
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35 infection; basocellular skin cancer; all malignancies excluding basocellular skin cancer; arterial
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37 hypertension; diabetes mellitus; hyperlipidaemia; total serum cholesterol; renal failure requiring
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39 haemodialysis; serum creatinine levels; neurotoxicity; hirsutism; and gingival hyperplasia. Our
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41 preselected subgroup analyses included 1) low-risk bias compared to high-risk bias trials 2) micro-
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43 emulsion cyclosporine compared to oil-based cyclosporine formulation 3) total population (adult
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45 and paediatric studies) compared to adult studies only (www.ctu.dk/protocols).
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54 *Search strategy*

56 We searched The Cochrane Central Register of Controlled Clinical Trials (CENTRAL),
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58 MEDLINE, EMBASE, and the Science Citation Index Expanded (to April 2010) [10]. Search terms
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4 were (c*closporin* or CyA or Neoral* or Sandimmun*) combined with (tacrolimus or FK506 or
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6 FK 506 or Prograf) and 'heart transplantation' [MESH term] and (random* or blind* or placebo* or
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8 meta-analysis).
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14 We scanned bibliographies of relevant articles for additional trials. We had no restrictions to
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16 blinding, language, or publication status.
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19 20 21 *Data extraction and quality assessment*

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23 Three authors independently assessed trial eligibility (LP, CHM and FG). We assessed the impact
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25 of bias risk by evaluating the trials with respect to generation of the allocation sequence, allocation
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27 concealment, blinding, and reporting of incomplete outcome data [11]. Generation of the allocation
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29 sequence was considered adequate when generated by a computer, random-number table, shuffling
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31 of cards, or something similar. Allocation concealment was considered adequate when allocation of
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33 patients involved a central independent unit, such as an on-site locked computer, sealed envelopes
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35 or something similar. Blinding was adequate if the trial was described as double-blind and the
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37 method of blinding involved identical active drugs. Post-randomization exclusion of patients was
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39 registered. When possible we converted per-protocol to intention-to-treat-analysis. Bias risk was
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41 assessed without blinding by 3 authors [11].
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50 *Quantitative data synthesis*

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52 We used Cochrane Collaboration Software (RevMan 5.0.22). Data were analysed with both fixed-
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54 effect and random-effects models. In case of discrepancy regarding significance between the two
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56 models both results were reported. Otherwise, only results from the random-effects model were
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58 reported. Data were presented as relative risk (RR) with values less than 1.0 favouring tacrolimus,
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4 and with 95% confidence intervals (CI). Heterogeneity was assessed with I^2 , which describes the
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6 percentage of the variability in effect estimates that is due to heterogeneity rather than sampling
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8 error (chance). I^2 lies between 0% (no heterogeneity) and 100% (maximal heterogeneity) [12]. Test
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10 of interaction was performed to evaluate the difference between the 2 estimates [13].
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13 14 15 16 *Trial sequential analysis* 17

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19 Trial sequential analysis was applied as cumulative meta-analyses are at risk of producing random
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21 errors because of sparse data or repetitive testing on accumulating data [14]. To minimize random
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23 errors we calculated the required information size (i.e., the number of participants needed in a meta-
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25 analysis to detect or reject a certain intervention effect) [14]. Information size calculation also
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27 accounted for the diversity present in the meta-analysis. In our meta-analysis, information size was
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29 based on the assumption of a plausible RR reduction of 20% [14]. The underlying assumption of
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31 trial sequential analysis is that significance testing may be performed each time a new trial is added
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33 to the meta-analysis. We added the trials according to the year of publication. On the basis of the
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35 required information size and risk for type I and type II errors trial sequential monitoring
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37 boundaries were constructed [14]. These boundaries will determine the statistical inference one may
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39 draw regarding the cumulative meta-analysis that has not reached the required information size. If a
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41 trial sequential monitoring boundary is crossed before the required information size is reached in a
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43 cumulative meta-analysis, firm evidence may have been established and further trials are
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45 superfluous. On the other hand, if the boundaries are not surpassed, it is most probably necessary to
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47 continue doing trials in order to detect or reject a certain intervention effect. We used as defaults a
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49 type I error of 5%, type II error of 20%, and adjusted the information size for diversity unless
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51 otherwise stated [14].
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Results

Figure 1 depicts the results of the search strategy. Database searches identified 450 references.

Exclusion of duplicates and irrelevant references left 11 randomized trials published in 25 publications [15-39]. One trial could not be included in the meta-analysis as none of the outcome measures were addressed [29]. We confirmed with the authors that all patients only participated once in the trials [28,30]

The meta-analyses involved 10 trials with a total of 952 patients (table 1): 486 patients were randomized to tacrolimus and 466 patients to cyclosporine [15,16,19,20,24-28,30]). Three trials with 192 patients compared tacrolimus with the old formula oil-based cyclosporine [24,26,27] and seven trials with 760 patients compared tacrolimus with the new formula microemulsion cyclosporine [15,16,19,20,25,28,30].

In 8 trials the population consisted of adult patients [15,19,20,24,26-28,30], while in 1 trial the population consisted of a combination of adult and paediatric patients [16], and in 1 trial only paediatric patients were included [25].

Concomitant immunosuppressive treatment was the same within all trials except for one where the patients were randomized to 3 groups: one receiving cyclosporine and mycophenolate mofetil, one receiving tacrolimus and mycophenolate mofetil, and one receiving tacrolimus and sirolimus [19].

We therefore excluded the latter group of our analyses. Immunosuppressive treatment varied within trials. All patients were treated with steroids. As antiproliferative agent azathioprine (7 trials [15,16,24-28]) or mycophenolate mofetil (3 trials [19,20,30]) was used. Induction therapy was used for all patients in 4 trials [15,25,26,28], for some of the patients in 3 trials [19,24,27], and for none

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4 in 3 trials [16,20,30]. In case induction therapy was used, either anti-thymocyte globulin (ATG) or
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6 muromonab-CD3 (OKT3®) was administered [15,25,26,28]. Patients were followed from 6 months
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8 to 5 years.
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11 Trial methodology was inadequately reported in the majority of trials (table 2). All trials were
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13 considered trials with high risk of bias.
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16 17 18 *Mortality*

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20 Ten trials reported on mortality, and overall no significant difference in mortality was found
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22 between tacrolimus and cyclosporine (relative risk (RR) 0.78; 95 % CI 0.54-1.13, $p = 0.19$).
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25 Tacrolimus was significantly superior to microemulsion cyclosporine (RR 0.64; 95% CI 0.42-0.96,
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27 $p=0.03$). No significant difference in mortality between tacrolimus and oil-based cyclosporine was
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29 found (RR 1.79; 95% CI 0.77-4.15, $p=0.17$). Test of interaction showed significant difference in
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31 intervention effect on mortality between the oil-based and microemulsion cyclosporine formulas
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33 (p=0.04).
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40 The significant difference in mortality between tacrolimus and microemulsion cyclosporine,
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42 however, disappeared when the studies including paediatric patients were excluded (RR 0.66; 95%
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44 CI 0.40-1.09, $p=0.10$). Test of interaction showed no significant differences in mortality between
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46 the paediatric and adult cyclosporine subgroups ($p=0.89$), This suggests that the lack of significance
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48 caused by withdrawing paediatric studies was solely caused by reducing the number of patients in
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50 the groups.
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53 54 55 *Acute rejection*

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4 No significant difference in grade 3A or higher rejection was found between tacrolimus and (both
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6 formula combined) cyclosporine (RR 0.86; 95% CI 0.62-1.20, p=0.38). However, tacrolimus was
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8 associated with a significant reduction in Grade 3 A or higher rejection compared to microemulsion
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10 cyclosporine (RR 0.71; 95% CI 0.56-0.90, p=0.004).
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14 Rejection causing haemodynamic instability was reported in 5 trials comparing tacrolimus versus
15
16 microemulsion cyclosporine and no significant difference was found (RR 0.96; 95% CI 0.34-1.38,
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18 p=0.29).
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21 22 23 *Infections*

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25 Infections were analysed as number of patients who experienced at least one episode of infection.
26
27 No significant difference in proportion of patients with infection was found between tacrolimus and
28
29 cyclosporine (RR 1.01; 95% CI 0.84-1.21, p=0.91). Neither was any significant difference found
30
31 when subgroup analysis for the microemulsion and oil-based cyclosporine was applied. In addition
32
33 2 trials compared number of patients with CMV infection for tacrolimus versus microemulsion
34
35 cyclosporine, and no significant difference was found (RR 1.03; 95% CI 0.75-1.42, p=0.85).
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41 42 *Malignancies*

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44 According to our protocol we analysed malignancies as basocellular skin cancer and all other
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46 cancers excluding basocellular skin cancer. Three trials found no significant difference between
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48 tacrolimus versus microemulsion cyclosporine on basocellular skin cancer (RR 1.20; 95% CI 0.29-
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50 4.93, p=0.80). Four trials reported on other cancers and found no significant difference between
51
52 tacrolimus and cyclosporine (RR 0.57; 95% CI 0.20-1.63, p=0.85). Nor did we find any significant
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54 difference when subgroup analysis for the microemulsion and oil-based cyclosporine was applied.
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Hypertension

Eight trials found significantly less hypertension in patients treated with tacrolimus compared with cyclosporine (RR 0.80; 95% CI 0.69-0.93, $p=0.003$). In addition subgroup analysis showed that hypertension was less common for tacrolimus compared with oil-based cyclosporine (RR 0.66; 95% CI 0.54-0.80, $p<0.0001$). An insignificant trend was seen towards less hypertension for tacrolimus compared with microemulsion cyclosporine (RR 0.88; 95% CI 0.77-1.01, $p=0.07$). The difference was significant when a fixed-effect model was applied (RR 0.86; 95% CI 0.78-0.95, $p=0.003$).

Hyperlipidaemia

Four trials reported on the number of patients treated pharmacologically for hyperlipidaemia and 5 trials reported on total serum cholesterol. Significantly less patients treated with tacrolimus received treatment for hyperlipidaemia compared with cyclosporine (RR 0.57; 95% CI 0.44-0.74, $p<0.0001$).

This was both seen for patients treated with oil-based cyclosporine (RR 0.57; 95% CI 0.38-0.87, $p=0.009$) and microemulsion cyclosporine (RR 0.57 95% CI 0.41-0.79, $p=0.0009$).

In addition, we found that tacrolimus significantly lowers total cholesterol compared with cyclosporine (mean difference 0.4 mmol/L; 95% CI -0.66 to -0.22 mmol/L, $p<0.0001$). This was both seen for the oil-based ($p=0.005$) as for the microemulsion ($p=0.002$) subgroups, and the effect was seen even though in some of the trials more patients in the cyclosporine group were on cholesterol lowering therapy.

Diabetes

Eight trials reported on diabetes. An insignificant trend towards more diabetes was seen in tacrolimus compared with cyclosporine (RR 1.35; 95% CI 0.93-1.94, $p=0.11$). No significant difference was seen for subgroup analyses on oil-based cyclosporine (RR 1.07, 95% CI 0.40-2.90,

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4 p=0.89) and microemulsion cyclosporine (RR 1.50, 95% CI 0.90-2.50, p=0.12). When the fixed-
5
6 effect model was applied, we found significant differences between tacrolimus and cyclosporine
7
8 (RR 1.24; 95% CI 1.02-1.49, p=0.03) and tacrolimus and microemulsion cyclosporine (RR 1.25
9
10 95% CI 1.03-1.51, p=0.02).
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13 14 15 16 *Renal function*

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18 No significant difference between tacrolimus and cyclosporine was seen concerning renal failure
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20 requiring haemodialysis (RR 1.45; 95% CI 0.50-4.26, p=0.49). In addition no significant difference
21
22 was seen for subgroup analyses on oil-based cyclosporine and microemulsion cyclosporine.
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25 Serum creatinine at end of the trials (n=6) was 8 $\mu\text{mol/L}$ lower in patients treated with tacrolimus
26
27 compared with cyclosporine, however, this difference was not significant (95% CI -18.3 to -1.7
28
29 $\mu\text{mol/L}$, p=0.11). Nor was any significant difference seen in serum creatinine for subgroup analyses
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31 on oil-based cyclosporine and microemulsion cyclosporine.
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34 35 36 37 *Chronic allograft vasculopathy*

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39 Five trials reported on chronic allograft vasculopathy, and no significant difference was found for
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41 tacrolimus compared with cyclosporine (RR 1.22; 95% CI 0.72-2.05, p=0.46). Nor was any
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43 difference found for subgroup analysis on oil-based cyclosporine and microemulsion cyclosporine.
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49 50 *Hirsutism and gingival hyperplasia*

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52 Hirsutism was reported in 2 trials and was significantly less frequent seen in patients treated with
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54 tacrolimus than microemulsion cyclosporine (RR 0.17; 95% CI 0.04-0.62, p=0.008). Gingival
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56 hyperplasia was reported in three trials and was significantly less frequent seen in patients treated
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58 with tacrolimus than with microemulsion cyclosporine (RR 0.07; 95% CI 0.01-0.37, p=0.002).
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Neurotoxicity

Neurotoxicity was reported in 5 trials and was analysed as number of patients who experienced at least one neurotoxic reaction or stroke. No significant difference was observed (RR 1.31; 95% CI 0.58-3.00, $p=0.50$). Nor was any significant difference detected when subgroup analysis for the microemulsion and oil-based cyclosporine was applied.

Adult patients

We performed subgroup analysis for only adult patients by excluding the 2 trials including only or partly paediatric patients [16,25]. We did not find any differences for any of the outcome measures except for the difference in mortality, which was described above.

Trial sequential analysis

Trial sequential analysis was performed for the statistical significant differences in mortality seen for both the tacrolimus vs. oil-based cyclosporine group as well for the tacrolimus versus microemulsion cyclosporine group. In none of the analyses was the required information size obtained and none of the trials sequential monitoring boundaries were broken by the cumulative Z-curve.

Discussion

Principal findings

Our systematic review has generated a number of important findings. Tacrolimus seems to be significantly superior to both types of cyclosporine as regards hypertension, hyperlipidaemia,

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4 hirsutism, and gingival hyperplasia. Furthermore tacrolimus seems to be significantly superior to
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6 microemulsion cyclosporine regarding mortality and acute severe biopsy proven rejection.
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10 11 *Strengths*

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13 Our systematic review of randomized trials offers a number of advantages. We conducted our
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15 review according to a protocol following the recommendations of the Cochrane Collaboration [7]
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17 and published our protocol before the conduct of the review (www.ctu.dk/protocols). We
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19 systematically searched a number of databases and reference lists for randomized trials, which
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21 should have reduced selection bias to a minimum [40]. We selected trials and extracted data in
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23 triplicate. We conducted sensitivity analyses using different models. We considered risk of
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25 systematic errors ('bias'), risks of random errors ('play of chance'), as well as risk of design errors
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27 (e.g., type of cyclosporine) [40]. We reported our findings in accordance with PRISMA [8].
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34 35 *Limitations*

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37 This systematic review also encompasses some limitations. The quality and quantity of available
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39 evidence limit our findings and interpretations. All trials had high risk of bias [40]. Moreover, only
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41 few patients with relatively few outcomes were included in the trials. Hence, risks of random errors
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43 are potential explanations of our findings as suggested by our trial sequential analyses on mortality.
44
45 In addition, patients included in randomized trials may not be representative of the general patient
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47 population. For instance most trials included in the current review did not include patients who were
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49 bridged to transplantation with a left ventricular device. The proportion of patients bridged to
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51 transplantation with an assist device has been increasing in the general heart transplantation
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53 population and consisted of 19% in a recent analysis [1]. Moreover, in recent years heart transplant
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55 recipients have become older during the technical evolution, with currently 25% of all heart
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4 transplants performed in people over 60 years of age [1]. These factors could potentially influence
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6 the external validity of the included trials.
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10 11 *Perspective*

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13 In our meta-analysis tacrolimus was found superior to the new microemulsion cyclosporine
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15 concerning mortality in terms of risk of dying, but no significant difference between tacrolimus and
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17 the old formula oil-based cyclosporine was observed. The question arises how this difference can be
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19 explained, as microemulsion cyclosporine was introduced to overcome the differences in absorption
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21 and oral bioavailability of the original oil-based formulation of cyclosporine [2]. Microemulsion
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23 cyclosporine results in higher maximum cyclosporine concentrations than oil-based cyclosporine
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25 even when cyclosporine trough (C₀) levels are similar, which might influence tolerability and
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27 toxicity. A randomized multicenter trial comparing both cyclosporine formulas found more
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29 consistent bioavailability of the microemulsion formula resulting in less severe acute rejection in
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31 patients treated with the new formula, however, no significant difference in mortality was found at
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33 24 months follow-up in 380 patients [41].
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42 Concerning the difference in mortality observed for the two different cyclosporine formulas in our
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44 meta-analysis, it should be noted that clinical experience with tacrolimus was more limited in the
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46 trials comparing this drug with oil-based cyclosporine compared to the trials comparing tacrolimus
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48 with microemulsion cyclosporine, and tacrolimus blood target levels were higher in the trials with
49
50 oil-based cyclosporine compared to the trials with microemulsion cyclosporine [15-21,21-28,30].
51
52 However, a randomized trial comparing low and high tacrolimus doses in heart transplant recipients
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54 did not find any significant difference in mortality, but a more favourable safety profile for the
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56 patients treated with a low tacrolimus dose [42].
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4 Traditionally cyclosporine dosing has been based on trough cyclosporine level (C0) monitoring
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6 [43]. Cyclosporine level at 2 hours post-dose (C2) has though been found to be the best single time-
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8 point point predictor of 0 to 4-hour abbreviated area under the absorption curve (AUC₀₋₄) in heart,
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10 lung, kidney and liver transplant recipients [43,44]. Clinical benefits have been shown for other
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12 solid organ recipients when C2 monitoring was applied compared to C0 [44]. For heart transplant
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14 recipients the picture is more unclear as one large trial did not find a correlation between C2 levels
15
16 and the incidence of rejection [44]. It appears though, that in general a lower C2 level may be
17
18 sufficient to prevent rejection, and with lower levels different adverse effects might be expected
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20 [43,44]. None of the trials in this meta-analysis applied C2 levels for therapeutic drug monitoring of
21
22 cyclosporine, and we were therefore not able to analyze C0 compared to C2 monitoring in our trial.
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30 The statistical significant difference in mortality between tacrolimus and microemulsion
31
32 cyclosporine disappeared when two trials including paediatric patients were excluded. However
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34 results for the different trials were consistent and none of the trials found tacrolimus to be inferior to
35
36 cyclosporine. No significant difference between adult and paediatric patients was found when test
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38 of interaction was performed. This suggests that the lack of significance caused by withdrawing
39
40 paediatric studies was caused by reducing the number of patients in the groups.
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47 Our meta-analysis found tacrolimus to be associated with less severe acute biopsy proven rejection
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49 compared with microemulsion cyclosporine. This is in line with observational data from The
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51 ISHLT showing that 19% of patients who at transplant discharge were receiving tacrolimus and
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53 mycophenolate mofetil suffered a treated rejection as compared to 27% of patients receiving
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55 cyclosporine and mycophenolate mofetil ($p < 0.0001$)[1].
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4 Another meta-analysis regarding tacrolimus versus microemulsion cyclosporine has been published,
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6 and results are slightly different from our systematic review [45]. This might be explained by the
7
8 following: we found additional randomized trials comparing tacrolimus with microemulsion
9
10 cyclosporine [30], we excluded studies which were not properly randomized [46], and we excluded
11
12 study groups where there were differences in concomitant immunosuppressive medication between
13
14 the tacrolimus and cyclosporine treatment groups [19]. Furthermore, we included trials comparing
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16 tacrolimus with oil-based cyclosporine [24,26,27].
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23 Traditionally, immunosuppressive treatment for heart transplantation has gained much experience
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25 from knowledge regarding other types of organ transplantation. A meta-analysis comparing
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27 tacrolimus versus cyclosporine in 3813 liver transplant recipients found tacrolimus to be superior to
28
29 cyclosporine in improving patient and graft survival and preventing acute rejection after liver
30
31 transplantation, however, tacrolimus was significantly more diabetogenic than cyclosporine [47]. A
32
33 meta-analysis comparing tacrolimus versus cyclosporine in 4102 kidney transplant recipients found
34
35 tacrolimus to be superior to cyclosporine in improving graft survival and preventing acute rejection
36
37 after kidney transplantation, however, tacrolimus was found to increase post-transplant diabetes,
38
39 neurological, and gastrointestinal adverse effects [48]. The reduction in acute rejection and
40
41 mortality seen in kidney and liver transplant recipients treated with tacrolimus was only seen in our
42
43 subgroup of heart transplant recipients where tacrolimus was compared to microemulsion
44
45 cyclosporine. In contrast to kidney and liver transplant recipients we did not find a significant
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47 difference on diabetes in heart transplant recipients, however, an insignificant trend towards more
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49 diabetes in the tacrolimus group was seen.
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59 ***Conclusion***

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4 Recognizing the limitations of the study due to the size and nature of the included trials, our
5
6 systematic review shows that tacrolimus seems superior to cyclosporine in heart transplant
7
8 recipients regarding hypertension, hyperlipidaemia, hirsutism and gingival hyperplasia. In addition,
9
10 tacrolimus seems to be superior to microemulsion cyclosporine regarding mortality and acute severe
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12 biopsy-proven rejection. Given the result of our analysis it appears that an appropriately sized,
13
14 randomized trial of tacrolimus versus microemulsion cyclosporine using contemporary target levels
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16 and adjunctive immunosuppression in cardiac transplantation is warranted to determine if the results
17
18 of the present meta-analysis can be confirmed.
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28 **Acknowledgements**

29
30 This work was supported by a grant from the Rigshospitalet Research Council to LP.

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33 Supplementary material

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35 Supplementary material (forrest plots of all meta-analyses and an additional table on
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37 immunosuppressive treatment strategies and drug target levels of the included studies) is available
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39 at *European Journal of Clinical Pharmacology* online.
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For Peer Review

Table 1. Characteristics of included trials.

<i>Trial</i>	<i>Number of patients</i>	<i>Age (Y) Tac/ Cyclo</i>	<i>Follow-up period (mo)</i>	<i>Adult/ Paediatric</i>	<i>Oil-based /micro-emulsion Cyclosporine</i>
Rinaldi 1997	25	49/53	12	A	Oil-based
Reichart 1998	82	50/52	36	A	Oil-based
Taylor 1999	85	53/53	12	A	Oil-based
Meiser 2004	60	55/55	24	A	Micro emulsion
Wang 2004	21	49/44	6	A	Micro emulsion
Pollock-BarZiv 2005	26	4/5	26	P	Micro emulsion
Grimm 2006	314	51/51	18	A	Micro emulsion
Kobashigawa 2006 A	67	34/28	60	A/P	Micro emulsion
Kobashigawa 2006 B	223	54/51	12	A	Micro emulsion
Wang 2008	49	Un known	36	A	Micro emulsion

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Table 2 Assessment of methodology quality

Trial	Allocation sequence	Allocation concealment	Blinding	Intention to treat	Incomplete outcome data addressed
Rinaldi 1997	Unclear	Unclear	No – only biopsies	Yes	Yes
Reichart 1998	Adequate	Adequate	No	Yes	Yes
Taylor 1999	Unclear	Adequate	No – only biopsies	Unclear	Unclear
Meiser 2004	Unclear	Unclear	No	Yes	Yes
Wang 2004	Unclear	Unclear	No	Yes	Unclear
Pollock-BarZiv 2005	Unclear	Unclear	No – only biopsies	Yes	Yes
Grimm 2006	Adequate	Adequate	No – only biopsies	Yes	Yes
Kobashigawa 2006 A	Unclear	Unclear	No –only biopsies and coronary angiography	Yes	Yes
Kobashigawa 2006 B	Unclear	Unclear	Unclear	Yes	Yes
Wang 2008	Unclear	Unclear	No	Yes	Yes

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9 Figure 1. Diagram of identification of randomized trials for inclusion
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11 Figure 2. Intervention effect of tacrolimus vs. cyclosporine on mortality
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13 Figure 3. Intervention effect of tacrolimus vs. cyclosporine on biopsy proven acute rejection $\geq 3a$
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15 Figure 4. Intervention effect of tacrolimus vs. cyclosporine on hypertension
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17 Figure 5. Intervention effect of tacrolimus vs. cyclosporine on hyperlipidaemia requiring treatment
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19 Figure 6. Intervention effect of tacrolimus vs. cyclosporine on post-transplant diabetes
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28 Figure legends to supplementary electronic material
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33 Figure 7. Intervention effect of tacrolimus vs. cyclosporine on rejection causing hemodynamic
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35 instability
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37 Figure 8. Intervention effect of tacrolimus vs. cyclosporine on infection rate
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39 Figure 9. Intervention effect of tacrolimus vs. cyclosporine on CMV-infection rate
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41 Figure 10. Intervention effect of tacrolimus vs. cyclosporine on malignancy
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43 Figure 11. Intervention effect of tacrolimus vs. cyclosporine on basocellular skin cancer
44

45 Figure 12. Intervention effect of tacrolimus vs. cyclosporine on renal failure requiring haemodialysis
46

47 Figure 13. Intervention effect of tacrolimus vs. cyclosporine on serum creatinine ($\mu\text{mol/L}$)
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49 Figure 14. Intervention effect of tacrolimus vs. cyclosporine on chronic allograft vasculopathy
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51 Figure 15. Intervention effect of tacrolimus vs. cyclosporine on hirsutism
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53 Figure 16. Intervention effect of tacrolimus vs. cyclosporine on gingival hyperplasia
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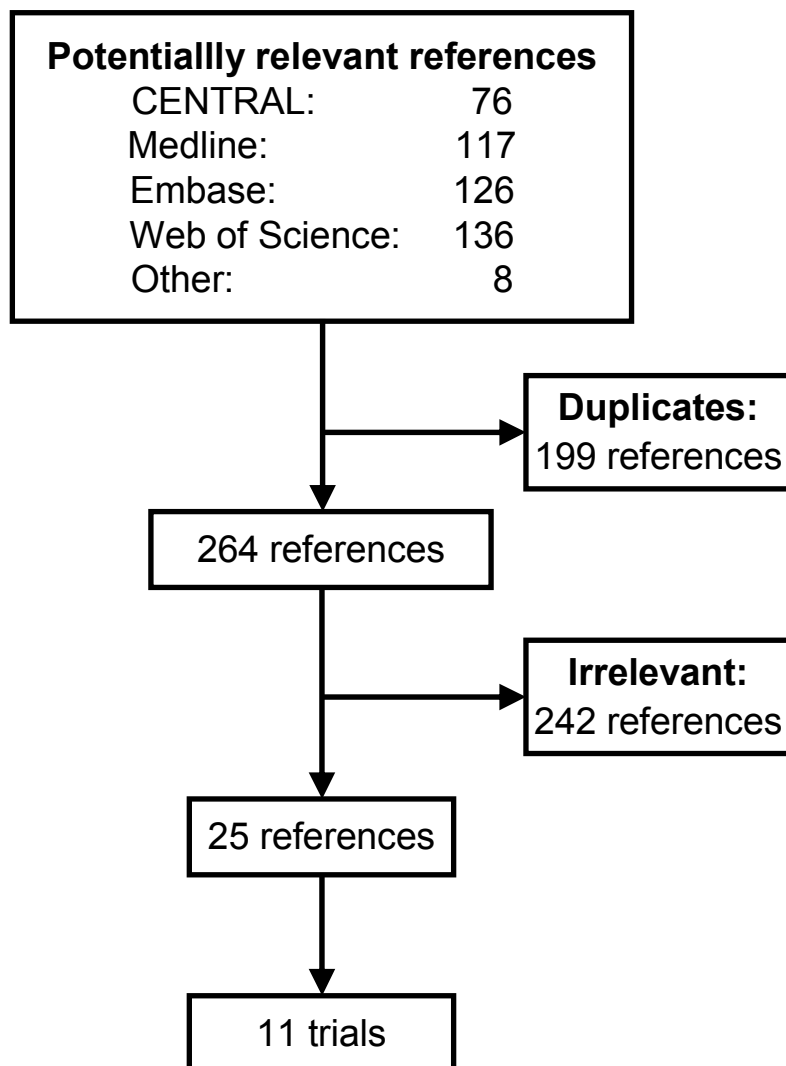
55 Figure 17. Intervention effect of tacrolimus vs. cyclosporine on neurotoxicity
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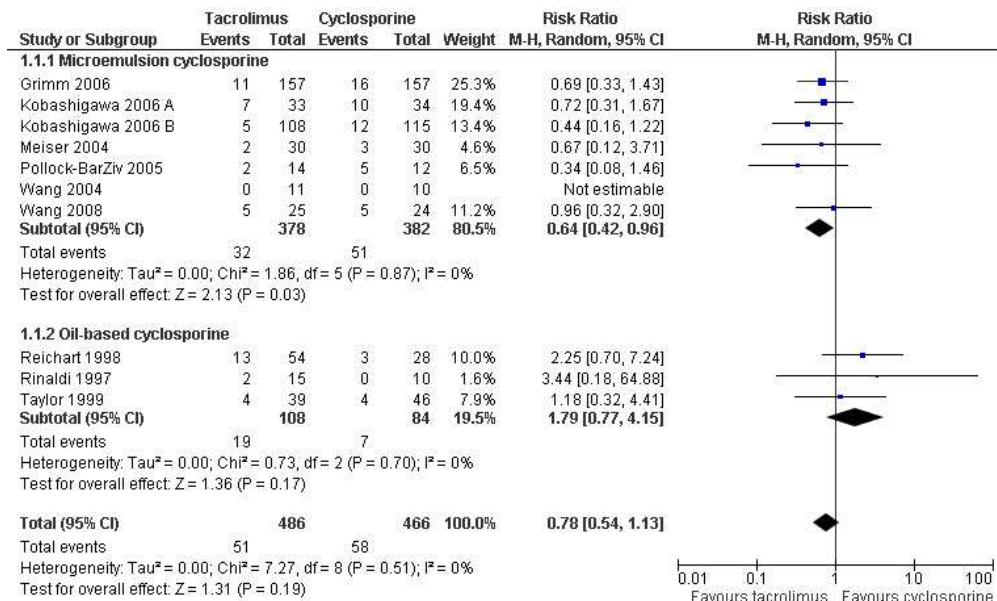
Figure 18. Intervention effect of tacrolimus vs. cyclosporine on total blood cholesterol (mmol/L)

<i>Trial</i>	<i>Type of cyclosporine</i>	<i>Cyclosporine trough target/dose</i>	<i>Tacrolimus dose (mg/ kg/d)/ Target (ng/dl)</i>	<i>Azathioprine /MMF dose</i>	<i>Inductiontherapy</i>
Rinaldi 1997	Oil-based	0-1 mo: 180-360 >1 mo: 80-180 (2-6 mg/kg/d)	0-12: 15-25 (0.15 mg/kg/d) Reduced during study	Azathioprine: Pre-operat: 4 mg/kg Maintenance: 1-2 mg/kg	Yes, Rabbit ATG
Reichart 1998	Oil-based	0-6 mo: 200-400 >6 mo: 150-250	0-28 d: 15-25 (0.3 mg/kg/d; >28 d: 10-20 Later adjusted to 0-12 mo: <15 (<0.3 mg/kg/d)	Azathioprine: 210 +/- 147 Cum. Dosis (Tacrolimus treatm gr.) 324 +/- 125 Cum. Dosis (Ciclosporin treatm gr.)	Partly, ATG
Taylor 1999	Oil-based	0-1 mo: 250-600 1-3 mo: 200-400 >3 mo: 150-250	0-1 mo: 10-20 1-3 mo: 10-15 >3 mo: 5-10	Azathioprine: Pre-operat: 4 mg/kg Maintenance: 2 mg/kg	Partly, OKT3 in high-risk patients
Meiser 2004	Micro emulsion	0-6 mo: 200-300 >6 mo: 100-200	0-6 mo: 13-15; >6 mo: 10-12	MMF: 0-6 mo: 2.5-4 ug/ml >3 mo: 5-15	No
Wang 2004	Micro emulsion	0-3 mo: 300 (6 mg/kg/d)	0-3 mo: 10-20 (0.15 mg/kg/d; >3 mo: 5-15	Azathioprine	Yes, Rabbit ATG
Pollock-BarZiv 2005	Micro emulsion	0-6 mo: 250-325 (6-10 mg/kg/d) >6 mo: 200-250	0-6 mo: 10-12 (0.1-0.3 mg/kg/d; >6 mo: 8-10	Azathioprine 2-3 mg/kg/d;	Yes, Polyclonal Rabbit ATG
Grimm 2006	Micro emulsion	1-3 mo: 200-350 >3 mo: 100-200	1-3 mo: 10-20; >3 mo: 5-15	Azathioprine 2-4 mg/kg/d; WBC>2000 cells/ul	Yes, ATG or OKT3
Kobashigawa 2006 A	Micro emulsion	0-1 mo: 250-350; >1 mo: 150-250	0-1 mo: 10-15; >1 mo: 5-10	Azathioprine 2 mg/kg/d; WBC>3500 cells/ul	No
Kobashigawa 2006 B	Micro emulsion	0-3 mo: 200-400 >3 mo: 100-300	0-3 mo: 10-20; >3 mo: 5-15	MMF: Start 3 g/d Target whole blood trough conc 3-5 ng/mL	Partly, ATGAM, OKT3 or RATG allowed, and only encouraged with renal dysfunction
Wang 2008	Micro emulsion	Unknown	Unknown	MMF	Yes, type unknown

Supplementary material:

Table 3 Immunosuppressive treatment strategies of included trials

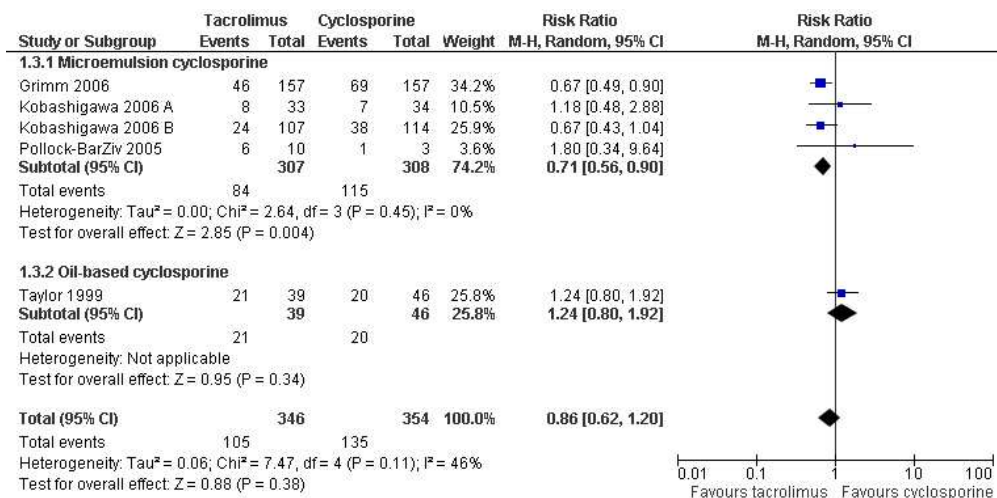




Intervention effect of tacrolimus vs. cyclosporine on mortality
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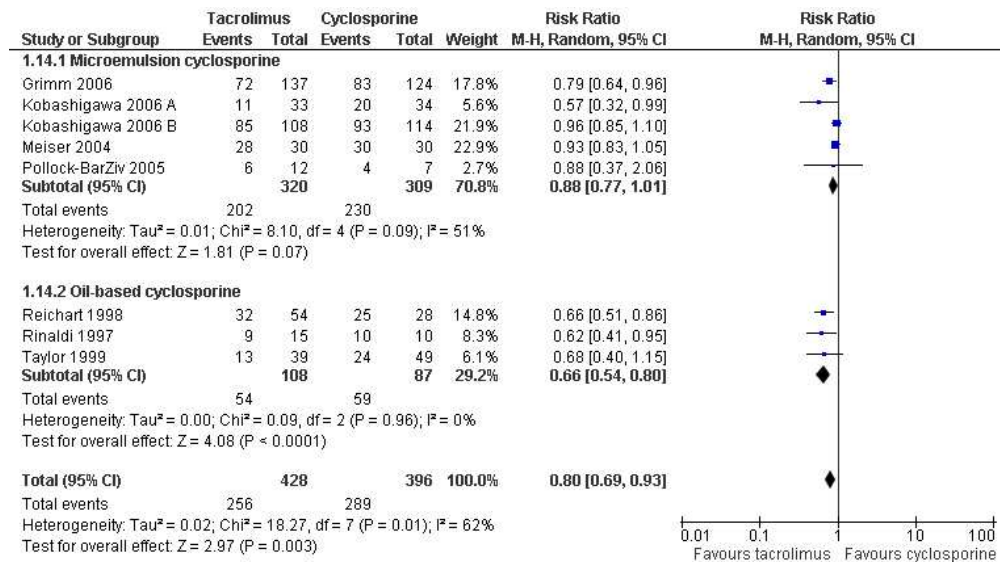
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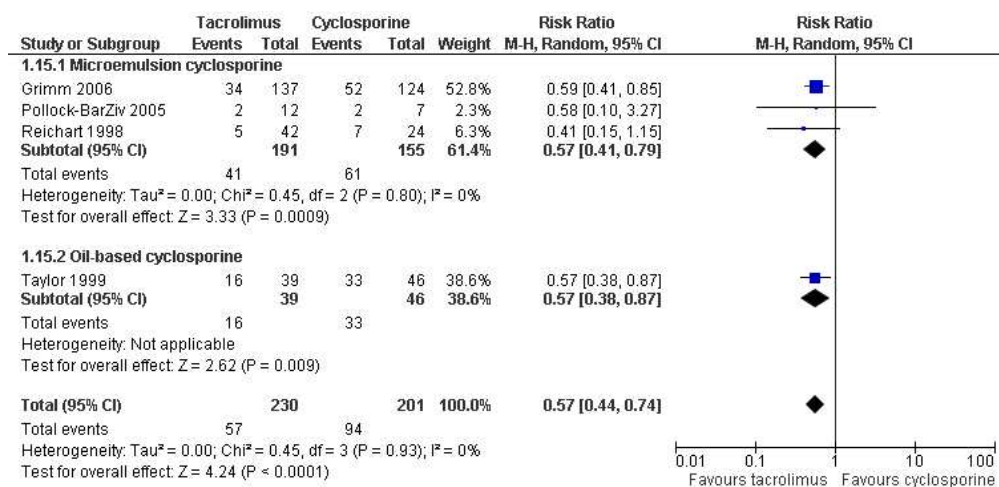
Intervention effect of tacrolimus vs. cyclosporine on biopsy proven acute rejection $\geq 3a$
64x32mm (300 x 300 DPI)

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Intervention effect of tacrolimus vs. cyclosporine on hypertension
64x36mm (300 x 300 DPI)

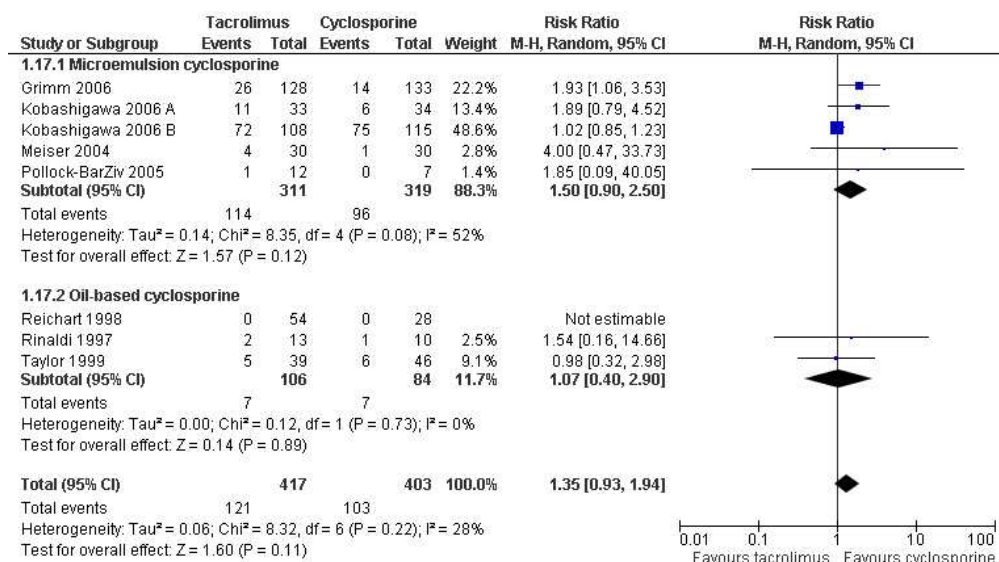
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Intervention effect of tacrolimus vs. cyclosporine on hyperlipidaemia requiring treatment
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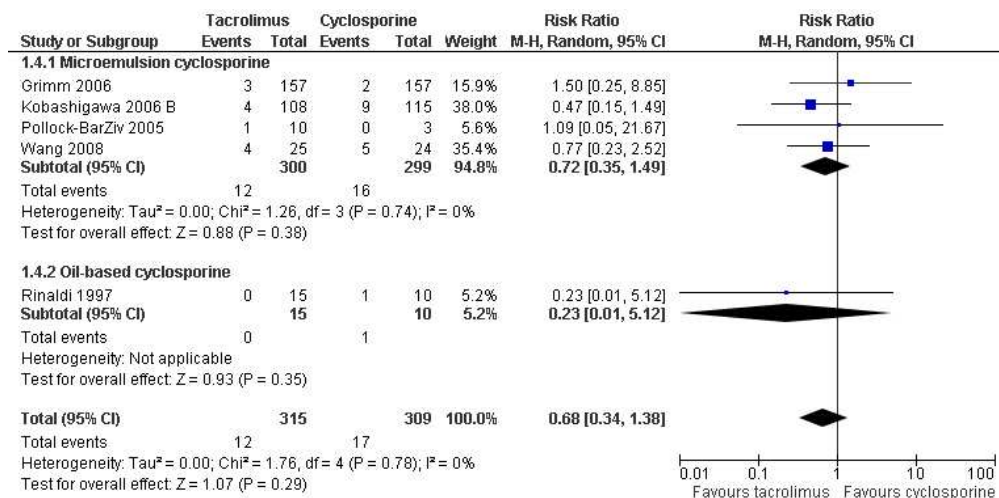
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Intervention effect of tacrolimus vs. cyclosporine on post-transplant diabetes
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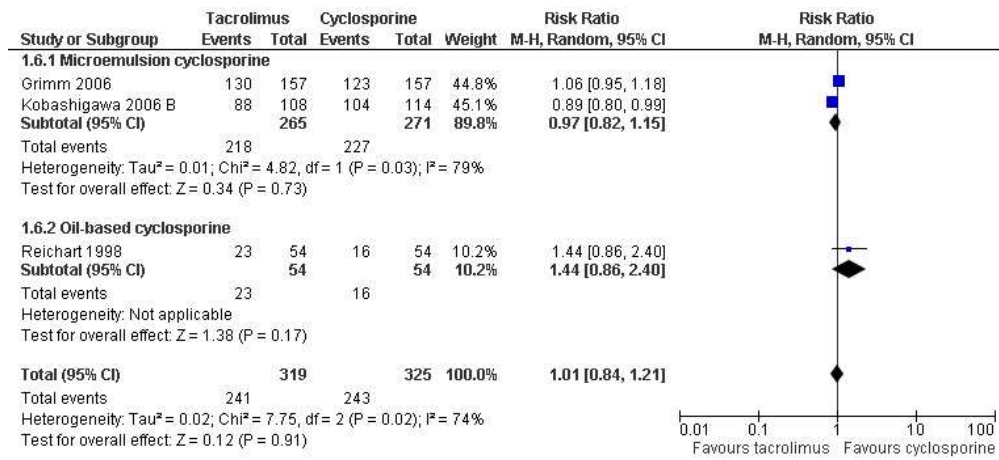
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Intervention effect of tacrolimus vs. cyclosporine on rejection causing hemodynamic instability
64x32mm (300 x 300 DPI)

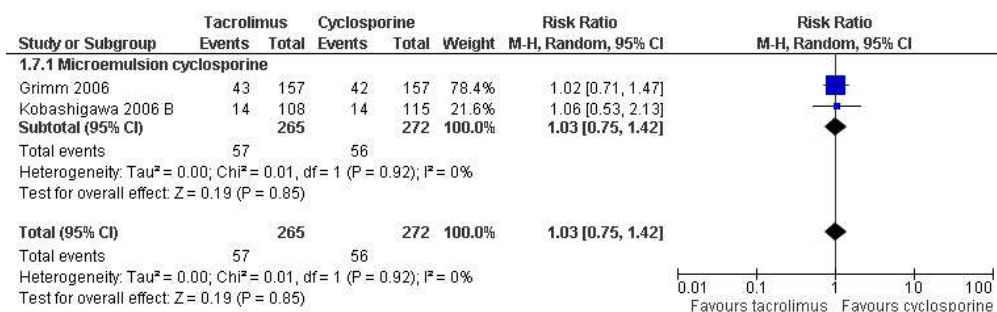
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Intervention effect of tacrolimus vs. cyclosporine on infection rate
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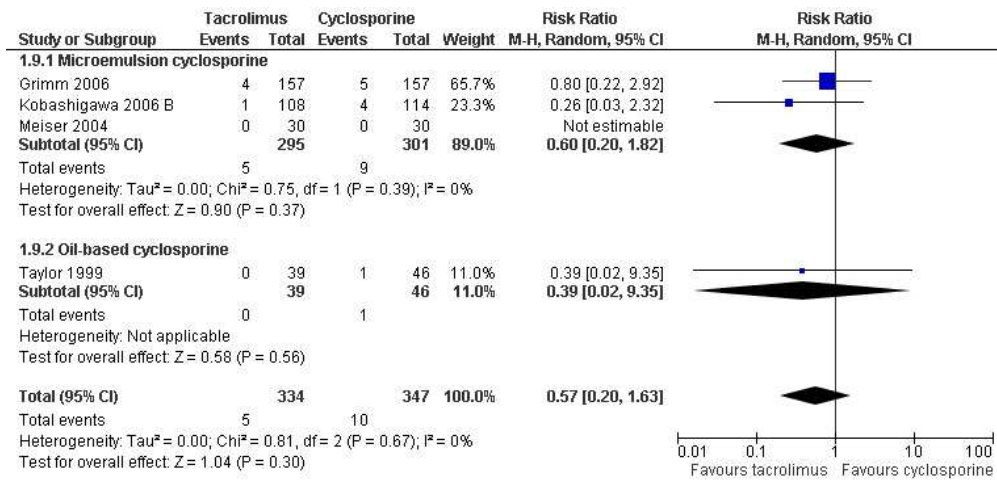
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Intervention effect of tacrolimus vs. cyclosporine on CMV-infection rate
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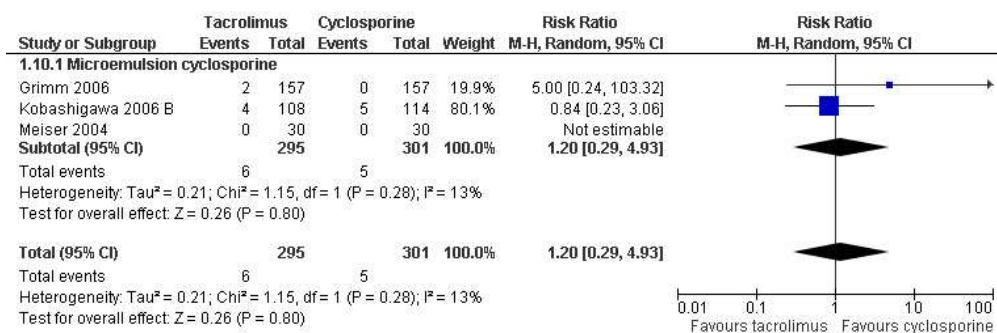
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Intervention effect of tacrolimus vs. cyclosporine on malignancy
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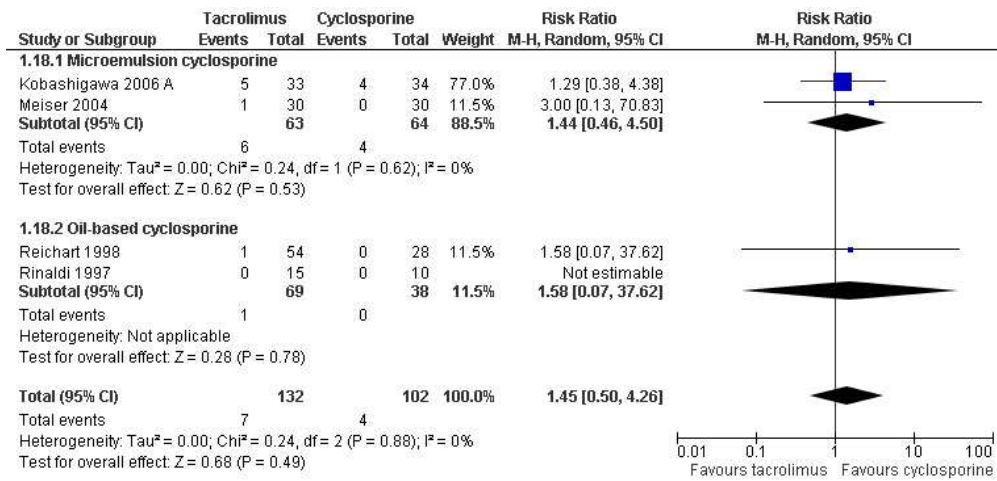
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Intervention effect of tacrolimus vs. cyclosporine on basocellular skin cancer
64x21mm (300 x 300 DPI)

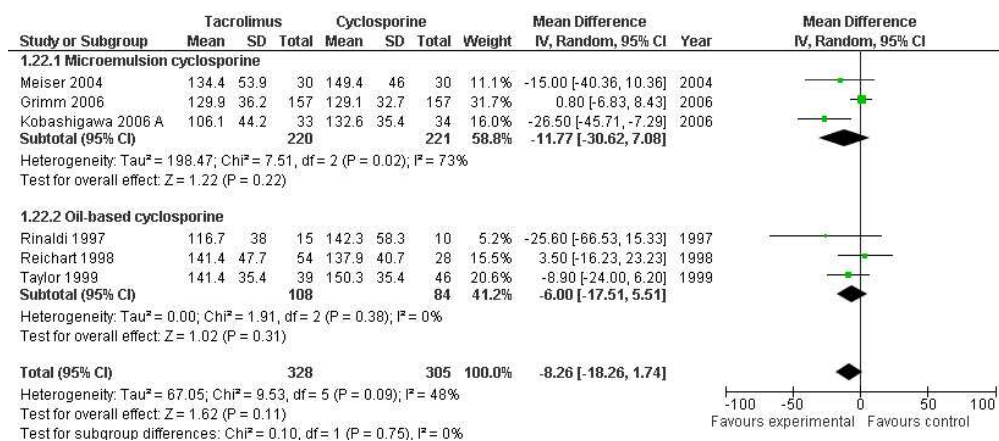
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Intervention effect of tacrolimus vs. cyclosporine on renal failure requiring haemodialysis
64x31mm (300 x 300 DPI)

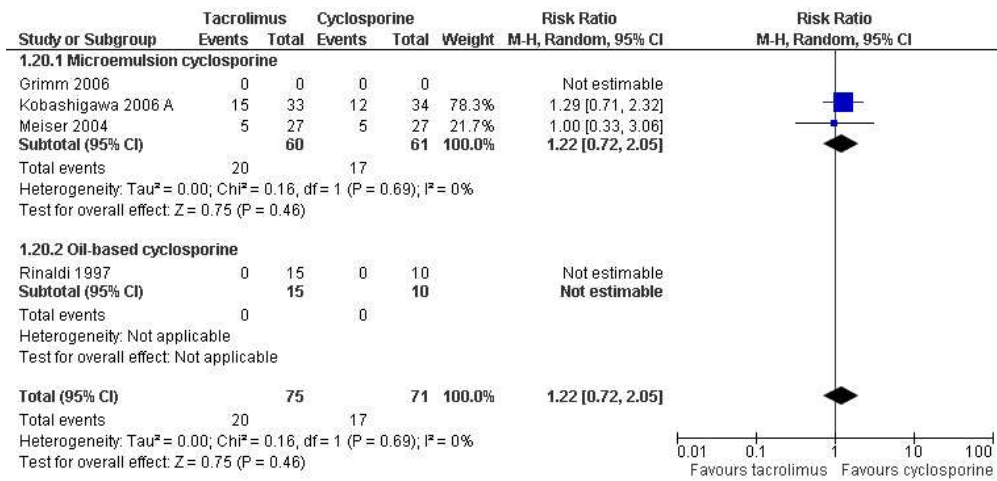
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Intervention effect of tacrolimus vs. cyclosporine on serum creatinine ($\mu\text{mol/L}$)
69x29mm (300 x 300 DPI)

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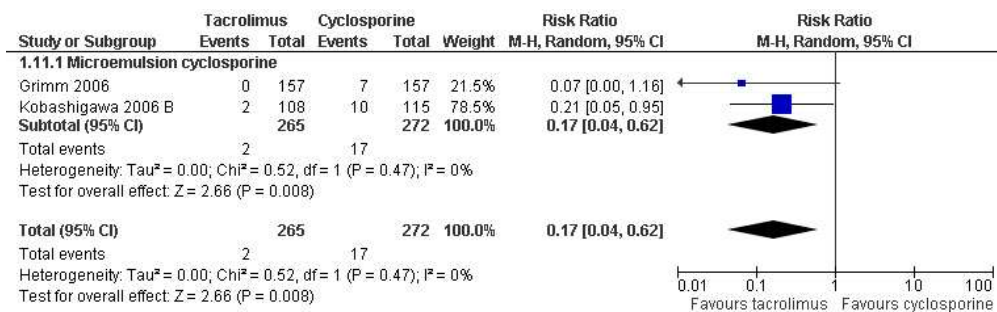
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Intervention effect of tacrolimus vs. cyclosporine on chronic allograft vasculopathy
64x31mm (300 x 300 DPI)

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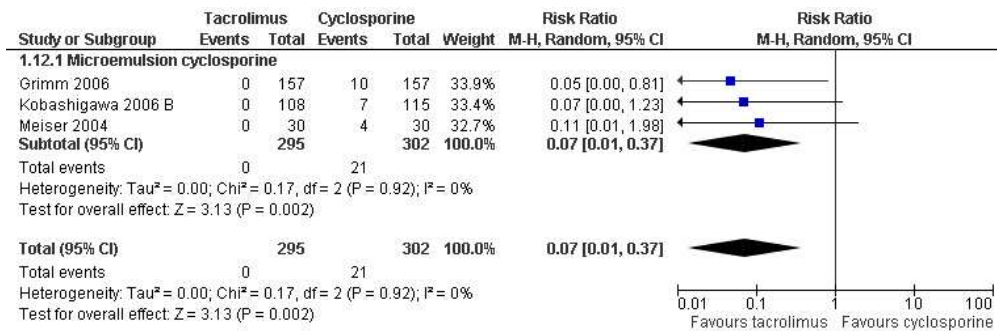
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Intervention effect of tacrolimus vs. cyclosporine on hirsutism
64x20mm (300 x 300 DPI)

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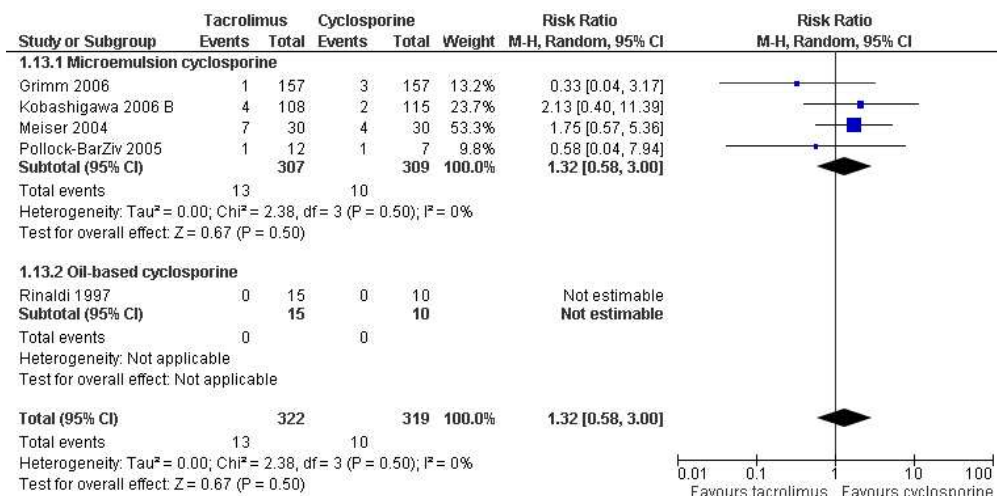
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Intervention effect of tacrolimus vs. cyclosporine on gingival hyperplasia
64x21mm (300 x 300 DPI)

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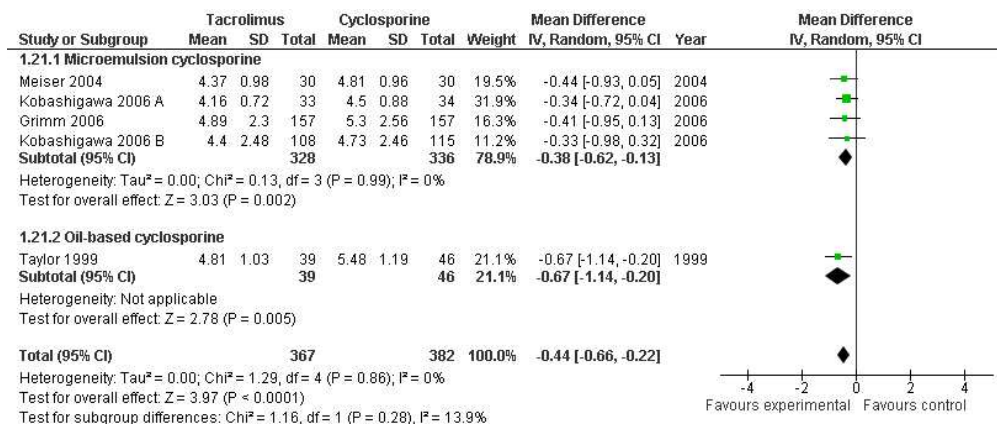
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Intervention effect of tacrolimus vs. cyclosporine on neurotoxicity
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Peer Review

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Intervention effect of tacrolimus vs. cyclosporine on total blood cholesterol (mmol/L)
68x28mm (300 x 300 DPI)

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