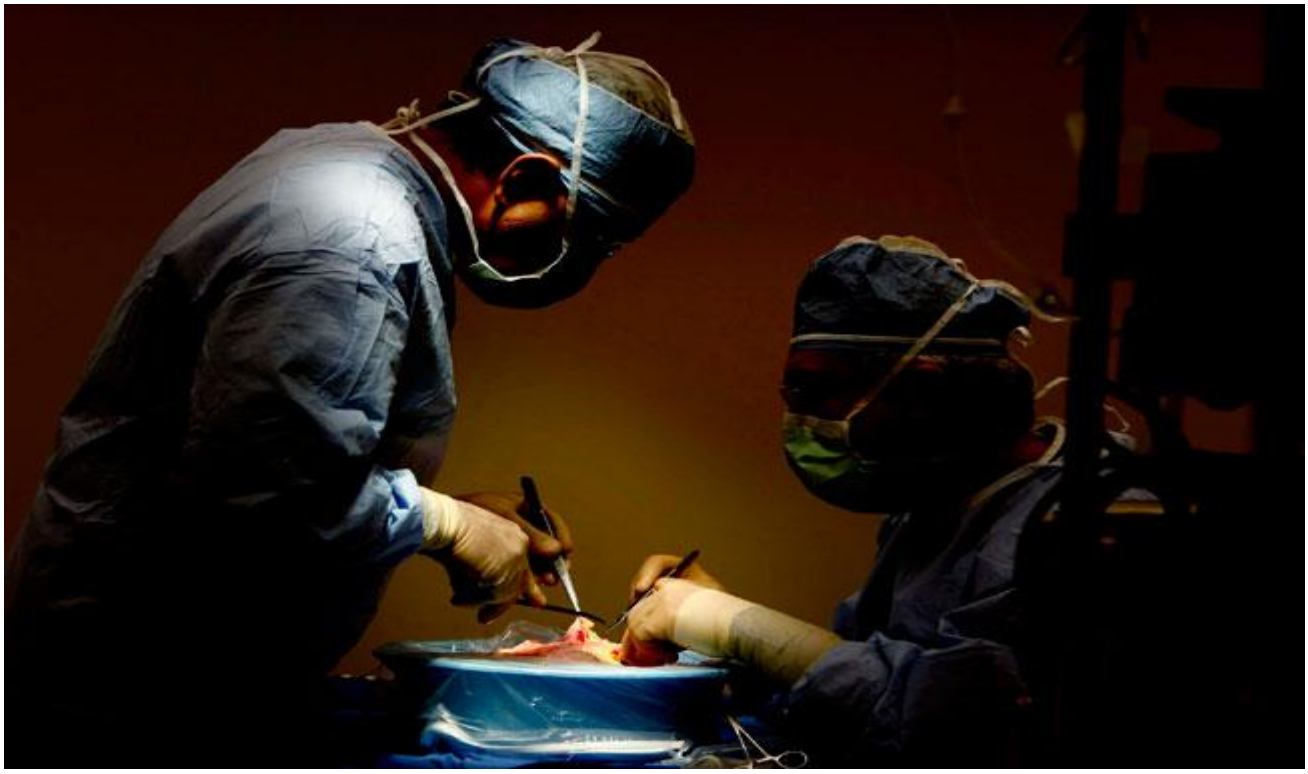
FACULTY OF HEALTH AND MEDICAL SCIENCES
UNIVERSITY OF COPENHAGEN

Immunosuppressive polyclonal and monoclonal T-cell antibody induction therapy for solid organ transplant recipients: systematic reviews with meta-analyses and trial sequential analyses of randomised clinical trials



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To study the phenomena of disease without books is to sail an uncharted sea, while to study books without patients is not to go to sea at all.

In taking up the study of disease, you leave the exact and certain for the inexact and doubtful and enter a realm in which to a great extent the certainties are replaced by probabilities.

SIR WILLIAM OSLER (1849-1919

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Ph.D. thesis

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Contents

Preface

The preparation of a Ph.D. thesis is not an individual project, and many people have contributed to this Ph.D. thesis in one way or another, and I would like to sincerely thank everybody for their help.

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Original papers

This Ph.D. thesis is based on the following papers:

Paper I. Penninga L, Møller CH, Gustafsson F, Gluud C, Steinbrüchel DA. Immunosuppressive T-cell antibody induction for heart transplant recipients. Cochrane Database Syst Rev. 2013 Dec 2;12:CD008842. doi: 10.1002/14651858.CD008842.pub2.

Paper II. Penninga L, Møller CH, Penninga EI, Iversen M, Gluud C, Steinbrüchel DA. Antibody induction therapy for lung transplant recipients. Cochrane Database Syst Rev. 2013 Nov 27;11:CD008927. doi: 10.1002/14651858.CD008927.pub2.

Paper III. Penninga L, Wettergren A. Wilson CH, Chan AW, Steinbrüchel DA, Gluud C. Antibody induction versus placebo, no induction, or another type of antibody induction for liver transplant recipients. Cochrane Database Syst Rev. 2014 June;6:CD010253. doi: 10.1002/14651858.CD010253.pub2.

Paper IV. Penninga L, Wettergren A. Wilson CH, Chan AW, Steinbrüchel DA, Gluud C. Antibody induction versus corticosteroid induction for liver transplant recipients. Cochrane Database Syst Rev. 2014 May;5:CD010252. doi: 10.1002/14651858.CD010252.pub2.

Summary

Background

Transplantation of heart, lung, liver, kidney, and pancreas are accepted treatment modalities for patients with end-stage organ failure. These recipients of solid organ transplants are subject to life-long immunosuppressive medication to avoid rejection of the transplanted organ. Immunosuppression after solid organ transplantation is a very complex medical intervention, and requires a balance between sufficient immunosuppression to avoid rejection of the transplanted organ, while avoiding the risks of over-immunosuppression, and the risks of drug-specific adverse effects.

Polyclonal and monoclonal T-cell specific antibodies can be used to induce immunosuppression shortly after transplantation for preventing rejection. Furthermore, the use of T-cell specific antibodies may allow for delayed introduction of maintenance immunosuppressive drugs. Currently, the harms and benefits of the different types of T-cell specific antibodies are unclear.

Cochrane reviews with meta-analyses and trial sequential analyses of randomised clinical trials generally provide the best available evidence for health care interventions and clinical practice. Such Cochrane reviews are used to assess and summarise benefits and harms of clinical interventions. Furthermore, Cochrane reviews will also reveal lack of evidence, and define the specific need for future randomised clinical trials. Cochrane reviews assessing T-cell specific antibody induction are needed to assess the role of T-cell specific antibodies for induction of immunosuppression after solid organ transplantation.

Objectives

To assess the benefits or harms of T-cell specific antibody induction in heart, lung, liver, pancreas and kidney-pancreas transplant recipients.

Methods

We performed five systematic reviews of all relevant randomised clinical trials. To quantify the estimated effect of various interventions, we performed meta-analyses using The

Cochrane Collaboration methodology and trial sequential analysis. All reviews were performed according to protocols published in the Cochrane Database of Systematic Reviews. Included trials were identified through The Cochrane Library, MEDLINE Ovid, EMBASE Ovid, and Science Citation Index Expanded. In addition, we searched the WHO International Trial Clinical Trial Register Platform, as well the reference lists from included trials and (systematic) reviews, meta-analyses, and health technology assessment reports. Two authors independently screened the retrieved titles and abstracts for inclusion. Data extraction and the assessment of risk of bias were conducted by two authors independently of each other.

Results

The five systematic reviews included a total of 70 randomised clinical trials with 6214 participants. All trials had high risk of bias regarding one or more bias domains (generation of the randomisation sequence, concealment of the randomisation sequence, blinding of patients and personnel, blinding of outcome assessors, incomplete outcome data, selective outcome reporting, and other biases). Therefore, interventional effects should be interpreted conservatively.

We included 22 trials with a total of 1427 heart transplant recipients. We found that acute rejection might be reduced by interleukin-2 receptor antagonists compared with no induction, and by polyclonal antibody induction compared with interleukin-2 receptor antagonists, though trial sequential analyses cannot exclude random errors, and the significance of our observations depended on the statistical model used. Furthermore, in heart transplant recipients no other clear benefits or harms were associated with the use of any kind of T-cell antibody induction compared with no induction, or when one type of T-cell antibody is compared with another type of antibody.

We included six trials with a total of 278 lung transplant recipients. We found no significant differences among interventions in terms of mortality, acute rejection, adverse effects, infection, pneumonia, cytomegalovirus infection, bronchiolitis obliterans syndrome, post-transplantation lymphoproliferative disease, or cancer.

We included 19 trials with 2067 liver transplant recipients comparing antibody induction versus no induction, placebo, or another type of antibody. We found no significant differences in mortality, graft loss including death, adverse events, infection, cancer, post-transplant lymphoproliferative disease, or hepatitis C virus recurrence. Acute rejection seemed to be reduced when any kind of T-cell specific antibody induction was compared with no induction. When trial sequential analysis was applied, the trial sequential monitoring boundary for benefit was crossed before the required information size was obtained.

We included 10 trials with 1489 liver transplant recipients which compared antibody induction versus corticosteroid induction, and no significant differences were found regarding, mortality, graft loss, or acute rejection outcomes. Cytomegalovirus infection may be reduced in patients receiving antibody induction compared with corticosteroid induction. However, when trial sequential analysis was applied, the required information size was not reached, and random errors could not be excluded. Furthermore, diabetes mellitus seemed to be less frequent when T-cell specific antibody induction was compared with corticosteroid induction, and the trial sequential monitoring boundary for benefit was crossed before the required information size was reached.

We could not identify trials with pancreas-transplant-alone recipients. We included 13 trials with 953 simultaneous kidney-pancreas transplant recipients, and we found no significant differences in mortality, pancreas graft loss, renal graft loss, acute pancreas rejection, acute renal rejection, adverse effects, or pancreas and kidney function for the different comparisons.

Conclusions

We did not find any significant differences in mortality and graft loss for the different types of investigated T-cell specific antibodies in heart, lung, liver, and kidney-pancreas transplant recipients. Acute rejection might be reduced in heart and liver transplant recipients when T-cell specific antibody induction was compared with no antibody induction. T-cell specific antibody induction in liver transplant recipients seemed to reduce diabetes mellitus and may reduce cytomegalovirus infection when compared with

corticosteroid induction. For the different comparisons, all trials had methodological limitations increasing the risks of overestimating benefits and underestimating harms (bias), small number of participants increasing the risks of random errors (play of chance), and short trial duration not informing on long-term outcomes. Many of the patient-important outcomes are reported differently in most of the trials. There is a need for well-designed randomised clinical trials with low risk of bias and low risk of play of chance to properly assess induction of immunosuppression with the different types of T-cell specific antibodies in heart, lung, liver, pancreas and kidney-pancreas transplant recipients.

Dansk resumé

Baggrund

Cochrane litteraturbedømmelser (engelsk: systematic reviews) med meta-analyser og sekventielle analyser (engelsk: trial sequential analysis) af randomiserede kliniske forsøg giver vejledning til klinisk praksis, sundhedsrelaterede beslutninger, og nationale og internationale retningslinier. Systematiske literaturoversigter af høj kvalitet opsummerer og giver overblik over eksisterende evidens, og kan fremme eller forhindre implementering af evidens i klinisk praksis. Derudover er systematiske literaturoversigter med til at klarlægge manglende evidens for klinisk praksis, og kan danne baggrund for fremtidige forsøg. Eftersom organtransplantation er blevet en etableret behandling for patienter med organsvigt, er det yderst vigtigt at reducere dødelighed og lidelserne efter transplantation og at øge langtidsfunktion af det transplanterede organ.

Formål

At vurdere gavn og skade af behandling med T-celle specifikke antistoffer for at forhindre afstødning af det transplanterede organ efter: 1) hjertertransplantation; 2) lungetransplantation; 3) levertransplantation i sammenligning med ingen antistoffer, eller i sammenligning med andre typer af T-celle specifikke antistofbehandlinger; 4) levertransplantation i sammenligning med induktion med kortikosteroider; og 5) pankreas og nyre-pankreas transplantation.

Metode

Vi gennemførte fem systematiske literaturoversigter indkluderende relevante randomiserede kliniske forsøg. Til at kvantificere den estimerede effekt af de undersøgte interventioner, udførte vi meta-analyser i henhold til Cochrane samarbejdets metodologiske anbefalinger samt sekventiel analyse af forsøgene. Alle publikationer var baseret på protocoller som vi publicerede i Cochrane Databasen for Systematiske Oversigsigtsartikler. Vi søgte systematisk efter randomiserede kliniske forsøg i The Cochrane Llibrary, MEDLINE, EMBASE, Science Citation Index Expanded, og WHO International Clinical Trial Platform. To forfattere screenede uafhængigt af hinanden

søgeresultaterne for om de opfyldte inklusionskriterierne. To forfattere foretog data ekstraktion og vurdering af risiko for bias uafhængigt af hinanden.

Resultater

Fem systematiske oversigtsartikeler inkluderede i alt 70 randomiserede kliniske forsøg med 6214 patienter. Alle forsøgene havde høj risiko for bias i en eller flere bias domæner (generering af randomiserings sekvens; skjult allokering; blinding af patienter og personale; blinding af effektmåls vurderer; inkomplette data om effektmål; selektiv rapportering af effektmål, andre bias risici). Derfor bør alle interventionseffekter tolkes konservativt.

Vi inkluderede 22 forsøg med 1427 hjertetransplanterede patienter. Akut afstødning bliver muligvis reduceret når interleukin-2 receptor antagonister (IL-2 RA) sammenlignes med ingen induktionsterapi, og når polyklonale antistoffer sammenlignes med IL-2 RA, dog kan sekventiel analyse af forsøgne ikke udelukke tilfældige fejl, og det signifikante fund var afhængig af den anvendte statistiske analyse model. Ingen andre signifikante forskelle fandtes ved anvendelsen af T-celle specifikke antistof induktionsterapi sammenlignet med ingen antistof induktion, eller en anden type af antistof induktion.

Vi inkluderede seks forsøg med 278 lungetransplanterede patienter, og vi fandt ingen statistisk signifikante forskelle angående mortalitet, akut afstødning, bivirkninger, infektioner, lungebetændelse, cytomegalovirus infektion, bronchiolitis obliterans syndrom, post-transplantation lymfoproliferativ sygdom, eller kræft.

Vi inkluderede 19 forsøg med 2067 levertransplanterde patienter som sammenlignede antistof induktion versus ingen antistof induktion, placebo, eller en andet type antistof induktion. Vi fandt ingen signifikante forskelle i mortalitet, levergraft overlevelse, bivirkninger, infektioner, kræft, post-transplantation lymfoproliferativ sygdom, og fornyet hepatitis C virus infektion. Akut afstødning ser ud til at være reduceret når antistof induktion sammenlignes med ingen antistof induktion, og sekventiel analyse af forsøgne viser at grænsen for gavn er overskreden.

Vi inkluderede 10 forsøg med 1489 levertransplanterede patienter som sammenlignede antistof induktionsterapi med induktion med kortikosteroider. Vi fandt ingen signifikante forskelle i dødelighed, levergraft overlevelse, og akut afstødning. Cytomegalovirus infektion ser ud til at være reduceret i patienter som får antistof induktion sammenlignet med kortikosteroid induktion. Sekventiel analyse af forsøgene viste at den nødvendige informationsstørrelse ikke var opnået, og tilfældige fejl kan ikke udelukkes. Desuden er diabetes mellitus signifikant mindre hyppigt når antistof induktion sammenlignes med induktion med kortikosteroider, og sekventiel analyse af forsøgene viser at grænsen for gavn er overskreden.

Vi fandt ingen randomiserede forsøg med isolerede pankreas transplanterede patienter. Vi inkluderede 13 forsøg med 953 nyre-pankreas transplanterede patienter. Vi fandt ingen signifikant forskelle i mortalitet, pankreasgraft overlevelse, nyregraft overlevelse, pankreasafstødning, nyreafstødning, bivirkninger, og nyre– og pankreasfunktion.

Konklusioner

Baseret på de tilgængelige randomiserede kliniske forsøg er der fortsat usikkerhed om de gavnlige og skadelige virkninger af T-celle specifikke antistoffer for induktionsbehandling efter organtransplantation. Vi fandt ingen statistisk signifikant forskel i dødelighed og overlevelse af det transplanterede organ for de forskellige typer T-celle specifike antistoffer hos hjerte, lung, lever, og nyre-pankreas transplanterede patienter. T-celle specifikke antistoffer anvendt hos lever og hjertet transplanterede patienter ser ud til at reducere akut afstødning. T-celle specifikke antistoffer anvendt hos levertransplanterede patienter ser ud til at reducere diabetes og cytomegalovirus i sammenligning med brug af induktion med kortikosteroider. Alle de inkluderede forsøg var af lav metodologisk kvalitet med risiko for overestimering af gavnlige effekter og underestimering af skadelige effekter (bias), inkluderede få patienter i forsøgene med risiko for tilfældige fejl (tilfældighedernes spil), og havde kort forsøgsvarighed og opfølgning hvilket umuliggør vurdering af langtidseffekter. Der er derfor fortsat et behov for veldesignede og veludførte randomiserede kliniske forsøg af høj metodologisk kvalitet for at evaluere behandlingen med T-celle specifikke antistoffer for at forhindre afstødning efter hjerte, lung, lever, og pankreas og nyre-pankreastransplantation.

Chapter 1

Introduction

Organ transplantation is a valuable treatment option for patients with end-stage organ failure[1]. These recipients of solid organ transplants are subject to life-long immunosuppressive medication to avoid rejection of the transplanted organ[2]. Transplant recipients are at high risk of rejection of the transplanted organ, especially early after transplantation, and rejection becomes less frequent as time passes after transplantation[3;4]. Therefore, clinical practice in solid organ transplantation includes potent high-dose immunosuppression on the day of transplantation, and during the early days and weeks after transplantation. This practice of administration of potent immunosuppressive agents during and shortly after the transplant procedure is called 'induction[5]. Strategies for 'induction' include administration of high doses of conventional maintenance immunosuppressive drugs like corticosteroids, calcineurin-inhibitors (cyclosporine and tacrolimus), or anti-metabolites (mycophenolate mofetil or azathioprine)[6].

T-cell specific antibodies are also frequently used for 'induction' of immunosuppression shortly after transplantation for preventing of rejection. Furthermore, the administration of T-cell specific antibodies may allow for delayed introduction of maintenance immunosuppressive drugs[7].

The best evidence for clinical practice

Cochrane reviews with meta-analyses and trial sequential analyses of randomised clinical trials generally provide the best available evidence for health care interventions and clinical practice[8;9]. Such Cochrane reviews are used to assess and summarise benefits and harms of clinical interventions[9]. Furthermore, Cochrane reviews will also reveal lack of evidence, and define the specific need for future randomised clinical trials[9].

Immunosuppression

In general, immunosuppression after solid organ transplantation is a very complex medical intervention, and requires a careful balance between sufficient immunosuppression to

avoid rejection of the transplanted organ, while avoiding the risks of over-immunosuppression, and the risks of drug-specific adverse effects[10;11].
Currently, the harms and benefits of the different types of T-cell specific antibodies are unclear[11-13]. T-cell specific antibodies are very potent drugs which cause profound immunosuppression, and some agents even remove all circulating T-cells from the circulation[12;14]. This may cause increased risks of infection, post-transplantation lymphoproliferative disease, and malignancy[14-17]. Prolonged use of these T-cell specific antibodies is considered harmful, and consequently T-cell specific antibodies are not used as maintenance immunosuppressive drugs[7;14-16].

The question remains whether T-cell specific antibodies should be used for induction of immunosuppression in solid organ transplants[10;18;19]. There is uncertainty regarding the use of T-cell specific antibody induction, and there is uncertainty regarding the type of T-cell specific antibody to use. This uncertainty is clearly reflected in recent journal articles: is induction therapy still needed for heart transplantation?[20;21]; induction therapy: why, when and which agent?[12]; induction therapy in heart transplantation: is there a role?[5]; the question of induction? maybe not all antibodies are equal….[13]; can antibody prophylaxis allow sparing of other immunosuppressives[22]; to induce or not to induce: do patients at greatest risk for fatal rejection benefit from cytolytic induction therapy[11]; anti-interleukin-2 receptor antibodies in transplantation; what is the basis for choice?[23]; induction therapy in renal transplant recipients; how convincing is the current evidence[24]; post-transplant lymphoproliferative disease, association with induction therapy[25]; acute rejection, T-cell-depleting antibodies, and cancer after transplantation[16].

Furthermore, it has been speculated and hoped for that T-cell specific antibodies could cause a kind of tolerance in the recipient for the transplanted organ[3;26;27]. The proposed mechanism was that a lower number of circulating T-cells at the time of transplantation might smoothen the contact between the donor organ and the recipient, and thereby facilitate the acceptance of the donor organ by the recipient[3;26;27]. Hence, it was hoped for that use of T-cell specific antibody induction might allow for total withdrawal of maintenance immunosuppressive treatment, or if total withdrawal was not feasible, that at least lower doses of maintenance immunosuppressive drugs could be used[22]. Being aware

of the hypothesis that antibody induction therapy can cause tolerance we searched for evidence for this in randomised clinical trials.

This Ph.D. thesis aimed to summarise the evidence from randomised clinical trials regarding the use of T-cell specific antibody induction in solid organ transplant recipients (heart; lung; liver) using meta-analysis and trial sequential analysis. We have not assessed the role of T-cell specific antibody induction in isolated kidney transplant recipients, as this has been done previously[28], but we report on the preliminary results of our Cochrane review on antibody induction for pancreas and kidney-pancreas transplant recipients.

Chapter 2

Polyclonal and monoclonal T-cell specific antibodies used for induction

Different types of T-cell specific antibodies have been used for induction of immunosuppression for solid organ transplant recipients[12;14;23]. In this chapter we shortly describe the different agents, their mechanism of action, and their current availability.

Polyclonal antibodies

Minnesota anti-lymphocyte globulin (ALG), and rabbit and horse anti-thymocyte globulin (ATG) are polyclonal antibodies which have been used for induction. Production and distribution of Minnesota ALG was stopped in 1992 in the United States.[29]. Rabbit ATG and horse ATG are still commercially available. These polyclonal antibodies recognise not only single T-cell epitopes, but a wide range of multiple T-cell epitopes[30]. Furthermore, even other immune cells than T-cells, like B-cells, natural killer cells, and monocytes might be affected by these polyclonal antibodies[30].

Minnesota anti-lymphocyte globulin (ALG)

In 1968, equine ALG was isolated from horses at the University of Minnesota, then purified and used for human transplantation. In 1970, Minnesota ALG received application as an investigational drug from the Food and Drug Administration (FDA) regulations in the US, which meant that the University could produce and distribute ALG, but not sell the drug. Minnesota ALG was then available for induction of immunosuppression as well as treatment of acute rejection in organ transplantation. A decade later many studies reported positive results and outcomes using Minnesota ALG. Minnesota ALG was often used as part of quadruple immunosuppressive drug therapy drug with corticosteroids, azathioprine, and cyclosporine maintenance therapy[6]. After these reports, some considered the outcomes so impressive that antibody induction was considered as a proven beneficial component of immunosuppression. The discussion changed from whether antibody induction should be used towards which type of antibody induction drug to use[13]. Since 1971, The University of Minnesota produced, distributed and sold Minnesota ALG. By the late 1980's the university was producing Minnesota ALG for more than 100 transplant centres around the world. For profit selling of the drug was against the FDA regulations.

Consequently, the FDA withdrew, Minnesota ALG from the market in 1992 due to charges of academic misconduct[29].

Equine anti-thymocyte globulin (ATG)

Equine ATG (ATGAM) is a polyclonal anti-lymphocyte globulin produced by immunisation of horses to human T-lymphocytes. Subsequently, the antibodies are harvested from the horse serum. Equine ATG was first developed in the laboratory of Sir Peter Medawar in the UK, and then used by Thomas Starzl in the 1960's. It has been registered In the US since 1981 for use in kidney transplantation, and was the first commercially available ATG formulation in Europe and the US[30]. It has not been registered for use in non-renal solid organ transplantation. Administration of equine ATG causes profound immunosuppression by a rapid reduction in the number of T-lymphocytes. This prevents, or at least delays, cellular rejection of the transplanted organs. This reduction in T-lymphocytes is due to complement-dependent cell-mediated cytotoxicity, and lysis of lymphocytes. This prevents, or at least delays, cellular rejection of the transplanted organs. Furthermore equine ATG affects other immune cells, and has complex actions involving cytokine pathways. Half-life of the drug is approximately 5 days, and administration is often daily for 14 days or shorter, but reports of 50 subsequent daily doses have been published[30]. Adverse effects associated with the administration of equine ATG are haematological effects like thrombocytopenia and leukopenia (including lymphopenia and neutropenia). Furthermore administration of equine ATG may cause cytokine release syndrome, and influenza-like symptoms may occur. Equine ATG should be administered slowly intravenously through a high-flow vein to avoid thrombophlebitis. Serious adverse effects occur in 5% and include serum-sickness, dyspnoea or apnoea, ARDS, arthralgia, pain, nausea, vomiting and diarrhoea. Filtration of Equine ATG before administration reduces adverse effects

Rabbit anti-thymocyte globulin (ATG)

Rabbit anti-thymocyte globulin is an IgG polyclonal anti-human thymocyte globulin. Rabbit ATG is marketed as two different formulas. Thymoglobulin (manufactured by Genzyme/Sanofi) is produced by immunisation of rabbits with human thymocytes. Rabbit ATG Fresenius is produced in rabbits by immunisation of rabbits with a human Jurkat cell line. Thymoglobulin was the first available rabbit ATG, and appeared on the market in 1984 in Europe, and 1999 in the US. It has been registered in the United States since

1998 for treatment of acute rejection in renal transplant recipients. Furthermore, it is used for treating aplastic anaemia, and graft-versus-host disease after bone marrow transplantation.

Rabbit anti-thymocyte globulin is an IgG polyclonal anti-human thymocyte globulin. After immunisation of the rabbit with either human thymocytes or human Jurkat cell line, the antibodies are obtained from the rabbit. Subsequently, the reactive antibodies to human cells are removed, and the product is pasteurised to avoid viral and bacterial contamination. Administration of rabbit ATG causes immunosuppression. Because of the similarity of thymocyte epitopes to those on mature T lymphocytes, the binding of antibodies to T lymphocytes results in complement-dependent and/or antibody-dependent cell-mediated cytotoxicity, and cell phagocytosis by macrophages. These mechanisms cause the depletion of T-cells from the circulation[30]. Rabbit ATG is given on a daily basis for 1-2 weeks, at a dose of 1.0–1.5 mg/kg daily. Administration of rabbit ATG may cause release of cytokines, and patients might complain about influenza-like symptoms and rashes. Furthermore, the 'cytokine release syndrome' may cause fever, chills, rashes, nausea, vomiting, diarrhoea, hypotension, and dyspnea. This can occur especially with the first infusion[6]. Premedication consisting of corticosteroids, antihistamines, and paracetamol is frequently administered to avoid these adverse effects. Haematological adverse effects, e.g. thrombocytopenia and leukopenia, occur frequently after rabbit ATG administration, and are normally treated by discontinuation of further ATG administrations.

Monoclonal antibodies

Muromonab-CD3 (OKT3) and alemtuzumab (Campath-1H) are monoclonal T-cell specific antibodies which have been used for induction[14]. In addition, a wide variety of monoclonal antibodies have been developed, and tested, like anti-CD2. Some of these monoclonal antibodies have been studied in very small trials, but have not ever been commercially available. Muromonab-CD3 was withdrawn from the market in 2010 due to decreasing demands, and after publication of a Cochrane review which highlighted the high number of adverse events associated with muromonab-CD3 administration[31]. Alemtuzumab is still commercially available[32].

Muromonab-CD3

Muromonab-CD3 (Orthoclone OKT3) is a monoclonal antibody which binds to the CD3 receptor complex at the T-cell. The CD3 molecule is present on all mature T-cells, and binding of Muromonab-CD3 to the CD3 receptor either destroys the T-cell directly or disables the T-cells allograft response. Muromonab-CD3 was the first monoclonal antibody registered in the USA, and was labelled for treatment of rejection in heart, liver and kidney transplant recipients. The good results of the use of Muromonab-CD3 for treatment of rejections initiated the use of muromonab-CD3 for induction of immunosuppresssion to prevent rejection. Muromonab-CD3 was given intravenously over 1 min at a dose of 5 mg/day with a half-life of 18 hours[6;14;17]. Nearly all patients develop the 'cytokine release syndrome' after first–time administration of muromonab-CD3. The cytokine release syndrome could cause influenza-like symptoms like fever, chills, malaise, nausea, headache, arthralgia, myalgia, vomiting, and/or diarrhoea, but might also cause severe hypotension, shortness of breath, hypoxia, and aseptic meningitis, and epileptic seizures [33]. Premedication with corticosteroids, antihistamines, and paracetamol before administration of muromonab-CD3 was a necessity. Human anti-mouse antibodies (HAMA) can develop in patients treated with muromonab-CD3. OKT3 was withdrawn from the US market in 2010 because of decreased demand after introduction of newer agents for antibody induction [6].

Alemtuzumab

Alemtuzumab (Campath or Lemtrada, Genzyme/Sanofi) is a humanised monoclonal IgG antibody against human CD52, a protein present on the surfaces of mature lymphocytes. Alemtuzumab was originally raised by injection of human lymphocyte proteins in rats by Dr. Waldman in 1983 at the department of Pathology at Cambridge University, hence the name Campath-1. To avoid potential complications with the rat proteins of the antibody, the product was humanised, and called Campath-1H.

Waldmann initially developed the drug as a tool to understand the immune system, though it was soon clinically tested for haematological malignancies, organ and bone marrow transplantation, and a variety of auto-immune diseases. Alemtuzumab was used to treat T-cell leukaemia and T-cell lymphoma, and was FDA approved for treatment of chronic

lymphocytic leukaemia in 2001[32]. Alemtuzumab has not been registered for use in organ transplantation. Alemtuzumab (MabCampath) was withdrawn from the market in 2012 in Europe and the US, and substituted by a higher price relaunch product Alemtuzumab (Lembrada) for treatment of multiple sclerosis. In 2013, alemtuzumab was registered for treatment of multiple sclerosis in Europe.

CD52 is a cell surface antigen found on T and B lymphocytes, macrophages, monocytes. and NK cells, as well as on some granulocytes[6;32]. Precisely how binding to CD52 results in lymphocyte depletion remains unclear, but it is likely to involve complement fixation, anti-body-dependent cellular cytotoxicity, and cross-linking of the CD 52 surface antigen[24]. Very mild cytokine release may occur after the initial dose of alemtuzumab. Alemtuzumab is given as an IV infusion over 2 hours, typically as a single dose of 30 mg, or as 20 mg given in two doses several days apart. Possible adverse effects include hypotension, fevers, rigors, chills, rash, bronchospasm, and shortness of breath; thus, premedication with antihistamines and paracetamol is recommended. Because of profound lymphopenia, there is an increased risk of opportunistic infections, and monitoring is recommended[14;32].

Interleukin-2 receptor antagonists

Daclizumab, basiliximab, BT-563, and Lo-Tact-1 are interleukin-2 receptor antagonists which have been used for induction. Interleukin-2 receptor antagonists are monoclonal antibodies, but as they are supposed to act more selectively compared with the other monoclonal antibodies they are often classified separately. Interleukin-2 receptor antagonists block the alpha chain of the high affinity interleukin-2 receptor on activated T-lymphocytes, and inhibit phosphorylation of Jak1, Jak3, and STAT5a/b components of the interleukin-2 receptor dependent activation pathway [23]. Currently, basiliximab is the only interleukin-2 receptor antagonist which is commercially available.

BT-563 and Lo-Tact-1

BT-563 and Lo-Tact-1 are interleukin-2 receptor antagonists used in the 1990's for antibody induction, and were considered less potent interleukin-2 receptor antagonists than daclizumab and basiliximab. Both BT563, and Lo-Tact-1 have not been used for antibody induction for more than a decade[34;35].

Daclizumab

Daclizumab (Zenapax, Roche Pharmaceuticals) is a recombinant mouse-human IL-2 receptor antagonist. Daclizumab was administrated intravenously at 1 mg/kg every 2 weeks for a total of 5 doses, and a period of 2 months, and the goal was to achieve blockade of the interleukin-2 receptor for 12 weeks. Daclizumab contains 10% mouse sequences, and may induce IgE-mediated hypersensitivity reactions. Daclizumab was withdrawn voluntarily from the market in 2009 by Roche. No safety concerns consisted [6;14;23;32].

Basiliximab

Basiliximab (Simulect, Novartis) is a chimeric human-mouse interleukin-2 receptor antagonist which also binds and blocks the alpha subunit of the IL-2 receptor similar to daclizumab. Basiliximab is similar to daclizumab a non-T-cell depleting antibody[6;13;23;32]. Basiliximab is administrated at 20 mg intravenously over 30 min; usually two doses at day 0 and 4 of transplantation, and the first dose should be given within 2 hours prior to transplantation. The goal of basiliximab treatment is to achieve blockade of the interleukin-2 receptor for 4-6 weeks. Basiliximab is generally assumed to be well-tolerated by patients [23]. Basiliximab contains 30% mouse sequences, which may induce IgE-mediated hypersensitivity reactions. Basiliximab is more immunogenic than daclizumab, but for both drugs the development of anti-idiotypic IgE antibodies is rare. Clinical differences between basiliximab and daclizumab are considered to be small[36]. Half-life of basiliximab in liver transplant recipients is severely decreased compared with kidney transplantation, was shown by pharmacokinetic studies, especially in liver transplant recipients with ascitic fluid drainage[23].

Chapter 3

Antibody induction for heart transplant recipients (paper I)

In this Cochrane review we assessed the benefits, harms, feasibility and tolerability of immunosuppressive T-cell antibody induction versus placebo, or no antibody induction, or another kind of antibody induction for heart transplant recipients.

We included all randomised clinical trials assessing immunosuppressive T-cell antibody induction for heart transplant recipients. Within individual trials, we required all participants to receive the same maintenance immunosuppressive therapy.

We assessed mortality, acute rejection, infection, *Cytomegalovirus* (CMV) infection, post-transplantation lymphoproliferative disorder, cancer, adverse events, chronic allograft vasculopathy, renal function, hypertension, diabetes mellitus, and hyperlipidaemia.

In this review, we included 22 randomised clinical trials that investigated the use of T-cell antibody induction, with a total of 1427 heart-transplant recipients. All trials were with high risk of bias. Five trials, with a total of 606 participants, compared any kind of T-cell antibody induction versus no antibody induction; four trials, with a total of 576 participants, compared interleukin-2 receptor antagonist versus no induction; one trial, with 30 participants, compared monoclonal antibody (other than interleukin-2 receptor antagonist) versus no antibody induction; two trials, with a total of 159 participants, compared interleukin-2 receptor antagonist versus monoclonal antibody (other than interleukin-2 receptor antagonist) induction; four trials, with a total of 185 participants, compared interleukin-2 receptor antagonist versus polyclonal antibody induction; seven trials, with a total of 315 participants, compared monoclonal antibody (other than interleukin-2 receptor antagonist) versus polyclonal antibody induction; and four trials, with a total of 162 participants, compared polyclonal antibody induction versus another kind or dose of polyclonal antibodies.

No significant differences were found for any of the comparisons for the outcomes of mortality, infection, CMV infection, post-transplantation lymphoproliferative disorder,

cancer, adverse events, chronic allograft vasculopathy, renal function, hypertension, diabetes mellitus, or hyperlipidaemia. Acute rejection occurred significantly less frequently when IL-2 RA induction was compared with no induction (93/284 (33%) versus 132/292 (45%); RR 0.73; 95% CI 0.59 to 0.90; I^2 57%; low-quality evidence) applying the fixed-effect model. No significant difference was found when the random-effects model was applied (RR 0.73; 95% CI 0.46 to 1.17; I^2 57%; low-quality evidence). In addition, acute rejection occurred more often statistically when interleukin-2 receptor antagonist induction was compared with polyclonal antibody induction (24/90 (27%) versus 10/95 (11%); RR 2.43; 95% CI 1.01 to 5.86; I^2 28%; low-quality evidence). For all of these differences in acute rejection, trial sequential alpha-spending boundaries were not crossed and the required information sizes were not reached when trial sequential analysis was performed, indicating that we cannot exclude random errors.

We observed some occasional significant differences in adverse events in some of the comparisons, however, definitions of adverse events varied between trials, and numbers of participants and events in these outcomes were too small to allow definitive conclusions to be drawn.

This review shows that acute rejection might be reduced by interleukin-2 receptor antagonist compared with no induction, and by polyclonal antibody induction compared with interleukin-2 receptor antagonist, though trial sequential analyses cannot exclude random errors, and the significance of our observations depended on the statistical model used. Furthermore, this review does not show other clear benefits or harms associated with the use of any kind of T-cell antibody induction compared with no induction, or when one type of T-cell antibody is compared with another type of antibody. The number of trials investigating the use of antibodies against T-cells for induction after heart transplantation is small, and the number of participants and outcomes in these randomised clinical trials is limited. Furthermore, the included trials are at a high risk of bias. Hence, more randomised clinical trials are needed to assess the benefits and harms of T-cell antibody induction for heart-transplant recipients. Such trials ought to be conducted with low risks of systematic and random errors.

Chapter 4

Antibody induction for lung transplant recipients (paper II)

In this review, we aimed to assess the benefits and harms of immunosuppressive T-cell antibody induction with ATG, ALG, interleukin-2 receptor antagonist, alemtuzumab, or muromonab-CD3 for lung transplant recipients.

We included all randomised clinical trials that compared immunosuppressive monoclonal and polyclonal T-cell antibody induction for lung transplant recipients. An inclusion criterion was that all participants must have received the same maintenance immunosuppressive therapy within each study.

Our review included six randomised clinical trials (representing a total of 278 adult lung transplant recipients) that assessed the use of T-cell antibody induction. All trials were with high risk of bias.

We conducted comparisons of polyclonal or monoclonal T-cell antibody induction versus no induction (3 studies, 140 participants); polyclonal T-cell antibody versus no induction (3 studies, 125 participants); interleukin-2 receptor antagonists (IL-2RA) versus no induction (1 study, 25 participants); polyclonal T-cell antibody versus muromonab-CD3 (1 study, 64 participants); and polyclonal T-cell antibody versus interleukin-2 receptor antagonist (3 studies, 100 participants). Overall we found no significant differences among interventions in terms of mortality, acute rejection, adverse effects, infection, pneumonia, cytomegalovirus infection, bronchiolitis obliterans syndrome, post-transplantation lymphoproliferative disease, or cancer.

We found a significant outcome difference in one trial that compared anti-thymocyte globulin versus muromonab-CD3 relating to adverse events (25/34 (74%) versus 12/30 (40%); RR 1.84, 95% CI 1.13 to 2.98; low-quality evidence). However, trial sequential analysis found that the required information size had not been reached, and the cumulative Z-curve did not cross the trial sequential alpha-spending monitoring boundaries.

None of the studies reported quality of life or kidney injury. Trial sequential analyses indicated that none of the meta-analyses achieved the required information sizes and the cumulative Z-curves did not cross the trial sequential alpha-spending monitoring boundaries, nor reached the area of futility.

We concluded that no clear benefits or harms associated with the use of T-cell antibody induction compared with no induction, or when different types of T-cell antibodies were compared were identified in this review. Few studies were identified that investigated use of antibodies against T-cells for induction after lung transplantation, and numbers of participants and outcomes were also limited. All trials were with high risk of bias.

Further randomised clinical trials are needed to perform robust assessment of the benefits and harms of T-cell antibody induction for lung transplant recipients. Such trials ought to be conducted with low risks of systematic and random errors.

Chapter 5

Antibody induction versus placebo, no induction, or another type of antibody induction for liver transplant recipients (paper III)

In this Cochrane review we assessed randomised clinical trials on immunosuppression with T-cell specific antibody induction compared with placebo, no induction, or another type of antibody induction in liver transplant recipients. Our inclusion criteria stated that patients within each included trial should have received the same maintenance immunosuppressive therapy. We planned to include trials with all the different types of T-cell specific antibodies which are or have been used for induction, i.e., polyclonal antibodies (rabbit or horse anti-thymocyte globulin (ATG), or anti-lymphocyte globulin (ALG)), monoclonal antibodies (muromonab-CD3, anti-CD2, or alemtuzumab), and interleukin-2 receptor antagonists (daclizumab, basiliximab, BT563, or Lo-Tact-1).

We included 19 randomised clinical trials with a total of 2067 liver transplant recipients. All 19 trials were with high risk of bias. Of the 19 trials, 16 trials were two-armed trials, and three trials were three-armed trials. Hence, we found 25 comparisons with antibody induction agents: interleukin-2 receptor antagonist (IL-2 RA) versus no induction (10 trials with 1454 patients); monoclonal antibody versus no induction (five trials with 398 patients); polyclonal antibody versus no induction (three trials with 145 patients); interleukin-2 receptor antagonist versus monoclonal antibody (one trial with 87 patients); and interleukin-2 receptor antagonist versus polyclonal antibody (two trials with 112 patients). Thus, we were able to compare T-cell specific antibody induction versus no induction (17 trials with a total of 1955 patients). Overall, there was no difference in mortality, graft loss including death, and adverse events outcomes between any kind of T-cell specific antibody induction compared with no induction when the T-cell specific antibody induction agents were analysed together or in separate. Acute rejection seemed to be reduced when any kind of T-cell specific antibody induction was compared with no induction (RR 0.85; 95% CI 0.75 to 0.96; I^2 20%; moderate-quality evidence) and when applying trial sequential analysis, the trial sequential monitoring boundary for benefit was crossed before the required information size was obtained. Furthermore, serum creatinine was statistically significantly higher when T-cell specific antibody induction was compared with

'no induction' (MD 3.77 µmol/L; 95% CI 0.33 to 7.21; low-quality evidence), as well as when polyclonal T-cell specific antibody induction was compared with 'no induction', but this small difference was not clinically significant. We did not find any statistical significant differences for any of the remaining pre-defined outcomes: infection, cytomegalovirus infection, hepatitis C recurrence, malignancy, post-transplant lymphoproliferative disease, renal failure requiring dialysis, hyperlipidaemia, diabetes mellitus, and hypertension when the T-cell specific antibody induction agents were analysed together or in separate. Limited data were available for meta-analysis on drug-specific adverse events like haematological adverse events for anti-thymocyte globulin.

When T-cell specific antibody induction agents were compared with another type of antibody induction, no statistical significant differences were found for mortality, graft loss, and acute rejection for the separate analyses. When interleukin-2 receptor antagonists were compared with polyclonal T-cell specific antibody induction, drug-related adverse events were less common in the patients treated with interleukin-2 receptor antagonists (RR 0.23; 95% CI 0.09 to 0.63; low-quality evidence), but this was caused by the results from one trial, and trial sequential analysis could not exclude random errors. We did not find any statistical significant differences for any of the remaining pre-defined outcomes: infection, cytomegalovirus infection, hepatitis C recurrence, malignancy, post-transplant lymphoproliferative disease, renal failure requiring dialysis, hyperlipidaemia, diabetes mellitus and hypertension

We conclude that the effects of T-cell antibody induction remain uncertain due to the low quality of the evidence, the small number of randomised clinical trials for certain comparisons which provide data to measure review outcomes, as well as the limited number of participants in the trials. T-cell specific antibody induction seems to reduce acute rejection when compared with no induction. No other clear benefits or harms were associated with the use of any kind of T-cell specific antibody induction compared with no induction, or when compared with another type of T-cell specific antibody. Furthermore, the included trials are all with high risk of bias. Hence, more randomised clinical trials are needed to assess the benefits and harms of T-cell specific antibody induction compared with placebo, and compared with another type of antibody for prevention of rejection in

liver transplant recipients. Such trials ought to be conducted with low risks of systematic and random errors.

Chapter 6

Antibody induction versus corticosteroid induction for liver transplant recipients (paper IV)

In this Cochrane review we assessed the benefits and harms of T-cell specific antibody induction compared with corticosteroid induction for liver transplant recipients.

We assessed all randomised clinical trials on immunosuppression with T-cell specific antibody induction compared with corticosteroid induction in liver transplant recipients. We stipulated in our inclusion criteria that, within each trial, patients should have received the same maintenance immunosuppressive therapy.

We included 10 randomised trials with a total of 1589 liver transplant recipients which studied the use of T-cell specific antibody induction versus corticosteroid induction. All trials were with high risk of bias. We compared any kind of T-cell specific antibody induction versus corticosteroid induction in 10 trials with 1589 participants, including interleukin-2 receptor antagonist induction versus corticosteroid induction in nine trials with 1470 participants, and polyclonal T-cell specific antibody induction versus corticosteroid induction in one trial with 119 patients.

Our analyses showed no significant differences regarding mortality, graft loss, and acute rejection, infections, hepatitis C virus recurrence, malignancy, and post-transplantation lymphoproliferative disorder when any kind of T-cell specific antibody induction was compared with corticosteroid induction. Cytomegalovirus infection was less frequent in patients receiving any kind of T-cell specific antibody induction compared with corticosteroid induction (RR 0.50; 95% CI 0.33 to 0.75; I^2 3%; low-quality evidence). This was also observed when interleukin-2 receptor antagonist induction was compared with corticosteroid induction (RR 0.55; 95% CI 0.37 to 0.83; I^2 0%; low-quality evidence), and when polyclonal T-cell specific antibody induction was compared with corticosteroid induction (RR 0.21; 95% CI 0.06 to 0.70; low-quality evidence). However, when applying trial sequential analysis regarding cytomegalovirus infection the required information size

was not reached. Furthermore, diabetes mellitus occurred less frequently when T-cell specific antibody induction was compared with corticosteroid induction (RR 0.45; 95% CI 0.34 to 0.60; I^2 0%; moderate-quality evidence), when interleukin-2 receptor antagonist induction was compared with corticosteroid induction (RR 0.45; 95% CI 0.35 to 0.61; I^2 0%; moderate-quality evidence), and also when polyclonal T-cell specific antibody induction was compared with corticosteroid induction (RR 0.12; 95% CI 0.02 to 0.95; low-quality evidence). When applying trial sequential analysis, the trial sequential monitoring boundary for benefit was crossed. We found no subgroup differences for type of interleukin-2 receptor antagonist (basiliximab compared to daclizumab). Definitions of adverse events were different between trials and this complicated pooling of data for this outcome. No data were found on quality of life.

We conclude that due to the low quality of the evidence, the effects of T-cell antibody induction remain uncertain. T-cell specific antibody induction seems to reduce diabetes mellitus and may reduce cytomegalovirus infection when compared with corticosteroid induction. No other clear benefits or harms were associated with the use of T-cell specific antibody induction compared with corticosteroid induction. For some of the analyses, the number of trials investigating the use of T-cell specific antibody induction after liver transplantation is small, and the numbers of patients and outcomes in these randomised trials are limited. Furthermore, the included trials are heterogeneous in nature and have applied different types of T-cell specific antibody induction therapy. All trials were with high risk of bias. Hence, more randomised clinical trials are needed to assess the benefits and harms of T-cell specific antibody induction compared with corticosteroid induction for liver transplant recipients. Such trials ought to be conducted with low risks of systematic and random errors.

Chapter 7

Antibody induction for pancreas and kidney-pancreas transplant recipients (abstract)

In this Cochrane review we assessed randomised clinical trials on T-cell specific antibody induction compared with placebo, no induction, or another type of antibody induction in pancreas and kidney-pancreas transplant recipients. Our inclusion criteria stated that patients within each included trial should have received the same maintenance immunosuppressive therapy. We planned to include trials with all the different types of T-cell specific antibodies which are or have been used for induction, i.e., polyclonal antibodies (rabbit or horse anti-thymocyte globulin (ATG), or anti-lymphocyte globulin (ALG)), monoclonal antibodies (muromonab-CD3, anti-CD2, or alemtuzumab), and interleukin-2 receptor antagonists (daclizumab, basiliximab, BT563, or Lo-Tact-1).

We identified randomised trials assessing antibody induction for kidney-pancreas transplant recipients, but not for isolated pancreas transplantation. We included 13 randomised clinical trials with a total of 953 kidney-pancreas transplant recipients. All 13 trials were with high risk of bias. Of the 13 trials, 12 trials were two-armed trials, and one trial was a three-armed trial.

We conducted comparisons of T-cell specific antibody induction versus no antibody induction in six trials, 716 participants); polyclonal T-cell specific antibody induction (anti-thymocyte globulin) versus no antibody induction (2 trials, 100 participants); interleukin-2 receptor antagonists versus no antibody induction (3 trials, 388 participants); polyclonal T-cell specific antibody induction versus interleukin-2 receptor antagonist induction (2 trials, 79 participants); polyclonal T-cell specific antibody induction versus monoclonal T-cell specific antibody induction (4 trials; 126 participants); low-dose muromonab-CD3 versus standard-dose muromonab-CD3 (1 trial; 10 participants).

Overall, there was no difference in mortality, graft loss including death, and acute pancreas and kidney rejections between any kind of T-cell specific antibody induction

compared with no induction when the T-cell specific antibody induction agents were analysed together or in separate. We did not find any statistical significant differences for any of the remaining pre-defined outcomes: infection, sepsis, cytomegalovirus infection, malignancy, post-transplant lymphoproliferative disease, treatment withdrawal, adverse events, pancreas graft function, and kidney graft function

We conclude that the effects of T-cell antibody induction for kidney-pancreas recipients remain uncertain due to the low quality of the evidence, the small number of randomised clinical trials for certain comparisons which provide data to measure review outcomes, as well as the limited number of participants in the trials. Furthermore, the included trials are all with high risk of bias. Hence, more randomised clinical trials are needed to assess the benefits and harms of T-cell specific antibody induction compared with placebo, and compared with another type of antibody for prevention of rejection in pancreas and kidney-pancreas transplant recipients. Such trials ought to be conducted with low risks of systematic and random errors.

Discussion

Summary of main results

Our systematic reviews investigated the benefits and harms of T-cell specific antibody induction in solid organ transplant recipients. We included data from 70 trials with a total of 6214 participants. All trials were with high risks of bias.

In each of the systematic reviews, we did not find statistical significant differences in mortality and graft loss for the different types of investigated T-cell specific antibodies in heart, lung, liver, and kidney-pancreas transplant recipients. We found a possible reduction in acute rejection when T-cell specific antibody induction was compared with no antibody induction in heart and liver transplant recipients.

T-cell specific antibody induction in liver transplant recipients seemed to reduce diabetes mellitus and may reduce cytomegalovirus infection when compared with corticosteroid induction.

We did not find evidence for benefit of harm for any of the other predefined outcomes infection, cytomegalovirus infection, post-transplantation lymphoproliferative disorder, cancer, adverse events, renal function, hypertension, diabetes mellitus, and hyperlipidaemia. Neither did we find evidence for benefit or harm for organ specific outcomes like chronic allograft vasculopathy for heart transplant recipients, bronchiolitis obliterans syndrome and pneumonia in lung transplant recipients, and hepatitis C recurrence for liver transplant recipients

For the different comparisons in the Cochrane reviews, a large proportion of the trials had methodological limitations, small number of participants, small number of events, and short trial duration. The different comparisons did not have sufficient power to draw firm conclusions. Many of the different comparisons had wide 95% confidence intervals, which might both hide beneficial and harmful effects.

We applied trial sequential analysis on the different outcomes for the different comparisons, and found that we lack firm evidence to draw firm conclusions both

regarding benefits and harms of the different type of T-cell specific antibodies. Therefore, we conclude that there is a need for well-designed, large randomised clinical trials with low risk of bias and low risk of play of chance to properly assess induction of immunosuppression with different types of T-cell specific antibodies in heart, lung, liver, pancreas and kidney-pancreas transplant recipients.

Quality of the evidence

Risk of bias is known to impact on the estimated intervention effect, with trials with high risk of bias tending to overestimate beneficial intervention effects and underestimate harmful intervention effects[37-43]. The risk of bias was high in all trials in our Cochrane reviews. Among the 70 trials included in our reviews, no trial was classified as having low risk of bias according to all bias domains (generation of the randomisation sequence, concealment of the randomisation sequence, blinding of patients and personnel, blinding of outcome assessors, incomplete outcome data, selective outcome reporting, for profit bias). Therefore, the estimated intervention effect may possibly be due to systematic errors. Most recent trials applied proper generation of the randomisation sequence, and adequate concealment of the randomisation sequence. Blinding was sparse in the included trials, and this might have influenced the outcomes of the trials. In some health care interventions like surgical procedures, appropriate blinding can be very challenging, if not impossible[44]. However, outcome assessors can nearly always be blinded. Antibody induction is though a short term pharmacological intervention where blinding through use of placebo is feasible.

We included data from 70 randomised clinical trials. Twenty-two of these trials were industry sponsored, and might be subject to industry bias. Industry sponsored studies more often have favorable efficacy results, less harm results, and conclusions in favour of the experimental intervention compared with non-industry supported studies[39].

We were not able to extract individual patient data from the identified randomised trials. Patients receiving heart transplants are transplanted for different reasons[45]. Indications for transplantation vary also among lung, liver and kidney transplant recipients, and patients transplanted for different indications (e.g., liver transplanted patients transplanted for either

autoimmune hepatitis, or hepatitis C virus, or metabolic liver disease) might react differently on antibody induction[11]. Meta-analysis of individual patient data might clarify whether harms and benefits of antibody induction are similar for patients transplanted for different indications[46].

Reporting outcomes that are not predefined in the trials gives rise to other concerns beside reporting bias. Follow-up in the included trials was in general between three months and five years. Hence, we have no evidence from randomised trials on long-term effects (> 5 years) of T-cell specific antibody induction on our outcome measures. Long-term effects would in particular be relevant for outcome measures like mortality, graft loss, infection, chronic allograft vasculopathy, bronchiolitis obliterans syndrome, hepatitis C virus recurrence, post-transplant lymphoproliferative disease, and cancer[15;16]. Furthermore, none of the trials investigated the hypothesis that antibody induction may cause tolerance to the transplanted organ.

Randomised clinical trials are a necessity to clarify the benefits and harms of health care interventions. These randomised clinical trials should be well-designed, well-reported, and transparent. To ensure adequate protocols for these randomised trails, the SPIRIT guidelines have been developed[47;48]. Furthermore, the International Committee of Medical Journal Editors decided in 2005 that these protocols should be published in a clinical trial registry in order to qualify for publication. Furthermore, to ensure adequate reporting with focus on patient important outcomes, The CONSORT (CONsolidated Standards of Reporting Trials) Statement was prepared already in 1996, and updated in 2010[49;50]. To summarize and pool data from single randomised trials, systematic reviews are required, and this reporting of systematic reviews should follow the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) statement[51].

We explored the presence of statistical heterogeneity by the chi-squared test and measured the quantity of heterogeneity by the I^2 test[52]. The chi-squared test has low power in the situation of a meta-analysis when trials have small sample size or are few in number as in this review. This means that while a statistically significant result may indicate a problem with heterogeneity, a non-significant result must not be taken as

evidence of homogeneity. To reflect our concern with heterogeneity, we looked at both fixed-effect model and random-effects model meta-analyses, in order to provide more conservative estimates of effect, and we reported both models when differences were found between the models[52]. Indeed, our review showed some significant results when the fixed-effect model was applied, which were not statistically significant when the random-effects model was applied. This makes our findings less robust.

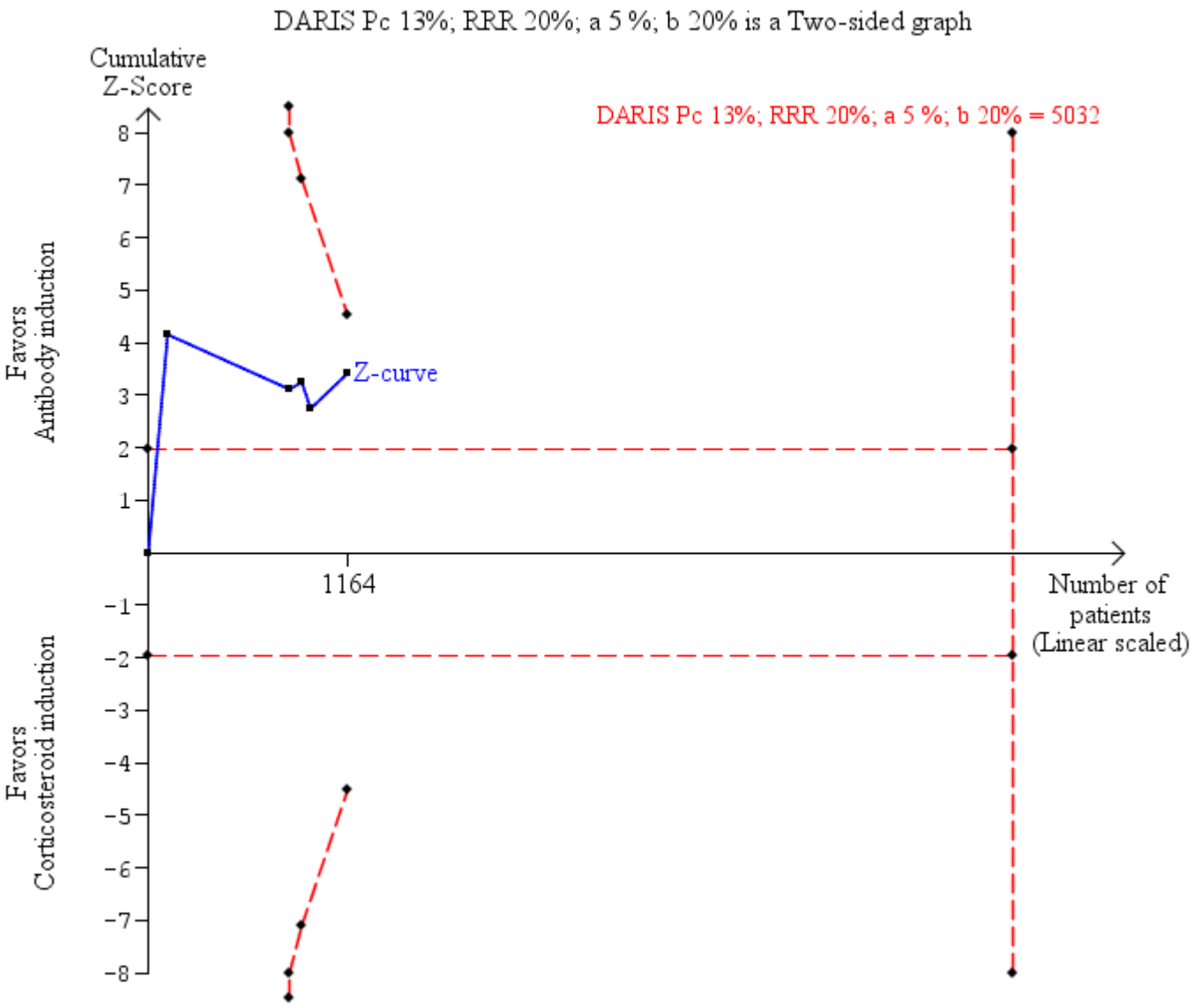
Risks of random errors

Random errors are unpredictable variations in outcome measures, i.e., the play of chance. The risk of random error is higher when data come from small information sizes (or 'sample sizes' for individual trials), so information sizes need to be sufficiently large for the risk of random error to be reduced and the chance of observing a true intervention effect to be increased[53]. Therefore, we also analysed the data using trial sequential analysis. Trial sequential analysis is a statistical method that assesses the risk of random error caused by sparse data and formal or informal repetitive testing of accumulating data. Trial sequential analysis allows us to calculate the required information size (i.e., the number of participants needed in a meta-analysis to detect or reject a certain intervention effect)[53;54]. The information size calculation should also account for the diversity present in the meta-analysis[54]. Trial sequential analysis helps us to assess whether a true intervention effect exists[55]. In our meta-analysis, the required information size was based on the assumption of a plausible RR reduction of 20%, or on the RR reduction observed in the included trials with low risk of bias. The underlying assumption of trial sequential analysis is that testing for significance may be performed each time a new trial is added to the meta-analysis. We added trials according to the year of publication, and when more than one trial was published in a year, we added trials alphabetically according to the last name of the first author. On the basis of the required information size and risk for type I (5%) and type II (20%) errors, we constructed trial sequential monitoring boundaries for benefits, harms, or futility. These boundaries determine the statistical inference that one may draw regarding a cumulative meta-analysis that has not reached the required information size; if a trial sequential boundary for benefit or harm was crossed before the required information size was reached, firm evidence may perhaps be established, and further trials may turn out to be superfluous. On the other hand, if a trial sequential boundary for futility was not

surpassed, it is most probably necessary to continue doing trials to detect or reject a certain intervention effect[53;54;56-60].

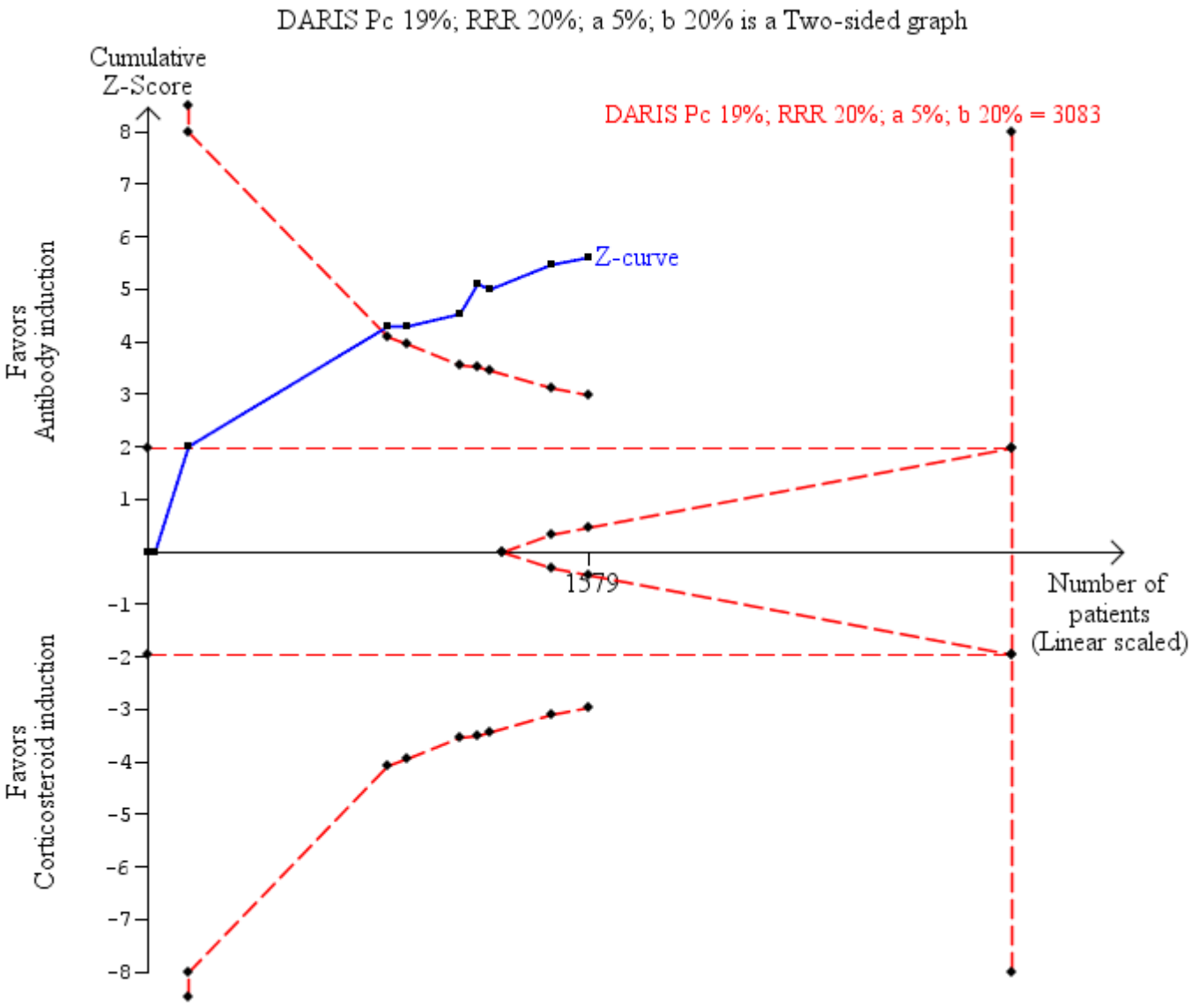
We performed trial sequential analyses for all the outcomes for the different comparisons in the different systematic reviews. Conventional meta-analysis found a statistically significant reduction in both cytomegalovirus infection and diabetes mellitus in liver transplant recipients receiving antibody induction compared with corticosteroid induction. Trial sequential analysis showed that the risk of random error is smaller for diabetes mellitus, as the boundary for benefit is crossed by the cumulative Z-curve

Figure 1



T-cell specific antibody induction versus corticosteroid induction. Outcome: cytomegalovirus infection. Trial sequential analysis of the effect of T-cell specific antibody induction versus corticosteroid induction on cytomegalovirus infection based on five trials with 1164 participants. The diversity-adjusted required information size (DARIS) of 5032 participants was calculated on the basis of type I error of 5%, type II error of 20%, and risk reduction of 20%, and information size was adjusted for diversity (3%). The cumulative Z-curve does not cross the trial sequential monitoring boundaries and the required information size was not reached.

Figure 2



T-cell specific antibody induction versus corticosteroid induction. Outcome: diabetes mellitus. Trial sequential analysis of the effect of T-cell specific antibody induction versus corticosteroid induction on diabetes mellitus based on 10 trials with 1579 participants. The diversity-adjusted required information size (DARIS) of 3083 participants was calculated on the basis of type I error of 5%, type II error of 20%, and risk reduction of 20%, and information size was adjusted for diversity (0%). The required information size was not reached but the cumulative Z-curve crosses the trial sequential monitoring boundary for benefit.

Agreements and disagreements with other studies and reviews

Traditionally, immunosuppressive treatment for heart, lung, liver, pancreas, and kidney-pancreas transplantation has gained much experience from knowledge regarding renal transplantation. New immunosuppressive agents are often first studied in kidney transplant recipients, and when proven effective then applied in non-renal solid organ transplant recipients. Many immunosuppressive agents are registered and approved only for use in kidney transplantation by the FDA and EMA, and are used off-label for non-renal solid organ transplant recipients. As off-label use is widespread, the manufacturers of the immunosuppressive agents have no economic incentive to actively apply for registration, and neither is there the necessity to initiate randomised clinical trials for heart, lung, liver and pancreas transplant recipients required for registration. Whether we can apply results from kidney transplant recipients to other solid organ transplant recipients is, however, unclear. It is assumed that drug-related adverse effects of immunosuppressive agents are similar in the different types of solid organ transplantation[10;12;61]. However, the incidence of acute rejection is different for the different types of solid organ transplant recipients, requiring different immunosuppressive treatments. Lung and pancreas recipients are at highest risk of rejection, heart and kidney at moderate risk, while liver transplant recipients have the lowest risk of rejection. Furthermore, the impact of episodes of acute rejection differs between organs. Acute rejection episodes in lung transplant recipients appear to increase the risk of the bronchiolits obliterans syndrome, though in heart transplant recipients there is no final proof that acute rejection episodes accelerate chronic allograft vasculopathy[10;45;62]. Chronic rejection is very seldom in liver transplant recipients, and it is unclear whether acute rejection episodes in liver transplant recipients have long-term consequences for the liver allograft.

In this Ph.D. thesis we did not assess T-cell specific antibody induction in kidney transplant recipients. A Cochrane review including 71 trials with a total of 10,537 patients studying the use of interleukin-2 receptor antagonists in kidney transplant recipients patients has been performed[28]. In this Cochrane review, interleukin-2 receptor antagonists compared

with placebo seemed to reduce graft loss including death with a functioning graft by 25% at six months (16 trials; RR 0.75, 95% CI 0.58 to 0.98) and one year (24 trials: RR 0.75, 95% CI 0.62 to 0.90), but not beyond these time points[28]. Furthermore, in kidney transplant recipients, interleukin-2 receptor antagonists compared with placebo reduced biopsy-proven acute rejection (14 trials; RR 0.72, 95% CI 0.64 to 0.81) and cytomegalovirus disease (13 trials; RR 0.81, 95% CI 0.68 to 0.97)[28]. Where interleukin-2 receptor antagonists were compared with anti-thymocyte globulin in kidney transplant recipients, biopsy-proven acute rejection at one year was increased in the interleukin-2 receptor antagonists group by 30%, but malignancies (7 trials; RR 0.25, 95% CI 0.07 to 0.87) and cytomegalovirus disease (13 trials; RR 0.68, 95 % CI 0.50 to 0.97) were reduced when interleukin-2 receptor antagonists were compared with anti-thymocyte globulin[28].Hence, the benefits of interleukin-2 receptor antagonist induction found in renal transplant recipients were not observed or were not that convincing in non-renal solid organ transplant recipients. This might be due to the limited number of patients and events, systematic errors, and design errors in non-renal reviews[24]. Alternatively, the Webster 2010 review included trials that were all with high risk of bias. This bias may not have been accounted sufficiently for in the analyses or in the conclusions drawn[28]. Moreover, Webster and colleagues did not employ trial sequential analysis or other measures to control for random errors[28]. Furthermore, the differences between kidney and non-kidney transplant reviews may be explained by organ specific differences.

Transplant registries are widely applied, and indeed almost every solid organ transplant recipient is registered in a transplant registry[63]. These transplant registries have the advantage of being near-complete, and have long-term follow-up. These transplant registries also clearly report which T-cell specific antibodies and other immunosuppressive agents are used for the different types of solid organ transplantation[16]. Furthermore, they report on outcomes for transplant recipients receiving different kind of immunosuppressive agents. Due to the observational nature of the data, these findings should be interpreted with caution because they might suffer from selection bias, confounding by indication, or other confounding factors[9]. For example, patients at high risk of acute rejection might be more likely to receive T-cell specific antibody induction.

Due to the completeness of the transplant registries, they should be used to obtain long-term follow-up after end of study on transplant recipients who have participated in randomised clinical trials[63].

Overall completeness and applicability of the evidence

To identify all available evidence from randomised trials, we conducted extensive searches for trials and included publications in all languages[64]. Furthermore, we included trials on all types of T-cell specific antibodies which are or have been used for induction in solid organ transplantation including both historically used antibodies, and currently available T-cell specific antibodies. We included trials regardless of dose and duration of treatment of the applied T-cell specific antibodies. We have included trials with different maintenance immunosuppressive drugs, but required that concomitant immunosuppressive treatment was similar in the intervention groups within the trial, to be able to properly assess the role of antibody induction. Participants of the included trials underwent organ transplantation for a large variety of indications, and represented a diverse sampling of solid organ transplant recipients. The heterogeneity of the patients in the different trials, and systematic reviews might indeed reflect the well-known heterogeneity in clinical practice. However, only few trials included patients who received an organ from a living or 'donation after cardiac' (DCD) donor, and none of these trials reported separately on outcomes for these patients. Data from paediatric patients were also very limited, as well as data from patients transplanted with donor specific antibodies, and ABO-blood group incompatibility. Furthermore, donor and recipient age have increased during the last decade.

Almost all trials reported on our primary outcomes mortality, graft loss, and acute rejection. The majority of trials reported on infections, cytomegalovirus infections, malignancy, and post-transplant lymphoproliferative disorder. None of the trials reported on quality of life, and only few trials reported on renal failure and function, with few and conflicting results. Limited data were available on drug-specific adverse events like cytokine release syndrome for muromonab-CD3 and haematological adverse events for anti-thymocyte globulin.

Not all types of T-cell specific antibody induction currently available have been studied in randomised clinical trials. Alemtuzumab for induction after solid organ transplantation has

been introduced during the last decade[32]. However, no evidence from randomised clinical trials regarding alemtuzumab in heart, lung, and liver was identified, though we identified a small trial which assessed alemtuzumab in kidney-pancreas transplantation. Other randomized trials with alemtuzumab are only available for kidney transplant recipients. Furthermore, the majority of trials are performed using interleukin-2 receptor antagonists, and trials investigating polyclonal antibodies versus placebo are sparse. In addition, many trials compare the use of T-cell antibody induction with no induction, while fewer trials compare one type of T-cell specific antibody compared with another type of T-cell specific antibody.

Strengths and limitations

We have already touched upon a number of strengths by conducting systematic reviews with bias assessment, meta-analyses and trial sequential analyses above.

Our systematic reviews tried to assess in detail the available evidence on T-cell specific antibody induction for solid organ transplant recipients. However the results are limited by the number, size, and quality of the trials. Furthermore, immunosuppression after solid organ transplantation is a very complex medical intervention involving often three or four other immunosuppressive agents, and a variety of other drugs they might interact with. Proper assessment of other immunosuppressive drugs is important to optimize immunosuppressive treatment[65].

When evaluating benefits and harms we used trial sequential analysis to control for random errors and we applied congruence, i.e., we requested the same thresholds for beneficial and harmful effects. This may not be ethically defensible. Usually we require more solid evidence for benefits than for harms. Moreover, many interventions that may offer benefits have been withdrawn from the market due to just some patients dying in association with the intervention. Accordingly, societies and regulatory agencies do not have congruent requirements for evidence on benefits and harms. Therefore, we may have been too stringent when declaring risks of harm.

One issue that has not been touched upon is the costs of using T-cell specific antibodies for induction[66;67]. The costs of T-cell specific induction agents vary in different countries. These costs might be small compared to the total costs of solid organ transplantation.

However, if T-cell antibody induction does not carry clear benefits on patient-relevant outcomes, then the additional costs of induction are hard to defend[66;67].

Conclusions

Overall, the evidence for making any recommendations for or against the use of T-cell specific antibody induction in recipients of heart, lung, liver, pancreas and kidney-pancreas is sparse. We could not find any statistical significant difference on mortality and graft loss for any of the investigated interventions. Acute rejection may be reduced when T-cell specific antibody was compared with no antibody in heart transplant recipients, however, trial sequential analysis showed that the required information size was not reached. Similar, acute rejection may be reduced when T-cell specific antibody was compared with no antibody in liver transplant recipients, and trial sequential analysis showed that the boundary for benefit was crossed. However, all trials were with high risks of bias.

We did not find any benefit or harm regarding adverse effects and our other pre-defined outcomes. Except from a possible reduction in diabetes mellitus and cytomegalovirus infection when T-cell specific antibody induction was compared with induction with corticosteroids. Trial sequential analysis showed that the boundary for benefit was crossed for diabetes mellitus, but trial sequential analysis could not exclude random errors regarding cytomegalovirus infection. However, all trials were with high risks of bias.
For most of the analyses, the number of trials investigating the use of T-cell specific antibody induction after solid organ transplantation is small, and the number of patients and outcomes in these randomised trials are limited.

Given the result of our analysis, it appears that appropriately sized randomised clinical trials comparing T-cell antibodies versus placebo in solid organ transplant recipients using contemporarily adjunctive immunosuppression and calcineurin-inhibitor sparing regimens are warranted. These trials should study intervention with basiliximab (currently the only interleukin-2 receptor antagonist commercially available), anti-thymocyte globulin, or alemtuzumab. Such trials ought to be conducted with low risks of systematic error (bias) and low risk of random error (play of chance), and should follow the 'SPIRIT' guidelines[47;48] and 'CONSORT' guidelines[49;50].

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