Bootcongres 2013

wetenschappelijke voorjaarsvergadering

Nederlandse Transplantatie Vereniging

13 en 14 maart 2013

i.s.m.

UMC St Radboud

Locatie:

Hotel Duiven

Inhoudsopgave

In het programma vindt u achter de titel van de voordracht een verwijzing naar de pagina waar u het betreffende abstract kunt vinden.

Welkom op het 25e Bootcongres!

Al voor de 25e maal een Bootcongres betekent dat het een traditioneel gebeuren is. Tegelijkertijd ook een goed moment om na te denken over vernieuwing en aanpassing aan actuele ontwikkelingen. Deze editie van het Bootcongres kent daarom drie noviteiten. Ten eerste worden programma en abstracts ook aangeboden op een app. Als dat goed bevalt is dit exemplaar van het programmaboekje wellicht het laatste dat in gedrukte vorm aan u is uitgereikt. Ten tweede kent dit Bootcongres als motto 'de patiënt als partner'. Niet geheel toevallig ook één van de centrale thema's van het organiserend centrum, het UMC St Radboud. Het thema komt tot uitdrukking doordat op woensdagmiddag een deel van het programma in samenwerking met drie patiëntenverenigingen is georganiseerd. De leden van deze verenigingen zijn ook voor het betreffende programmadeel uitgenodigd. Dit biedt de onderzoeker de gelegenheid om over zijn bevindingen te communiceren met hen om wie het uiteindelijk te doen is. Tenslotte zijn de traditionele posterpresentaties vervangen door meer eigentijdse laptoppresentaties.

De organisatiecommissie heeft geprobeerd een gevarieerd en aantrekkelijk programma samen te stellen. Uit 126 ingezonden abstracts zijn ruim 80 voordrachten geselecteerd. Daarnaast is een aantal gerenommeerde sprekers uitgenodigd over diverse onderwerpen, varierend van e-health tot nieuwe orgaanperfusietechnieken. Een van de hoogtepunten van het congres is de uitreiking door erelid Gauke Kootstra van de prijs die naar hem vernoemd is. Ook worden er prijzen uitgereikt voor het beste artikel op transplantatie-gebied (basaal en klinisch), de aanmoedigingsprijs voor translationeel transplantatie onderzoek en een award voor onderzoek dat leidt tot op patiëntenkarakeristieken aangepaste behandeling. Daarnaast zijn er ook dit jaar weer prijzen voor de beste presentaties van iedere sessie, waarbij de sessievoorzitters als jury fungeren.
Het programma wordt afgesloten met de algemene ledenvergadering van de Nederlandse Transplantatie Vereniging. Het bestuur van de NTV heeft tijdens deze vergadering nog een verrassing in petto.
Een gekoesterde traditie van het Bootcongres is de feestavond. De gezelligheid en saamhorigheid die kenmerkend zijn voor de Nederlandse transplantatie-gemeenschap vieren hierbij immer hoogtij. Vanuit de congreslocatie wordt u met bussen vervoerd naar een 150 jaar oud fort aan de oever van de Waal.

Namens de organisatiecommissie wens ik u een inspirerend en onderhoudend Bootcongres toe!

Luuk Hilbrands, voorzitter organisatiecommissie

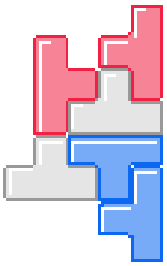
Organisatiecommissie Bootcongres 2013

Marlies Cornelissen
Frank d'Ancona
Mary Hakvoort
Marjo van Helden
Frits Hendrix
Luuk Hilbrands
Andries Hoitsma
Michel van der Jagt
Hans Koenen
Michiel Warlé

Vanuit het secretariaat NTV te Haarlem
Jeanine Gies
Marie José van Gijtenbeek
Marja Weber

Participerende patiëntenverenigingen
Harten Twee
Nederlandse Leverpatiënten Vereniging
Nierpatiënten Vereniging Nederland

Accreditatie is aangevraagd* / toegekend door de volgende verenigingen:



Nederlandse Vereniging voor Heelkunde	12 punten*
Nederlandse Vereniging voor Immunologie	12 punten*
Nederlandse Vereniging van Maag-Darm-Leverartsen	12 punten*
Nederlandse Internisten Vereniging	12 punten*
Nederlandse Vereniging voor Kindergeneeskunde	11 punten

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Op individuele basis kan accreditatie worden aangevraagd bij:

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Nederlandse Vereniging voor Cardiologie

Hotel Duiven (bij Arnhem A12)
Impuls 2
6921 RK Duiven
Telefoon 026-318 8888

Routebeschrijving

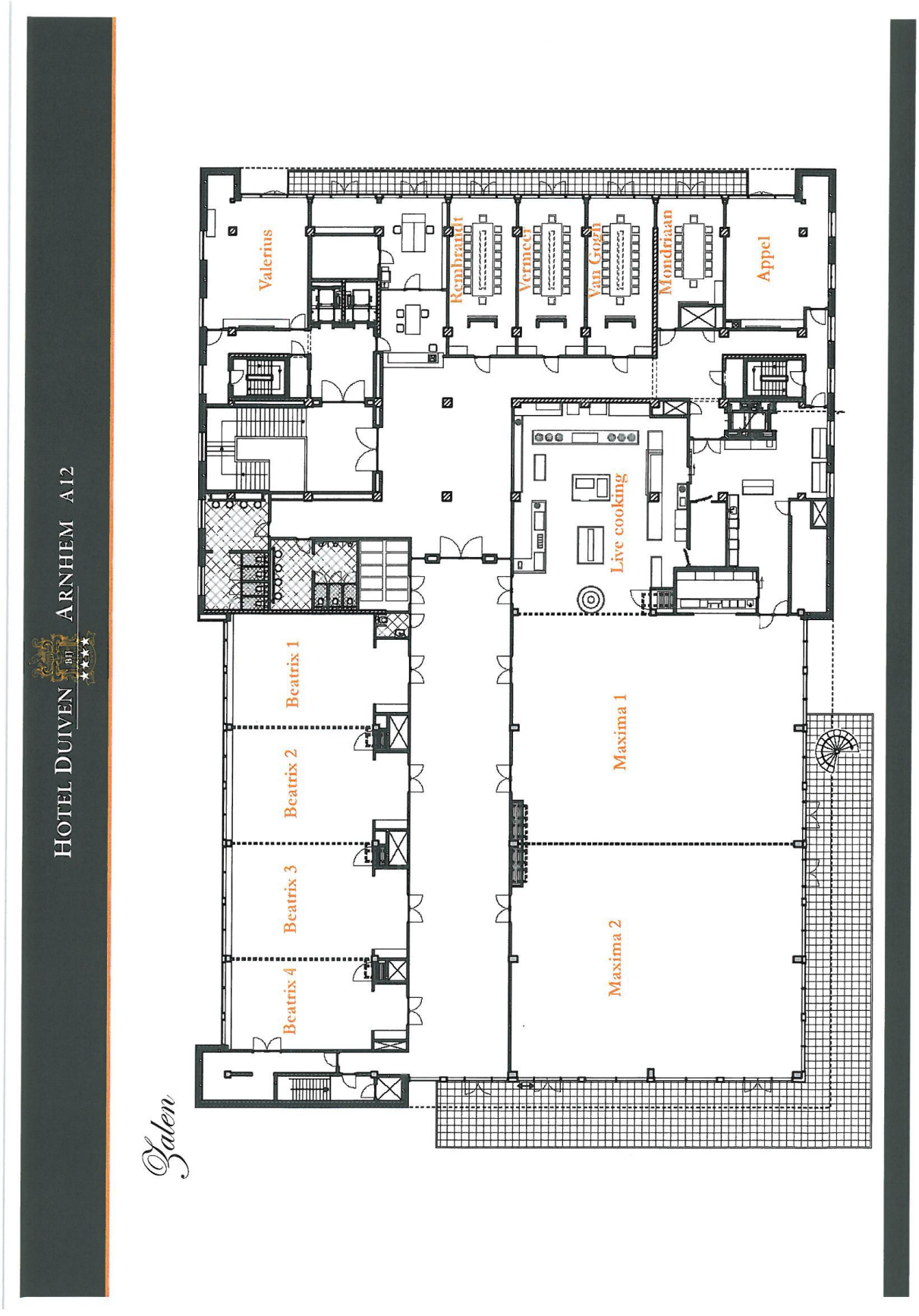
Per auto

Via de A12 rijdt u richting Oberhausen tot afslag Duiven/Zevenaar-west, afrit 28. U kunt het Hotel Duiven bij Arnhem A12 al zien aan de rechterzijde. Aan het einde van deze afrit gaat u bij de stoplichten rechtsaf. Bij de eerstvolgende stoplichten gaat u nogmaals rechtsaf, vervolgens neemt u de eerste afslag rechts en bent u gearriveerd bij het Hotel.

Per Openbaar Vervoer

U reist met de trein naar station Duiven. Vanuit het station brengt buslijn 65 u in 10 minuten naar het hotel.

Plattegrond zalen



Locatie en tijdstippen van de maaltijden

Woensdag

Ontbijt	07.00 - 09.30 uur
Lunch	12.15 - 13.30 uur
Lunch onderwijssessie Beatrixzaal (lunchpakket)	11.50 - 13.05 uur
Diner en feestavond in het Wijnfort te Lent	19.15 - 00.30 uur

Donderdag

Ontbijt	07.00 - 08.30 uur
Lunch	12.30 - 13.30 uur

Bijeenkomsten tijdens Bootcongres

Dinsdag 12 maart 2013

12.00 – 14.00	Landelijk Overleg Transplantatie Thoracale Organen *Locatie: Rembrandtzaal*
14.00 – 16.00	Landelijk Overleg Regionale Uitnameteams *Locatie: Vermeerzaal*
16.00 – 17.30	NTS en NTV (voorzitters commissies) *Locatie: Van Goghzaal*
17.30 – 20.00	Landelijk Overleg Levertransplantatie *Locatie: Beatrixzaal 2*
17.30 – 20.00	Landelijk Overleg Niertransplantatie *Locatie: Beatrixzaal 3*
17.30 – 20.00	Landelijke Werkgroep Transplantatie Verpleegkunde *Locatie: Beatrixzaal 1*

Woensdag 13 maart 2013

08.00 – 10.00 uur	Transplantatie Werkgroep Nederland *Locatie: Beatrixzaal 2*

Schematisch overzicht programma
Woensdag 13 maart 2013

Woensdag 13 maart 2013		**Maxima 2**	**Beatrix 1 en 2**	**Beatrix 3 en 4**
10.00 – 11.45		Plenaire sessie I Prof. dr. J. de Vries: "Imaging van de immuunrespons" Prof. dr. B. Tönshoff: "Treatment of antibody-mediated rejection" Drs. M. van Agteren: "Uitkomsten van ABO-incompatibele niertransplantaties" Drs. M. van den Hoogen: "Inductietherapie met Rituximab bij niertransplantaties"		
11.45 – 12.15		Vervolg plenaire sessie I Basaal-wetenschappelijke abstracts		
11.50 – 13.15			Onderwijssessie	
12.15 – 13.15	Lunchpauze			
		Maxima 1 en 2	**Beatrix 1 en 2**	**Beatrix 3 en 4**
13.30 – 15.30		Plenaire sessie II Samen met patiëntenverenigingen P.A. Zillén: "The patient as partner in health care" Dr. K. Schipper: "De patiënt als partner in onderzoek"		
15.30 – 16.00	Theepauze			
16.00 – 17.45		Parallelsessie III – Klinisch/Verpleegkundig Samen met patiëntenverenigingen	Parallelsessie IV – Klinisch/Basaal	Parallelsessie V – Basaal
18.00 – 18.45	Plenaire afsluiting dag 1, gevolgd door hapje en drankje			
18.45 – 19.00	Busvervoer naar het Wijnfort Dinerbuffet en feestavond			

Schematisch overzicht programma
Donderdag 14 maart 2013

Donderdag 14 maart 2013		**Maxima 2**	**Beatrix 1 en 2**	**Beatrix 3 en 4**
08.30 – 9.30		Plenaire sessie VI L. Engelen: "E-health, wat hebben we eraan?" Dr. A. Rahmel: "Integere allocatie van donororganen"		
09.30 – 11.00	Koffiepauze met laptoppresentaties in Maxima 1, Rembrandt, Vermeer en Van Gogh			
11.00 – 12.30		Parallelsessie VII – Klinisch	Parallelsessie VIII – Allocatie	Parallelsessie IX - Basaal
12.30 – 13.30	Lunchpauze			
13.30 – 14.00		Plenaire sessie X Dr. C. Fondevila: "New perfusion strategies to increase the number and quality of donor organs"		
14.00 – 15.30		Parallelsessie XI – Klinisch		Parallelsessie XII - Basaal
15.30 – 16.00	Theepauze			
16.00 – 17.45		Uitreiking prijzen Ledenvergadering Nederlandse Transplantatie Vereniging		
17.45 – 18.00		Sluiting en vaarwel Vertrek congresdeelnemers		

Sessie I **Plenair** **Maxima 2**

Voorzitters: *L. Hilbrands en D. Roelen*

09.00 Ontvangst en registratie

10.00 Opening

10.05 "**Imaging van de immuunrespons**"
Prof. dr. J. de Vries, hoogleraar Translationele tumorimmunologie
UMC St Radboud Nijmegen

10.35 "**Treatment of antibody-mediated rejection**"
Prof. dr. B. Tönshoff, Professor of Pediatrics and Pediatric Nephrology
University Children's Hospital Heidelberg, Germany

11.05 "**Uitkomsten van ABO-incompatibele niertransplantaties**"
Drs. M. van Agteren, internist-nefroloog
Erasmus MC, Rotterdam

11.25 "**Inductietherapie met Rituximab bij niertransplantaties**"
Drs. M. van den Hoogen, afdeling nefrologie
UMC St Radboud Nijmegen

De deelnemers voor de onderwijssessie kunnen zich begeven naar de Beatrixzaal

Sessie I **Plenair** - vervolg **Maxima 2**

Voordrachten in het Nederlands of Engels, spreektijd 8 minuten, discussietijd 2 minuten.

11.45 CD3 antibody-induced transplant tolerance relies on in situ TGF-beta and PD-1 signaling in T cells (p. 49)
M.C. Baas[1,2,3], A. Besançon[1,2], C. Kuhn, C. Mangez[1,2], F. Valette[1,2], T. Goncalves[1,2], L. Chatenoud[1,2] and S. You[1,2], [1]National de la Santé et de la Recherche Médicale, Unité 1013, Paris, France, [2]Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Paris, France, [3]Present address: Dept. of Nephrology, Radboud University Medical Centre Nijmegen, The Netherlands

11.55 High expression of S100 calcium binding proteins A8 and A9 in macrophages during acute transplant rejection is associated with a beneficial immune response (p. 50)
N.V. Rekers[1], I.M. Bajema[2], M. Mallat[3], J.D.H. Anholts[1], C. Kerkhoff[4], J. Roth[5], G.M.J.S. Swings[1], M.C. van Groningen[6], J.W. de Fijter[3], F.H.J. Claas[1], M. Eikmans[1], [1]Dept of Immunohematology and Blood Transfusion, [2]Dept of Pathology, and [3]Dept of Nephrology, Leiden University Medical Center, Leiden, The Netherlands, [4]Dept of Immunology, Fraunhofer Institute for Cell Therapy and Immunology, Rostock, Germany, [5]Institute of Immunology, University Hospital, Münster, Germany, [6]Dept of Pathology, Erasmus MC, Rotterdam, The Netherlands

12.05 Kidney transplantation does not reverse the premature T cell ageing in end- stage renal disease patients (p. 51)
R.W.J. Meijers[1], N.H.R. Litjens[1], L.E.A. de Wit[1], A.W. Langerak[2], C.C. Baan[1], W. Weimar[1] and M.G.H. Betjes[1], [1]Dept. of Internal Medicine, section Nephrology and Transplantation, [2]Dept. of Immunology, Erasmus Medical Center, Rotterdam, the Netherlands

12.15 Lunchpauze

Onderwijssessie **Beatrixzaal 1, 2, 3 en 4**

11.50 Lunchpakket bij ingang van de zaal
voor alle **geregistreerde** deelnemers aan de onderwijssessie

Voorzitters: L.J.W. van der Laan en M.J.C. Wessels

11.50 "**Infectieuze complicaties na transplantatie**"
Prof. dr. Willem J. van Son
Afd. Interne geneeskunde, Universitair Medisch Centrum Groningen

12.15 "**Chirurgische aspecten van leverdonatie en levertransplantatie**"
Prof. dr. Geert Kazemier
Afd. Heelkunde, VU Medisch Centrum Amsterdam

12.40 "**Dosering van immunosuppressiva in het tijdperk van 'personalized medicine'**"
Prof. dr. Teun van Gelder
Afd. Farmacologie, Erasmus MC Rotterdam

13.05 Einde onderwijssessie

Sessie II Plenair Maxima 1 en 2

Plenaire sessie samen met patiëntenverenigingen

Voorzitters: M. van Helden en H. Bart

13.30 "**The patient as partner in health care**"
P.A. Zillén, ervaringsdeskundige, Saltsjö-Boo, Zweden

14.00 "**De patiënt als partner in onderzoek**"
Dr. K. Schipper, onderzoeker/gezondheidszorgpsycholoog
Afd. Metamedica, VU medisch centrum, Amsterdam

Voordrachten in het Nederlands, spreektijd 8 minuten, discussietijd 4 minuten.

14.30 Unspecified living kidney donors in The Netherlands: an overview (p. 52)
M. de Klerk[1,2], W Zuidema[1] on behalf of the 8 Kidney Transplant Centers in The Netherlands, [1]Dept. of Internal Medicine – Transplantation, Erasmus MC Rotterdam, [2]Dutch Transplant Foundation, Leiden, The Netherlands

14.42 First results of A Randomized Controlled Trial on A Home-Based Educational Intervention (p. 53)
S.Y. Ismail[1], A.E. Luchtenburg[2], W.C. Zuidema[2], W. Weimar[2], J.J. V. Busschbach[1], E.K. Massey[2], Dept. of [1]Medical Psychology and Psychotherapy and [2]Internal Medicine: Kidney Transplant Unit, Erasmus MC, Rotterdam, The Netherlands

14.54 NiCe: Patient-centered care for live kidney donors (p. 54)
E.N. Bossenbroek[1], K.C.K. Tran[1], J.N.T. Sattoe[2], F.J.M.F. Dor[1], W. Weimar[3], J.N.M. IJzermans[1], [1]Dept. of Surgery, division of Transplant Surgery, Erasmus MC, University Medical Center, Rotterdam, The Netherlands, [2]Rotterdam University of Applied Science, Rotterdam, The Netherlands, [3]Dept. of Internal Medicine, division of Nephrology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

Sessie II **Plenair** - vervolg **Maxima 1 en 2**

15.06 Low pressure pneumoperitoneum during laparoscopic donor nephrectomy to optimize live donors' comfort (p. 55)
M.C. Warlé[1], A.W. Berkers[1], J.F. Langenhuijsen[2], M.F. van der Jagt[1], Ph.M. Dooper[3], H.J. Kloke[3], D. Pilzecker[3], S.H. Renes[4], K.E. Wever[1], A.J. Hoitsma[3], J.A. van der Vliet[1], F.C.H. D'Ancona[2], [1]Dept. of Surgery, Division of Vascular- and Transplant Surgery, [2]Dept. of Urology, [3]Dept. of Nephrology, [4]Dept. of Anesthesiology, Radboud University Nijmegen Medical Center, The Netherlands

15.18 28 Years of pancreas transplantation: the Leiden experience (p. 56)
M.J. Verhagen[1,2], J.J. Blok[1,2], J. Ringers[1], J.W. de Fijter[3], A.F. Schaapherder[1], A.G. Baranski[1], J. Dubbeld[1], H. Putter[4], A.E. Braat[1], Dept. of [1]Transplant Surgery, [3]Nephrology and [4]Medical Statistics, Leiden University Medical Center, Leiden, The Netherlands, [2]Eurotransplant International Foundation, Leiden, The Netherlands

15.30 Theepauze

Parallelsessie III - Klinisch/Verpleegkundig Maxima 1 en 2

Parallelsessie samen met patiëntenverenigingen

Voordrachten in het Nederlands, spreektijd 8 minuten, discussietijd 4 minuten.

Voorzitters: H. Bakker en A. Hoitsma

16.00 Kwaliteitsverbetering in de zorg voor kinderen rondom niertransplantatie door inzet gespecialiseerd verpleegkundige (p. 57)
B.G.I. van Gaal[1], J.L. Knoll[2], E.A.M. Cornelissen[2], T. van Achterberg[1], [1]Institute for Quality of Healthcare, [2]Dept. of Pediatric Nephrology, UMC St Radboud, Nijmegen, The Netherlands

16.12 Tacrolimus predose concentrations do not predict the risk of acute rejection after renal transplantation: a pooled analysis from three randomised-controlled clinical trials (p. 58)
R. Bouamar[1], N. Shuker[1], D.A. Hesselink[2], W. Weimar[2], H. Ekberg[3], B. Kaplan[4], C. Bernasconi[5], T. van Gelder[1,, 4, 1]Dept. of Hospital Pharmacy, Clinical Pharmacology Unit, [2]Dept. of Internal Medicine, Renal Transplant Unit, Erasmus MC, Rotterdam, The Netherlands, [3]Dept. of Nephrology and Transplantation, Skane University Hospital, Malmö, Sweden, [4]Dept. of Medicine, Dept. of Surgery, Dept. of Pharmacology, University of Arizona School of Medicine, Tucson, AZ, [5]Limites Medical Research Ltd., Vacallo, Switzerland

16.24 Delayed trough level measurement of tacrolimus QD requires an adjusted target range (p. 59)
G.A.J. van Boekel[1], R.E. Aarnoutse[2], K.E.J. Hoogtanders[3], T. Havenith[3], L.B. Hilbrands[1], [1]Dept. of Nephrology and [2]Dept. of Clinical Pharmacy, Radboud University Nijmegen Medical Centre, The Netherlands, [3]Dept. of Clinical Pharmacy, Maastricht University Medical Center, The Netherlands

16.36 Knowledge about dialysis, transplantation and living donation among prospective living kidney donors and recipients (p. 60)
L. Timmerman[1], S.Y. Ismail[2], A.E. Luchtenburg[1], T. Royaards[1], J.A. Kal - van Gestel[1], W.C. Zuidema[1], J.N.M. IJzermans[3], J.J. van Busschbach[2], W. Weimar[1], E.K. Massey[1], Depts of [1]Internal Medicine, [2]Medical Psychology and Psychotherapy, [3]General Surgery, Erasmus Medical Centre Rotterdam, The Netherlands

Parallelsessie III - Klinisch/Verpleegkundig - vervolg **Maxima 1 en 2**

16.48 Treatment Efficacy of Hypertension in Dutch Kidney Transplant Patients (p. 61)
L.C. Dobrowolski, R.J.M. ten Berge, F.J. Bemelman, C.T. P. Krediet, Dept. of Nephrology - Renal Transplant Unit, Academic Medical Center, Amsterdam, The Netherlands

17.00 The influence of region on expected patient survival of patients starting with renal replacement therapy (p. 62)
A.C. Hemke[1], M.A. van den Dorpel[2], M.B.A. Heemskerk[1], A.J. Hoitsma[1,3], [1]Dutch Transplant Foundation, Leiden, [2]Maasstad Ziekenhuis Rotterdam, [3]Radboud University Medical Centre Nijmegen, The Netherlands

17.12 Health literacy and self-management among kidney transplant patients (p. 63)
L. Maasdam[1], E.K. Massey[1], M. Tielen[1], M.C. van Buren[1], J.A. Kal–van Gestel[1], M.G.H. Betjes[1], W. Weimar[1], [1]Kidney transplant unit, Erasmus Medical Center Rotterdam, The Netherlands

17.24 **"Patiënt en wetenschap"**
P. van Boheemen, Science Alliance

18.00 Ludieke afsluiting van het programma

Parallelsessie IV - Klinisch/Basaal **Beatrix 1 en 2**

Voordrachten in het Nederlands of Engels, spreektijd 8 minuten, discussietijd 2 minuten.

Voorzitters: M. Baas en J. van der Vlag

16.00 Immune reactivity of an individual against a virus induces a broad repertoire of HLA-alloreactive memory T cells (p. 64)
H. van den Heuvel[1], P.M.W. van der Meer-Prins[1], K.M. Heutinck[2,3], R.J.M. ten Berge[3], I.I.N. Doxiadis[1], F.H.J. Claas[1], [1]Dept of Immunohaematology and Blood Transfusion, Leiden University Medical Centre, Leiden, [2]Dept of Experimental Immunology, and Renal Transplant Unit, [3]Dept of Internal Medicine, Academic Medical Centre, Amsterdam, The Netherlands

16.10 Angiopoietin2: a prognostic marker in kidney transplantation (p. 65)
W.H. Westendorp[1], L.G. Koudstaal[1,2], J. Damman[1], J.G. Burgerhof[3], M.A. Seelen[4], H. van Goor[2], R.J.Ploeg[1,5], H.G. Leuvenink[1], [1]Dept of Surgery, [2]Dept of Pathology, [3]Dept of Epidemiology, [4]Dept of Nephrology, University Medical Center Groningen, The Netherlands, [5]Nuffield Dept. of Surgical Sciences, University of Oxford, United Kingdom

16.20 Renal ischemia/reperfusion induces release of angiopoietin-2 from human grafts of living and deceased donors (p. 66)
D.K. de Vries[1], M. Khairoun[2], J.H.N. Lindeman[1], I.M. Bajema[4], E. de Heer[4], M. Roest[5], A.J. van Zonneveld[2,3], C. van Kooten[2], T. J. Rabelink[2,3], A.F. Schaapherder[1], M.E.J. Reinders[2,3], Dept. of [1]Surgery, [2]Nephrology, [3]Einthoven laboratory for experimental vascular medicine and [4]Pathology, Leiden University Medical Center, Leiden, The Netherlands, [5]Dept. of Clinical Chemistry and Hematology, University Medical Center Utrecht, Utrecht, The Netherlands

Parallelsessie IV - Klinisch/Basaal - vervolg **Beatrix 1 en 2**

16.30 Severe ischemia-reperfusion injury defined by peak alanine amino-transferase after orthotopic liver transplantation is a strong risk factor for development of nonanastomotic strictures in donor livers from donation after cardiac death (p. 67)
K.S. Korkmaz[1], B.-J.F. de Rooij[1], M.J. Coenraad[1], A. Inderson[1], J. Dubbeld[2], H.W. Verspaget[1], B. van Hoek[1], [1]Dept of Gastroenterology and Hepatology, [2]Dept of Surgery, Leiden University Medical Center, Leiden, The Netherlands

16.40 MicroRNA profiling in urinary sediment of transplant recipients with acute rejection (p. 68)
M. Eikmans[1], J.D.H. Anholts[1], S. Heidt[1], J.W. de Fijter[2], F.H.J. Claas[1], [1]Dept of Immunohematology and Blood Transfusion, [2]Dept of Nephrology, Leiden University Medical Center, Leiden, The Netherlands

16.50 Circulating microRNAs correlate with diabetic nephropathy and systemic microvascular damage and normalize after simultaneous pancreas-kidney transplantation (p. 69)
R. Bijkerk[1,2], M. Khairoun[1], J.M.G.J. Duijs[1,2], C.J.H. ter Horst[1], A.P.J. de Vries[1], E.J.P. de Koning[1], J.W. de Fijter[1], T.J. Rabelink[1,2], A.J. van Zonneveld[1,2] , M.E.J. Reinders[1,2], [1]Dept. of Nephrology and [2]Einthoven Laboratory for Experimental Vascular Research, Leiden University Medical Center, The Netherlands

17.00 Mobilization and priming of mesenchymal stromal cells from human liver grafts: differences between non-heart beating versus heart beating donors (p. 70)
S.R.R Hall[1], J. de Jonge[1], E.L.D. de Mare-Bredemeijer[2], J. Kwekkeboom[2], H.J. Metselaar[2], R.W.F. de Bruin[1], H.W. Tilanus[1], L.J.W. van der Laan[1], [1]Dept. of Surgery and Laboratory of Experimental Transplantation and Intestinal Surgery, [2]Dept. of Gastroenterology & Hepatology, Erasmus MC, Rotterdam, The Netherlands

Parallelsessie IV - Klinisch/Basaal - vervolg **Beatrix 1 en 2**

17.10 The mannan-binding lectin-associated serine proteinase-2 D120G mutation is a common pathophysiological denominator for non-anastomotic biliary strictures after orthotopic liver transplantation and the Budd-Chiari syndrome (p. 71)
B.-J. de Rooij[1], K. Korkmaz[1], H. Verspaget[1], M. Coenraad[1], R. Porte[2], B. van Hoek[1], [1]Leiden University Medical Center, Leiden, [2]University Medical Center Groningen, Groningen, The Netherlands*

17.20 A single dose of rituximab results in a long lasting B-cell depletion in peripheral blood, without affecting the peripheral T-cell compartment (p. 72)
E.G. Kamburova[1], H.J.P.M. Koenen[1], M.W.F. van den Hoogen[2], I. Joosten[1], L.B. Hilbrands[2], [1]Dept. of Laboratory Medicine - Medical Immunology, [2]Dept. of Nephrology, Radboud University Medical Centre, Nijmegen, The Netherlands

17.30 Identification of two pathways by which intravenous immune-globulin modulates dendritic cells in humans in vivo (p. 73)
A.S.W. Tjon[1], H. Jaadar[1], R. van Gent[1], P. M. van Hagen[2], L.J.W. van der Laan[3], P.A.W. te Boekhorst[4], H.J. Metselaar[1], J. Kwekkeboom[1], Depts of [1]Gastroenterology and Hepatology, [2]Internal Medicine and Immunology, [3]Surgery and [4]Hematology, Erasmus MC-University Medical Centre, Rotterdam, The Netherlands

17.45 Einde parallelsessie

Parallelsessie V - Basaal — Beatrix 3 en 4

Voordrachten in het Engels, spreektijd 8 minuten, discussietijd 2 minuten.

Voorzitters: H. Koenen en N. Litjens

16.00 Mannan-binding lectin reduces ER-stress sensor GRP78/BIP in tubular epithelial cells following renal ischemia/reperfusion (p. 74)
P. van der Pol[1], N. Schlagwein[1], D.J. van Gijlswijk[1], H.C. de Boer[1,2], J. O'flynn[1] and C. van Kooten[1], [1]Dept. of Nephrology and [2]Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, The Netherlands

16.10 Hepatocyte and cholangiocyte-derived microRNAs in serum as early markers for ischemia & reperfusion injury in pigs (p. 75)
C.J. Verhoeven[1], J. de Jonge[1], J. Kwekkeboom[2], H.J. Metselaar[2], R.W.F. de Bruin[1], H.W. Tilanus[1], G. Kazemier[1, 3], L.J.W. van der Laan[1], [1]Dept. of Surgery and Laboratory of Experimental Transplantation and Intestinal Surgery, [2]Dept. of Gastroenterology & Hepatology, Erasmus MC-University Medical Center, Rotterdam, [3]Dept. of Surgery, VU Medical Center Amsterdam, The Netherlands

16.20 Mesenchymal stem cells control allo-reactive CD28- T cells that are unaffected by belatacept treatment (p. 76)
A.U. Engela, C.C. Baan, N.H.R. Litjens, M. Franquesa, M.G.H. Betjes, W. Weimar, M.J. Hoogduijn, Dept. of Internal Medicine, Section Nephrology and Transplantation, Erasmus MC, University Medical Center Rotterdam, The Netherlands

16.30 Differential effects of the innate vs. adaptive immunity in lysing human renal tubular epithelial cells (p. 77)
M.W.H.J. Demmers[1], S.S. Korevaar[1], J.N.M. IJzermans[2], M. G. H Betjes[1], W. Weimar[1], A.T. Rowshani[1] and C.C. Baan[1], Depts of [1]Internal Medicine and [2]Surgery, Erasmus MC, University Medical Center Rotterdam, The Netherlands. A.T. Rowshani and C.C. Baan contributed equally to this study

Parallelsessie V - Basaal - vervolg **Beatrix 3 en 4**

16.40 KIR and HLA associated genotypic risk of Cytomegalovirus (CMV) infection and disease in renal transplantation (p. 78)
C.M. Michelo[1], A. van der Meer[1], H.J Tijssen[1], R. Zomer[1], L.B. Hilbrands[2], I. Joosten[1], [1]Dept. of Laboratory Medicine - Laboratory of Medical Immunology, [2]Dept. of Laboratory Medicine - Nephrology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

16.50 Epigenetic analysis of the TSDR of FOXP3 demonstrates that natural Treg infiltrate the cardiac allograft already before an acute rejection episode (p. 79)
K. Boer[1], A.M.A. Peeters[1], A.P.W.M. Maat[2], K. Caliskan[3], A.H.M.M. Balk[3], W. Weimar[1], C.C. Baan[1], Dept of [1]Internal Medicine, [2]Thoracic Surgery and [3]Cardiology, Erasmus University Medical Center Rotterdam, The Netherlands

17.00 Immunogenicity of HLA-DRB3 after kidney transplantation and the development of a tool for epitope discovery using mutagenized recombinant HLA-fusion proteins (p. 80)
T.H.P.M. Habets[1], E. Bouwmans[2*], J.J.Y. Frijns[1], E. Bielen[1], M.H.L. Christiaans[3], S.L. Morley[4], C.E.M. Voorter[1], J. Vanderlocht[1*] , M.G.J. Tilanus[1*], [1]Dept of Transplantation Immunology, Tissue Typing Laboratory, [2]Dept of Internal Medicine, Division of Haematology, [3]Dept of Internal Medicine, Division of Nephrology, Maastricht UMC, Maastricht, The Netherlands, [4]NHS Blood and Transplant University of Cambridge, Cambridge, United Kingdom*

17.10 CD137, a marker to detect the total alloreactive T cell compartment? (p. 81)
N.H.R. Litjens, E.A. de Wit, C.C. Baan, M.G.H. Betjes, Erasmus Medical Center, Dept. of Internal Medicine, Section Nephrology & Transplantation, Rotterdam, The Netherlands

17.20 Phenotypical characterisation of peripherally circulating HLA-A02-restricted polyomavirus BK (BKV)-specific CD8+ cytotoxic T lymphocytes (CTL) in healthy adults (p. 82)
M.C. van Aalderen[1], E.B.M. Remmerswaal[1], K.M. Heutinck[1], N.D. van der Bom-Baylon[1], A. ten Brinke[2], R.A.W. van Lier[2], R.J.M. ten Berge[1,3], [1]Dept. of Experimental Immunology, [2]Sanquin Research, [3]Renal Transplant Unit, Academic Medical Centre, Amsterdam, The Netherlands

Parallelsessie V - Basaal - vervolg **Beatrix 3 en 4**

17.30 Alloantigen-specific Tregs can be identified by activation-induced CD154 expression (p. 83)
N.H.R. Litjens, C.C. Baan, M.G.H. Betjes, Erasmus Medical Center, Dept. of Internal Medicine, Section Nephrology & Transplantation, Rotterdam, The Netherlands

17.45 Einde parallelsessie

Sociaal programma **Maxima 1 en 2 en Foyer**

18.00 Plenaire afsluiting dag 1, gevolgd door hapje en drankje

18.45 Vertrek bussen voor het Wijnfort

19.15-0.30 Diner en feestavond

Sessie VI Plenair — Maxima 2

Voorzitter: *M. Cornelissen*

08.30 "**E-health, wat hebben we eraan?**"
L. Engelen, Radboud Reshape & Innovation Center
UMC St Radboud, Nijmegen

09.00 "**Integere allocatie van donororganen**"
Dr. A. Rahmel, Eurotransplant International Foundation, Leiden

09.30 Koffiepauze en laptoppresentaties

Laptoppresentaties — Maxima 1

Laptoppresentaties – basaal en klinisch

1. Epitope hunting in HLA-DQ: modeling, predicting and chasing (p. 131)
J. Vanderlocht, M. Groeneweg, T.E. Latuhihin, M.H.L. Christiaans, C.E.M. Voorter, M.G.J. Tilanus, Dept. of Transplantation Immunology, Tissue Typing Laboratory, Maastricht University Medical Center, The Netherlands

2. Rare HLA alleles within the CWD groups redefined by a new SBT typing strategy (p. 132)
C.E.M. Voorter, C. Meertens, F. Palusci, M.G.J. Tilanus, Transplantation Immunology, Tissue Typing Laboratory, Maastricht University Medical Center

3. Predicted Indirectly Recognizable HLA epitopes presented by HLA-DRB1 correlate with the de novo development of donor-specific HLA IgG antibodies after kidney transplantation (p. 133)
H.G. Otten[1], J.J.A. Calis[2], C. Keşmir[2], A.D. van Zuilen[3], E. Spierings[1], [1]Dept. of Immunology, University Medical Center Utrecht, Utrecht, The Netherlands, [2]Dept. of Theoretical Biology/Bioinformatics, Utrecht University, Utrecht, The Netherlands, [3]Dept. of Nephrology, University Medical Center Utrecht, Utrecht, The Netherlands

Laptoppresentaties - vervolg **Maxima I**

4. A validated rapid LC-MS/MS method for the determination of free fraction of tacrolimus (p. 134)
A.L. van Dapperen[1], M.A. Sikma[1], N.A. Stienstra[2], H.M. Koudijs[1], J. Meulenbelt[1], E.M. van Maarseveen[2], [1]National Poisons Information Center, Intensive Care Center of the division of Anesthesiology, Intensive Care and Emergency Medicine, [2]Clinical Pharmacy, University Medical Center of Utrecht, The Netherlands

5. MnTMPyp, a selective superoxide dismutase mimetic, reduces oxidative stress in brain dead donors (p. 135)
D. Hoeksma[1], L.F.A. van Dullemen[1], P.J. Ottens[1], S. Veldhuis[1], R.J. Ploeg[1,2], H.G.D. Leuvenink[1], [1] Dept. of Surgery, University Medical Center Groningen, Groningen, The Netherlands, [2] Dept. of Surgery, University of Oxford, Oxford, United Kingdom

6. Hypothermic machine perfusion of the pancreas for islet transplantation (p. 136)
M. Leemkuil[1], M. de Nijs[2], E. van Rossenberg[2], C. Vermeulen[2], J. Sijtsma[2], M. Engelse[3], E.J.P. de Koning[4], C. Krikke[1], H. Leuvenink[1], R.J. Ploeg[1,5], [1]Dept. of surgery, University Medical Center Groningen, [2]Dept. of nephrology, Leiden University Medical Center, [3]Dept. of nephrology and immunohematology, Leiden University Medical Center, [4]Dept. of nephrology and endocrinology, Leiden University Medical Center, The Netherlands, [5]Dept. of transplantation, Oxford transplant centre, United Kingdom

7. Preoperative fasting protects aged obese male and female mice against renal ischemia-reperfusion injury (p. 137)
F. Jongbloed[1,2], R.W.F. de Bruin[1], S. van den Engel[1], L.J.W. van der Laan[1], H. van Steeg[2], J.N.M. IJzermans[1], M.E.T. Dollé[2], [1]Dept. of Surgery, Laboratory for Experimental Transplantation and Intestinal Surgery, Erasmus Medical Center, Rotterdam, [2]Laboratory of Health Protection Research, National Institute of Public Health and the Environment, Bilthoven, The Netherlands

Laptoppresentaties - vervolg **Maxima I**

8. Oxidative damage in clinical ischemia/reperfusion injury: a re-appraisal (p. 138)
D.K. de Vries[1], L.G.M. Wijermars[1], K.A. Kortekaas[2], D. Tsikas[3], R.J.M. Klautz[2], A.F.M. Schaapherder[1], J.H.N. Lindeman[1], Depts. of [1]Surgery and [2]Cardiothoracic Surgery, Leiden University Medical Centre, The Netherlands, [3]Institute of Clinical Pharmacology, Hannover Medical School, Germany

9. Angiopoietin-1 administration in a brain death rat model (p. 139)
W.H. Westendorp[1], S. Veldhuis[1], H. van Goor[2], R.J.Ploeg[1,3], H.G. Leuvenink[1], [1]Dept of Surgery, [2]Dept of Pathology, University Medical Center Groningen, The Netherlands, [3]Nuffield Dept of Surgical Sciences, University of Oxford, United Kingdom

10. Five year graft survival and delayed graft function predicted by pre-existent renal damage (p. 140)
W.H. Westendorp[1], H. Tent[2], M.L.C. Bulthuis[3], H.S. Hofker[1], R.J.Ploeg[1,4], H.G. Leuvenink[1], M.C.R.F. van Dijk[3], H. van Goor[3], [1]Dept of Surgery, [2]Dept of Nephrology, [3]Dept of Pathology, University Medical Center Groningen, The Netherlands, [4]Nuffield Dept of Surgical Sciences, University of Oxford, United Kingdom

11. Donor pre-treatment with the heat shock protein-inducer geranylgeranylacetone reduces brain death-associated inflammation in the kidney at organ retrieval (p. 141)
L.F.A. van Dullemen[1], D. Hoeksma[1], P.J. Ottens[1], S. Veldhuis[1], R.J. Ploeg[1,2], H.G. D. Leuvenink[1], [1]Dept. of Surgery, University Medical Center Groningen, The Netherlands, [2]Dept. of Surgery, University of Oxford, United Kingdom

12. Opposite effects of prednisolone treatment on liver and kidney graft from brain dead rat donors (p. 142)
R.A. Rebolledo, MD[1,2], B. Liu, MD[1], A. Van Erp[1], P. Ottens[1], S. Veldhuis[1], H.G.D Leuvenink, PhD[1], [1]Dept of Surgery, University Medical Center Groningen, Groningen, The Netherlands, [2]Facultad de Medicina, Universidad de Chile, Santiago, Chile

Laptoppresentaties - vervolg **Maxima 1**

13. Anti-inflammatory effects of prednisolone treatment did not improve organ quality in brain dead rats (p. 143)
R.A. Rebolledo, MD[1,2], B. Liu, MD[1], A. Van Erp[1], P. Ottens[1], S. Veldhuis[1], H.G.D Leuvenink, PhD[1], P. Romanque[2], [1]Dept of Surgery, University Medical Center Groningen, Groningen, The Netherlands, [2]Facultad de Medicina, Universidad de Chile, Santiago, Chile

14. The effect of the β-Human Chorionic Gonadotropin-Related Peptide (AQGV) on a Brain Death Induced Inflammation in Rats (p. 144)
R.A. Rebolledo, MD[1,2], B. Liu, MD[1], A. Van Erp[1], P. Ottens[1], S. Veldhuis[1], H.G.D Leuvenink, PhD[1], P. Romanque[2], [1]Dept. of Surgery, University Medical Center Groningen, Groningen, The Netherlands, [2]Facultad de Medicina, Universidad de Chile, Santiago, Chile

15. Duplicated ureters and renal transplantation: a single-center experience and review of the literature (p. 145)
V.P. Alberts[1], R.C. Minnee[1], M. Zijp[1], K.A.M.I. van Donselaar - van der Pant [2], F.J. Bemelman[2], P.J. Zondervan[3], M.P. Laguna Pes[3], M.M. Idu[1], [1]Dept. of Surgery, Academic Medical Center, Amsterdam, The Netherlands, [2]Renal Transplant Unit, Dept. of Nephrology, Academic Medical Center, Amsterdam, The Netherlands, [3]Dept. of Urology, Academic Medical Center, Amsterdam, The Netherlands

16. Risk factors for wound-related complications after renal transplantation (p. 146)
M.M. Fockens[1], V.P. Alberts[1], F.J. Bemelman[2], K.A.M.I. van Donselaar – van der Pant[2], M.M. Idu[1], [1]Dept. of Surgery, Academic Medical Center, Amsterdam, The Netherlands, [2]Renal Transplant Unit, Dept. of Nephrology, Academic Medical Center, Amsterdam, The Netherlands

17. Late fulminant antibody mediated rejection of a blood group ABO-incompatible kidney transplant during Serratia marcescens urosepsis (p. 147)
A.E. de Weerd[1], A.G. Vonk[2], H. van der Hoek[3], W. Weimar[1], M.G.H. Betjes[1], M. van Agteren[1], [1]Dept of Nephrology, [2]Dept of Medical Microbiology, Dept of Haematology, Erasmus Medical Center, Rotterdam, The Netherlands

Laptoppresentaties - vervolg **Maxima 1**

18. Nierfunctie na een longtransplantatie (p. 148)
L. Küsters, L. van Zandvoort, B. van den Blink, R.A.S. Hoek, P.Th.W. van Hal, Dept. of Respiratory Medicine, Erasmus MC Rotterdam, The Netherlands

19. Een Buschke Lowenstein tumor bij een transplantatiepatient: is interferon de oplossing? (p. 149)
N.E. Wester, E.M. Hutten, M. Freulings, M. Seelen, C. Krikke, University Medical Center Groningen, The Netherlands

20. Chronic hepatitis E in a renal transplant recipient (p. 150)
M.A. de Vries[1], J. Roodbol[2], R.A. de Man[3], A. van der Eijck[4], J.P.A. Samijn[2], J.M.M. Boots[1], [1]Dept of Internal Medicine and Nephrology, [2]Dept of Neurology, Maasstad Ziekenhuis Rotterdam, [3]Dept of Gastro-enterology and Hepatology, [4]Dept. of Virology, Erasmus Medical Center Rotterdam, The Netherlands

21. Vitamin D status in children pre- and post renal transplantation: hyperparathyroidism and interaction with calcineurin inhibitors (p. 151)
A.A. Prytuła[1], A.H. Bouts[2], M. van Gorkom[1], E. Dorresteijn[1], K. Cransberg[1], [1]Pediatric Nephrology Dept., Erasmus MC - Sophia Children's Hospital, Rotterdam, The Netherlands, [2]Pediatric Nephrology Dept., Emma Children's Hospital, Amsterdam, The Netherlands

22. Uitkomsten van niertransplantatie in de diverse bevolkingsgroepen van de regio Groot Amsterdam (p. 152)
M. Kiran[1], K. van Donselaar-van der Pant[1], N. van der Weerd[1], M. Idu[2], R.J.M.. ten Berge[1], F. Bemelman[1], [1]Niertransplantatie afdeling, Afdeling Interne Geneeskunde, [2]Afdeling Chirurgie Academisch Medisch Centrum, Universiteit van Amsterdam, The Netherlands

23. Is dysfunction of the HNF-1 beta gene involved in the pathogenesis of NODAT? (p. 153)
G.A.J. van Boekel, M.D. van den Beukel, B. de Kort, C. Lamper, M.G.M. Strik, L.B. Hilbrands, Dept. of Nephrology, Radboud University Nijmegen Medical Centre, The Netherlands

24. Soluble CD30 levels do not predict acute rejection after withdrawal of tacrolimus in renal transplant patients (p. 154)
L.L.F.G. Valke[1,2], B. van Cranenbroek[1], L.B. Hilbrands[2] and I. Joosten[1], [1]Dept. of Laboratory Medicine, Laboratory of Medical Immunology, [2]Dept. of Nephrology, Radboud University Medical Centre, Nijmegen, The Netherlands

25. Novel biomarkers for the prediction of acute kidney injury in patients undergoing liver transplantation (p. 155)
B. Dedeoglu[1], H. R. H. de Geus[2], G. Fortrie[1], M.G.H. Betjes[1], [1]Dept of Nephrology, Erasmus University Medical Center, Rotterdam, [2]Dept of Intensive Care, Erasmus University Medical Center, Rotterdam, The Netherlands

26. MMP-2 CT/TT genotype is a risk factor for mortality or liver transplantation in primary sclerosing cholangitis (p. 156)
K.S. Korkmaz[1], B.-J. de Rooij[1], M. Janse[2], M.J. Coenraad[1], J.J. van der Reijden[1], R.K. Weersma[2], R.J. Porte[3], A.G. Baranski[4], H.W. Verspaget[1], B. van Hoek[1], [1]Dept. of GI & Hepatology, Leiden University Medical Center, Leiden, The Netherlands, [2]Dept. of GI & Hepatology, University Medical Center Groningen, The Netherlands, [3]Dept. of Hepatobiliary Surgery and Liver Transplantation, University Medical Groningen, The Netherlands, [4]Dept. of Surgery, Leiden University Medical Center, Leiden, The Netherlands

27. Psychological factors associated with medication adherence among young adult kidney transplant recipients (p. 157)
K.M.E. Meys[1], J. Roodnat[2], W. Weimar[2], R. Kerner[2], M.G.H. Betjes[2], K. Cransberg[1], E.K. Massey[2], [1]Dept. of Pediatric Nephrology, Erasmus MC Sophia Children's Hospital, Rotterdam, The Netherlands, [2]Dept of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands

28. Psychological processes that contribute to psychological well-being and social participation among young adult kidney transplant recipients (p. 158)
K.M.E. Meys[1], J. Roodnat[2], W. Weimar[2], R. Kerner[2], M.G.H. Betjes[2], K. Cransberg[1], E.K. Massey[2], [1]Dept. of Pediatric Nephrology, Erasmus MC Sophia Children's Hospital, Rotterdam, [2] Dept. of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands

Laptoppresentaties - basaal

29. JAK inhibitor tofacitinib interferes with interferon alpha mediated inhibition of hepatitis C replication (p. 159)
P.E. de Ruiter[1], C.C. Baan[2], Q. Pan[3], J. Kwekkeboom[3], H.J. Metselaar[3], R.W.F. de Bruin[1], H.W. Tilanus[1], L.J.W. van der Laan[1], [1]Dept. of Surgery and Laboratory of Experimental Transplantation and Intestinal Surgery, [2]Internal Medicine, [3]Dept. of Gastroenterology & Hepatology, Erasmus MC-University Medical Center, Rotterdam, The Netherlands

30. Dendritic cell-derived CXCL1: no role in T cell activation or differentiation (p. 160)
M. Kouwenberg, J. van der Vlag, L.B. Hilbrands, Dept of Nephrology, Radboud University Medical Centre, Nijmegen, The Netherlands

31. hCMV-specific CD8+ T cells in lymph nodes from renal transplant recipients contain 'true' memory cells (p. 161)
E.B.M. Remmerswaal[1,2], P.L. Klarenbeek[3], M.E. Doorenspleet[3], B.D.C. van Schaik[4], K. van Donselaar[2], F. Bemelman[2], R.E.E. Esveldt[3], A.H. van Kampen[4], F. Baas[5], A. ten Brinke[6], R.A.W. van Lier[1], N. de Vries[3], R.J.M. ten Berge[2], [1]Dept of Exp Immunology, [2]Renal Transplant Unit, Dept of Nephrology, Div of Int Medicine, [3]Dept of Clin Immunology and Rheumatology, [4]Dept of Clin Epidemiology, Biostatistics and Bioinformatics, [5]Dept of Genome Analysis, [6]Sanquin Research, Dept of Immunopathology, AMC, Amsterdam, The Netherlands

32. Primary CMV infection has a limited effect on the immunological age of kidney transplant recipients (p. 162)
R.W.J. Meijers[1], N.H.R. Litjens[1], L.E.A. de Wit[1], A.W. Langerak[2], C.C. Baan[1], W. Weimar[1] and M.G.H. Betjes[1], [1]Dept. of Internal Medicine, section Nephrology and Transplantation, [2]Dept. of Immunology, Erasmus Medical Center, Rotterdam, The Netherlands

33. Towards optimal monitoring of memory B cell responses in sensitized patients (p. 163)
G. Karahan[1], FHJ. Claas[1], S. Heidt[1] , [1]Dept. of Immunohaematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands

Laptoppresentaties - vervolg **Rembrandt**

34. Dietary restriction and fasting arrest B and T cell development and recruits mature B and T cells to the bone marrow (p. 164)
S.Shushimita[1], M.J.W. de Bruijn[2], R.W.F. de Bruin[1], J.N.M. IJzermans[1], R.W. Hendriks[2] and F.J.M.F. Dor[1], [1]Dept. of Surgery, division of Transplant Surgery, [2]Dept. of Pulmonology, Erasmus MC, Rotterdam, The Netherlands

35. Natural regulatory T-cells impair the donor-specific cytotoxic T-Cell response long after transplantation (p. 165)
N.M. van Besouw, R. de Kuiper, M. Klepper, M.G.H. Betjes, W. Weimar, C.C. Baan, Dept. of Internal Medicine – Transplantation, Erasmus Medical Center, Rotterdam, The Netherlands

Laptoppresentaties **Vermeer**

Laptoppresentaties - donatie

36. A systematic review identifying psychosocial risk factors for living kidney donors (p. 166)
L. Wirken[1], H. van Middendorp[1], C.W. Hooghof[2], A.J. Hoitsma[2], A.W.M. Evers[1], [1]Dept. of Medical Psychology, [2]Dept. of Nephrology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

37. Modifiable factors in Access to Living Donor Kidney Transplantation among Diverse Populations (p. 167)
S.Y. Ismail[1,2], A.E. Luchtenburg[2], J.A. Kal - van Gestel[2], W.C. Zuidema[2], W. Weimar[2], E.K. Massey[2], J.J.V. Busschbach[1], Depts of [1]Medical Psychology and Psychotherapy and [2]Internal Medicine: Kidney Transplant Unit, Erasmus MC, The Netherlands

38. Knowledge as a predictor for having a living kidney donor? (p. 168)
A.E. Luchtenburg[1], S.Y. Ismail[1,2], W.C. Zuidema[1], W. Weimar[1], E.K. Massey[1], J.J.V. Busschbach[2], Depts. of [1]Internal Medicine: Kidney Transplant Unit and [2]Medical Psychology and Psychotherapy, Erasmus Medical Centre Rotterdam, The Netherlands

39. A Psychometric Analysis of the Rotterdam Renal Replacement Knowledge-Test (R3K-T) using Multidimensional Item Response Theory (MIRT) (p. 169)

S.Y. Ismail[1,2], L. Timmerman[2], R. Timman[1], A.E. Luchtenburg[2], P.J.H. Smak Gregoor[3], R.W. Nette[4], R.M.A. van den Dorpel[5], W.C. Zuidema[2], W. Weimar[2], E.K. Massey[2], J.J.V. Busschbach[1], [1]Medical Psychology and Psychotherapy, [2]Internal Medicine: Kidney Transplant Unit, Erasmus MC, Rotterdam, [3]Internal Medicine, Albert Schweitzer Ziekenhuis, Dordrecht, [4]Internal Medicine, Sint Franciscus Gasthuis, Rotterdam, [5]Internal Medicine, Maasstad Ziekenhuis, Rotterdam, The Netherlands

40. Public solicitation of organs from living donors – an ELPAT view (p. 170)

M. Frunza[1], A. Lennerling[2], F. Citterio[3], R. Johnson[4], N. Mamode[5], S. Sterckx[6,7], W. Zuidema[8], K. van Assche[6], W. Weimar[7], F. Dor[9], [1]Babes-Bolyai University, Cluj, Romania, [2]University Hospital Göteborg, Sweden, [3]Catholic University, Rome, Italy, [4]NHSBT, Bristol, UK, [5]Guys Hospital, London, UK, [6]Free University of Brussels, Brussels, [7]Ghent University, Belgium, [8]Kidney Transplant Unit and [9]Transplant Surgery, Erasmus MC, Rotterdam, The Netherlands

41. Psychosocial screening of the unspecified living kidney donors in The Netherlands (p. 171)

M. de Klerk[1,2], W. Zuidema[1], E.K. Massey[1] on behalf of the 8 Kidney Transplant Centers in The Netherlands, [1]Dept. of Internal Medicine - Transplantation, Erasmus MC Rotterdam, [2]Dutch Transplant Foundation, Leiden, The Netherlands

Laptoppresentaties - verpleegkundig

42. Altruïstische donatie (p. 172)
R.A.M. Meijer-Vogt, D.P. Jansen, A.M.S. Roelofs, C. Krikke, J.S.F. Sanders, University Medical Center Groningen, The Netherlands

43. Painscore and patients preferences in the repetitive use of the Dried Blood Spot method in comparison to venous puncture in renal transplant recipients (p. 173)
H.J.M. Mullens, K. Hoogtanders, L. Stolk, M.H.L. Christiaans, Vakgroep Interne Geneeskunde, onderafdeling Nefrologie en Laboratorium Klinische Farmacie en Toxicologie, Academisch Ziekenhuis Maastricht, Nederland

44. Who has high expectations of donation? Exploring the psychological profile of living kidney donors (p. 174)
L. Timmerman[1], M. Laging[1], W.C. Zuidema[1], J.N.M. IJzermans[2], M.G.H. Betjes[1], J.J. van Busschbach[3], W. Weimar[1], E.K. Massey[1], Depts of [1]Internal Medicine, [2]General Surgery, [3]Medical Psychology and Psychotherapy, Erasmus Medical Centre Rotterdam, The Netherlands

45. MijnKinderNierNet (p. 175)
J.L. Knoll, E.A.M. Cornelissen, H. de Jong, Dept. of Pediatric Nephrology, Universitair Medisch Centrum St Radboud, Nijmegen, The Netherlands

Parallelsessie VII – Klinisch — Maxima 2

Voordrachten in het Nederlands of Engels, spreektijd 8 minuten, discussietijd 2 minuten.

Voorzitters: M. van den Hoogen en F. d'Ancona

11.00 RUNSMART: 15 year follow-up of a multicentre, randomised, calcineurin inhibitor withdrawal study in kidney transplantation (p. 84)
J.I. Roodnat[1], L.B. Hilbrands[2], P.J.H. Smak Gregoor[3], R.J. Hené, R.G.L. de Sévaux[2], J. Kal van Gestel[1], C. Konijn[4], A. van Zuilen[5], T. van Gelder[1], A.J. Hoitsma[2], W. Weimar[1], [1]Department of Internal Medicine, EMC Rotterdam, [2]Dept. of Nephrology, Radboud UMC Nijmegen, [3]Dept. of Internal Medicine, Albert Schweitzer Hospital, Dordrecht, [4]Dutch Transplantation Foundation, Leiden, [5]Dept. of Nephrology, UMC Utrecht, The Netherlands

11.10 Do differences in the use of induction therapy affect outcome after heart transplantation, a comparison of centres (p. 85)
M.B.A. Heemskerk [1], O.C. Manintveld[2] , J. Brügemann[3], N. de Jonge [4], [1]Dutch Transplant Foundation, [2]Dept of Cardiology, Erasmus MC, [3]Dept of Cardiology, University Medical Centre Groningen, [4]Dept of Heart Failure and Heart Transplantation, University Medical Centre Utrecht, The Netherlands

11.20 Blood Pressure and Sodium Excretion in Kidney Transplant Recipients on Different Immunosuppressive Regimens (p. 86)
L.C. Dobrowolski, F.J. Bemelman, R.J.M. ten Berge, C.T. P. Krediet, Dept. of Nephrology - Renal Transplant Unit, Academic Medical Center, Amsterdam, The Netherlands

11.30 First in human trial of ischemic postconditioning in kidney transplantation from donations after cardiac death (p. 87)
E.K. van den Akker[1], D.A. Hesselink[2], O.C. Manintveld[3], J.A. Lafranca[1], W.Weimar[2], J.N.M.IJzermans[1], F.J.M.F. Dor[1], [1]Dept. of Surgery, Division of Transplant Surgery, [2]Dept. of Internal Medicine, division of Nephrology and Renal Transplantation, [3]Dept. of Cardiology, Heart Transplant Unit, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

Parallelsessie VII – Klinisch - vervolg **Maxima 2**

11.40 Determinants of improved long-term survival after liver transplantation in a single center (p. 88)
K. Sebib Korkmaz[1], B. J.F. de Rooij[1], J.J. Blok[2], H.W. Verspaget[1], P.W.J. Maljaars[1], M.J. Coenraad[1], J. Ringers[2], J. Dubbeld[2], A. Inderson[1], B. van Hoek[1], Depts of [1]Gastroenterology and Hepatology and [2]Surgery, Leiden University Medical Center, Leiden, The Netherlands

11.50 Hepatic encephalopathy is an independent risk factor for mortality in patients awaiting liver transplantation (p. 89)
M.J. Coenraad[1], L. Verbruggen[1], M. Navasa[2], H.W. Verspaget[1], B. van Hoek[1], J. Bosch[2], [1]Dept. of Gastroenterology and Hepatology, Leiden University Medical Centre, Leiden, The Netherlands, [2]Liver Unit, Hospital Clínic-IDIBAPS, CIBERrehd, University of Barcelona, Barcelona, Spain

12.00 Increase in RBC transfusion requirements during orthotopic liver transplantation after market withdrawal of aprotinin (p. 90)
F. Arshad[1], A. Westerkamp[1], T. Lisman[1], R. Porte[1], M. de Boer[1], [1]Dept of Hepatobiliary Surgery and Liver Transplantation, University Medical Center Groningen, The Netherlands

12.10 The use of proton pump inhibitors does not increase the risk of acute rejection after renal transplantation (p. 91)
G.A.J. van Boekel, C.H.H. Kerkhofs, F. van de Logt, L.B. Hilbrands, Dept. of Nephrology, Radboud University Nijmegen Medical Centre, The Netherlands

12.20 Predictive value of anti-PLA2r antibodies at time of transplantation for kidney failure in patients with membranous nephropathy (p. 92)
B.P. Rietveld[1], H.G.Otten[2], M.C.Verhaar[1], A.D. van Zuilen[1], *Depts of [1]Nephrology and Hypertension and [2]Immunology, University Medical Center Utrecht, The Netherlands*

12.30 Lunchpauze

Parallelsessie VIII – Allocatie — Beatrix 1 en 2

Voordrachten in het Nederlands of Engels, spreektijd 8 minuten, discussietijd 2 minuten.

Voorzitters: F. Hendriks en M. van der Jagt

11.00 De KIT verlagen door de voorbereidingen voor allocatie van NHB-III nieren te vervroegen (p. 93)
A.S. Ramlochan Tewarie - Ramsaransing [1], B.G. Hepkema[2], [1]Stafarts Nederlandse Transplantatiestichting, [2]Medisch immunoloog UMC Groningen, Nederland

11.10 15 years of DCD3 in the Netherlands: increasing efforts in donor activities for a small increase of transplants (p. 94)
H.A van Leiden[1], N.E. Jansen[1], B.J.J.M Haase – Kromwijk[1], A.J. Hoitsma[2], Dutch Transplant Foundation, Leiden[1], University Medical Center St. Radboud, Nijmegen[2], The Netherlands

11.20 Donor organ temperature after systemic flush out is far from cold (p. 95)
C.H. Vrijenhoek[1], J.E. Buiter[2], J. Ringers[1], C. Krikke[2], H.G.D. Leuvenink[2], [1]Leiden University Medical Center, [2]University Medical Center Groningen, The Netherlands

11.30 Early deterioration of PELD score in young children with biliary atresia predicts poor outcome (p. 96)
M.I. Sijsling, N.S.S. Kuiken, F.A.J.A. Bodewes, H.J. Verkade, P.F. van Rheenen, Dept. of Pediatric Gastroenterology, Hepatology and Nutrition, Beatrix Children's Hospital, University Medical Center Groningen, The Netherlands

11.40 The Eurotransplant Donor Risk Index distribution amongst MELD categories within the Eurotransplant region (p. 97)
J.J. Blok[1,2], J. Ringers[1], A.O. Rahmel[2], X. Rogiers[4], H. Putter[3], A.E. Braat[1], [1]Dept. of Transplant Surgery, Leiden University Medical Center, Leiden, The Netherlands, [2]Eurotransplant International Foundation, Leiden, The Netherlands, [3]Dept. of Medical Statistics, Leiden University Medical Center, Leiden, The Netherlands, [4]Dept. of Surgery, Ghent University Hospital, Ghent, Belgium

Parallelsessie VIII – Allocatie - vervolg **Beatrix 1 en 2**

11.50 Training 'Communication about Donation' successful in facilitating medical professionals and in increasing the donation consent rate (p. 98)
N.E. Jansen[1], H.A. van Leiden[1], J. de Graauw[1], A.J. Hoitsma[2], B.J.J.M. Haase-Kromwijk[1], [1]Dutch Transplant Foundation, Leiden, [2]University Medical Center St. Radboud, Nijmegen

12.00 Effect of donor risk factors on islet yield in pancreatic islet isolation (p. 99)
J.J. Blok[1,2], M.A. Engelse[3], M. J. Verhagen[1,2], H. Putter[4], J. Ringers[1], E.J. de Koning[3], A.E. Braat[1], [1]Dept. of Transplant Surgery, Leiden University Medical Center, Leiden, [2]Eurotransplant International Foundation, Leiden, [3]Dept. of Nephrology, Leiden University Medical Center, Leiden, [4]Dept. of Medical Statistics, Leiden University Medical Center, Leiden, The Netherlands

12.10 Transplanted kidneys from donors after cardiac death type 3 who are older than 60 years of age (p. 100)
M.B.A. Heemskerk[1], A.C. Hemke[1], B.J.J.M. Haase Kromwijk[1], A.J. Hoitsma[1,2], [1]Dutch Transplant Foundation, [2]Dept of nephrology, University Medical Centre St. Radboud, The Netherlands

12.20 Deceased donor factors and non-viable kidneys (p. 101)
R.A. Poldervaart, J. Hagenaars, M. van der Hoeven, M.J. Poldervaart, J. Kal van Gestel, I. Tieken, J.N.M. IJzermans, M. Betjes, W. Weimar, J.I. Roodnat, Dept of Internal Medicine and Surgery EMC Rotterdam, Eurotransplant Leiden, The Netherlands

12.30 Lunchpauze

Parallelsessie IX – Basaal — Beatrix 3 en 4

Voordrachten in het Engels, spreektijd 8 minuten, discussietijd 2 minuten.

Voorzitters: S. Heidt en A. van der Meer

11.00 Effects of human alpha-1-Antitrypsin (hAAT) therapy in a murine model of bilateral kidney ischemia-reperfusion injury (p. 102)
N. Maicas[1], J. van der Vlag[1], C. Dinarello[2], R. Masereeuw[3], M. Bakker-vanBebber[1], L.A. Joosten[4], L.B. Hilbrands[1], [1]Dept of Nephrology, [3]Dept of Pharmacology and Toxicology, [4]Dept of Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, [2]Dept of Medicine, University of Colorado Health Sciences Center Denver, USA

11.10 A preoperative amino acid free diet protects against hepatic ischemia reperfusion injury (p. 103)
T.C Saat, T.M van Ginhoven, M. Verweij, L.J.W. van der Laan, J.N.M. IJzermans, R.W.F. de Bruin, Laboratory for Experimental Transplantation and Intestinal Surgery (LETIS) – University Medical Center Rotterdam, The Netherlands

11.20 Mesenchymal stem cell-derived trophic factors promote liver regeneration but does not protect against ischemia reperfusion injury (p. 104)
S.M.G. Fouraschen[1], L.J.W. van der Laan[1], J. Wolf[2], H.J. Metselaar[3], R.W.F. de Bruin[1], H.W. Tilanus[1], K.M. Olthoff[2], J. de Jonge[1], [1]Dept. of Surgery and Laboratory of Experimental Transplantation and Intestinal Surgery, Erasmus MC-University Medical Center, Rotterdam, The Netherlands, [2]Penn Transplant Institute, University of Pennsylvania, Philadelphia, USA, [3]Dept. of Gastroenterology & Hepatology, Erasmus MC-University Medical Center, Rotterdam, The Netherlands

11.30 Functional analysis of virus-specific T-cells with cross-reactivity to allo-antigens (p. 105)
K.M. Heutinck[1,2], H. van den Heuvel[3], S.L. Yong[1,2], E.B.M. Remmerswaal[1,2], F.H.J. Claas[3], R.J.M. ten Berge[2], [1]Dept of Experimental Immunology, [2] Renal Transplant Unit, Dept of Internal Medicine, Academic Medical Centre, Amsterdam, [3]Dept of Immunohematology and Blood transfusion, Leiden University Medical Centre, Leiden, The Netherlands

11.40 Disappearance of immunoglobulin producing plasmablasts in kidney transplant patients is associated with a diminished cytokine producing capacity by peripheral follicular T-lymphocytes (p. 106)
G.N. de Graav, MD[1], M. Dieterich[1], N. Litjens, PhD[1], D.A. Hesselink, MD,PhD[1], M.G.H. Betjes, MD, PhD[1], W.W. Weimar, MD, PhD[1], R. Bouamar[1], C.C. Baan, PhD[1]. Internal Medicin & Transplant Immunology[1], Erasmus MC, Rotterdam, The Netherlands

11.50 Tubular injury markers KIM-1 and NGAL represent different pathophysiological processes during delayed graft function (p. 107)
C. van Kooten[1], P. van der Pol[1], S.W. van der Kooij[1], D.J. van Gijlswijk-Jansen[1], N. Schlagwein[1], M.J. Mallat[1], I.M. Bajema[2], J.W. de Fijter[1], Dept of [1]Nephrology and [2]Pathology, Leiden University Medical Center, Leiden, The Netherlands

12.00 The TNF-alpha -238 G-allele predisposes to severe bacterial infection in patients with end-stage liver disease enlisted for liver transplantation (p. 108)
E. de Mare-Bredemeijer[3], R. Bartáková[1]*, S. Fraňková[1], D. Roelen[4], T. Visseren[3], P. Trunečka[1], J. Špičák[1], H. Metselaar[3], M. Jirsa[2], J. Kwekkeboom[3], J. Šperl[1], [1]Dept. of Hepatogastroenterology, Inst for Clin and Exp Med and [2]Laboratory of Experimental Medicine, Prague, Czech Republic, [3]Dept. of Hepatogastroenterology, University Medical Center, Rotterdam, [4]Dept. of Immunohematology and Blood Transfusion, University Medical Center, Leiden, The Netherlands. *These authors contributed equally*

12.10 Alemtuzumab as anti-rejection therapy in kidney transplant patients induces homeostatic T cell proliferation and impairs IL7 but not IL2 responses (p. 109)
A.P. Bouvy, D.A. Hesselink, M. Klepper, W. Weimar and C.C. Baan, Dept. of Nephrology and Transplantation, Erasmus MC, University Medical Center Rotterdam, The Netherlands

12.20 Mature dendritic cells are superior stimulator cells for expansion of human alloreactive regulatory T cells (p. 110)
N.H.R. Litjens, C.C. Baan, M.G.H. Betjes, Erasmus Medical Center, Dept. of Internal Medicine, Section Nephrology & Transplantation, Rotterdam, The Netherlands

12.30 Lunchpauze

Sessie X Plenair Maxima 2

Voorzitter: M. Warlé

13.30 **"New perfusion strategies to increase the number and quality of donor organs"**
Dr. C. Fondevila, Associate Professor of Surgery, Institute of Digestive Diseases, Hospital Clinic, University of Barcelona, Spain

Parallelsessie XI – Klinisch Maxima 2

Voordrachten in het Nederlands of Engels, spreektijd 8 minuten, discussietijd 2 minuten.

Voorzitters: M. Warlé en K. van Donselaar

14.00 Successful ex-vivo normothermic machine perfusion and viability testing of discarded human donor livers (p. 111)
S. op den Dries[1,2], N. Karimian[1,2], M.E. Sutton[1,2], A.C. Westerkamp[1,2], M.W.N. Nijsten[3], A.S.H. Gouw[4], J. Wiersema-Buist[2], T. Lisman[2], H.G.D. Leuvenink[2], R.J. Porte[1], [1]Section of Hepatobiliary Surgery and Liver Transplantation, Dept. of Surgery, [2]Surgical Research Laboratory, [3]Dept. of Critical Care, [4]Dept. of Pathology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

14.10 Auxiliary and orthotopic liver transplantation for acute liver failure. A single center experience (p. 112)
T.P. Brouwer[1], A. Inderson[2], J.J. Blok[1], M.J. Coenraad[2] ,A.E. Braat[1], B. van Hoek[2], J. Ringers[1], [1]Dept. of Transplant Surgery, [2]Dept. of Gastroenterology and Hepatology, Leiden University Medical Center, Leiden, The Netherlands

14.20 Duct-to-duct biliary reconstruction in liver transplantation for primary sclerosing cholangitis is associated with less biliary complications, compared with Roux-en-Y hepatico-jejunostomy (p. 113)
M.E. Sutton[1], R. Bense[1], T. Lisman[1], E. van der Jagt[2], A.P. van den Berg[3], R.J. Porte[1], [1]Dept of Surgery, [2]Dept of Radiology, [3]Dept of Gastroenterology and Hepatology, University Medical Center Groningen, The Netherlands

14.30 Outcome of kidney transplantation in HIV positive recipients in The Netherlands (p. 114)
W. van Snippenburg[1], T. Mudrikova[1], J.W. De Fijter[2], T. van Gelder[3], K.A.M.I. van Donselaar[4], A.J. Hoitsma[5], A.D. van Zuilen[6], [1]Dept of Internal and Infectious Diseases, UMC Utrecht, [2]Dept of Nephrology, Leiden UMC, [3]Dept of Internal Medicine, Erasmus Medical Center Rotterdam, [4]Dept of Nephrology, AMC Amsterdam, [5]Dept of Nephrology, Radboud UMC Nijmegen, [6]Dept of Nephrology and Hypertension, UMC Utrecht, The Netherlands

14.40 Conversion from Prograft® to Advagraf® after kidney transplantation is safe (p. 115)
M.M. Cadogan, W. Zuidema, N.J. de Leeuw van Weenen, D.A. Hesselink, M.G.H. Betjes, W. Weimar, Dept. of Internal Medicine, Division of Nephrology and Transplantation, Erasmus MC Rotterdam, The Netherlands

14.50 Transplant nephrectomy: what are the surgical risks? (p. 116)
V.P. Alberts[1], R.C. Minnee[1], F.J. Bemelman[2], K. A.M.I. van Donselaar–van der Pant[2], M.M. Idu[1], [1]Dept. of Surgery, Academic Medical Center, Amsterdam, The Netherlands, [2]Renal Transplant Unit, Dept. of Nephrology, Academic Medical Center, Amsterdam, The Netherlands

15.00 Increased mortality in elderly recipients of non-heart beating kidneys (p. 117)
S.P. Berger[1], J.I Roodnat[1], J.N. Ijzermans[2], M.G. Betjes[1], W. Weimar[1], [1]Dept. of Internal Medicine, [2]Dept. of Surgery, Erasmus MC University Medical Center, Rotterdam, The Netherlands

Parallelsessie XI – Klinisch - vervolg **Maxima 2**

15.10 Immuno adsorption and risk of bleeding of recipients receiving a blood group ABO-incompatible kidney transplant (p. 118)
A.E. de Weerd[1], M. van Agteren[1], P.A.W. te Boekhorst[2], W. Weimar[1], M.G.H. Betjes[1], Dept of Nephrology[1], Dept of Haematology[2], Erasmus Medical Center, Rotterdam, The Netherlands

15.20 Gastroparesis after Lung transplantation: Prevalence, reversibilty and relation with outcome (p. 119)
S.H.Smit[1], A.W.J.M. Glaudemans[2], W. van der Bij[1], M.E. Erasmus[1], E.A.M. Verschuuren[1], [1]Dept of Pulmonary Diseases, University Medical Center Groningen, [2]Nuclear Medicine & Molecular Imaging, University Medical Center Groningen, The Netherlands

15.30 Theepauze

Parallelsessie XII – Basaal — Beatrix 3 en 4

Voordrachten in het Engels, spreektijd 8 minuten, discussietijd 2 minuten.

Voorzitters: I. Joosten en E. Remmerswaal

14.00 Downregulation of complement activity via the mannan-binding lectin (MBL) pathway by dietary restriction and fasting (p. 120)
S. Shushimita[1], P. van der Pol[2], R.W.F. de Bruin[1], J.N.M. IJzermans[1], C. van Kooten[2], F.J.M.F. Dor[1], [1]Dept. of Surgery, division of Transplant Surgery, Erasmus MC, University Medical Center, Rotterdam, The Netherlands, [2]Dept. of Nephrology, Leiden University Medical Center, Leiden, The Netherlands

14.10 Targeting PKC in human T cells using Sotrastaurin stabilizes regulatory T cells and prevents IL-17 production (p. 121)
X. He[1], H.J.P.M. Koenen[1,], R.L. Smeets[2,*], R. Keijsers[3], E. van Rijssen[1], P.C. van de Kerkhof[3], A.M.H. Boots[4,**], I. Joosten[1,**], [1]Laboratory of Medical Immunology, [2]Laboratory of Clinical Chemistry, Dept. of Laboratory Medicine, [3]Dept. of Dermatology, Radboud University Medical Centre, Nijmegen, The Netherlands, [4]Dept. of Rheumatology and Clinical Immunology, University Medical Centre Groningen, Groningen, The Netherlands, */** Authors contributed equally to this study*

14.20 Bone Marrow and Adipose Tissue Derived Mesenchymal Stem Cells Induce HLA-specific Lysis by CD8+ T Cells (p. 122)
M. Roemeling-van Rhijn[1], M.E. Reinders[3], M. Franquesa[1], A.U. Engela[1], S.S. Korevaar[1], C.C. Baan[1], H. Roelofs[4], J.N.M. IJzermans[2], M.G.H. Betjes[1], W. Weimar[1], M.J. Hoogduijn[1], Depts of [1]Internal Medicine, [2]General Surgery, Erasmus MC, Rotterdam, The Netherlands, Depts of [3]Nephrology, [4]Immunohematology and bloodtransfusion, Leiden University Medical Center, Leiden, The Netherlands

14.30 A comparison of inflammatory, cytoprotective and injury gene expression in heart beating and non-heart beating donor kidney (p. 123)
T.C. Saat, D. Susa, H.P. Roest, N.F.M. Kok, S. van den Engel, J.N.M. IJzermans, R.W.F. de Bruin, Dept. of Surgery, University Medical Centre, Rotterdam, The Netherlands

14.40 Evidence for a possible role of memory B cells in acute kidney graft rejection (p. 124)
S. Heidt[1], M. Vergunst[1], J. Anholts[1], M. Eikmans[1], J.W. de Fijter[2], F.H.J. Claas[1], [1]Dept. of Immunohaematology and Blood Transfusion, [2]Dept. of Nephrology, Leiden University Medical Center, Leiden, The Netherlands

14.50 Human alloantigen induced regulatory T cells suppress alloreactivity in an antigen specific manner independent of CD127 expression (p. 125)
K. Boer[1], A.M.A. Peeters[1], R.Kraaijeveld[1], W.Schoordijk[1], N.H.R. Litjens[1], M.G.H. Betjes[1], W.Weimar[1], C.C. Baan[1], [1]Dept. of Internal Medicine, Erasmus University Medical Center Rotterdam, The Netherlands

15.00 CXCR5+CD4+ follicular helper T cells accumulate in resting human lymph nodes and have superior B cell helper activity (p. 126)
E.B.M. Remmerswaal[1,2], S.H.C. Havenith[1,2], M.M. Idu[3], K.A.M.I. van Donselaar[2], N. van der Bom[1], F.J. Bemelman[2], E.M.M. van Leeuwen[1], I.J. M. ten Berge[2], and R.A.W. van Lier[4], [1]Dept. of Experimental Immunology, Academic Medical Center, Amsterdam, The Netherlands, [2]Renal Transplant Unit, Dept. of Internal Medicine and [3]Dept. of Surgery, Academic Medical Center, Amsterdam, the Netherlands, [4]Sanquin Research and Landsteiner Laboratory, Academic Medical Centre, Amsterdam, The Netherlands

15.10 Rabbit antithymocyte globulin induction therapy induces donor-specific HeliosnegFOXP3pos regulatory T cells in kidney transplant patients (p. 127)
A.P. Bouvy[1], M. Klepper[1], M.M.L Kho[1], J.N.M. IJzermans[2], N.H.R. Litjens[1], M.G.H. Betjes[1], W. Weimar[1], C.C. Baan[1], [1]Dept. of Internal Medicine, [2]Dept. of Surgery, Erasmus MC, University Medical Center Rotterdam, The Netherlands

Parallelsessie XII – Basaal - vervolg **Beatrix 3 en 4**

15.20 Differences in mobilization of rare hematopoietic stem cells from human liver grafts of non-heart beating and heart beating donors (p. 128)
S.R.R Hall[1], Q. Pan[2], A. Pedroza-Gonzalez[2], R.W.F. de Bruin[1], J. Kwekkeboom[2], H.J. Metselaar[2], H.W. Tilanus[1], G. Wagemaker[3], J. de Jonge[1], L.J.W. van der Laan[1], [1]Dept. of Surgery and Laboratory of Experimental Transplantation and Intestinal Surgery, [2]Dept. of Gastroenterology & Hepatology, [3]Dept. of Hematology, Erasmus MC-University Medical Center, Rotterdam, The Netherlands

15.30 Theepauze

Prijsuitreikingen **Maxima 2**

16.00 Uitreiking ATRP prijs 2013
gevolgd door lezing door winnaar van de ATRP 2012

16.10 Uitreiking Novartis Transplantation Grant 2013
Uitreiking Novartis Personalized Immunosuppression Award

16.20 Uitreiking Gauke Kootstraprijs 2013
gevolgd door lezing door de prijswinnaar

Ledenvergadering **Maxima 2**

16.30 Ledenvergadering Nederlandse Transplantatie Vereniging

17.45 Sluiting en vaarwel

CD3 antibody-induced transplant tolerance relies on in situ TGF-beta and PD-1 signaling in T cells

M.C. Baas[1,2,3], A. Besançon[1,2], C. Kuhn, C. Mangez[1,2], F. Valette[1,2], T. Goncalves[1,2], L. Chatenoud[1,2] and S. You[1,2], [1]National de la Santé et de la Recherche Médicale, Unité 1013, Paris, France, [2]Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Paris, France, [3]Present address: Dept. of Nephrology, Radboud University Medical Centre Nijmegen, The Netherlands

CD3 antibody therapy, successfully used to treat patients with recent onset type 1 diabetes, can restore self-tolerance to autoantigens. We extented these tolerogenic properties to the field of transplantation by demonstrating that short-term, low-dose course with CD3-specific antibodies at the time of effector T cell priming to the alloantigens induced tolerance to fully mismatched allografts. CD3 antibodies primarily targeted antigen-activated T cells while sparing Tregs which can transfer donor-specific tolerance. Of potential clinical relevance, we recently found that humanized CD3 antibodies also promote permanent islet graft acceptance when applied in mice transgenic for the human CD3epsilon chain. The aim of the present study was to further elucidate the immune mechanisms driving CD3 antibody-mediated tolerance. C57Bl/6 mice, rendered diabetic after an injection with streptozotocin, were grafted with fully MHC-mismatched BALB/c pancreatic islets and treated with CD3-specific antibodies at day 7 after transplantation, which induced allograft tolerance. Although after treatment, the percentage of Foxp3+ Tregs increased in secondary lymphoid organs and in the graft itself, Foxp3+ Tregs were not mandatory for tolerance induction. Furthermore, de novo generation of Foxp3+ Tregs was not evidenced in our model. However, tolerance induction relied on in situ PD-1 and TGF-beta signaling in T cells. In the allograft but not in secondary lymphoid organs, CD3 antibody treatment induced strong expression of the inhibitory receptor PD-1, particularly on CD8+ T cells that also acquire the capacity to produce high amounts of TGF-beta as shown by single cell PCR. Blocking these signaling pathways by neutralizing antibodies abrogated tolerance induction.
In conclusion, CD3 antibody therapy establishes an intragraft tolerogenic environment via signaling through the inhibitory PD-1 pathway and local production of TGF-beta by both CD4+ and CD8+ T cells.

High expression of S100 calcium binding proteins A8 and A9 in macrophages during acute transplant rejection is associated with a beneficial immune response

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In patients, who had been transplanted between 1986 and 1995 and who suffered from acute rejection, a high expression of S100 calcium binding proteins A8 and A9 was found to be associated with favorable graft outcome. We here confirmed this finding in a 1995-2005 transplant cohort: high S100A8 and A9 mRNA expression during acute rejection (>2 times the median; n=36) led to a 12-year graft survival of 91.5%, whereas low expression (n=61) was related to 69.2% graft survival. S100A9 mRNA expression significantly correlated with the extent of S100A9 protein by immunohistochemistry ($P<0.005$). S100A8 and A9 expression was associated with a significantly elevated expression of anti-inflammatory IL-10 ($P<0.01$) and regulatory T cell marker FoxP3/CD3 ($P<0.0001$), and with significantly decreased levels of kidney injury molecule ($P<0.05$). As S100A8 and S100A9 are mainly expressed by monocytes and macrophages, we investigated protein expression of macrophage activation marker CD163 and pan-marker CD68. Triple immunostaining on paraffin slides showed that the majority of CD68+ macrophages within the tissue were either positive for S100A9 or for the macrophage activation marker CD163, indicating the presence of different macrophage subtypes during acute rejection. In line with the in vivo findings, overexpression of the S100A8 and S100A9 genes in the monocytic cell line THP-1 led to de novo production of IL-10. S100A8 and S100A9 are markers of a distinct macrophage subpopulation during acute transplant rejection, which may have regulatory properties leading to a beneficial graft outcome.

Kidney transplantation does not reverse the premature T cell ageing in end-stage renal disease patients

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Background: End-stage renal disease (ESRD) patients have a defective T cell mediated immune system which is related to excessive premature ageing of the T cell compartment. This is believed to be caused by the uremia-induced pro-inflammatory conditions. Kidney transplantation (KTx) reduces this pro-inflammatory environment and the aim of this study is to investigate whether KTx is able to reverse premature immunological ageing. Methods**:** For this purpose, we followed 140 KTx recipients excluding cytomegalovirus (CMV)pos donor/CMVneg recipient combinations as primary CMV infection influences T cell differentiation status. KTx recipients were followed prior to and at 3, 6 and 12 months post KTx and their circulating T cells studied using several T cell ageing parameters. First, thymic output was assessed by determining the T cell receptor excision circle (TREC) content together with % $CD31^+$ naïve T cells. Relative telomere length (RTL) was determined as a measure for proliferative history and immunophenotyping was used to establish the differentiation status of circulating T cells. Results: The TREC content, % of $CD31^+$ naïve T cells as well as the RTL of $CD4^+$ and $CD8^+$ T cells remained unaltered within the first year post KTx. The absolute number of $CD4^+$ as well $CD8^+$ T cells decreased 3 months after KTx mainly due to a decrease in memory T cells and in particular the more differentiated T cells ($p<0.001$ for both $CD4^+$ EM and $CD8^+$ EMRA population). At 12 months post transplantation the number of $CD8^+$ EMRA T cells reached again pre-KTx values. Remarkably, the number $CD4^+$ EM T cell population remained significantly low ($p<0.05$) within the first year post KTx (i.e. 189.4±15.3 cells/µl pre vs 136.3±13.7 cells/µl at 12 months).
Conclusion**:** The prematurely aged T cell compartment of ESRD patients is not reversed by kidney transplantation. Loss of thymic function and excess of highly differentiated aged T cells seem permanent and therefore remain important determinants of a dysfunctional immune system after transplantation
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Unspecified living kidney donors in The Netherlands: an overview

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Background: The first unspecified, formerly known as, altruistic or good Samaritans, was performed in 2000 in Rotterdam. After the 'Big Donor Show' in 2007, these unspecified living kidney donors are a rapidly growing source of living donors. Here we describe the unspecified living kidney donors who have been reported from 2000 until now in all 8 kidney transplant centers in The Netherlands. Methods: We asked the centers about the registered unspecified donors, the number of donors with a contraindication and their reasons and the number of unspecified donors who actually donated. Results: From January 2000 till January 2013, 284 unspecified donors started with the screening evaluation in all 8 centers. The median numbers of registered donors per center was 24 (range 7-121). After the screening process 106 donors have a contraindication to become a living donor. The reasons for refusal were 35 on their own initiative, 42 for medical reasons, 24 for psychological, and 5 for social-economic. 142 donors have already donated their kidney, 10 donors are still waiting for an operation and 26 donors are in screening on the out-patient clinic. The blood type of these 142 donors was 75 times O (53%), 52 times A (37%), 8 times B (5%) and 7 times AB (5%). 50% of the donors were female. 56 donors donated directly to a recipient on the wait list and 86 in a domino-paired procedure: 68 made 2 transplants possible, 13 donors donated in a triplet construction and 5 donors in a quartet procedure. So these 86 unspecified donors were enrolled in chain constructions which resulted in 195 kidney transplants.
Conclusion: In total 142 unspecified donors made 251 kidney transplants possible. With 86 chain constructions we have increased the number of kidney transplants by 127% from 86 to 195. Including all unspecified donors in domino-paired procedures would increase the number of transplants even more. All unspecified donors should be enrolled in chain constructions.

First results of A Randomized Controlled Trial on A Home-Based Educational Intervention

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Objective: Living donor kidney transplantation (LDKT) is the most successful form of renal replacement therapy (RRT). We observed a significant inequality between Western and non-Western patients in the access to LDKT. In a randomized controlled trial we investigated the effectiveness and efficacy of a home-based educational intervention to reduce this inequality by providing accessible and adequate information and to establish well-informed decision making through a guided communication with patients and individuals from their social network (invitees). Methods: In this trial 160 patients who were on the wait list for a deceased donor kidney transplantation were urn-randomized over two conditions. The control group received standard care: hospital education (informational video and handbook) (n=80). The experimental group received standard care plus an educational intervention in their homes using multisystemic therapy (n=80). The intervention was a European translation of the programme developed by Rodrigue in the USA. A questionnaire was administered to all patients in both conditions and invitees as a baseline and post-measurement on: knowledge, risk perception, subjective norm, self-efficacy, and communication. Results: The ratio of Western and non-Western patients in the control group was (40/40) and in the experimental group (22/43) (p= 0.074). Compared to the control group, patients who received the home-based education showed significant improvements in their overall knowledge on kidney disease and RRT's (p< 0.001) and communicated more with their loved ones about RRT (p= 0.048). On average patients invited 5 invitees for the educational session. These invitees showed improvements in their overall knowledge (p< 0.001) and their self-efficacy regarding discussing RRT's with the patient (p= 0.032). In addition the invitees showed a decrease in their risk perception towards LDKT (p< 0.001) and they were more willing to donate a kidney (p= 0.016) after the educational intervention.
Conclusions: We argue that these improvements in knowledge, communication, risk perception and willingness to donate support well-informed decision making regarding patient's optimal treatment option.

NiCe: Patient-centered care for live kidney donors

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Introduction: In our experience, live kidney donors and their care providers often indicate that cure and care were not in line with the donors' biomedical and contextual factors. "Kidney donor-centered care" (NiCe) is an intervention to align this care. NiCe defines how nurse practitioners (NPs) provide patient-centered care to kidney donors and therefore substitute the care given by the surgical residents. Purpose: Aim of this study was to determine whether the implementation of NiCe, which connects the care and support with the biomedical and contextual factors for live kidney donors, would result in an increased patient satisfaction. Methods: The NiCe study was a quantitative study, with a qualitative component. The control group (N=27) consisted of donors admitted between October 16th 2011 and February 12th 2012 and the intervention group (N=26) of donors between February 13th 2012 and April 30th 2012. Three different methods of data collection were used. A validated four-point Likert scale questionnaire of Consumer Quality Index was used to measure patient satisfaction. Donors' medical files were screened for complications and mortality. The qualitative part consisted of a log book in which professionals could report their experiences in order to evaluate the process. Results: The care by the surgical team was significant better appreciated in the intervention group versus the control group (2.87 vs 3.3, $p<0.05$). Also the care was more aligned after the implementation of NiCe ($p<0.05$).There were no significant differences regarding complications, readmissions, communication and care by NPs and nurses. According to the care providers involved in this study, there were no difficulties in the process of NiCe implementation.
Conclusion: A greater patient satisfaction was reached in the intervention group. Therefore the most important recommendation for the future is to continue NiCe for live kidney donors.

Low pressure pneumoperitoneum during laparoscopic donor nephrectomy to optimize live donors' comfort

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Nowadays laparoscopic donor nephrectomy (LDN) has become the gold standard to procure live donor kidneys. As the relationship between donor and recipient loosens, it becomes of even greater importance to optimize safety and comfort of the surgical procedure. Low pressure pneumoperitoneum has been shown to reduce pain scores after laparoscopic cholecystectomy. Live kidney donors may also benefit from the use of low pressure during LDN. To evaluate feasibility and efficacy to reduce postoperative pain, we performed a randomized double blind study. Twenty donors were randomly assigned to standard (14 mmHg) or low (7 mmHg) pressure during LDN. One conversion from low to standard pressure was indicated by protocol due to lack of progression. Intention-to-treat analysis showed that low pressure resulted in a significantly longer skin-to-skin time (149±86 *versus* 111±19 minutes), higher urine output during pneumoperitoneum (23±35 *versus* 11±20 mL/hr), lower cumulative overall pain score after 72 hours (9.4±3.2 *versus* 13.5±4.5) and a lower cumulative overall referred pain score (1.8±1.9 *versus* 4.2±3). Donor serum creatinine levels, complications and quality of life dimensions were not significantly different. Our data show that low pressure pneumoperitoneum during LDN is feasible and may contribute to increase live donors' comfort during the early postoperative phase.

28 Years of pancreas transplantation: the Leiden experience

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Introduction: The past decades there has been a lot of development in the field of pancreas transplantation. Here we describe the experiences of our center over the past 28 years. Objective: Evaluation of all pancreas transplantations performed at our center. Methods: All pancreas transplantations performed at our center from 1984 till 2011 were analysed. Univariate and multivariate analysis were performed to identify significant donor, transplant and recipient risk factors. This cohort was divided into two groups, using the start of modern immunosuppressive therapy (induction therapy) in 1999 as dividing point. Results: A total of 322 pancreas transplants were performed, of which 304 primary pancreas transplants (298 SPK, 4 PAK and 2 PTA) and 18 re-transplants (3 SPK, 14 PAK and 1 PTA). Donor factors were: age 35 years (median), BMI 23 (median), CVA-related COD 56% and DCDD donors 1.6%. Transplant factors were: 65% regional allocation and 91% UW perfusion fluid. Recipient factors were: age 42 years (median) and 43% pre-emptive transplantation. In the more recent group recipients were significantly older (43 years vs. 39 years; p=0.026) and a higher rate of re-transplants was performed (7% vs. 3%; p=0.067). Median P-PASS was16 and PDRI was 1.24. Throughout the years there was a trend in increasing (median) PDRI ranging from 1.1 to 1.4 in more recent years (p=0.12). Overall (death-censored) pancreas graft survival was 77% at 5 years and 73% at 10 years follow-up. When analysing the two groups separately, group I had 70% at 5 years and 67% at 10 years, group II had 81% at 5 years and 75% at 10 years. Overall 5- and 10 year patient survival rates were 86% and 73%. For SPK transplants results were even better (group II 5- and 10-year graft survival 83% and 76%). Thrombosis rates were not significantly different: group I 11% and group II 8% (p=0.36). Results of the univariate and multivariate analysis for risk factors are currently processed.
Conclusion: Over the past 28 years, pancreas transplantation has developed tremendously. Regardless of the more liberal acceptance concerning donor quality and recipient condition, results improved and are currently at a high level. Furthermore, despite improved outcome of the more recent pancreas transplant cohort, there is still 8% graft thrombosis, which remains a significant concern, as is also described in the literature (10-15%).

Kwaliteitsverbetering in de zorg voor kinderen rondom niertransplantatie door inzet gespecialiseerd verpleegkundige

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In het UMC St Radboud werd een kwaliteitsslag ervaren door de inzet van een verpleegkundig specialist binnen de zorg voor kinderen in de predialyse en transplantatiefase. In navolging hierop werd in vier centra een verpleegkundige aan het kindernefrologisch team toegevoegd en geëvalueerd of de kwaliteit van zorg toenam. Methode: Een niet gecontroleerde voor en nameting in vier centra voor de behandeling van kinderen met een chronische nierziekte: het AMC, het Erasmus MC, het UMC Utrecht en het VU MC. Samen met de ouders en de professionals van de deelnemende centra werden eerst indicatoren ontwikkeld aan de hand van de beschikbare richtlijnen. Nadat 149 ouders van kinderen schriftelijk met het onderzoek hadden ingestemd werden de dossiers van deze kinderen driemaandelijks gescreend. Daarnaast werden interviews afgenomen met de ouders, de professionals van het kindernefrologisch team. Resultaten: In de vier deelnemende centra gaven de aan het team toegevoegde verpleegkundigen voorlichting over de behandeling, de werking en inname van medicatie, voeding en bewegen en sporten. Daarnaast droegen zij zorg voor veel praktische zaken zoals het meten van lengte, gewicht en bloeddruk, het doorbellen van laboratoriumuitslagen, het regelen van incontinentiemateriaal en thuiszorg en het onderhouden van contacten met de patiënt (case manager). De bereikbaarheid van het kindernefrologisch team verbeterde volgens de ouders en zij hadden het gevoel sneller antwoord te krijgen op gestelde vragen. De ouders waren tevreden over de verpleegkundige, de kindernefroloog en het kindernefrologisch team. Volgens de artsen vervulde de verpleegkundige een belangrijke functie tijdens de spreekuren waardoor zij zich nu meer op de medisch- inhoudelijke zorg konden richten.
Concluderend laat deze evaluatie zien dat in de deelnemende centra de kwaliteit van zorg licht verbeterde waarbij de verpleegkundige een actieve rol had. Zij had vooral voorlichtende taken en was belast met regel- en organisatie zaken. Daarnaast verbeterde de bereikbaarheid van het kindernefrologisch team en waren ouders tevreden over het hele kindernefrologisch team inclusief de verpleegkundige en hadden de kindernefrologen meer tijd voor de medisch-inhoudelijke zorg. Andere verbeteringen kunnen met het nog sterker neerzetten van de gespecialiseerde verpleegkundige als coördinator van de zorg en behandeling mogelijk binnen handbereik komen.

Tacrolimus predose concentrations do not predict the risk of acute rejection after renal transplantation: a pooled analysis from three randomised-controlled clinical trials

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Introduction: Therapeutic drug monitoring for tacrolimus (Tac) is universally applied. However, the concentration-effect relationship for Tac is poorly defined. This study investigated whether Tac concentrations are associated with acute rejection in kidney transplant recipients. Methods: Data from three large clinical trials in kidney transplantation (the Symphony-Elite, Opticept, and FDCC trials) were pooled. We used univariate and multivariate analysis to investigate the relationship between BPAR and Tac predose concentration at 5 time points (day 3, 10, and 14, and month 1 and 6 after transplantation). Results: 1304 kidney transplant recipients were included. A total of 136 patients experienced biopsy-proven acute rejection (BPAR), giving an overall incidence of 10.4%. The majority of BPAR occurred within the first month after transplantation (7%). We did not find any significant correlations between Tac predose concentrations and the incidence of BPAR at the different time points. In the multivariate analysis, only delayed graft function (DGF) and the use of induction therapy were independently correlated with BPAR, with an odds ratio of 2.7 [95% CI: 1.8 - 4.0; $p < 0.001$] for DGF and 0.66 [95% CI: 0.44 - 0.99; $p = 0.049$] for induction therapy. The other variables, including the Tac predose concentrations, were not statistically significantly associated with BPAR.

Conclusion: We did not find an association between the Tac predose concentrations measured at 5 time points after kidney transplantation and the incidence of acute rejection occurring thereafter. Based on the results of this study it is not possible to define the optimal target concentrations for Tac.

Delayed trough level measurement of tacrolimus QD requires an adjusted target range

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Background: Tacrolimus has a narrow therapeutic window and therefore requires therapeutic drug monitoring (TDM), which is usually performed by measuring trough levels before ingestion of the morning dose. In daily clinical practice, it is logistically difficult to measure trough levels in patients who use tacrolimus twice daily and visit the outpatient clinic in the afternoon. The slow-release formulation of tacrolimus (QD) is taken once daily in the morning and has a terminal elimination half-life time of 37.8 h. Therefore, trough levels measured in the afternoon may be close enough to morning trough levels, which would be convenient for patients visiting the outpatient clinic in the afternoon. Alternatively, the target range for trough levels measured in the afternoon should be adjusted. Aim: To assess whether measurement of the trough level can be delayed from the morning to the afternoon in renal transplant patients with tacrolimus QD taken in the morning. Methods and patients: We measured tacrolimus pharmacokinetics in 13 patients using tacrolimus QD (ADVAGRAF®). The target range of the trough level was between 5 and 10 ug/L. In each patient, eleven blood samples were drawn during a period of 32 hours after the intake of tacrolimus QD by use of the validated dried blood spot method. Tacrolimus levels were measured with HPLC-tandem mass spectrometry. Results: The mean interval after transplantation was 74 months (range 25-178). The mean daily dose of tacrolimus QD was 5 mg (1.5-9). The 24-hour exposure (area under the curve) to tacrolimus was 308.5 ug.h/L (standard deviation 72.0). The trough levels at 24, 26, 28, 30 and 32 hours after ingestion were 9.1 ug/L (2.1), 8.2 ug/L (1.9), 8.1 ug/L (1.8), 7.7 ug/L (1.9), and 7.1 ug/L (1.6), respectively. Trough levels at 24 hours and at 32 hours differed significantly ($p<0.001$). The Pearson correlation coefficient between trough levels and area under the curve was 0.91, 0.90, 0.89, 0.92 and 0.89 for trough levels taken at 24, 26, 28, 30 and 32 hours, respectively ($p<0.001$ at each timepoint).
Conclusion: Delayed trough level measurement provides significantly lower values and therefore requires adjustment of the target range. However, the persisting strong correlation between trough levels taken until 32 hours after ingestion and 24-hour exposure warrants the use of delayed trough level measurement to improve patient convenience.

Knowledge about dialysis, transplantation and living donation among prospective living kidney donors and recipients

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Objective: In order to give informed consent, living kidney donors and recipients must have a good understanding of renal replacement therapies (RRT) options and risks. The purpose of this study was to explore the knowledge level about dialysis, transplantation and living donation among donors and recipients. Method: Eighty-five living kidney donors and 82 living donor kidney recipients completed the self-report Rotterdam Renal Replacement Knowledge-Test (R3K-T) in their native language (available in 9 languages) one day before surgery. This questionnaire consists of 10 items about Dialysis & Transplantation (DT, score:0-10) and 11 items about Living Donation (LD, score:0-11). Results: Recipients scored significantly higher on the DT-subscale (*M*=8.40, *SD*=2.53) than donors (*M*=6.36, *SD*=2.87). Donors scored significantly higher on the LD-subscale (*M*=8.36, *SD*=1.43) than recipients (*M*=6.93, *SD*=2.44). Using multiple linear regression analyses we found that recipients knew less about DT if their native language was not Dutch and if they were undergoing pre-emptive transplantation. Recipients knew less about LD if they were female, if their native language was not Dutch and if they had a religious affiliation. Donors knew less about LD if their native language was not Dutch.
Conclusion: It appears that recipients and donors retain different information even when informed together. The finding that donors did not answer all questions about LD correctly is in line with conclusions of earlier studies that some living kidney donors do not make the decision to donate based on consideration of risks and benefits. Despite patient education and the questionnaire being offered in various languages, non-Dutch speaking donors and recipients scored lower on RRT knowledge. Therefore, extra efforts should be made to ensure that these donors and recipients understand the information given.

Treatment Efficacy of Hypertension in Dutch Kidney Transplant Patients

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Background: Hypertension (HT) in kidney transplant recipients (KTR) may reach prevalence up to 90% and jeopardizes graft and patient survival. The risk for graft loss increases 15% for every 10mmHg systolic BP. Although HT in KTRs is often defined as ≥140/90 mmHg, the KDIGO target for BP in KTRs is <130/80 mmHg. For this purpose KDIGO advises a daily sodium excretion target of 100 mmol. Treatment efficacy of HT in KTRs is largely unknown. With the advent of new, but potentially dangerous approaches to therapy resistant HT such as native sympathetic kidney denervation, insight into treatment efficacy is of vital importance. We evaluated the HT treatment efficacy among Dutch KTR and aimed to identify possibilities for improvement of current therapy. Methods: We retrieved data on office BP and use of antihypertensive drugs (number of medications, dosages not available) from the Dutch Organ Transplant Registration (NOTR) on 6070 KTRs registered in 2011 (55.9% deceased donor; median time since transplantation 7.8 years; 59.4% male; median age 45 years). We also collected data from the 539 KTRs currently cared for at our center, focussing on dosages of antihypertensive drugs and 24h urinary sodium excretion.

Results Average BP in the NOTR registered patients was 134/78 mmHg (SD 18/10). In 40.2% of the NOTR patients BP was ≥140/90 mmHg of whom 8.2% had no registered use of antihypertensives, 28.5% used one, 34.2% used two and 29.1% used ≥3 antihypertensive drugs. 30.6% had a BP >130/80 but lower than 140/90 mmHg of whom 14.4% had no registered use of antihypertensive drugs, 32.8% used one, 32.0% used two and 20.9% used ≥3 antihypertensives.

In our center these parameters were comparable, with an average BP of 136/81mmHg (SD 17/10). In 43.4% BP was ≥140/90 mmHg of whom 10.2% used no, 25.6% used one, 30.8% used two and 32.9% used ≥3 antihypertensive drugs. 75.3% of the KTRs with BP ≥140/90 mmHg while on ≥3 drugs were prescribed their antihypertensive drugs at dosages that were lower than the highest permitted dose. Maximum dosages of ≥3 antihypertensives were prescribed in 8.1% of patients with BP ≥140/90 mmHg. Mean daily urinary sodium excretion was 156 mmol (SD 70.4).

The influence of region on expected patient survival of patients starting with renal replacement therapy

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Renal transplantation is the optimal treatment modality for most patients with end stage renal disease (ESRD). However, the transplant policy (donor and patient selection) among the Dutch transplant centres is different. We know already that there are regional differences in registration on the waiting list and in transplantation rate, according to the patient's residence. We wondered whether these differences in transplantation policy affect expected patient survival for all patients starting renal replacement therapy (RRT), instead of focussing on graft survival of transplanted patients only. We also wondered whether there are regional differences with respect to expected patient survival from the start of RRT. We obtained data from the Dutch Renal Replacement Registry (RENINE), on patients between 18 and 70 years old, who started RRT in the period 2004-2008 (N=5026). The 5 year survival was analysed with Kaplan Meier, and (uni- and multivariate) Cox regression analysis. Included variables in the analysis were: age, primary renal disease, sex, therapy at 90 days, transplant region and municipal health service (GGD) region. The transplant region was derived from the centre where the patient started RRT treatment and the GGD-region was derived from the address of the patient. In Kaplan Meier analysis RRT-patient survival was different for the different transplant regions (P=0.032) as well as for the different GGD regions (p=0.001). Multivariate Cox regression analysis, with age, primary renal disease and transplant region, showed that transplant region is no longer related to 5 year patient survival independently (p=0.18). A similar analysis with GGD region instead of transplant region, showed that this GGD region is also an independent predictor for survival when we correct for age and primary renal disease (p= 0.003).

Based on the analysed data we conclude that there are remarkable regional differences in the patient survival after the start of RRT. However, this cannot be related to differences in transplant policy in the different transplant centres. Reasons for regional differences should be subject of further research.

Health literacy and self management among kidney transplant patients

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Introduction: Previous research showed that almost 60% of the patients are re-hospitalized within the first year after kidney transplantation. The extent to which patients understand health advice and can identify symptoms and act accordingly, are factors that may contribute to complications after transplantation. The aim of this study was to investigate the relationships between health literacy (HL), self-management (SM), patient characteristics and socio-economic characteristics among a cohort of kidney transplant patients. Methods: From May 2012 to November 2012, we invited patients who were recently transplanted to participate. Inclusion criteria were reading and understanding the Dutch language and age > 18. We measured SM using an adapted version of the Partners in health scale (PIH, 24 items) and HL using the Dutch Newest vital sign (NVSD, 6 items). Other data (socioeconomic status, work, donor type, medical data, and ethnicity) were collected from the medical status. Results: Of the 99 potential participants, 80 (80%) were included. Socio demographic data: 65% male, mean age 55, 74% living donor recipients, and 24% of Non-European descent. Patients who scored statistically significantly lower on HL were of Non-European descent, were not transplanted pre-emptively, and were retired. Also, a significant negative correlation was found between HL and age. No statistically significant associations were found between SM and socio-demographic and patient characteristics. There was a significant positive correlation between HL and 3 subscales of SM: emotional social aspects, knowledge and aftercare, and physical care.
Conclusions: We identified a number of subgroups among kidney transplant patients who appear to have a lower HL: older patients, retired patients and Non-European patients. In turn, lower HL was related to poorer coping with emotional and social consequences, poorer monitoring of physical care and lower perceived knowledge and aftercare after kidney transplantation.

Immune reactivity of an individual against a virus induces a broad repertoire of HLA-alloreactive memory T cells

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Background: Alloreactive CD8+ memory T cells are present in all individuals, regardless of prior interaction with allogeneic cells. This finding is explained by heterologous immunity: virus-specific T cells can recognize multiple antigens through cross-reactivity of their TCR. In line with this, virus-specific TCRs have been shown to recognize not only multiple viral peptides, but also allo-HLA molecules by cross-reactivity. In a recent study we could demonstrate that allo-HLA reactivity of virus-specific T cells is common, and several research groups have succeeded in identifying allo-HLA reactivity by virus-specific T cells. However, this research focusses primarily on identification of allo-HLA reactive CD8 T cells on a clonal level, whereas most individuals develop a polyclonal response upon a viral infection. As a consequence, the impact of a broad virus-specific immune response on the allorepertoire within an individual is currently unknown. The aim of the present study was therefore to determine the impact of virus-specific polyclonal immune responses on the overall alloimmune response within an individual. Methods: An inventory was made of the incidence and specificity of virus-specific allo-HLA reactive T cells within a large group of healthy individuals. Hereto we used a panel of tetramers, consisting of 8 CMV-, 13 EBV-, 7 HSV-, and 2 Influenza virus-specific tetramers. PBMCs of multiple tetramer-positive healthy donors were labeled with CFSE and stimulated with irradiated allogeneic cells in a mixed lymphocyte reaction *in vitro*. Upon 8 days of culture, the cells were again stained with the relevant tetramers. Proliferation of tetramer positive cells was measured by FACS analysis, as identified by the tetramer+CFSE^{low}CD8+ subset. Results: Preliminary results show that *in vitro* stimulation of a responder with a single stimulator can induce several memory CD8 T cell responses with different specificities. Even with the restricted amount of tetramers, we could identify several different responding specificities upon stimulation with a single donor. The tested responder contained memory CD8 T cells specific for 8 different viral epitopes, and 5 of these specificities -originating from three different viruses- responded upon stimulation with a single stimulator.
Conclusion: Our results show that polyclonal immune responses directed against a virus can induce several virus-specific memory CD8 T cell responses with alloreactive potential against a single donor.

Angiopoietin2: a prognostic marker in kidney transplantation

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Organs derived from deceased brain dead (DBD) donors show worse organ function and more acute rejection episodes than organs derived from living donors. Human studies have provided evidence that DBD donors suffer from bacterial translocation and higher endotoxin load. A link between endotoxemia and Angiopoietin 2 (Ang2) is established. In humans, LPS triggers Ang2 release, which binds to the Tie2 receptor. Use of Ang2 as biomarker of endothelial integrity has gained much attention since Ang2 reflects the immunogenic state of an organ and could therefore be used as a predictor of organ quality and survival. We measured serum Ang2 by ELISA in 100 DBD and 220 living donors (LD). Serum was obtained immediately after the declaration of brain death (T0) and just before organ retrieval (T1). Serum from living kidney donors retrieved after start of the operation (T0) and just prior to organ retrieval (T1) was used as control. Serum Ang2 levels in DBD donors are higher at T0 and T1 (T0: 1925 ± 198.9 pg/ml and T1: 2418 ± 305.2 pg/ml) compared to living donors (T0: 690.7 ± 47.60 pg/ml and T1: 1384 ± 102.9 pg/ml). In LD, Ang2 levels increased at T1 compared to T0 ($p<0.05$). In LD, T1 Ang2 levels are associated with glomerular filtration rate (GFR) at 12 months after transplantation (Spearman's ρ -0.193 p=0.019). In DBD donors, T0 Ang2 levels are associated with serum creatinin 14 days after transplantation (Spearman's ρ -0.333 p=0.017).

These results show elevated Ang2 levels in DBD compared to LD, which is illustrative of an inflammatory response. A clinical validated Ang2 test and therapeutic interventions that modulate the Ang2 response in the DBD donor might be novel tools to improve organ quality.

Renal ischemia/reperfusion induces release of angiopoietin-2 from human grafts of living and deceased donors

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Recent insights suggest that endothelial cell (EC) activation plays a major role in renal ischemia/reperfusion (I/R) injury. Interactions between ECs and pericytes via signaling molecules, including angiopoietins, are involved in maintenance of the vascular integrity. Experimental data have shown that enhancement of Angiopoietin (Ang)-1 signaling might be beneficial in renal I/R injury. However, little is known about the role of angiopoietins and pericyte/EC interactions in human renal I/R injury. In this study, EC activation and changes in pericytes and angiopoeitins are assessed in human living and deceased donor kidney transplantation. Local release of angiopoietins was measured by unique, dynamic arteriovenous measurements over the reperfused kidney. Results demonstrate that renal I/R is associated with acute EC activation shown by a vast Ang-2 release from both living and deceased donors shortly after reperfusion. Its counterpart Ang-1 was not released. Histological analysis of kidney biopsies showed EC loss, accompanied by diminished NG2 pericyte expression after reperfusion. Baseline protein and mRNA Ang-1 expression was significantly reduced in deceased compared to living donors and decreased after reperfusion. Interventions aimed at maintenance of vascular integrity by Ang-2 blockade or Ang-1 administration may provide a tool for donor pretreatment and interventions around human clinical transplantation.

Severe ischemia-reperfusion injury defined by peak alanine aminotransferase after orthotopic liver transplantation is a strong risk factor for development of nonanastomotic strictures in donor livers from donation after cardiac death

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Introduction: Nonanastomotic strictures (NAS) are considered the Achilles heel in orthotopic liver transplantation (OLT). Donor livers from donation after cardiac death (DCD) are an important source but they are more prone to ischemia/reperfusion (IR) injury and biliary complications after OLT. Aim: Evaluation of IR-injury parameters peak aspartate aminotransferase (AST) and peak alanine aminotransferase (ALT) post-OLT as potential predictors for the development of NAS. Data such as patient, donor and operative characteristics were obtained from local anesthetic and surgical patient charts and endoscopy reports. Methods: IR-injury was defined using peak AST or peak ALT evaluated at day 4 to day 7 post-OLT. A low peak AST or ALT of < 1500 IU/L was defined as mild IR-injury whereas a high peak AST or ALT >1500 IU/L was considered as severe IR-injury. NAS was considered as any stricture or irregularity of the intra- or extrahepatic bile ducts positioned at least 1 cm above the surgical notch occurring within four years post-OLT. Results: A total of 45 OLTs were performed using DCD donors were included. Median recipient age at OLT was 57 years with a predominance of male recipients (80%). Median MELD score was 18. NAS developed in 37.8% of donor livers after OLT. DCD livers developing NAS occurred significantly more often in the group with severe IR-injury based on high peak ALT compared to the mild IR-damage evaluated by lower peak ALT (88.2% vs 11.8%, respectively, $\chi 2=14.3$, $p< 0.01$). Four-year cumulative incidence of NAS in the severe IR-injury group was 70% compared to 11.1% in the mild IR-injury group ($p< 0.001$). Multivariate analysis showed severe IR-injury, as evaluated by peak ALT >1500 IU/L, to be an independent significant risk factor for development of NAS post-OLT adjusted for CIT, WIT, peak AST, PSC as indication for OLT, recipient gender and recipient age (peak ALT >1500 IU/L aHR=11.83, 95% CI , p=0.01).
Conclusion: Severe IR-injury as evaluated by high peak ALT >1500 IU/L is a very significant independent risk factor for the development of NAS post-DCD donation OLT.

MicroRNA profiling in urinary sediment of transplant recipients with acute rejection

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RNA assessment in urine sediments from kidney transplant recipients may represent a non-invasive tool for detecting acute rejection. MicroRNAs are small, non-coding RNA molecules that negatively regulate mRNA expression, and which have gained interest for their role in rejection of kidney transplants. Urinary sediment cells are prone to generate low-quantity and -quality RNA, the latter being a result of degradation of the RNA. Therefore, we first tested microRNA stability of artificially degraded RNA from isolated leukocytes and found that microRNA molecules remained stable, regardless of the extent of RNA degradation. Furthermore, microRNA expression could be reproducibly quantified in extremely low amounts of RNA (10 pg per reaction) with the help of locked nucleic acid (LNA)-enhanced PCR primers. In a pilot study, we aimed to discover in an unbiased manner urinary microRNAs that best distinguish conditions of acute rejection. Expression of all known human microRNAs (Exiqon, PCR panel, n=742) was profiled in RNA from urinary sediment samples of 6 kidney transplant recipients with biopsy-supported acute rejection (80.0 ± 46.9 days posttransplant; 4 with T-cell mediated rejection, 2 with vascular rejection) and of 6 patients without rejection (84.0 ± 18.8 days posttransplant). For each patient, microRNA signals were standardized to the average signal of five reference microRNAs. For the 499 microRNAs detected, the level of 15 microRNAs significantly discriminated acute rejections from controls (Mann-Whitney rank test, $P<0.05$). MicroRNAs 451 and 25 represented the most promising discriminative analytes, since their expression levels were high in the sediments and were at least 13-fold increased ($P\leq0.017$) during acute rejection compared to the control group.
In conclusion, we identified microRNAs in the urinary sediment, of which the expression levels are associated with acute rejection. Predictive and diagnostic value of these levels for acute rejection will be verified in a larger patient cohort.

Circulating microRNAs correlate with diabetic nephropathy and systemic microvascular damage and normalize after simultaneous pancreas-kidney transplantation

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Introduction: Simultaneous pancreas-kidney transplantation (SPK) is an advanced treatment option for patients with type I diabetes with extensive microvascular disease and nephropathy (DN). Circulating microRNAs (miRNAs) can be sensitive biomarkers and their functional properties could provide insight into disease state. By assessing miRNA profiles in healthy control subjects and patients with type I diabetes before and following SPK we aimed to identify differentially expressed subsets of miRNAs that associate with microvascular destabilization and disease state. Methods: Circulating miRNA expression was determined using TaqMan® Human MicroRNA Array Card in plasma of DN (n=8) and SPK patients (n=8) and compared with healthy controls (n=3). In addition, the SPK patients were studied longitudinally before, 1, 6 and 12 months after SPK. Microvascular morphology and mean capillary density were visualized using sidestream darkfield imaging of the oral mucosa. Furthermore, circulating levels of angiogenic factors, including angiopoietin-1 (Ang1), angiopoietin-2 (Ang2), VEGF and soluble thrombomodulin (sTM) were measured using ELISA. Results: In our study we identified miR-25, miR-27a, miR-126, miR-130b, miR-132, miR-152, miR-181a, miR-320 and miR-660 to have elevated expression levels in plasma of DN patients as compared to healthy controls, whereas miR-223 and miR-574-3p expression was decreased. After SPK, expression levels of these miRNAs normalized and positively correlated with glomerular filtration rate and Hba1c levels. Interestingly, the expression of miR-126, miR-130b and miR-132, which are known to be pro-angiogenic, correlated with Ang2 levels. In addition, miR-130b showed a strong correlation with increased tortuosity of the microvasculature and sTM levels.
Conclusion: Circulating miRNAs correlate with DN and systemic microvascular destabilization. Following SPK these profiles normalized concomitant with microvascular stabilization.

Mobilization and priming of mesenchymal stromal cells from human liver grafts: differences between non-heart beating versus heart beating donors

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Background: Mesenchymal stromal cells (MSCs), a prominent nonhematopoietic cell type with immunomodulatory and regenerative properties. There is evidence to support that MSCs require priming stimuli with inflammatory cytokines to be able to elicit their immunomodulatory function. However, so far no evidence for this MSCs priming has been shown *in vivo*. Since profound inflammatory changes are observed in liver grafts from non-heart beating (NHB) and heart beating (HB) donors, the aim of this study was to quantify differences in mobilization of MSCs and their immune suppressive capacity between NHB and HB donors. Methods: During liver transplantation, graft preservation solutions (perfusates) of nine HB and seven NHB donor livers were collected. Mononuclear cells (MNCs) from perfusates were stained with a cocktail of antibodies specific for human CD45, CD146, CD73, and CD44 to quantify percentage MSCs using multi-colour flow cytometry. Liver MSCs obtained from the adherent MNC fraction underwent expansion. Following expansion, MSCs were stimulated with 10 ng/mL of human IFN-γ and 10 ng/mL of human TNF-α and examined for expression of T-cell suppressive receptor, PD-L1. MSCs obtained from healthy bone marrow (BM) were used as positive controls. Results: No difference in the growth potential of liver perfusate-derived MSCs from NHB versus HB donors was observed. However, mobilization of CD45-CD146+CD44+ cells was more pronounced in perfusate from HB (mean 0.62% $\pm$ 0.5 SD) versus NHB (0.16% $\pm$ 0.25) donors. Moreover, baseline PD-L1 expression in *ex vivo* expanded MSCs from HB donors (24% $\pm$ 20.5) was elevated when compared to NHB donors (4.7% $\pm$ 8.6, n=5). PD-L1 in BM-MSCs isolated from healthy subjects was 3.6% $\pm$ 3.2 (n=5). Following stimulation with IFN-γ/TNF-α, PD-L1 expression was upregulated on 95% $\pm$ 6.4, 96.6% $\pm$ 1.5 and 96.3% $\pm$ 4 of MSCs from HB, NHB and BM, respectively. Discussion: MSCs mobilized from liver grafts may contribute to systemic immunomodulation of alloresponses after liver transplantation. Tissue injury associated with the donor type plays an important role in mobilization of MSCs. Moreover, MSCs from HB donors had elevated level of PD-L1 expression compared to MSCs from NHB donors, suggesting *in vivo* priming of the immune regulatory capacity. The effect of upregulation of PD-L1 on liver perfusate-derived MSCs in modulating T cell-mediated immune responses is currently being investigated.

The mannan-binding lectin-associated serine proteinase-2 D120G mutation is a common pathophysiological denominator for non-anastomotic biliary strictures after orthotopic liver transplantation and the Budd-Chiari syndrome

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Background and aims: Microthrombus formation in the microvascular supply of the biliary tree during ischemia intervals throughout the transplantation procedure are thought to be the cause of non-anastomotic biliary strictures (NAS) after liver transplantation. In the Budd-Chiari syndrome (BCS), thrombosis develops in the hepatic venous outflow tract in patients with an underlying thrombotic diathesis and hypofibrinolysis. Compelling data shows that the coagulation and complement systems are co-regulated and highly integrated. Mannose-binding lectin-associated protease (MASP)-2 is a liver-derived essential component of the lectin complement activation pathway and activates pro-urokinase plasminogen activator. About 5% of the Caucasian population is heterozygous for an inherited deficiency of MASP-2. We therefore hypothesized that deficiency of MASP-2 might decrease fibrinolytic potential. Patients and methods: We explored the impact of the MASP2 D120G mutation on the development of NAS in a cohort of 147 liver transplantations. The risk of NAS was assessed by multivariate Cox regression analysis. The analysis was replicated in a validation cohort involving 167 liver transplantations. The risk of BCS was explored in all patients combined. Results: In the discovery study the MASP2 mutation in the donor was accompanied by a significantly increased risk of NAS (adjusted hazard ratio, 6.4; 95% CI, 2.2-19.0; P=0.008), adjusted for factors with p< 0.15 in univariate analysis: recipient age, primary liver disease and ischemia time. In the validation study the MASP2 mutation also increased the risk of NAS (adjusted hazard ratio, 3.0; 95% CI, 1.0-8.9; P< 0.05). Three out of the four patients (75%) with BCS as indication for liver transplantation were heterozygous for MASP2 D120G compared to 4% in patients with other indications for OLT (P=0.0004). In addition, after transplantation two out of three patients who developed BCS received a donor liver heterozygous for the minor G-allele of MASP2 compared to only 5% in patients with other complications after OLT (P=0.009).
Conclusion: This study revealed a strong association between the MASP2 D120G mutation and the development of BCS and NAS post-liver transplantation. Based on our findings, there is evidence that BCS and NAS share a similar MASP2-associated pathophysiological mechanism which causes disordered coagulation or fibrinolysis.

A single dose of rituximab results in a long lasting B-cell depletion in peripheral blood, without affecting the peripheral T-cell compartment

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The B-cell depleting antibody rituximab is widely used for the treatment of B-cell malignancies and autoimmune disorders. In organ transplantation, it is especially applied to reduce the level of alloantibodies. Substantial evidence indicates that the mechanism of action of rituximab is not confined to reducing antibody production, but also involves other functions of B cells, such as antigen presentation and cytokine production. Rituximab may thereby also affect T-cell activation and differentiation. In a randomized double-blind placebo-controlled study for the prevention of rejection after renal transplantation, we studied the effects of rituximab in combination with standard immunosuppressive therapy on the peripheral T- and B-cell population. Renal transplant patients received immunosuppression consisting of tacrolimus, mycophenolate mofetil, prednisolone, and a single dose of 375 mg/m^2 rituximab or placebo during transplant surgery. In 20 patients (10 rituximab, 10 placebo) without any rejection or CMV infection, we analyzed the PBMCs by multicolor flow cytometry before transplantation, and at 3, 6, 12, and 24 months after transplantation. A single dose of rituximab resulted in a long lasting B-cell depletion. At 24 months after treatment, the CD19$^+$ B-cell counts were still below 20 cells/µl in 88% of the rituximab treated patients, while the median value was 127 (range 50-380) in the placebo group. Interestingly, at 12 months the percentage of CD24^{++}CD38^{++} transitional B cells was significantly higher in rituximab-treated patients compared to the placebo group. The expression of Th1- (CXCR3, IFNγ, TNFα), Th2- (CCR4, IL-4), Th17- (CCR6, RORγt, IL-17), and Treg-associated (CD25, FOXP3) markers in the peripheral T-cell population as well as the numbers of naïve and memory T cells were not influenced by rituximab treatment. Also, in iliac lymph nodes obtained during surgery from renal transplant patient who had received rituximab 4 weeks earlier in preparation for an ABO-incompatible transplantation, we found no change in the phenotype of T cells as compared to lymph nodes from control patients.
In conclusion, although a single dose of rituximab results in a long lasting B-cell depletion in peripheral blood, this is not accompanied by changes in the T-cell compartment.

Identification of two pathways by which intravenous immunoglobulin modulates dendritic cells in humans in vivo

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Background: High-dose intravenous Immunoglobulin (IVIg) is a safe and effective immunosuppressive therapy in autoimmune diseases. Previously, we observed that treatment with anti-HBsAg IVIg reduces the acute rejection incidence after liver transplantation, but the mode of action is not fully understood. Since myeloid dendritic cells (mDCs) play a crucial role in the initiation of cell-mediated acute rejection, we studied whether and how IVIg modulates mDCs in humans *in vivo*. Methods: From 28 patients treated with IVIg (indications: immunodeficiency or autoimmune disease), blood was collected before and at 1 and 7 days after IVIg infusion. *In vitro*, mDCs purified from blood of healthy subjects were cultured with IVIg or rIL-4 for 24hr. Surface receptors on mDC were measured by flowcytometry and plasma cytokine concentrations by ELISA. Results: Expression of the activating Fcγ receptor (FcγR) IIa (CD32a) on circulating mDCs was reduced at 1 and 7 days after IVIg treatment (T=0→T=1: -30%, p=0.03; T=0→T=7: -22%, p=0.02), while expression of the inhibitory FcγRIIb (CD32b) remained unchanged. In addition, 7 days after IVIg treatment, expression of the signalling part of the IFN-γ receptor (IFNGR2) on circulating mDC was 2.5-fold diminished (p=0.002). Hence, IVIg significantly balances mDC towards an inhibitory status. Recently it has been described that IVIg can modulate FcγR expression on macrophages in mice by stimulating the IL-33 - IL-4 cytokine axis. Remarkably, we observed in our patients a rise in plasma IL-33 levels after IVIg treatment (T=0→T=1: +179%, p<0.0001; T=0→T=7: +98%, p<0.0001), which was positively correlated with a rise in IL-4 plasma levels (r= 0.577, p<0.01). *In vitro*, rIL-4 inhibited CD32a and IFNGR2 expression on mDC, like we had observed in patients. In addition, IVIg also appeared to have direct effect on both CD32a and IFNGR2 expression on mDC (CD32a: -96%, p<0.01; IFNGR2: -57%; p=0.2 after 24 hrs culture in presence of IVIg). Functionally, IL-4- or IVIg-treated mDCs were less responsive to immune complex stimulation. Conclusion: IVIg treatment results in decreased CD32a and IFNGR2 expression on mDCs, which renders them refractory to immune complexes and IFNγ. This modulation may occur by stimulation of the IL-33-IL-4 cytokine axis or by a direct effect of IVIg. Hence, by modulating the most potent antigen presenting cells via two different pathways, IVIg may be a promising candidate for immunosuppression after organ transplantation.

Mannan-binding lectin reduces ER-stress sensor GRP78/BIP in tubular epithelial cells following renal ischemia/reperfusion

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Ischemia/reperfusion (I/R) injury is a key event in kidney transplantation. Recently, we demonstrated a crucial role for Mannan-binding lectin (MBL) in the pathogenesis of renal I/R injury. Ischemia is an important factor leading to impaired protein folding and consequent endoplasmic reticulum (ER) stress. GRP78/BIP, a stress-inducible ER chaperone, serves as a master modulator for the ER-stress response. In the present study we explored the role of MBL in the GRP78-induced ER-stress response. Exposure of human tubular epithelial cells (TEC) to MBL *in vitro* resulted in binding and internalization of MBL. Confocal microscopy revealed strong colocalization with ER-stress sensor GRP78. Within two hours of MBL exposure, assessment of ER-stress markers sXBP-1 and CHOP, revealed a twenty- and tenfold induction respectively, which was accompanied by a strong IL-6 protein expression, another hallmark of ER-stress. Assessment of rat sXBP-1 following I/R *in vivo* revealed an extensive induction of ER-stress within 2 hours, which was accompanied by an elevated expression of IL-6 and intra-epithelial presence of MBL. Interestingly, staining for GRP78 revealed a complete loss of protein expression in the cortico-medullary region already 2 hours after reperfusion, which was followed by loss of TEC adhesion and cell death within 24 hours. Importantly, inhibition of MBL completely prevented degradation of GRP78 in the cortico-medullary region, diminished the early IL-6 expression and protected against TEC injury.
In conclusion, inhibition of MBL prevents degradation of GRP78 and reduces IL-6 expression. Loss of GRP78 could be a possible mechanism by which MBL induces tubular epithelial injury following reperfusion.

Hepatocyte and cholangiocyte-derived microRNAs in serum as early markers for ischemia & reperfusion injury in pigs

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Background and aims: Ischemia and reperfusion injury (IRI) of the liver graft is an important factor determining long-term transplantation success. At different stages of graft procurement, transportation and directly post-engraftment, ischemic injury of the graft can occur. Due to a lack of biomarkers however, it remains difficult to assess graft quality and the degree of ischemic injury during liver transplantation. Recently, hepatocyte and cholangiocyte-derived micro-RNAs (HDmiRs and CDmiRs) have been identified as sensitive markers for liver injury in patients' serum after liver transplantation. The aim of this study was to investigate whether HDmiRs and CDmiRs in serum can accurately assess the amount of hepatic IRI in a porcine model. Methods: Ten female Yorkshire pigs were subjected to hepatic IRI using a total vascular exclusion model by clamping the hepatic artery, portal vein and hepatic veins for 30 minutes. Two vascular probes continuously monitored the absence of blood flow during ischemia. Serum and liver biopsies were collected prior to ischemia and up to 90 minutes after reperfusion. Samples were tested for levels of AST, ALT, gamma-GT, total bilirubin and LDH. Through real-time qPCR serum samples were analysed for HDmiRs HDmiR-122 & HDmiR-148a, and CDmiRs CDmiR-30e & CDmiR-222. As control miRNAs, the muscle-derived miR-133a and blood-derived miR-191 were analysed. Results: Both HDmiRs and CDmiRs were detectable in porcine serum. Of the conventional injury markers, only AST showed an elevation in serum with a maximum of three-fold after reper-fusion (64 ± 30 U/L at baseline vs. 64 ± 10 U/L at reperfusion and 135 ± 50 at 60 minutes after reperfusion, P=0.012). In contrast to this, serum HDmiR- and CDmiR-levels were already significantly higher at time of reperfusion (4.9 ± 3.7 fold change, P=0.02) and their levels remained elevated longer after reperfusion compared to AST, reaching up to 90-fold (32.7 ± 29.9 fold change). MiRNA-191 and miR-133a, which served as control miRNAs, showed neither significant changes after ischemia/reperfusion, suggesting that our findings are related to hepatic IRI.
Conclusion: In this study we demonstrate that microRNAs are an early and sensitive serum marker for hepatic IRI in pigs. To further investigate the correlation between miRNA release in serum and tissue damage, pre- and post-reperfusion biopsies will be analysed on liver and bile duct histology and miRNA expression levels.

Mesenchymal stem cells control allo-reactive CD28- T cells that are unaffected by belatacept treatment

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Introduction: CD28/B7 co-stimulation blockade with belatacept is used to prevent allo-reactivity in kidney transplant patients. Cells that lose CD28 during differentiation are not sufficiently controlled by belatacept. Yet, these CD28- T cells have cytotoxic capacities and pathogenic potential. Mesenchymal stem cells (MSC) possess various immunosuppressive properties, inhibit effector cell proliferation and therefore are interesting candidates for cellular therapy in organ transplantation. Our study aimed to investigate the effect of MSC on allo-reactive CD28- T cells and to elucidate the relevant mechanisms of action.
Methods: MSC were isolated from perirenal adipose tissue of kidney donors; PBMC were derived from blood bank donors. Mixed lymphocyte reactions (MLR; 7-days) were used to mimic allo-reactivity. Flow cytometric analyses were performed (n = 5). Results: Belatacept (1µg/mL) and MSC (1:10; ratio MSC : effector cells) reduced effector cell proliferation by 40% (mean; $p = 0.002$) and 63% ($p = 0.001$), respectively. The combination of both inhibited proliferation by 78% ($p < 0.0001$). Belatacept treatment did not influence the proliferation of CD4+CD28- T cells and CD8+CD28- T cells when compared to MLR. MSC, however, reduced the proliferation of CD4+CD28- T cells by 67% ($p = 0.002$). The same effect was observed when MSC were separated from the MLR by permeable cell culture inserts. In both systems the influence of MSC on the proliferation of CD8+CD28- T cells was less profound. Combined treatment of belatacept and MSC did not impair the suppressive function of MSC on CD4+CD28- T cells or CD8+CD28- T cells.
Conclusion: Allo-activated CD28- T cells that remain unaffected by belatacept treatment are inhibited by MSC in a cell-cell-contact independent manner. Although further investigation of specific contributing mechanisms is required, the anti-proliferative effect of MSC on CD28- T cells strongly emphasizes the potential of a MSC-belatacept combination therapy to prevent graft rejection.

Differential effects of the innate vs. adaptive immunity in lysing human renal tubular epithelial cells

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Introduction: At present, the relative contribution of the innate and adaptive cytotoxic immunity to human renal tubular epithelial cell injury (TECs) remains poorly defined. Cells of the innate immune system (NK cells) play a role in late antibody-mediated rejection and cells of the adaptive immune system (T cells) are main players during cellular rejection. Here we studied the differences in net lysis-efficacy of TECs by NK cells and CD8+ T cells during alloreactivity in vitro. Material and Methods: First, we performed europium release kill assays using FACS sorted NK and CD8 T cells. Recipient PBMCs were cocultured with donor TECs in the presence of IL-2 and IL-15. In addition, we performed autologous kill assays. Second, the proliferative response of PKH labelled lymphocytes was measured using flow cytometry. Results: Only 5-10% of TECs were lysed by CD8+ T cells at a high effector:target ratio (40:1), while NK-cell lysis of TECs reached up to 57% of TECs under the same conditions. In control experiments T cells showed 75% lysis. We also studied the expression of the surface marker CD107a which is a marker for functional cytotoxic activity. TEC activated CD8+ T cells expressed CD107a up to only 1.6%, while 14.0% of the NK cells expressed CD107a in the same setting. This refers to a more potent functional cytotoxic activity of NK cells. Allogeneic proliferative response was induced in 6.6% ± 3.1 of CD8+ T-cell population after TEC co-culture. Autologous TEC did not lead to a CD8+ T-cell proliferative response. In addition, transwell experiments revealed that the TEC induced CD8+ T-cell proliferation was cell-cell contact dependent.
Conclusion: Our data show a stronger cytolytic activity of NK cells against TECs compared to CD8 + T cells. This phenomenon may underlie the differences in clinical manifestation causing more renal tubule damage by NK rich graft infiltration during ischemia –reperfusion injury or late acute humoral rejection.

KIR and HLA associated genotypic risk of Cytomegalovirus (CMV) infection and disease in renal transplantation

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Natural Killer (NK) cells are critical regulators of anti-viral immune reactions alongside the adaptive immune response. We hypothesized that NK cells are crucial in the anti-viral response after renal transplantation, and either directly or indirectly affect the allo-immune response. NK cell express Killer Immunoglobulin-like Receptors (KIR), which specifically recognize HLA class I antigens on target cells. These KIR/HLA interactions contribute to the complex balance between activating and inhibitory receptors signalling which determine whether or not NK cell effector functions are initiated. We studied the association of NK cell KIR and HLA genotype with risk of Cytomegalorvirus (CMV) primary infection/disease and rejection episodes after renal transplantation. Renal transplant patients between 2002 and 2011 at UMC St. Radboud Nijmegen were included and organized into four groups defined by the CMV sero-status of the recipient (R) and donor (D). Inclusion criteria were >19yrs, first transplant, immunosuppressive regimen (Tacro/MMF/Pred). First, we analyzed the association of KIR genotype and risk of CMV disease in the R-/D+ cohort. A total of 90 R-/D+ patient-recipient pairs were included in this cohort of which about half received the kidney from a living donor. In this cohort of 90 donor-recipient pairs the incidence of CMV disease was 43.3% and the rejection rate was 30% in the first year of transplantation. The average time to CMV disease was 142 ± 45 days after transplantation. KIR typing results revealed a similar gene distribution in our R-/D+ recipients as compared to previous data on European renal transplant cohorts. The frequency of KIR haplotypes AA and BA/BB (BX) was 31% and 69%, respectively. Preliminary risk analysis showed no association between recipient's HLA Bw4/Bw6 and C1/C2 epitopes and the risk of CMV disease. CMV disease was not a risk factor for the rejection episodes observed in this cohort. Neither were the recipient 's KIR haplotypes and HLA Bw4/Bw6 and C1/C2 epitopes significantly correlated with the observed rejection episodes. However, in contrast to some earlier studies, recipients with the KIR AA haplotype showed a better, albeit not statistically significant, outcome in CMV disease incidence when compared to recipients with the KIR BX haplotype. Analysis of additional cohorts and functional studies should provide us with a rationale for our observed findings.

Epigenetic analysis of the TSDR of FOXP3 demonstrates that natural Treg infiltrate the cardiac allograft already before an acute rejection episode

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Two subtypes of FOXP3+ regulatory T cells (Treg) have been identified. Natural (n) Treg originate in the thymus, while antigen specific induced (i) Treg are induced in the periphery by antigen exposure and cytokines. Their role in regulating allogeneic responses in immunosuppressed heart transplant patients is unknown. Therefore we investigated the origin of graft infiltrated FOXP3+ Treg and examined their immunosuppressive capacities by studying *FOXP3* characteristics during acute rejection (AR) and immunological quiescence. In total, 91 endomyocardial biopsies (EMB) were analyzed of 42 patients, 28 patients experienced at least 1 rejection episode requiring anti-rejection therapy (ISHLT rejection grade 2R; rejectors) and 14 patients remained free from rejection (non-rejectors). The percentage of demethylated TSDR (Treg Specific Demethylated Region) in the *FOXP3* gene represents the percentage of nTreg. *FOXP3* mRNA levels represent the total regulatory T cell population. In time-zero biopsies, no evidence for nTreg was detected. In grade 1R EMB of non-rejectors no significant accumulation of nTreg was observed. However, in rejectors, in all grade 1R EMB (taken before AR) and 2R EMB (AR) nTreg were detected with a significant higher percentage compared to EMB of non-rejectors ($p \leq 0.001$). *FOXP3* mRNA levels significantly increased after transplantation with the highest expression in grade 2R EMB. Remarkably, no significant difference was observed in the *FOXP3* mRNA levels in 1R EMB of non-rejectors compared to 1R EMB of rejectors ($p = 0.32$), while the latter had significant evidence for a higher percentage nTreg ($p = 0.001$), suggesting more iTreg in the non-rejectors.
These data show that nTreg infiltrate the cardiac allograft already before histologically proven AR, indicating that nTreg are unable to prevent the rejection process. The characteristics of the *FOXP3* gene in EMB of non-rejectors support a role for antigen specific iTreg in the prevention of rejection.

Immunogenicity of HLA-DRB3 after kidney transplantation and the development of a tool for epitope discovery using mutagenized recombinant HLA-fusion proteins

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In contrast to the DRB1 gene other DRB genes are only present in a subset of individuals and these genes show only modest diversity (e.g. DRB3, 58 alleles). Although antibodies recognizing these other DRB genes are frequently encountered in many transplant centres, diversity in these DRB3,4,5 genes is not considered when determining minimal matching criteria for organ transplantation. Notably, at present there is limited insight in the immunogenicity of proteins encoded by these other DRB genes. Moreover, the immune dominant epitopes and the immunogenicity of the allelic variation remain elusive. In the current study we aim to provide information on the immunogenicity of DRB3 and its allelic diversity using the patient database of our centre. In addition we aim to develop methodology for the discovery of epitopes in DRB3. We showed in a selection of our patient cohort that 39% (23 of 59) DRB3 negative patients developed an antibody response (MFI>1000) against DRB3 after transplantation with a DRB3 positive organ. Additionally we identified 12 DRB3 positive individuals with an antibody response against the DRB3 allele of their organ donor with no crossreactivity with their own DRB3 protein. Taken together this does not only illustrate the immunogenicity of DRB3, but it also means that the allelic variation in DRB3 is immunogenic. In addition the identification of such intra-allele antibodies opens venues for mapping the immunogenic epitopes, because by definition these antibodies will not react with conserved regions of the DRB3 molecule but to the epitopes which are unique to the donor allele. Because there is currently no tool for DRB3 epitope discovery, we are developing a novel methodology using recombinant fusion proteins of DRA and DRB3*01:01 in which we inserted single amino acid substitutions. These fusion proteins were successfully produced in a monomeric form by means of the Drosophila S2 expression system. These fusion proteins were checked for correct folding and we are currently setting up an ELISA detection system. Besides the identification of an epitope for intra-allele antibodies this methodology may also provide insight in the immunodominance of certain epitopes in DRB3 negative individuals. So, we anticipate that the identification of immunodominant regions in different DRB3 alleles may allow the definition of permissive and non-permissive mismatches that can be taken along in organ matching criteria.

CD137, a marker to detect the total alloreactive T cell compartment?

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Introduction: Alloreactive T cells are important mediators of rejection or tolerance of the transplanted organ. Detection and isolation of viable alloreactive T cells at the single cell level requires a cell surface marker specifically induced upon T cell receptor activation. In this study, a member of the TNFR-family, CD137 (4-1BB) was investigated for its potential to identify circulating alloreactive T cells in combination with phenotypic and functional analysis. Methods: Optimal conditions for sensitive and specific detection of allogeneic-induced CD137 expression on circulating T cells were established. Thereafter, $CD137^+$ alloreactive T cells were phenotypically and functionally characterized by multi-parameter flow-cytometry. Results: Alloantigen-induced CD137 expression identified both alloreactive $CD8^+$ T cells (0.21±0.07%) and $CD4^+$ T cells (0.21±0.05%). $CD137^+$ alloreactive T cells were detected in differrent T cell subsets, including naïve T cells, but were preferentially found in $CD28^+$ T cells and not in the terminally differentiated T cell subsets. Upon allogeneic stimulation, the cytokine producing, but not proliferating capacity of T cells mainly resided within the CD137-expressing fraction. A minority of the $CD137^+$ alloreactive cytokine producing T cells (<10%) produced any combination of IFN-γ IL-2 and TNF-α. Poly-functional alloreactive T cells, defined by multiple cytokine expression, were infrequently observed. Numbers of alloreactive $CD137^+$ $cytokine^+$ T cells were positively associated with the number of HLA mismatches, although a substantial variation was observed.
Conclusions: Activation-induced CD137 expression allows for detection of the total cytokine producing, but not the total proliferating, alloreactive T cell compartment at the single cell level by multi-parameter flow-cytometry. CD137 expression might be a useful marker to gain more insight into the development of alloreactive T cells following kidney transplantation.

Phenotypical characterisation of peripherally circulating HLA-A02-restricted polyomavirus BK (BKV)-specific CD8+ cytotoxic T lymphocytes (CTL) in healthy adults

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Introduction 70-100% of healthy individuals are latently infected with BKV. In renal transplant recipients (RTR) treated with immunosuppressive drugs, BKV systemically reactivates in ~30%, as defined by positive plasma PCR. In ~5% of these RTRs, BKV causes interstitial nephritis of the allograft, responsible for increased graft loss. CD8+ CTLs are essential in the defence against viruses. So far, peripherally circulating human BKV-specific CD8+ CTLs in healthy individuals have not been phenotypically characterized, due to their low frequencies in the circulation. Methods and Results We used four different HLA-A02-tetramers (2 VP1 and 2 large T antigen epitopes) and applied steps that lower the detection limit: an extended incubation period with tetramers, and the measurement of large numbers of lymphocytes by flowcytometry. Therewith, we could show that BKV-specific CTLs were detectable directly ex vivo in 5 of 20 buffy coats from healthy HLA-A02-positive individuals, and are mainly VP1-specific. Phenotypic analysis showed that they are CD27int/hi CD28int/hi CD45RAint/lo CD127hi KLRG1int/lo and CCR7lo. Furthermore, they were CXCR3hi CD49dhi and CD38lo HLA-DRlo PD-1int/lo Ki67lo. Lastly, they were granzyme Kint, granzyme Blo and perforinlo, and T-betint and Eomesoderminlo/int. Conclusion Peripherally circulating HLA-A02-restricted BKV-specific CD8+ CTLs in healthy individuals can be considered as KLRG1int/lo non-activated 'effector memory' T-cells that express some granzyme K, but no other cytotoxic effector molecules. These cells are important in suppressing BKV infection in healthy individuals. As such, it will be interesting to see how they differ from CD8+ CTLs in RTRs suffering from BKV pathology.

Alloantigen-specific Tregs can be identified by activation-induced CD154 expression

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Introduction: Antigen (Ag)-specific T cells can be recognized using cell surface expression of markers specifically induced upon T cell receptor interaction with peptides presented in the context of HLA molecules on Ag-presenting cells. CD154 is a member of the TNF-superfamily and its expression is specifically induced upon T cell activation. CD154 cell surface expression allows viable isolation of Ag-specific $CD4^+$ T cells and detailed phenotypic as well as functional analysis. To use Tregs as cell therapy in kidney transplantation, it is desirable to use alloAg-specific Tregs to prevent unwanted suppression of immune responses. In this study, we investigated whether CD154 cell surface expression is able to detect Ag-specific regulatory T cells upon allogeneic stimulation. Methods: Highly enriched fractions of Tregs and T cells depleted for Tregs (Teff) were FACS sorted. These Tregs and Teff were stimulated with HLA-mismatched (MM) PBMC in the presence of co-stimulatory antibodies (αCD28 and αCD49d) as well as αCD40 to maintain CD154 expression on the cell surface. AlloAg-specific, $CD154^+$ Tregs were studied extensively using phenotypic as well as functional analyses, testing their suppressive capacity in a mixed lymphocyte reaction (MLR) either immediately upon isolation or following expansion. Results: Maximal CD154 expression within Tregs was observed upon 24 hour stimulation. AlloAg-specific $CD154^+$Tregs consisted of both naïve (mean$\pm$SEM; 38$\pm$12%) as well as memory (62$\pm$12%) T cells and FOXP3 expression (>80% of the Tregs are $FOXP3^+$, median fluorescence intensity: 4928) remained high. There was no association between percentages of $CD154^+$ Tregs and the number of HLA-MM. Sorted $CD154^+$Tregs were superior ($P<0.05$) in suppressing Ag-specific responses when compared to $CD154^-$ Tregs and total Tregs, i.e. at a 1:5 (Treg:Teff) ratio the median percentages of inhibition (IH) in a MLR amounted to 57% versus 25% and 31%, respectively. $CD154^+$Tregs could be efficiently expanded in an Ag-specific manner, which enhanced their suppressive capacity. At a 1:5 ratio, the median % of IH in a MLR increased to 98%.
Conclusions: These data show for the first time that alloAg-specific Tregs can be detected using CD154 expression. $CD154^+$ alloAg-specific Tregs can be isolated and efficiently expanded increasing their Ag-specific suppressive capacity. These alloAg-specific $CD154^+$ Tregs may be of potential benefit for cellular immunotherapy in kidney transplantation.

RUNSMART: 15 year follow-up of a multicentre, randomised, calcineurin inhibitor withdrawal study in kidney transplantation

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Background: Calcineurin inhibitors (CNI) are essential immunosuppressives in the early phase after renal transplantation. As they are nephrotoxic, withdrawal at a certain timepoint has been a challenge ever since their introduction. We aimed to analyse the influence of CNI withdrawal at 6 months after transplantation on long term results. Methods: 212 patients transplanted between 1997 and 1999 participated in a randomized multicentre trial. All patients were initially treated with Mycophenolate mofetil (MMF), Cyclosporine A (CsA) and prednisone (pred). At six months after transplantation, 63 patients were randomised for MMF/Pred (group 1), 76 for MMF/CsA (group 2) and 73 for MMF/CsA/Pred (group 3). Follow-up data until November 2012 were extracted from the NOTR database. Results: At randomisation groups were not signifycantly different for recipient, donor and transplantation characteristics. Within 6 months after randomisation 24 patients had a rejection episode (28,6%, 6.8%, and 1.4% in groups 1, 2, and 3, respectively, $p<0.001$). The frequency of CsA use at 1 and 2 years after transplantation was 12.7% and 19.0% in group 1, 97.4% and 86.8% in group 2, and 95.9% and 79.5% in group 3. At the end of follow-up 67 patients have died with a functioning graft, 43 patients have lost their graft, and 102 are alive with a functioning graft. Graft survival censored for death was 67%, 76% and 81% in groups 1,2 and 3: difference not significant. In multivariate analysis, graft survival censored for death was significantly associated with serum creatinine at 6 months after transplantation and with acute rejection after randomisation, but not with randomisation group. Also patient survival and uncensored graft survival did not differ between the groups.
Conclusion: This intention to treat analysis did not show long term graft or patient survival benefit for patients withdrawn from calcineurin inhibitor treatment at six months after kidney transplantation.

Do differences in the use of induction therapy affect outcome after heart transplantation, a comparison of centres

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The use of induction therapy is different in the 3 Dutch heart transplant centres. One centre uses ATG per protocol and other centres IL-2 receptor antagonists only in case of moderate to severe kidney dysfunction in the intended recipients. We questioned whether these differences result in different outcomes after transplantation. Follow-up data were obtained of 341 adult patients transplanted between 2002 and 2011 (Centre A: n=165, Centre B: n=158 and Centre C: n=18). We compared patient survival, treated rejection episodes, malignancies and kidney function. The outcome of 25 paediatric transplants was analysed separately. In the comparisons Centre C has been left out of consideration because of the low number of transplants and comparisons are between adult patients. For statistical analysis Chi square test, T-test, Kaplan Meier, Log Rank, and Cox regression were used. Recipients were older in Centre A than in Centre B (p=0.03), mean age 49 yrs (±SD=10) vs 46 yrs (±SD=12). The distribution of primary heart disease was similar: coronary artery disease 35 vs 35%, cardiomyopathy 59 vs 57% and other disease 6 vs 8%. Survival after 5 years was 79% in adult patients without a difference between Centre A and Centre B (p=0.3); survival in centre C was comparable. In the paediatric group survival was 90% at 5 years. Centre A treated significantly more acute rejection episodes than Centre B (Hazard ratio = 9.7; 95%CI: 5,6 -16,8; p‹0.001) within the first year. Skin malignancies occurred in 11 and 6 patients in Centre A and Centre B respectively. Other malignancies occurred in respectively 7 and 7 patients. Higher serum creatinine levels were found in Centre A after the first and second year after transplantation: 153µmol/l vs 107µmol/l (p‹0.01) and 133µmol/l vs 105µmol/l (p‹0.05) respectively. Later on these levels were comparable.
Survival after heart transplantation in the Netherlands is better than reported by the International Society of Heart & Lung Transplantation and is the same in the older two centres. Dutch centres differ in the use of induction therapy. More rejection treatments are given in Centre A which do not result in more malignancies. After 1 and 2 years kidney function is worse in the Centre A. Creatinine levels equal Centre B levels later on. The differences found do not support the use of induction therapy and raise questions about the interpretation of endomyocardial biopsies and /or the treatment of rejection on hemodynamic changes only.

Blood Pressure and Sodium Excretion in Kidney Transplant Recipients on Different Immunosuppressive Regimens

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Background: Hypertension is a well-known side effect of the immunesuppressive calcineurinine inhibitors (CNI) cyclosporine and tacrolimus. Whereas from cyclosporine it has been well established that it causes a sodium-loading sensitive form of hypertension, for tacrolimus this is not the case. Tacrolimus is assumed to exert a similar effect on salt sensitivity as cyclosporine. Therefore we hypothesized that among kidney transplant recipients using tacrolimus there would be a higher correlation between daily sodium excretion and pulse pressure than among patients on other (i.e. non tacrolimus or cyclosporine) immunosuppressive regimens. Methods: We retrieved data from our clinical database on the 539 KTRs currently followed in our center. We stratified the patients in tacrolimus (n= 285), cyclosporine (n= 114) or non-CNI users (n= 139). For our primary analysis we included pulse pressure (i.e. an integrated measure for both diastolic and systolic pressure, which may correlate better with volume status than mean arterial pressure) and 24-hour ambulatory urine sodium excretion in a univariate linear regression analysis in each of the three groups. In secondary analyses we controlled for serum creatinine, age and time after transplantation. Results: Systolic blood pressure was higher in the tacrolimus (135mmHg) and cyclosporine (136mmHg) group than in the non-CNI group (130 mmHg, p = 0.004); pulse pressure was similar (54, 51, 50 mmHg, p = 0.07). Sodium excretion was higher in the tacrolimus (152 mmol) and cyclosporine (150 mmol) group than in the non-CNI group (137 mmol, p =0.038). The correlation between sodium excretion and pulse pressure was only significant in the tacrolimus group (R 0,14; p=0.02). In the multivariate linear regression analysis, sodium excretion (p= 0,031) and age (p <0,001) but not serum creatinin or time after transplantation, resulted in a combined R to predict pulse pressure of 0.539.
Conclusion: Among kidney transplant recipients using tacrolimus, daily sodium excretion is correlated to pulse pressure. This suggests hypertension in these patients is salt sensitive and that they will benefit more in terms of blood pressure effect from lowering salt intake than patients on non-CNI regimens.

First in human trial of ischemic postconditioning in kidney transplantation from donations after cardiac death

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Objective: Ischemic postconditioning (IPoC) may reduce renal ischemia-reperfusion injury (IRI) after kidney transplantation. We performed a first human pilot trial to study the feasibility and safety of IPoC in human deceased-after-cardiac death (DCD) kidney transplantation (KT). Methods: All patients undergoing DCD KT were eligible. The IPoC algorithm consisted of 1 minute reperfusion followed by 1 minute of ischemia, repeated three times. All complications of this procedure were listed. The primary outcome was the incidence of delayed graft function (DGF). Secondary outcome was renal function at 12 weeks. Data were compared to a historical control group (n=40), consisting of our most recent cohort of DCD KT patients before trial initiation. Follow up was 12 weeks. Results: A total of n=20 patients was included. Mean donor age and serum creatinine were higher in the experimental than in the historical control group: 61 yr (20-71) versus 51.5 yr (24-74) (p=0.01) and 79 umol/L ± 34.2 versus 63.8 umol/L ± 23.4 (p=0.047), respectively. In the experimental group, more kidneys had massive atherosclerosis: 25% vs 2.5% (p=0.01). IPoC was successfully applied in all patients. One patient suffered from a venous tear due to clamp manipulation. The incidence of DGF was 85% vs 62.5% (p= 0.07). Renal function was comparable between groups at 12 weeks after transplantation: 161 umol/L (109-536) vs 149 umol/L (81-315) (p=0.10). Postoperatively, no additional risks or complications were seen as a consequence of IPoC.
Conclusion: We demonstrate for the first time that IPoC is feasible and appears to be safe in human KT. No benefit in terms of reduced DGF or better renal function was observed as a result of IPoC. This may have been caused by poorer donor organ quality.

Determinants of improved long-term survival after liver transplantation in a single center

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Aims: To analyze long-term outcome of nearly two decades of auxiliary (AXLT) and orthotopic (OLT) liver transplantation (LT) in a single center. Methods: A retrospective analysis was performed on patient and graft survival and its determinants in the 348 consecutive LTs performed in nearly 20 years at our institution. Sept. 1992- Dec. 2001 was considered the first decade and Jan. 2002-1 March 2011 the second decade. Results: A total of 335 OLTs en 13 AXLTs were performed in the studied time-period using 303 donor livers from donation after brain death (DBD) and –from 2001 on- 45 with donation after cardiac death (DCD). Hepatocellular carcinoma (HCC) was the main reason for OLT in both decades (13.4% vs 19.5%, p=0.11) followed by alcoholic liver disease (ALD). Hepatic artery thrombosis (HAT) was the main reason for retransplantation in both decades for DBD-LTs (5-year cumulative incidence (CI) HAT 6.8% vs 3.9%, p=0.22) whereas retransplantation due to biliary strictures was significantly higher for DCD-LTs compared to DBD-LTs (5-year CI 14% vs 5.1%, respectively, p<0.01). Patient and graft survival had significantly improved in the second decade versus the first decade (10-year patient survival 83% vs. 52%; log rank p<0.01, graft survival 68% vs. 41% respectively; log rank p<0.01). Post-LT infection was the main cause of patient mortality in both decades although a significant decrease was noticed in the second decade (10-year cumulative incidence13.3% vs 3.8%, respectively p<0.01). Death from recurrent primary disease (mainly HCC) decreased from 11.6% to 1.4% (p<0.01). DBD livers had lower cold ischemic times (CIT) and lower recipient warm ischemic times (RWIT) in the second decade compared to the first decade (CIT 709 minutes vs. 591 minutes; p<0.01 and RWIT 42 min vs. 35 min; p<0.01, respectively) and DCD donors had lower CITs compared to DBD donors in the second decade (487 minutes vs. 591 minutes, respectively; p<0.01). With multivariate analysis over all 20 years acute liver failure as transplantation indication(HR=2.80, p=0.03), blood loss during surgery (HR=1.06, p=<0.01) and decade of liver transplantation (HR=2.54, p=0.01) were significant determinants of patient survival.
Conclusions: Long-term patient- and graft-survival significantly improved in 20 years of liver transplantation, with less death due to infection, less recurrent primary disease and shorter ischemia times. Hepatic artery thrombosis and biliary strictures remain important causes for retransplantation.

Hepatic encephalopathy is an independent risk factor for mortality in patients awaiting liver transplantation

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Introduction: Hepatic encephalopathy (HE) is a severe complication of liver cirrhosis. HE is not accounted for in the MELD score, which is widely being used for organ allocation. Aim of this study was to assess the impact of encephalopathy on survival of patients awaiting liver transplantation. Methods: Retrospective analysis of consecutive adult patients listed for liver transplantation between 2007 and 2011 in a tertiary care centre. Clinical data were retrieved from patient records and MELD and MELDNa score were calculated. Survival analysis was performed using Kaplan Meier and Cox proportional hazard regression analysis with death as event, censored for liver transplantation or last visit. Log-rank analysis was performed to exclude competing risk of transplantation. Univariate analysis was performed for presence of HE, MELD score, MELDNa score, age, ascites, prior SBP or variceal hemorrhage and hepatocellular carcinoma. Parameters with $p<0.10$ were included in multivariate analysis. Results: 168 Patients were included; 25/51 patients with HE (49%) and 64/117 (54%) patients without HE underwent liver transplantation after a mean of 7.0 ± 7.8 (HE) vs. 9.7 ± 7.8 months (no HE) ($p= 0.158$). HE patients had a higher MELD score at listing than patients without HE (20 ± 9 vs. 12 ± 5, $p<0.001$). The chance to receive a liver transplantation showed a trend towards earlier OLT in patients with HE ($p=0.063$). The presence of HE was independently associated with increased mortality before transplantation (HR 3.702 (95% CI 1.496-9.162), $p=0.005$), also after adjusting for MELD and MELDNa score in multivariate analysis. MELD (HR 1.095 (95% CI 1.031-1.163), $p=0.003$) and MELDNa score (HR 1.124 (95% CI 1.051-1.202) were also independent predictors of mortality, whereas prior SBP and ascites were not. More severe HE was associated with a higher mortality risk, i.e., grade 2 HR 4.973 ($p<0.001$) grade 3-4 HR 28.413 ($p<0.001$). Mortality was not increased in patients with HE grade 1 (HR 1,094).
Conclusion: Hepatic encephalopathy is an independent risk factor for mortality in patients awaiting liver transplantation. Objective biomarkers for assessment of HE are needed as HE patients might deserve higher priority.

Increase in RBC transfusion requirements during orthotopic liver transplantation after market withdrawal of aprotinin

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Background: Blood loss during orthotopic liver transplantation (OLT) remains a topic of concern. Blood loss is currently managed by transfusion of red blood cells (RBC), fresh frozen plasma (FFP), platelet concentrates, fibrinogen and/or antifibrinolytic drugs. Besides evolvement in surgical techniques, including the piggyback implantation, a restrictive transfusion policy and a better overall understanding of hemostasis mechanisms has led to a significant decrease in blood loss and transfusion requirements in the past decades. We, amongst others, previously reported transfusion free OLT in around 30% of primary liver transplantations. Unfortunately, in the past years we are observing an increase in RBC transfusion requirements in our center. We hypothesized that this increase is related to the withdrawal of the antifibrinolytic drug aprotinin from the market. Objective: Aim of this study was to analyze recipient, donor, and surgical factors that can explain the recent increase in transfusion requirements. Methods: Blood transfusion records and clinical outcomes for 373 consecutive adult patients undergoing primary OLT 's in our center between 2000 and 2012 were analyzed. We considered a variety of potential causes that may, including the discontinuation of the antifibrinolytic drug aprotinin in 2007. Results: The proportion of patients without any RBC transfusion decreased from 39% to 19 % before and after 2007 ($p<0,001$). The median amount of RBC transfusion increased from 2,3 (IQR 0- 5,7) to 4,6 units(IQR 1,8-10,0) before and after 2007 ($p<0,001$). In a uni- and multivariate analysis, using binary logistic regression models, aprotonin became highly significant in predicting RBC transfusion (OR 2,371, 95%CI: 1,350-4,166, $p=0,003$). Other significant factors in the multivariate model were age recipient, cold ischemia time (cit), female recipient gender and MELD score.
Conclusion: RBC transfusion has significantly increased in the era after market withdrawal of aprotinin in 2007. These results plea for reconsideration of aprotinin in OLT.

The use of proton pump inhibitors does not increase the risk of acute rejection after renal transplantation

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Background: Mycophenolate mofetil (MMF), is the pro-drug of mycophenolic acid (MPA), which is formed after de-esterification of MMF in the stomach. Adequate exposure to MPA is associated with a decreased rate of acute rejection after renal transplantation. Several studies indicate that concomitant use of proton pump inhibitors (PPI) might impair the exposure to MPA due to incomplete de-esterification of MMF at elevated gastric pH. This could result in an increased risk of acute rejection. In our centre, renal transplant patients are prophylactically treated with a PPI (pantoprazole) or an H_2 antagonists (ranitidine) for at least three months after renal transplantation. Aim: To investigate whether MMF-treated renal transplant patients who concomitantly used pantoprazole, had a higher risk of acute rejection within the first 3 months after transplantation than those who used ranitidine. Patients and methods: We performed a retrospective study in adult patients, who underwent a kidney transplantation between January 2007 and December 2011. Their immunosuppressive therapy consisted of steroids, tacrolimus and MMF. Exclusion criteria were a history of bowel surgery, the use of a phosphate binder, the switch between PPI and H_2 antagonist, and the combined use of PPI and H_2 antagonist. Results: 207 patients were included: 126 with pantoprazole and 81 with ranitidine. Both groups were comparable regarding age, body weight, dose of prednisone and tacrolimus, retransplantations, and donor type. In the first 3 months after transplantation, the cumulative dose of MMF was 142,894±17,718 mg in patients with pantoprazole and 144,289±18,097 mg in patients with ranitidine (NS). The number of patients with a clinical diagnosis of acute rejection within three months after transplantation did not differ between both groups: 26 (19.5%) in the pantoprazole group versus 15 (20,0%) in the ranitidine group. There was also no difference in the number of patients with biopsy-proven acute rejection (13 [10,0%] versus 7 [9,1%]). Logistic regression analysis did not reveal a correlation between the cumulative dose of pantoprazole and the risk of acute rejection. Three months after renal transplantation, the mean estimated glomerular filtration rate did not differ significantly: 49.4±12.5 ml/min/1.73m^2 versus 50.7±12.5 ml/min/1.73m^2, respectively.
Conclusions: There was no evidence for an increased incidence of acute rejection in patients who concomitantly use MMF and pantoprazole.

Predictive value of anti-PLA2r antibodies at time of transplantation for kidney failure in patients with membranous nephropathy

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Introduction: The M-type phospholipase A2 receptor (PLA2r) is the major target antigen in idiopathic membranous nephropathy (iMN). Recently it was shown that anti-PLA2r antibodies (ab) were present at time of transplantation in a patient who developed recurrent iMN in the kidney graft. We assessed the presence of anti-PLA2r ab at the time of transplantation in patients with a reported iMN who underwent kidney transplantation between 1990 and 2008 in the UMC Utrecht and evaluated whether anti-PLA2r presence at the time of transplantation was associated with graft function. Methods: In this cohort of 897 kidney transplant recipients, all patients were identified with iMN according to the Renine database. Of these charts were reviewed, the actual cause of glomerulonephritis was determined from the chart, and urine protein/creatinine ratio, serum creatinine, rejection episodes and biopsies were recorded for the first five years after transplantation. Protein/creatinine ratio and createnine during follow-up were analysed using linear mixed models. Results: In our cohort 13 patients were identified with iMN according the Renine database (8 males, mean age at transplant=43). Chart review indicated that 4 patients had biopsy proven iMN, whereas 6 patients had FSGS, 2 MPGN and in 1 patient no classifying diagnosis was formulated. Anti-PLA2r ab were present in 5 of 13. All patients with documented iMN had anti-PLA2r ab (1 FSGS). In patients with iMN and positive anti-PLA2r ab (compared to the other nephrotic syndrome patients), the mean five year urine protein/creatinine ratio and serum creatinine was not significantly different (0.005 g/mmol (p=0.905) and -20 µmol/l (p=0.555)). No patients developed recurrent iMN. Two of the 4 patients with iMN were biopsied after transplantation; both did not show a histologic recurrence.
Discussion: We found that anti-PLA2r detectable at the time of transplantation identifies patients with iMN even if the disease is not active. Our data suggest that the presence of anti-PLA2r antibody at the time of transplantation in iMN does not predict disease recurrence nor influence graft function during a five year follow-up after kidney transplantation.

De KIT verlagen door de voorbereidingen voor allocatie van NHB-III nieren te vervroegen

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Achtergrond: voor NHB-III nierdonatie geldt helaas nog steeds dat de allocatie (in de huidige praktijk: het draaien van een matchlijst) pas mag starten na het vaststellen van de circulatiestilstand. Vanwege logistiek rondom HLA-typering en het verrichten van kruisproeven staat een nier vaak onnodig lang geparkeerd wat de KIT niet ten goede komt. Doel: inzicht verkrijgen in het logistieke proces van NHB-III nierdonatie en –transplantatie en analyseren hoeveel tijdswinst er te behalen is door de voorbereidingen van de allocatie van NHB-III nieren te vervroegen. Methode: retrospectief data collectie uit de periode 2000 t/m 2011 van alle postmortale Nederlandse NHB-III donoren waarbij 1 of meerdere nieren werd getransplanteerd in een Nederlands transplantatiecentrum. Resultaten: Van de 876 NHB-III geëffectueerde nierdonaties werd in 312 gevallen een HLA-typering gedaan. Bij 78% (n=242) van de donorprocedures werd de HLA-typering bekend ná de daadwerkelijke switch off, met een gemiddelde wachttijd van 2 uur en 52 minuten. Na gemiddeld 11 minuten werd een 1e kruislijst gedraaid. En na gemiddeld 3 uur en 17 minuten werd de nier geaccepteerd (dit geeft een schatting weer van de duur van een kruisproef). Na acceptatie van de nier werd de nier na gemiddeld 11 uur en 30 minuten getransplanteerd. De gemiddelde transplantatietijden per centrum variëren van 7 tot 14 uur.
Conclusie: Indien de huidige werkwijze wordt aangepast en de matchlijst wél voor de switchoff gedraaid mag worden dan zou het inzetten van de kruisproef simultaan kunnen lopen met het wachten op circulatiestilstand en explantatie. Dit zou circa 5 à 6 uur tijdswinst opleveren in het pre-explantatieproces, mits de HLA-typering tijdig word ingezet. Het verdient ook onze aandacht dat het gemiddeld 11 uur en 30 minuten duurt tot de nieren in het transplantatiecentrum worden getransplanteerd. Nederland heeft een gemiddelde KIT van 16 uur. De KI-tijden in de ons omringende landen zijn echter aanmerkelijk korter. Er valt dus naast het vervroegen van de voorbereidingen van de allocatieprocedure ook nog een flinke optimaliseringslag te behalen in het logistieke post-explantatieproces.

15 years of DCD3 in the Netherlands: increasing efforts in donor activities for a small increase of transplants

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Objective: To evaluate organ donation after circulatory death (DCD) category 3 in the Netherlands. Methods: Data on DCD3 organ donors in the Netherlands over the period 1998-Nov.2012 were analyzed per year; number reported to Eurotransplant, percentage used for transplantation and the age distribution. In medical record reviews of patients died in intensive care units during 2007-2011 we evaluated the reasons why organ procurement was not realized, after consent was given for DCD3 by the Donor Registry or the family. Results: The number of DCD3 donors from the Netherlands that were reported increased from 21 in 1998 to 163 in 2012, leading to an increase of DCD3 among reported deceased donors from 10% to 56%. Meanwhile the number of reported donation after brain death (DBD) donors fluctuated between 111 and 170 (mean 138 per year) and the number of other DCD category (1, 2 or 4) donors fluctuated between 6 and 24 (mean 12 per year). The percentage of reported deceased donors that were actually used for transplantation decreased from 94% in 1998 to 79% in 2012 and was mainly caused by a lower use of reported DCD3, and use in DCD3 even decreased from 90% to 67% in these 15 years. During these years the DCD3 group got older and the percentage of used donors decrease considerably with age in this group. Main reason for non procurement was, that potential DCD donors did not die within the time limit of 2 hours after withdrawal of treatment. The percentage of multi-organ donors among used DCD3 increased from 0% in 1998 to 48% in 2012.

Conclusions: Introduction of DCD3 in the Netherlands resulted in a higher rate of donation activities, but a lower increase of used donors and transplants. It is caused by the 2 hours time limit rule for DCD3 and the relatively low percentage of used organs from an increasing number of older DCD3 donors.

Donor organ temperature after systemic flush out is far from cold

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Introduction: Reduction of metabolic rate is the key factor in organ preservation. Systemic flush-out with ice-cold preservation solutions is considered to be the start of Cold Ischemia Time. Cooling to 0-4°C is however likely first reached when organs are packed and stored on ice. Methods: In this study liver and kidney temperature during flush-out and retrieval until removal were measured during MOD procedures by the Leiden team. Both Brain-Dead (n=8) and Non-Heart-Beating (n=5) donors were studied. Standard retrieval procedures including crushed ice and topical cooling were followed. Results: For BD-donors significantly less preservation solution (UW: 6.9±0.35 liters) was used compared to NHB-donors (HTK: 12.6±1.2 liters). Total flush time for BD and NHB donors tended to be different (BD:17.8±3.1 minutes; NHB: 25.8±4.5 minutes). Flow rate was not significantly different between donor types (BD: 454±66 ml/min; NHB: 580±138 ml/min). In BD-donors, liver temperature decreased rapidly to 11.3±0.7°C after 15min but increased again to 17.5±0.5°C after 60min. For NHB-donors no temperature dip was observed (17.9±2.6°C at 15min vs 16.4±2.3°C at 60min). Renal temperatures dropped to 11.2°C (n=1) after 15min in the BD-group and in he NHB-group mean temperature was 15±2.6°C. After 60 minutes, in the BD-donors 18.7±1.9°C was measured and 17.6±1.0°C in the NHB donors.
Discussion: The incomplete cooling during flush-out as well as the warm-up before organ removal observed in this study may lead to ischemic damage and may therefore explain the inferior outcome of deceased donors compared to living donors. The use of more fluid in the NHB-donors did not lead to lower temperatures questioning the assumption that HTK is preferable in NHB donation. Distribution of the preservation solution to organs may be more relevant illustrated by the lower liver temperature in the UW flushed HB-donors although other donor factors can not be ruled out.

Early deterioration of PELD score in young children with biliary atresia predicts poor outcome

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Background: The Pediatric End-stage Liver Disease (PELD) score is designed to prioritize children with biliary atresia for liver transplantation, based on the severity of chronic liver disease. High scores at listing predict poor outcome, including death. Periodic calculation of the PELD score in children awaiting liver transplantation may be an important additional predictor of pre-transplantation mortality. We aimed to determine the PELD change or cut-off score in young children with biliary atresia (BA) on the waiting list that could assist clinicians in identifying those with a high risk of dying before transplantation. Methods: A national cohort of children younger than 5 years with BA, screened for liver transplantation between 2000 and 2012 was retrospectively analyzed. PELD scores and change scores were calculated at listing and then 2-monthly until death or liver transplantation. Results: A total of 71 children with BA-associated end-stage liver disease were included, of which 12 (17%) died before transplantation. At the time of listing the optimum PELD score cut point to differentiate high from low risk patients was 21 points. In children with a PELD score of 21 points or higher mortality risk before transplantation increased to 40% (10/25), whereas a score of 20 points or below reduced this risk to 4% (2/46). Two months after listing, when 47 patients were still waiting for a donor liver, the optimum cut point to differentiate high from low risk patients was 24 points. Children with a score of 24 or more had a 56% (9/16) risk of death, whereas a score of 23 or below gave a 0% risk (0/31; 95% confidence interval (CI) 0 to 9%). A deterioration of PELD score of 5 points or more in the first two months after listing indicated a 50% (9/18) risk of death before transplantation, whereas a change score of 4 points or less reduced mortality to 0% (0/29; (95% CI 0 to 10%).
Conclusions: Periodic calculation of the PELD-score in young children with BA-associated end-stage liver disease awaiting transplantation facilitates recognition of those with a high risk of pre-transplantation demise. Early transplantation (e.g. with a living related donor organ) may prevent this tragedy.

The Eurotransplant Donor Risk Index distribution amongst MELD categories within the Eurotransplant region

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Introduction: Recently we introduced the Eurotransplant Donor Risk Index (ET-DRI) as indicator of donor quality within the Eurotransplant region. Since donor quality is only one side of the story, a combination of the ET-DRI with recipient characteristics (e.g. MELD) would possibly better predict outcome after orthotopic liver transplantation (OLT). Objective: Investigation of the distribution of the ET-DRI throughout different liver transplant recipient groups and combination of ET-DRI with several recipient factors as predictor of outcome after OLT. Methods: All liver transplants within the Eurotransplant region in adult (≥18) recipients performed from 1.1.2008 till 31.12.2010 were retrospectively analysed. Results: A total of 4489 liver transplants were performed. Distribution of transplantations with lab-MELD and exception-MELD were 73% (N=3290) vs. 26% (N=1176) (unknown N=23). Donor and recipient risk were (median): ET-DRI 1.91, DRI 1.78, lab-MELD 23 (N=3290), exception-MELD 26 (N=1176), overall lab-MELD 18 overall and match-MELD 25. Distribution of lab-MELD transplantations was: MELD6-14 23%, MELD15-20 20%, MELD21-25 11%, MELD26-30 12%, MELD 31-35 14% and MELD≥35 20%. Distribution of exception-MELD categories was: MELD20-25 49%, MELD26-30 37%, MELD31-35 10% and MELD>35 4%. ET-DRI significantly (p=0.001) predicted outcome in Kaplan-Meier survival analysis and in multivariate Cox-regression analysis (p<0.001) (when correcting for all available recipient factors). ET-DRI (median) distribution among lab-MELD categories was respectively: ET-DRI 2.02, ET-DRI 1.88, ET-DRI 1.87, ET-DRI 1.87, ET-DRI 1.88 and ET-DRI 1.87. The use of high-risk ET-DRI allografts (ET-DRI>1.91) led to a significant (p≤0.004) difference in outcome in the low-MELD (6-14) and medium-MELD (26-30) groups. In the exception-MELD categories the ET-DRI distribution was respectively: ET-DRI 1.94, ET-DRI 1.92, ET-DRI 1.93 and ET-DRI 1.72. The use of high-risk ET-DRI allografts did not led to a significant difference in outcome in any of the groups.
Conclusion: The ET-DRI was validated as risk score in this recent database. There is a trend towards the use of high ET-DRI allografts in lower (lab and exception) MELD categories and lower ET-DRI allografts in the high exception MELD category. The use of high-risk allografts leads to a significant difference in outcome in the lowest lab-MELD category. It is questionable whether high ET-DRI allografts should be used for lower lab-MELD categories.

Training 'Communication about Donation' successful in facilitating medical professionals and in increasing the donation consent rate

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Introduction: The training 'Communication about Donation' (CaD), developed by the Dutch Transplant Foundation in 2007, facilitates professionals to discuss the subject of organ and/or tissue donation with bereaved families and guide them in the donation decision-making process. Requesting for donation is difficult and complex and frequently results in an objection by relatives. The CaD training started in a few hospitals in 2008 and is now embedded in a large number of hospitals in the Netherlands, but is the training effective? Methods: The training was first implemented in 2008 in a pilot setting in 5 hospitals and afterwards psychologists were trained, according to a Train the Trainer module, to become a trainer in their local hospital. For measuring the effect of the CaD training a questionnaire was completed by medical professionals after each training. The consent rate for donation was assessed by comparing consent rates between hospitals with or without the CaD training. Results: From 2008 to 2012 the number of participating hospitals increased from 5 to 53. In total 115 psychologists participated in the 'Train the Trainer module' to become a qualified CaD trainer. In total, more than 2500 medical professionals are trained in approximately 263 CaD trainings. Medical professionals highly appreciate the CaD training with an average score of 8 on a range of 1 to 10. The consent rate increased in top clinical hospitals who implemented the CaD training and are active in training medical professionals, compared to top clinical hospitals without the CaD training (4.1% increased consent rate for tissue donation and 5.1% for organ donation).
Conclusion: What started as a small initiative to facilitate professionals in discussing donation with relatives, the CaD training now plays a prominent role in more than half of the Dutch hospitals. The training is effective, not only to help professionals discussing the subject of donation with relatives but also in improving the consent rate for donation.

Effect of donor risk factors on islet yield in pancreatic islet isolation

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Introduction: In the field of clinical islet transplantation certain donor risk factors, such as BMI, have been shown to have impact on islet yield. For vascularized pancreas transplantation risk models have been proposed (PDRI, P-PASS), but are not validated in contrast to certain liver donor risk indices (DRI, ET-DRI). Objective: Identification of donor and transplant risk factors that affect islet yield after pancreatic islet isolation. Methods: All consecutive pancreatic islet isolations performed at the LUMC from January 2008 till October 2012 were analyzed. P-PASS, PDRI, DRI, ET-DRI, donor and transplant factors were analyzed with multivariate models to identify risk factors that affect islet yield. Results: In total 195 isolations were performed. Donor and transplant factors were (median): age 53 years, female 50%, BMI 25, COD (CVA 64%, trauma 18%), DCDD 29%, cardiac arrest 29%, history of alcohol abuse 10%, hypertension 22%, smoking 56%, lipase 22U/L, amylase 103U/L, HbA1c 5.5%, creatinin 65µmol/L, cold ischemia time (CIT) 8.7h, perfusion fluid (UW 61%, HTK 33%), P-PASS 18 and PDRI 1.82. Median islet yield was 435.000 IEQ and 3872 IEQ/gram. P-PASS and PDRI did not correlate with IEQ (PDRI $p=0.74$ and 'reversed' P-PASS $p<0.001$) nor IEQ/gram (PDRI $p=0.24$ and reversed P-PASS $p<0.001$). Interestingly (liver) DRI and ET-DRI were significant for IEQ and IEQ/gram (DRI $p=0.025$ and $p=0.013$; ET-DRI $p=0.039$ and $p=0.005$). After multivariate analysis of donor and transplant factors, BMI ($p<0.001$), latest bilirubin ($p=0.015$) and CIT ($p=0.009$) were significantly associated with islet yield. Multivariate analysis of DRI and ET-DRI showed significance for both models (resp. $p<0.001$ and $p=0.019$).
Conclusion: Analysis of donor factors showed that donor BMI, bilirubin and CIT have a significant impact on islet yield after pancreatic islet isolation. Two risk models for whole pancreas transplantation (P-PASS, PDRI) did not significantly correlate with islet yield, interestingly the (liver) DRI and ET-DRI did.

Transplanted kidneys from donors after cardiac death type 3 who are older than 60 years of age

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In the Netherlands organ donation after cardiac death type 3 (DCD3) is common practice. Recently, we observe an increase in the percentage of DCD3 donors older than 60 years of age (60+DCD3). In 2007 there were only 23 (26%) 60+DCD3 donors, while in 2012 (cut-off date 21-11-2012) this number is already 42 (40%). We question whether the use of 60+DCD3 kidneys has an impact on short term graft function and survival. We analyzed short-term graft function and survival in first kidney transplantations performed in the Netherlands between 1-1-2007 and 1-1-2012 with kidneys from donors after brain death over 60 years of age (60+DBD), 60+DCD3 donors and DCD3 donors of 60 years of age or younger (60-DCD3). Data was obtained from the Dutch Transplant Follow up Registry (NOTR). The results are based on 157 transplanted 60+DCD kidneys, 446 60-DCD3 kidneys and 258 60+DBD kidneys. For statistical analysis the Chi-Square, Kaplan Meier, Log Rank and Cox regression analysis were used. The 60+DCD3 kidneys have 62% delayed graft function (DGF) and 13% never function, 60-DCD3 kidneys have 56% DGF, 7% never function, and 60+DBD kidneys 24% DGF, 5% never function; $p<0.001$. Death censored half year graft survival was 85% for 60+DCD3 kidneys, 92% for 60-DCD3 kidneys and 93% for 60+DBD donors ($p=0.02$). To include warm ischemia time, the multivariate Cox regression was performed on only the DCD3 kidneys. 60+DCD3 kidney had a Hazard ratio of 2.4 (95%CI: 1.2 – 4.5, $p=0.01$), warm ischemia time above 20 minutes had a HR of 2.2 (95%CI: 1.2 – 3.9, $p=0.01$), cold ischemia time above 20 hours a HR of 1.7 (95%CI: 0.9.2 – 3.2, $p=0.09$), donor hypertension a HR of 1.8 (95%CI: 0.99 – 3.2, $p=0.055$), donor serum creatinine a HR of 0.99 (95%CI: 0.98 - 1.01, $p=0.3$) and recipient age a HR of 0.99 (95%CI: 0.99 – 1.02, $p=0.49$).
In conclusion, 60+DCD3 kidneys perform less than younger DCD3 kidneys after transplantation. Limiting warm ischemia time and choosing donors without hypertension can reduce poor outcome.

Deceased donor factors and non-viable kidneys

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Background: As the need for transplantable organs is high, acceptance criteria for presumed deceased donor quality have been relaxed through the years. However, we are confronted with non viable kidneys (NVKs). Is it possible to identify factors that predict NVK? Methods: Recipients included received a deceased donor kidney transplant between January 2000 and April 2012. Deceased donor information was extracted from ET database using donor ET number. Missing values were requested from local Donor centres. Cox was performed with event NVKs. Donor variables included are: ischemia times, age, gender, BMI, type, cause of death, atherosclerosis, hypertension, diabetes, cardiovascular disease, cardiac arrest, hypotension, proteinuria and first serum creatinin. Recipient factors studied were: age, gender, waiting time, PRA, HLA-mismatches. Results: 582 patients were transplanted in the period studied: 366 heart-beating (HB, 63%) and 216 non-heart-beating (NHB) donations. 13 of the latter were NHB-II. 78.4% of donor procedures were done in the Netherlands. Amongst 38 NVKs the prevalence of NHB and HB donor kidneys was not significantly different. Donor age was above 45 years in all cases. NVKs were significantly more often from males, with more atherosclerosis, more hypertension, longer cold ischemia time (CIT), first warm ischemia time (WIT1), second warm ischemia time (WIT2), higher donor and recipient age and there were more HLA mismatches. In multivariate Cox analysis only donor age, CIT and hypertension had a significant influence on the prevalence of NVKs ($p<0.001$, <0.001 respectively 0.001). There was no interaction between these variables. The NVK risk of a 50 year old donor kidney after 24 hours CIT is equivalent to that of 16 hours for a 60 year old and 8 hours for a 70 year old donor kidney. Donor hypertension has a RR for NVK of 3.3 on top of that. However, in our population donor age>65 years ánd CIT>1300 minutes prevailed in only 18% of recipients of NVKs.
Conclusion: No single set of factors can predict NVKs. Donor type did not influence the prevalence of NVKs. As CIT is the only adjustable factor: ultra-short CIT should be aimed at in elderly donors, especially in the presence of donor hypertension.

Effects of human alpha-1-Antitrypsin (hAAT) therapy in a murine model of bilateral kidney ischemia-reperfusion injury

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Aims: Several lines of evidence have demonstrated the anti-inflammatory and cytoprotective effects of alpha-1-Antitrypsin (AAT), the major serum serine-protease inhibitor. The aim of the present study was to investigate the effects of human AAT (hAAT) monotherapy in a mouse model of renal ischemia-reperfusion (I/R) injury. Methods: Renal I/R injury was induced in male C57/Bl6 mice by bilateral clamping of the renal artery and vein for 21 min. After clamp removal, kidneys were inspected for restoration of blood flow. hAAT (80 mg/kg/day, Prolastin®, Bayer) was administered daily i.p. from day -1. Control animals received the same amount of human serum albumin. Mice (*n*=8 animals per group) were placed in metabolic cages at day 1, 2, 7 or 14 after surgery to collect urine. After 24h, blood samples were obtained and animals were sacrificed by cervical dislocation. Kidneys were harvested for further analysis. Serum creatinine and urea levels were measured using standard clinical chemistry methods. Measurement of the renal injury markers kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) in urine and plasma was performed by ELISA. Results: Kidney ischemia significantly impaired renal function, causing a marked increase in plasma creatinine and urea levels. Furthermore, renal I/R significantly increased the protein levels of both KIM-1 and NGAL, in urine and plasma. The observed increase of these parameters peaked at days 2 and 3. hAAT treatment ameliorated renal IRI, reducing the levels of plasma creatinine (13.7 ± 1.4 *vs* 22.4 ± 4.1 µmol/L, $P = 0.09$) and urea (11.9 ± 3.8 *vs* 28.2 ± 6.1 mmol/L, $P = 0.05$) at day 2. Interestingly, treatment with hAAT significantly decreased the NGAL protein levels in urine at day 3 (626 ± 177 *vs* 2135 ± 536 ng/mL, $P < 0.05$) and in plasma at days 2 (485 ± 71 *vs* 901 ± 118 ng/mL, $P < 0.05$) and 3 (448 ± 100 *vs* 1101 ± 196 ng/mL, $P < 0.05$).
Conclusions: These data indicate a protective effect of hAAT in renal ischemia reperfusion injury and support the potential of this natural protein to ameliorate ischemic and inflammatory conditions.

A preoperative amino acid free diet protects against hepatic ischemia reperfusion injury

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Background: Ischemia and reperfusion (I/R) injury is a serious and common complication after liver surgery and transplantation. We previously showed that three days of preoperative fasting protects against hepatic I/R injury. The protective effect was induced by the absence of protein, since a protein-free diet induced similar protection. To investigate whether total protein or single essential amino acids are responsible, we investigated the effects of a three-day preoperative amino acid free diet on hepatic I/R injury in mice. Materials and methods: Male C57BL/6 mice were randomized into four groups (n=6/group). Three days before the induction of hepatic I/R injury they received either a control diet, a leucin-free, methionine-free or tryptophan-free diet. Bodyweight was recorded during the dietary intervention and after surgery. Hepatic I/R injury was induced by clamping 70% of the liver for 75 minutes. Serum ALAT and LDH levels and the percentage of necrosis in liver tissue were used to assess damage. Results: Control diet fed mice gained 3% in weight. A leucin-free diet led to a reduction in bodyweight of 6.2%, tryptophan-free of 8.4% and methionine-free 9.5%. Postoperatively the control mice lost 10% of their body weight, while the mice on the deficient diets lost 0.5% on the first postoperative day. Six hours after reperfusion ALAT and LDH levels were significant lower in the leucin-free and tryptophan-free groups compared to the controls ($P<0.05$), and 24 hours after reperfusion liver enzymes were lower in the leucin-free, methionine-free and tryptophan-free groups ($P<0.01$). Liver tissue 24 hours after reperfusion showed significantly less necrosis in the methionine-free group (33.3%±sem6.2%) compared with the control group (77.5% ± sem10.8%) ($P<0.05$). The leucine-free (34.4%) and tryptophan-free (45.8%) diets showed a reduction that did not reach statistical significance.
Conclusion: A preoperative amino acid free diet limits the damage caused by hepatic I/R injury similar to fasting and a protein-free diet. Compared to fasting an amino acid free diet reduce weight loss. Therefore an amino acid free diet is a promising strategy to apply in the clinical setting.

Mesenchymal stem cell-derived trophic factors promote liver regeneration but does not protect against ischemia reperfusion injury

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Objectives: Mesenchymal stem cells (MSC) and their secreted factors represent a potential new therapeutic strategy to stimulate liver regeneration in living-donor liver transplantation to prevent small-for-size syndrome. Our previous data showed increased liver weight gain and hepatocyte proliferation in mice treated with MSC-derived factors after 70% partial hepatectomy (PH). In the current study we investigated if MSC-derived factors protect mice against ischemia and reperfusion injury (IRI) alone or in combination with 50% PH. Methods: In the IRI alone model, C57BL/6 mice were subjected to IRI by clamping the median and left lateral liver lobes for 90 minutes. In the IRI+PH model, mice were subjected to 60 minutes of IRI after which a 50% PH was performed by ligation of the right lateral lobe, caudate lobes and the right part of the median lobe. In both groups, animals were treated immediately after surgery (and 24 hours later if sacrificed after 2 days) with serum-free concentrated conditioned culture medium of liver-derived MSC (MSC-CM). Concentrated unconditioned medium (UM) was used as vehicle control. Animals in the IRI group were sacrificed after 6 or 24 hours to investigate effects on serum transaminases and hepatocyte damage. Animals of the IRI+PH group were sacrificed after 48 hours to also investigate effects on hepatocyte proliferation. Results: In the IRI model, serum ALT and AST levels after 6 and 24 hours showed no differences between the MSC-CM and UM treatment group. Similar, in the IRI+PH model, ALT and AST levels were not significantly different between both treatment groups. In this combined group, however, a significant reduction in tissue damage after MSC-CM treatment was observed, accompanied by reduced inflammatory cell infiltration. The average damage score in the MSC-CM group was 0.63 versus 1.40 in the UM group ($p=0.04$). This was furthermore accompanied by a significant increase in hepatocyte proliferation in the MSC-CM group as compared to the UM group (13.5% vs. 5.0% BrdU-positive nuclei, $p=0.002$).
Conclusion: This study shows that MSC-derived factors do not protect against the early effects of ischemia and reperfusion injury, but significantly stimulate hepatocyte proliferation and improve liver regeneration after liver resection, despite combined ischemia and reperfusion injury. MSC-derived factors therefore represent a promising strategy to expand options in living-donor liver transplantation.

Functional analysis of virus-specific T-cells with cross-reactivity to allo-antigens

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Background: Part of the antigen-specific memory T-cells generated against pathogens, such as cytomegalovirus (CMV) and Epstein-Barr virus (EBV) can also respond to allo-antigens, so called heterologous immunity. In kidney transplant recipients, the presence of virus specific T-cells with cross-reactivity to allo-antigens of donor origin might impair allograft function and survival. To study the role of heterologous immunity we developed a method to analyze the frequency and function of virus-specific T-cells with cross-reactivity against allo-antigens. Methods: We used peripheral blood mononuclear cells (PBMCs) from 5 healthy individuals known to have circulating CMV- or EBV-specific T-cells, which could be detected by tetramer-staining. PBMCs were used directly, or were labelled with CFSE and cultured for 6 days either with allogeneic stimulator cells in a mixed lymphocyte reaction (MLR) or with viral peptide and IL-2 to expand respectively allo- and virus-specific T-cells. Cells were (re-) stimulated for 6h with allogeneic cells or peptide loaded-autologous PBMCs and analyzed by flow cytometry. We identified cross-reactive CD8+ T-cells as tetramer+ and CFSEdim in MLRs. Results: Viral-antigen dose-dependently induced cytokine production and expression of the degranulation marker CD107α in virus-specific T-cells analyzed directly or expanded by either viral- or allo-antigen stimulation. Interestingly, EBV-specific T-cells, which have an effector-memory phenotype, produced both IFNγ and IL-2, whereas effector type CMV-specific T-cells produced only IFNγ. Re-stimulation with allo-antigen induced IFNγ in a small but specific percentage of T-cells which had expanded upon stimulation by viral peptide. These findings confirm that cross-reactive T cells responding to both allo- and viral- antigens can be detected *in vitro* by proliferation, IFNγ production and degranulation. We could not identify cross-reactive allo-specific T-cells by intracellular cytokine staining directly *ex vivo*, suggesting that the activation state of cells determines their capacity to secrete cytokines.
Conclusion: Together these results show that frequency and function of cross-reactive T-cells can be analyzed *in vitro*.

Disappearance of immunoglobulin producing plasmablasts in kidney transplant patients is associated with a diminished cytokine producing capacity by peripheral follicular T-lymphocytes

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Introduction: Follicular T-helper cells (Thf-cells) play a pivotal role in the differentiation of B-lymphocytes into antibody secreting cells, i.e. plasmablasts (CD19+CD20-CD27+CD38++). CD4+CXCR5+ T-cells are their peripheral blood counterparts and share functional properties with Thf-cells. They comprise IFNγ, IL17 and IL21 producing cells. B-cell mediated alloreactivity has been recognized as common cause of allograft dysfunction and predicts both early and late graft loss. Here we examined the frequency and functions of peripheral Thf-cells critical for B-cell activation before and after kidney transplantation (Tx). Methods: Peripheral blood samples of patients (N=30) before and after kidney Tx and of healthy controls (N=16) were studied. Patients were treated with Tacrolimus, MMF and steroids. By flow cytometry the absolute numbers of CD4+CXCR5+ T-cells, and their cytokine producing profile (IL17, IL21 and IFNγ) as well as the numbers of plasmablasts were measured. Functional interaction was studied by co-culture experiments (N=6) of cell sorted CD4+CXCR5+ and CD4+CXCR5- T-cells with sorted endotoxin activated B-cells. B-cell differentiation and IgM and IgG production were determined in the presence and absence of Tacrolimus (10 ng/ml). Results: Before Tx the absolute numbers of CD4+CXCR5+ T-cells were significantly lower than in healthy controls, and these numbers remained stable after Tx. However, after Tx the IL21 and IFNγ, but not the IL17 production capacity by the CD4+CXCR5+ T-cells was significantly reduced compared to before Tx (both $p<0.01$). Moreover, patients had a significantly lower plasmablast count than healthy controls ($p<0.02$). Interestingly, after Tx a complete vanishing of plasmablasts was observed ($p<0.0001$). The co-cultures studies revealed that only CD4+CXCR5+ T-cells, but not CD4+CXCR5- T-cells, provided help to B-cell differentiation into IgM and IgG producing plasmablasts ($p<0.05$). This CXCR5+ dependent T-cell help to B-cells was completely inhibited by Tacrolimus. Differentiation of B-lymphocytes into plasmablasts was highly correlated with IgM and IgG production, $R_s=0.91$, $p<0.0001$ and $R_s=0.85$, $p<0.0001$, respectively.

Conclusion: We showed that the functionality of peripheral Thf-cells (CD4+CXCR5+), critical for the B-cell mediated immunity, is inhibited in kidney transplant patients. Moreover, Tacrolimus completely blocked B-cell differentiation into Ig secreting plasmablasts.

Tubular injury markers KIM-1 and NGAL represent different patho-physiological processes during delayed graft function

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Kindey injury molecule (KIM)-1 and Neutrophil gelatinase-associated lipocalin (NGAL) are promising tubular injury markers in the context of various forms of acute kidney injury, including delayed graft function (DGF) and transplantation. Both markers can be expressed by renal tubular epithelial cells and released in the urine. Here, we investigated and quantified the presence of KIM-1 and NGAL in paired samples of urine and tissue in a cohort of patients, collected 10 days after transplantation. Regular urinary samples were collected in a prospective study of 92 patients receiving a CDD kidney. At day 10, protocol biopsies were obtained and frozen sections of 71 patients were available for analysis. Immunohistochemistry for KIM-1 and NGAL on all biopsies and a double staining on a smaller subgroup were performed. Urinary KIM-1 and NGAL were measured using specific ELISAs. At day 10, KIM-1 concentrations in urine showed a wide distribution (range 0,1-79,8 ng/ml, mean 10,1 ng/ml), which was also observed for the values corrected for urinary creatinin (range 0,43-254 ng/mg creat). When comparing both analyses, a very strong correlation was shown (r = 0,91). A similar distribution was found for urinary NGAL concentrations (range 0,3-6269 ng/ml, mean 748 ng/ml). Importantly, KIM-1 and NGAL concentrations only showed a weak correlation (r = 0,37). Next, KIM-1 and NGAL stainings (n=71) were performed on consecutive slides. In many cases, KIM-1 was abundantly expressed on tubular epithelial cells (0,1-27,6 %, median 7,7% of surface), with a tubular expression pattern including cytoplasmic and brush border staining and a strong expression on dedifferentiated epithelial cells. NGAL staining (0,1-25,9%, median 1,9 % of surface) was more restricted to a cytoplasmic staining. Importantly, there was no relation between the quantitative scores of these stainings. Finally, a fluorescent double staining was performed and confirmed that KIM-1 and NGAL mostly showed a distinct and separate tissue distribution, and colocalization was a rare event.

Our results indicate that both KIM-1 and NGAL are interesting biomarkers in the context of DGF, but seem to represent different biological processes and might be actively involved in a protective or repair process. Our study should allow a further evaluation of the clinical importance of measuring these biomarkers, either at the tissue or urine level.

The TNF-alpha -238 G-allele predisposes to severe bacterial infection in patients with end-stage liver disease enlisted for liver transplantation

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Background: Augmented susceptibility to infections increases mortality in patients with end-stage liver disease (ESLD). Activation of pro-inflammatory cytokine production via TLR4, which is a major defense mechanism against bacterial infections, shows inter-individual differences. We aimed to determine the contribution of genetic variants in TLR4 and pro-inflammatory cytokines to severe infections in patients with ESLD. Methods: We retrospectively assessed incidence of severe bacterial infections (SBI) (spontaneous bacterial peritonitis, pneumonia, urinary infection, erysipelas, bacteremia) requiring hospitalization and i.v. antibiotics administration in a cohort of 243 adult cirrhotic ESLD patients enlisted for orthotopic liver transplantation (OLT) from 1995 to 2010. Patients with hepatocellular carcinoma corresponding to Child-Pugh´s classification A, patients with metabolic liver disorders with normal liver function, patients with primary sclerosing cholangitis (PSC), and patients with acute liver failure were excluded. All patients were genotyped for TLR4 +1196C/T, CD14 -159C/T, TNFA -238G/A, TNFA -863C/A, IL-1B -31C/T and IL-1RA variable number of tandem repeats (VNTR) allelic variants. Associations were validated in a second cohort of 237 cirrhotic ESLD patients enlisted for OLT from 1995 to 2011 from another center. Results: Sixty nine (69/243, 28%) patients with ESLD presented with SBI while enlisted for OLT in the first cohort. Patients homozygous for TNFA -238 (GG genotype; (n=221)) showed a significantly increased risk of SBI (OR 9.33, P=0.009) compared to patients with the TNFA -238GA genotype, which is supposed to have increased the transcriptional activity of TNFA. In the second cohort, seventy two (72/237, 30%) ESLD patients suffered from SBI while enlisted for OLT. In this cohort, the association between TNFA -238GG and increased risk for SBI was confirmed (OR 3.76, p=0.032). The association was independent of clinical variables that were also included in multivariate analysis.
Conclusion: Our results indicate that a genetic variant in the TNFA gene that increases its transcriptional activity independently modifies the risk of SBI in patients with ESLD. These findings may help to identify those patients who are predisposed to SBI.

Alemtuzumab as anti-rejection therapy in kidney transplant patients induces homeostatic T cell proliferation and impairs IL7 but not IL2 responses

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Introduction: Induction therapy with alemtuzumab is known to result in T cell depletion followed by a slow immune recovery leading to hypo-responsiveness to the graft. Whether the same effects occur when alemtuzumab is given during immune activation, i.e. anti-rejection therapy is unknown. In this study we investigated the repopulation, phenotype and cytokine responsiveness of T cells after alemtuzumab anti-rejection therapy. Methods: Patients (n=11) were treated with alemtuzumab (2 days 30mg sc) for steroid-resistant rejection. Flow cytometric analysis was performed on whole blood to measure the expression of the proliferation marker Ki67 and to study interleukin 2 (IL2) and IL7 mediated phosphorylation of STAT5, a nuclear transcription factor for T cell activation and proliferation, in CD4 and CD8 naïve (CD45RO-CCR7+), central memory (CM; CD45RO+CCR7+), effector memory (EM; CD45RO+CCR7-) and EMRA (CD45RO-CCR7-) T cells, before and 3 months after treatment. Results: Three months after alemtuzumab therapy the T cell population recovered to 5% of baseline level (570 vs. 47 cells/μL, $p<0.01$), with no differences between CD4 and CD8 T cell recovery. The CD4 T cell pool showed a phenotypic shift towards memory T cells (median 70%, range 42-88% vs. 98% range 75-100%, $p=0.01$). In the CD8 T cells only the percentage of effector memory T cells increased (20% range 3-42% vs. 32 range 21-44%, $p=0.04$). Ki67 expression was significantly higher in both CD4 and CD8 T cells at 3 months when compared to baseline levels ($p<0.01$). For CD4 T cells higher percentages of Ki67+ T cells were both present in CM and EM T cells ($p<0.01$). Where for CD8 T cells only an increased percentage in the EM subset ($p=0.02$) was measured. At the functional level repopulated CD4 and CD8 T cells were not affected in their capacity to phosphorylate STAT5 when stimulated by IL2. However, in response to IL7 the STAT5 phosphorylation capacity was decreased in the total CD4 population ($p=0.02$) as well as in the CD4 memory subsets (all $p<0.03$).
Conclusion: After alemtuzumab anti-rejection therapy, repopulation takes place via homeostatic proliferation with a subsequent phenotypic shift towards memory T cells. In addition, CD4 T cells showed impaired STAT5 phosphorylation capacity in response to IL7 but not to IL2. This can be explained by tachyphylaxis, exhaustion or down-regulation of the IL7 receptor.

Mature dendritic cells are superior stimulator cells for expansion of human alloreactive regulatory T cells

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Introduction: Antigen (Ag)-specific regulatory T cells (Tregs) have a great therapeutic clinical potential. However, large numbers are needed before Tregs can be used as cellular immunotherapy in kidney transplant patients. Therefore, optimal expansion protocols are needed to generate these cell numbers from peripheral blood isolated Tregs, but are presently lacking. In this study, mature monocyte-derived dendritic cells (moDC) were studied for their capacity to generate alloAg-specific Tregs and compared with PBMC-induced and polyclonal Treg expansion. Methods: For this purpose highly pure Tregs ($CD4^+CD25^{bright}CD127^-$ T cells) and T cells depleted for Tregs (Teff= responder) were obtained using FACS sorting. The Tregs were expanded using αCD3/αCD28-coated microbeads (polyclonal) or an Ag-specific expansion protocol using HLA-mismatched PBMC or mature moDC as stimulator cells. The different expanded Tregs were stained for FOXP3 and their suppressive capacity was tested in a mixed lymphocyte reaction (MLR) using different ratios of Treg:Teff from 1:5 to 1:320. As a control, suppression was tested in a MLR using fully mismatched PBMC to both the responder as well as stimulator cells (third party). Results: Mature allogeneic moDC were on average 40-times more potent in inducing Treg expansion when compared to PBMC. For example, at a ratio of 1:10 (DC:Tregs), a similar fold expansion was observed during a period of 11 days, compared to a ratio of 4:1 (PBMC:Tregs), i.e. 16±6 and 14±6, respectively. With a 1:1 DC to Tregs ratio, it was possible to get >100-fold expansion of Tregs. The presence of rapamycine did not change the Treg expansion potency of DC. Remarkably, the presence of exogenous IL-15 but not IL-2 was needed for optimal DC-induced Treg expansion. Stable high FOXP3 expression was observed in all expanded Tregs. Both allogeneic DC and PBMC expanded Tregs were potent suppressors. For example, at a Treg to Teff ratio of 1:320, the allogeneic proliferation was inhibited by 60-70%. The proliferation to third party-stimulators was not inhibited at this ratio of Tregs. Polyclonal-expanded Tregs were less potent suppressors when compared to Ag-specific expanded Tregs and no suppressive activity was observed below a ratio of 1:320. Conclusions: Mature allogeneic DC are highly efficient cells for expansion of potent alloAg specific Tregs. This observation opens a new avenue for generation of Tregs on a large scale for immunotherapy.

Successful ex-vivo normothermic machine perfusion and viability testing of discarded human donor livers

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Ongoing organ donor shortage has led to expansion of donor criteria and the transplantation of livers that would previously have been declined. These so called extended criteria donor livers have a higher risk of failure after transplantation. In contrast to traditional static cold preservation, normothermic machine perfusion may reduce preservation injury, improve graft viability, and potentially allows *ex-vivo* assessment of liver graft viability prior to implantation. To date, successful normothermic perfusion of livers has been reported only in animal models. We report the first case series of four discarded human livers, which have been successfully perfused normothermically for 6 hours. Normothermic machine perfusion was successful in all four human donor livers included in this study. There were no technical failures of the perfusion machine and all livers were well perfused and oxygenated. The livers, although discarded for varying reasons, functioned well beyond expectations. Distinct oxygen consumption, continuous bile production (possibly the most important outcome parameter for a well-functioning liver) and normalization of the biochemical parameters in perfusion fluid were noted over time in all procedures. Histological examinations confirmed adequate presservation of liver viability.
This study shows that normothermic machine perfusion of human donor livers is feasible and offers a great future perspective in the field of liver transplantation. Normothermic perfusion of ECD livers allows assessment of graft viability before transplantation, and opens new avenues for selection, therapeutic interventions and preconditioning.

Auxiliary and orthotopic liver transplantation for acute liver failure. A single center experience

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Introduction: Auxiliary liver transplantation (AXLT) is an attractive alternative to orthotopic liver transplantation (OLT) in patients with acute liver failure (ALF). In the acute setting the auxiliary graft is expected to act as a bridge to recovery of the own liver. Methods: All AXLTs and OLTs performed for ALF in our center between September 1992 and June 2012 were analysed retrospectively. Donor-, recipient- and transplant factors were evaluated. We looked at patient death, need for re-transplantation and withdrawal of immunosuppressive therapy. Results: A total of 13 AXLTs (10 after 2002) and 10 OLTs (2 after 2002) for ALF were performed in our center. For AXLT donors were younger (median age 34 years) and liver allografts were transplanted into younger recipients (median age 37 years). Most frequent cause of ALF was acute hepatitis B (N=6). One-year patient survival was 7/13 (54%) for AXLT and 7/10 (70%) for OLT ($p<0.01$). Out of the 7 AXLT survivors 5 (71%) are off all immunosuppression (4 after removal of the graft, in one immunosuppression was slowly withdrawn with deliberate chronic rejecttion of the graft). In 4 cases the auxiliary graft was re-used for OLT in a cirrhotic patient. Of the 13 AXLT-patients 2 underwent an orthotopic re-transplantation (reOLT) after 6 and 8 days for primary non-function of the graft. Of OLT-patients 2 underwent reOLT, but later, after 3 and 6 years for hepatic artery thrombosis and chronic rejection respectively. All four reOLT patients are alive. All patients who died did so within the first month after transplantation. Primary nonfunction and/or vascular problems were present in 7/13 AXLT (1reOLT alive, 6 died), and in 0/10 OLTs ($p<0.01$).
Conclusion: Patient- and graftsurvival was better after OLT than after AXLT for ALF, mainly due to PNF and vascular problems after AXLT, while 71% of surviving patients after AXLT were off all immunosuppression. Better patient selection and further technical improvements may further improve AXLT results. However, validation of these findings in a larger patient cohort is warranted before final conclusions can be drawn.

Duct-to-duct biliary reconstruction in liver transplantation for primary sclerosing cholangitis is associated with less biliary complications, compared with Roux-en-Y hepatico-jejunostomy

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Background: Although duct-to-duct anastomosis has been shown as a feasible option for biliary reconstruction in patients undergoing orthotopic liver transplantation (OLT) for end-stage primary sclerosing cholangitis (PSC), it remains unclear whether there is any difference in long-term outcome compared with biliary reconstruction using a Roux-en-Y hepatico-jejunostomy. Aim: The aim of this study was to evaluate the long-term outcome after OLT for PSC using either duct-to-duct anastomosis or Roux-en-Y hepatico-jejunostomy for biliary reconstruction. Methods: Between January 1, 1991 and December 31, 2011, a total of 98 adult patients underwent OLT for PSC in our center with either a duct-to-duct anastomosis or a Roux-en-Y hepatico-jejunostomy. Median duration of follow up was 8.2 years (interquartile range 3.9 – 14.5 years). Patient characteristics, postoperative complications, patient and graft survival rates, as well as short-term and long term biliary outcome parameters were compared between the two groups in a retrospective analysis of a prospectively collected database. Results: Duct-to-duct biliary reconstruction was performed in 45 patients and Roux-en-Y hepatico-jejunostomy in 53 patients. There were no significant differences in patient demographics and general surgical variables between the two groups. Overall patient and graft survival rates were similar in the two groups. The incidence of biliary strictures and biliary leakage within one year after transplantation did not differ among the two groups, however, significantly more patients in the Roux-en-Y group suffered at least one episode of cholangitis within the first year (9% in duct-to-duct versus 25% in Roux-en-Y group; $p = 0.04$). In addition, Roux-en-Y reconstruction was associated with a significant higher rate of late onset (>1 year post-transplant) non-anastomotic biliary strictures compared to the duct-to-duct group (24% versus 7% at 5 years and 30% versus 7% at 10 years; $p = 0.01$). Conclusion: The use of duct-to-duct biliary reconstruction in patients with PSC is associated with lower incidences of post-transplant cholangitis and late-onset non-anastomotic biliary strictures, compared to Roux-en-Y hepatico-jejunostomy. If technically and anatomically feasible, duct-to-duct anastomosis should be the preferred technique of biliary reconstruction in patients undergoing OLT for PSC.

Outcome of kidney transplantation in HIV positive recipients in The Netherlands

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Introduction: Following the introduction of highly active antiretroviral therapy, life expectancy of HIV patients has improved and chronic complications such as kidney failure are seen more frequently. As a result an increasing number of HIV patients is accepted for kidney transplantation. The need for both sufficient immune suppression and HIV control gives rise to problems not encountered in the HIV negative population. Here we evaluate the outcome of all HIV positive patients who underwent a kidney transplantation in The Netherlands.
Methods: We retrospectively collected data up to August 2012 from patient files and hospital databases in the five university medical centers where HIV positive patients had received a kidney transplantation. The outcomes were compared to those described in international literature. Results are reported as median with their corresponding range. Results: 21 HIV positive patients have been transplanted in The Netherlands. 17 were male, 7 were Caucasian, 12 African and 2 African-American. Age at transplantation was 45 yrs (26-65), with a history of 5.0 yrs (0.2-10.2) of kidney replacement therapy. Pre-transplantation CD4 count was 470 cells/mm^3 (190-1318), plasma HIV RNA was undetectable (< 50 copies/mL) in all but one patient. Post-transplantation follow-up at the time of analysis was 2.3 years (0.4-17.6), patient and death-censored graft survival were 85.7% and 90.5%, respectively. Delayed graft function was present in 7 patients (33%), with a median of 14 days (5-51). There have been 7 viral reactivations, 1 opportunistic infection, 1 Kaposi sarcoma and 1 PTLD. 10 patients suffered a total of 14 acute rejections. Creatinine at 6, 12 and 18 months after transplantation was 160 (57-215), 152 (76-365) and 170 (78-346) umol/L respectively. CD4 count at 6, 12 and 18 months was 248 (70-699), 255 (120-693) and 309 (87-597) cells/mm^3. One patient showed HIV virological failure due to poor drug compliance, 3 others had transient low level viremia or viral blips.
Conclusion: In our cohort we find excellent patient and graft survival with a high rejection rate, which is comparable to results reported in literature. Good survival and the absence of serious HIV-related morbidity support the eligibility of HIV positive patients for kidney transplantation.

Conversion from Prograft® to Advagraf® after kidney transplantation is safe

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Background: In contrast to the conventional tacrolimus (Tac) formulation (Prograft®), the slow-release Tac formulation (Advagraf®) can be taken only once a day, which may result in improved adherence to medication. We studied the effect of conversion from Prograft to Advagraf on clinical and laboratory parameters in a large cohort of kidney transplant recipients. Methods: Patients who were taking Tac-Prograft and were at least 6 months after transplantation were asked to participate. Tac-Prograft to Tac-Advagraf conversion was done on a 1:1 daily dose basis. Follow-up time was one year. Clinical and laboratory data were retrospectively collected for visits at -3 and -6 months and prospectively at months 0 (conversion), 3, 6, 9, and 12 after conversion. Results: A total of 311 patients were asked to participate of whom 252 agreed to undergo a conversion of their Tac formulation. Patients were enrolled between September 2009 and October 2011. Twenty-six patients did not complete the study: 3 never started Advagraf (1.2%); 3 died (1.2%); 8 did not adhere to all follow-up visits (3.2%), while 11 patients (4.4%) discontinued Advagraf because of adverse events. The 226 remaining patients were median 51 (range 18-80) years of age. 76% were male and median time after transplantation was 3 (range 0.5-26) years. Conversion had no effect on median serum creatinine, haemoglobin and cholesterol levels. Fasting blood sugar levels remained stable and no new cases of new-onset diabetes were diagnosed. Also no effect on blood pressure and weight was found. Advagraf dose did not change over time, but median Tac predose concentrations decreased with 12% from 5.8 to 5.1 ng/ml. No acute rejections were observed.
Conclusions: Conversion from Tac-Prograft to Tac-Advagraf appears to be safe as it did not affect renal function nor resulted in late acute rejections. Only 4.4 % of the patients discontinued medication because of self reported complaints. The 1:1 daily dosage conversion resulted in a 12% decrease in Tac predose concentrations.

Transplant nephrectomy: what are the surgical risks?

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Introduction: Whether or not to remove a failed renal graft has been subject of much debate. One reason for a cautious approach to graft removal is its high morbidity and mortality rates. We analyzed the morbidity, mortality and risk factors of transplant nephrectomy at our center. Methods: We included 157 cases of transplant nephrectomy in 143 patients, performed between January 2000 and May 2012. Patient data were collected retrospectively. Results: A total of 32 surgical complications occurred after transplant nephrectomy (20%) and 16 patients needed surgical re-intervention (10%). Haemorrhage and infection are the most frequent causes of surgical complications (14%). The mortality rate was 3.2%. There were no significant differences in respect to characteristics and timing of transplant nephrectomy between the group with surgical complications and the group without. A total of 59 re-transplantations were performed in 57 patients (38%).
Conclusions: Transplant nephrectomy is associated with high morbidity and mortality rates. We found no significant risk factors for surgical complications following transplant nephrectomy and no significant association between timing of transplant nephrectomy and surgical complications. Steps to reduce these complications need further investigation.

Increased mortality in elderly recipients of non heart-beating kidneys

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Background: In the Netherlands a considerable number of non heart-beating kidneys (NHB) are transplanted to elderly recipients following the European Senior Program allocation principles. However, there is sparse data on the outcome of elderly recipients receiving NHB kidneys from elderly recipients. Methods: We examined the outcomes of NHB versus heart-beating (HB) kidney transplantation in recipients aged either above or below 65 years transplanted between 2000 and 2011 at our center. Results: In the studied period a total of 519 deceased donor transplantations were performed. The recipients below 65 years received 248 HB and 154 NHB kidneys while 89 HB and 28 NHB procedures were performed in recipients above 65 years. No significant difference for death censored graft survival was detected when HB kidneys were compared with of NHB kidneys in both age groups. When examining death with a functioning graft, survival was comparable in recipients below 65 years who received either NHB or HB kidneys. However, in recipients above 65 years patient survival was markedly lower after NHB transplantation compared to HB transplantation (median survival 3.6 vs 8.4 years, P=0.006). In a limited multivariate analysis for patient death (including patient and donor age, delayed graft function, rejection and donor category as covariates), the presence of delayed graft function was an independent predictor of death with a functioning graft in the elderly patient group (HR 3.2, CI 1.3-8.1), while delayed graft function was not associated with survival in younger patients.
Conclusions: This small study points towards the important role of organ quality in older recipients. The outcome of NHB transplantation may be markedly influenced by recipient age. Our findings suggest that untoward effects of delayed graft function may play a role in the increased mortality after NHB transplantation in elderly recipients in our cohort. Further studies on the interaction of donor quality with acceptor age are warranted. Additionally our findings emphasize the importance of thoughtful care for elderly patients experiencing delayed graft function after transplantation

Immuno adsorption and risk of bleeding of recipients receiving a blood group ABO-incompatible kidney transplant

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Introduction: Pretransplant removal of anti-blood group ABO antibodies is the cornerstone of all current ABO-incompatible transplantation programs. It involves plasmapheresis (PF) with plasma exchange or followed by specific immuno adsorption. Plasmapheresis by centrifugation is associated with an increased bleeding tendency because of loss of platelets. In our protocol, plasmapheresis is performed with a plasmafilter followed by immunoabsorption of anti-ABO antibodies using the Glycorex device. The bleeding complications of this technique are not known. Material and Methods: We analyzed recipients of an ABO-i kidney transplant between March 2006 and May 2012 (n=51). Controls were recipients of a ABO-compatible kidney transplant matched for age of donor and recipient and PRA in this period (n=102). Cases differed from controls in the preoperative regimen which included immuno adsorption, rituximab, tacrolimus, mycophenolate mofetil, prednisone and immunoglobulines. All patients received tacrolimus, mycophenolate mofetil and prednisone after transplantation. Data on the number of thrombocytes before and directly after immuno adsorption, red blood cell transfusions (EC) during two postoperative weeks were collected. Results: In the ABOi group on average 4 PF were performed before transplantation. In the first 30 patients PF was also performed postoperatively. After the last preoperative PF the number of platelets had an average decrease of 32%. In 23% of patients the platelets dropped below 100 x109/L, specifically in patients that had low numbers of platelets before PFs started (191 vs 238 x10e9/L). ABO-i recipients received 2.2 (EC) versus 0.7 in controls. Major bleeding (hemodynamic instability or >2 EC per episode) was observed in 25% of patients. The majority of these major bleedings occurred the day of surgery (8/13 patients). The number of immuno adsorptions was positively correlated ($r=0.35$, $p=0.013$) with the number of EC. In patients with a platelet count $<100 \times 10^9$/L after the last PF the average number of PF was 7.17 versus 5.26 when platelets remained $>100 \times 10^9$/L ($p<0.05$).
Discussion: ABO-i kidney transplants recipients are at increased risk of bleeding, especially when more immuno adsorption procedures are performed. We hypothesize that the thrombocytopenia seen in our patients is caused by adherence of platelets to the plasma membrane in combination with the lack of recovery by the intensity of the immuno adsorption scheme.

Gastroparesis after Lung transplantation: Prevalence, reversibilty and relation with outcome

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Introduction: Gastro paresis, a known complication after Lung transplantation (Ltx), is related to gastro-esophageal reflux disease (GERD) and might as such be responsible for Bronchiolitis Obliterans Syndrome (BOS). We studied the prevalence, risk factors and reversibility of gastro paresis in our Ltx patients and the relation with long term outcome. Patients and methods: 108 patients transplanted from 2006 to 2010, who underwent at least one gastric emptying scintigraphy were included. Routine scintigraphy was done 1 month and 6 months after Lung transplantation. Results: 54 of 108 patients had delayed gastric emptying of > 90 minutes 1 month after Ltx. Median gastric emptying time was 204 minutes (93-807) versus 64 minutes (17-90). Patients with delayed emptying were younger 45.4 yrs (20-63yrs) vs. 52.69 (18-67 yrs), had more often CF (18,5 % vs. 5,6%) or pulmonary fibrosis (22,2% vs. 14.8%). No relation was found between gatroparesis and operation type, duration of oparation, use of cardiopulmonary bypass, sex or diabetes. At 6 months 27 of the 54 patients still had delayed gastric emptying. They were compared to 40 patients with normal gastric emptying results in both tests. No relation with mortality (14,8% vs. 18,4%) or BOS (25.9% vs. 23,7%) was found.
Conclusion: Half of the patients after Lung transplantation have gastro paresis, and in half of them this spontaneously resolves. Age, CF and pulmonary fibrosis are risk factors for gastro pareses. No relation with BOS was found.

Downregulation of complement activity via the mannan-binding lectin (MBL) pathway by dietary restriction and fasting

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Background: Seventy-two hours of preoperative fasting (F) or 2 weeks of 30% dietary restriction (DR) offers robust protection against renal ischemia-reperfusion injury (IRI) in mice. However, the mechanism remains to be elucidated. We hypothesize that immunomodulation plays a pivotal role. Innate immunity, especially the complement system, is crucial in the pathophysiology of IRI. Therefore, we investigated the impact of F and DR on complement activation pathways. Materials and Methods: Male C57Bl/6 mice were fed *ad libitum* (AL) or underwent 72 hours F or 30% DR for 2 weeks (n=8/group), after which blood was drawn and serum was aliquoted and stored at -80°C. Functional activity of the complement activation pathways (classical (CP), lectin (LP) and alternative pathway (AP)) was assessed by ELISA using immobilized ligands. Deposition of C3 and C9 as a measure of complement activity along with concentration of upstream complement initiating proteins (Mannan-binding lectin (MBL)-A and –C, and C1q) was determined. Messenger RNA studies were also performed on both kidney and liver tissues of the experimental mice for all the important upstream and downstream complement proteins. Results: A significant downregulation in CP and LP activity by DR and in CP, LP and AP activity by F was observed compared to the AL group. The activation of both C3 and C9 in the DR and F group was signifycantly downregulated ($p \leq 0.002$) in CP, LP and AP (except for C3 activation in the AP of the dietary restriction group). Both MBL-A and –C concentrations were significantly lower ($p \leq 0.001$) after DR and F compared to that of AL group. C1q concentration was only significantly lower in the fasted group ($p \leq 0.0001$). The mRNA studies showed that there was a significant downregulation in the liver MBL expression in both DR and F ($p \leq 0.004$) groups. However, C3 mRNA levels (both in kidney and liver) were found to be elevated in both DR ($p \leq 0.03$) and F ($p \leq 0.05$) groups.
Conclusion: Dietary interventions downregulate complement activation pathways. The most prominent effect of DR and F was observed on the MBL pathway. To our knowledge, our data for the first time show that DR and F cause downregulation of complement activation pathways. We conclude that complement downregulation via the MBL pathway may be one of the mechanisms by which dietary interventions protect against renal IRI.

Targeting PKC in human T cells using Sotrastaurin stabilizes regulatory T cells and prevents IL-17 production

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Sotrastaurin (AEB071) is a small-molecular weight immunosuppressant that selectively targets PKC including the PKCα,β and theta isoforms. In experimental heart and kidney allo-transplantation animal models Sotrastaurin markedly prolonged graft survival times. Although a phase II clinical trial using Sotrastaurin plus MPA in renal transplant recipients was associated with increased rejection from week 4 onwards, this calcineurin inhibitor (CNI)-free regimen showed an acceptable safety profile and improved renal function. These results support further study of Sotrastaurin as a component of multi-drug immunosuppressive regimens. Here, we show that the pan-PKC inhibitor Sotrastaurin differentially affected human Treg and Tconv (conventional T-cells). Sotrastaurin dose-dependently prevented TCR/CD28-induced Tconv activation characterized by pro-inflammatory cytokine production and expression of activation markers. Also, Sotrastaurin neither favored the generation of induced Treg nor the induction of T-cell anergy.
In contrast, in $CD4^{pos}$ $CD25^{high}CD127^{low}Foxp3^{pos}$ Treg Sotrastaurin preserved a stable Treg phenotype as evidenced by maintenance of suppressive capacity, high Foxp3 and CD25 expression, and lack of IL-17A and IFNγ production, even when stimulated with the Th17-enhancing cytokine IL-1β. Thus, next to inhibiting Tconv, pharmacological inhibition of PKC by Sotrastaurin preserves Treg stability and may serve as a powerful tool to improve Treg-based immunotherapy.

Bone Marrow and Adipose Tissue Derived Mesenchymal Stem Cells Induce HLA-specific Lysis by CD8+ T Cells

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Introduction Mesenchymal Stem Cells (MSC) are of interest as cell therapy for clinical organ transplantation and are commonly isolated from bone marrow or adipose tissue of either autologous or allogeneic origin. Allogeneic cell use seems convenient but might harbor the risk of sensitization against foreign HLA. Therefore, we evaluated whether bone marrow and or adipose tissue derived MSC are capable of induction of HLA specific lysis by CD8+ T cells. Methods MSC were isolated from bone marrow (BM-MSC) or adipose tissue (A-MSC) of healthy individuals. HLA class I mismatched BM-MSC and A-MSC were used in parallel experiments (n=5) as stimulators and as targets for CD8+ T cells. MSC were either stimulated with 50ng/ml IFNγ or unstimulated before co-culture with IL-2 activated HLA class-I mismatched PBMC. Effector CD3+CD8+ T cells were obtained from these co-cultures via FACS sorting. Unstimulated and IFNγ-stimulated target BM-MSC and A-MSC were labeled with Europium and incubated for 4 hours with effector cells. Lysis of MSC was determined by spectrophotometric measurement of Europium release. Results Co-culture of PBMC and BM-MSC resulted in the induction of CD8+ T cells capable of lysing this BM-MSC and not of the mismatched A-MSC, demonstrating that lysis occured in a HLA class I specific manner. The CD8+ mediated lysis was increased when stimulator MSC and/or target MSC were IFNγ-stimulated, which augmented HLA class I expression, and ranged from a mean of 10.9% (effector: target (E:T) ratio of 5:1) till 75.1% (E:T ratio 40:1) if both stimulator and target were IFNγ-stimulated. Using A-MSC in the co-culture resulted in CD8+ mediated lysis of A-MSC in an HLA class I specific manner. Yet, this effect was less prominent than for BM-MSC with a maximal lysis of 30.5% (mean, E:T ratio 40:1) using IFNγ-stimulated stimulator and target MSC.
Conclusion Both BM-MSC and A-MSC can induce HLA class I specific lysis by CD8+ T cells. These results indicate a potential risk of allogeneic MSC use of sensitization against HLA and plea in favor of autologous cell usage for clinical application.

A comparison of inflammatory, cytoprotective and injury gene expression in heart beating and non heart beating donor kidney

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Background: The superior long-term survival of kidneys from living donors (LD) compared to brain death (BD) or non heart beating (NHB) donors is now well established. The pathophysiology that leads to organ damage following BD is relatively well established. In contrast, experimental studies on the changes that occur in organs are sparse. The aim of this study therefore was to compare expression levels of genes indicative of inflammation, cytoprotection and injury at the time of explantation, after clinically relevant cold ischemia times and over a time-course during cold storage. Material and methods: Male Brown Norway rats were randomly assigned to a LD, BD or NHB group (n=7/group). LD rats, control group, were mechanically ventilated after 1 hour nephrectomy was performed. In the BD a frontolateral trepanation was made and a balloon catheter was introduced and inflated which led to BD. After 6 hours both kidneys were removed. In the NHB group cardiac arrest was induced by isoflurane overdose. The NHB period lasted 20 minutes before starting with retrieval of NHB donor kidneys. After explantation kidneys of all groups were flushed and stored (4°C) in UW solution. At 0, 2, 4, 6, 12, 18 and 24 hours they were collected and snap frozen in liquid nitrogen. Results: At explantation, mRNA levels of the inflammatory genes IL-1β, IL-6, TNF-α, MCP-1, TLR4, P-selectin and E-selectin were massively upregulated in kidneys from BD compared to LD. IL-1β, IL-6, P-selectin and E-selectin were, to a lesser extend, increased in NHB donors. HMGB1 showed no significant difference. This inflammation resulted in significant injury of the kidney, as shown by the 319-fold increase of the kidney injury marker Kim-1 in BD as compared to only 5.3-fold in NHB. The cytoprotective gene HO-1 and cell cycle control gene p21were strongly increased in BD. During an 18 hour cold storage period HO-1, HIF-1α, Bcl-2, Bax, VEGF, TLR4 and HMGB1 were analysed. At the end of the cold storage period gene expression levels reflected those at the time of explantation.
Conclusion: Following BD a massive up-regulation of inflammatory, injury and cytoprotective genes in kidneys is present at the time of graft explantation. These expression levels do not change during cold preservation. In contrast, kidneys from NHB donors show only mild inflammation and injury. These results may explain why delayed graft function in NHB kidneys has not the deleterious effect on graft survival as it has in BD donors.

Evidence for a possible role of memory B cells in acute kidney graft rejection

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In organ transplantation, the composition of the peripheral B cell compartment is increasingly identified as an important determinant for graft outcome. Whereas naïve and transitional B cells have been associated with long-term allograft survival and operational tolerance, memory B cells have been linked to decreased allograft survival. We studied the peripheral B cell phenotype of kidney transplant recipients before transplantation, at discharge and at time of rejection using flow cytometry. Furthermore, isolated $CD19^+$ B cells were polyclonally activated and analysed for their immunoglobulin production capacity by ELISPOT assays. The study was performed in 21 patients who experienced an acute rejection episode within the first 6 months after transplantation and 22 patients without acute rejection in our study. Flow cytometric analysis of memory and naïve B cell subsets, classified according to IgD and CD27 staining, revealed a significant decrease in peripheral memory B cells at time of acute rejection ($p<0.0001$). In contrast, in patients without acute rejection, the B cell phenotype remained stable in time. The results of these phenotypic analyses were confirmed by functional data. When peripheral B cells were analysed for their capacity to produce immunoglobulins, we observed an increase in the ratio of IgM/IgG producing B cells upon acute rejection ($p=0.0003$). Coinciding with this phenotypic and functional shift towards naïve B cells at time of acute rejection we observed a strong decrease in peripheral B cells bearing the homing receptor CXCR5 ($p=0.0004$), which was not observed in patients without rejection. This suggests that the change in B cell composition may be due to homing of memory B cells towards lymphoid organs or the graft. The latter is supported by our findings in a separate patient cohort ($n=89$) showing that during acute rejection mRNA expression of CD20 and the chemoattractant CXCL13 (ligand for CXCR5) in kidney biopsies is significantly elevated ($p=0.047$ and $p=0.004$, respectively).
In conclusion, our data suggest that memory B cells may be involved in acute cellular kidney allograft rejection. These observations warrant more research into the exact phenotype of the graft infiltrating B cells during acute rejection and their possible interaction with T cells.

Human alloantigen induced regulatory T cells suppress alloreactivity in an antigen specific manner independent of CD127 expression

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In addition to thymically derived natural regulatory T cells (nTreg), Treg can be induced in the periphery (iTreg) by antigen exposure and cytokines. It is currently unknown whether iTreg are induced during alloreactivity and whether these iTreg specifically suppress alloreactive T cells. Therefore, we studied the generation of iTregs and examined their immunosuppressive capacity and specificity in an alloreactive setting. Sorted CD4+CD25-FOXP3- T cells from healthy blood bank donors (n=7) were stimulated with alloantigen and the induced CD4+CD25+FOXP3+ Treg were subdivided into CD127- iTreg and CD127+ iTreg by FACS sorting. Their suppressive capacity and specificity was examined in a secondary MLR. To validate the induced character of the iTreg, the methylation status of the TSDR (Treg Specific Demethylated Region) in the *FOXP3* gene was measured. After stimulation of CD4+CD25-FOXP3- T cells with alloantigen for 1 week, 78 ± 9% (mean ± SD) of the CD4+ cells became CD25+FOXP3+ (iTreg). Both CD127+ iTreg and CD127- iTreg robustly inhibited the proliferation of CD25- T cells in the secondary MLR (CD127+ iTreg: 93 ± 5%; CD127- iTreg: 95 ± 3%; ratio iTreg : CD25- T cells = 1:5) by restimulation with the same alloantigen. By stimulation with a third party alloantigen (fully mismatched at HLA-DR) in the secondary MLR, the proliferation of CD25- T cells was significantly less inhibited (iCD127+ iTreg: 68 ± 17%, p=0.004; iCD127- iTreg: 68 ± 13%, p=0.0003). By stimulation with a comparable alloantigen (1 HLA-DR mismatch) in the secondary MLR, the proliferation of CD25- T cells was inhibited with 96 ± 1% by CD127+ iTreg and 93 ± 2% by CD127- iTreg, which was comparable to the inhibition found by restimulation with the same alloantigen (p=0.4 and p=0.2). Non-activated CD3+CD4+CD25- T cells were unable to inhibit the proliferation of CD25- T cells. Both iTreg subsets were fully methylated at the TSDR in the *FOXP3* gene, confirming their induced character. Alloantigen specific iTreg are induced during alloreactivity. Even though the mechanism by which suppression is mediated needs further investigation, these data indicate that iTreg could play an important role in controlling alloreactive T cells after transplantation.

CXCR5+CD4+ follicular helper T cells accumulate in resting human lymph nodes and have superior B cell helper activity

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Although many relevant immune reactions are initiated in the lymph nodes (LN), this compartment has not been systematically studied in humans.
Analyses have been performed on immune cells derived from tonsils, but as this tissue is (chronically) inflamed, generalization of these data is difficult. Here, we analyzed the phenotype and function of human $CD4^+$ T cell subsets and lineages in 21 paired resting lymph node and peripheral blood (PB) samples. Naive, central memory and effector memory cells as well as Th1, Th2, Th17 and Tregs were equally represented in both compartments. On the other hand, cytotoxic $CD4^+$ T cells were strikingly absent in LN (LN 0.42%; PB 4.4%; $p<0.01$). The percentage of CXCR5 expressing $CD4^+$ T cells, representing putative follicular T helper (Tfh) cells, was significantly higher in LN than in PB (LN 27%; PB 12%; $p<0.005$). LN $CXCR5^+$ $CD4^+$ T cells also expressed higher levels of Tfh markers than their PB counterparts. Last but not least LN derived $CXCR5^+CD4^+$ T cells were superior in providing help to B cells, as assessed by the induction of IgG and IgM production, when co-cultured with LN B cells (IgG: LN 87% of max; PB 14% of max; IgM: LN 76% of max; PB 16% of max) or PB B cells (IgG: LN 15% of max; PB 2% of max; IgM: LN 38% of max; PB 10% of max).
Thus, functionally competent Tfh accumulate in resting human lymph nodes providing a swift induction of naive and memory antibody responses upon antigenic challenge.

Rabbit antithymocyte globulin induction therapy induces donor-specific HeliosnegFOXP3pos regulatory T cells in kidney transplant patients

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Introduction: Repopulation of regulatory T cells (Treg) after rabbit antithymocyte globulin (rATG) induction therapy in kidney transplant patients occurs via either thymopoiesis or homeostatic proliferation. In addition, rATG converts effector T cells into induced Tregs in vitro. In this study, we investigated the origin and the compensatory mechanisms by which the immune system repopulates Tregs as well as their controlling function after T cell depletion therapy. Methods: Patients were treated with rATG (3x2mg/kg/day, n=9) or non-depleting anti-CD25 antibody (Basiliximab: day 0, 4; 20mg, n=10) induction therapy in combination with tacrolimus, MMF and steroids. Flow cytometric analysis was performed to analyze Tregs (CD4+CD25+CD127-FOXP3+) for the expression of Ki67 (marker for cell division), CD31 (marker for recent thymic emigrants), and Helios (marker for natural Tregs). Functional studies were performed with FACSsorted CD4+CD25+CD127- Treg in co-culture with effector T cells activated by irradiated donor or 3rd party cells. Results: In the first six months after rATG induction therapy, the percentage of Tregs gradually increased ($p=0.003$ vs. baseline). At one month after rATG induction a higher proportion of Ki67 expressing Tregs was found than before transplantation ($p<0.01$) and compared to basiliximab treated patients ($p<0.0001$). The majority of proliferating cells expressed the memory marker CD45RO. No differences were observed in the percentage of thymic CD31+CD45RO-Treg between time points and treatment arms. The proportion of Helios+ Tregs was lower in the rATG group than in the basiliximab group (mean±sd, 62±19% vs. 81±14%, $p=0.03$) at one month. Particularly, fewer Ki67+CD45RO+ Treg expressed Helios (57±27% vs. 77±9%, $p<0.05$). At the functional level a clear difference was found between the rATG and basiliximab arm. After rATG therapy Tregs inhibited proliferation of donor antigen activated T cells while the proliferation of 3rd party activated T cells was not inhibited ($p<0.05$). The inhibition of proliferation by Tregs in the basiliximab group was comparable for both donor and 3rd party.
Conclusion: rATG therapy allows the induction of functional donor specific regulatory T cells via the conversion of effector T cells which is followed by homeostatic proliferation of both induced and natural Tregs.

Differences in mobilization of rare hematopoietic stem cells from human liver grafts of non-heart beating and heart beating donors

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Background: Profound physiological changes following organ donation results in liver injury. However, these same physiological changes may induce trafficking of hematopoietic stem/progenitor cells (HSPCs) as a restitutive response to limit tissue injury. The aim of this study was to determine if there are differences in the mobilization of HSPCs from liver grafts procured from heart beating (HB) versus non-heart beating (NHB) donors at time of transplantation.
Methods: During liver transplantation, graft preservation solutions (perfusates) of nine HB and seven NHB donor livers were collected. Liver perfusate mononuclear cells (MNCs), isolated using ficoll, were stained with a cocktail of mAbs specific for human lineage markers (Lin)-FITC, CD34-PE, CD90-APC, CD38-APC-Cy7 and CD45RA-PB to examine HSPC populations by poly-chromatic flow cytometry. Additionally, *in vitro* colony-forming unit assays were performed to assess the lineage-restriction of the HSPCs. Results: Polychromatic flow cytometric analysis revealed a difference in the mobilization of liver-perfusate derived live Lin-CD34+CD38- stem/progenitor cells from HB (0.36% $\pm$ 0.48, mean$\pm$SD) versus NHB donors (0.035% $\pm$ 0.038). Within the total $CD34^+$ subset, we found 0.14% $\pm$ 0.06 $CD34^+CD11b^-$ and 0.09% $\pm$ 0.07 $CD34^+HLA\text{-}DR^-$ single positive cells, generally considered as undifferentiated HSCs. The colony forming assays revealed that 325 $\pm$ 115 erythroid colonies (CFU-e), 103 $\pm$ 67 erythroid burst-forming units (BFU-e) and 36 $\pm$ 28 granulocyte progenitors units (CFU-c) were present per million mononuclear cells. This frequency of colony forming cells is almost 10-fold higher than those found in peripheral blood and 10-fold lower than in bone marrow. After liver transplantation, donor HSC continue to migrate from the graft in the first months as was shown by the approx. 2-8% of circulating $CD34^+$ cells being of donor origin.
Discussion: We found an enhanced mobilization of HSPCs from livers procured from HB donors compared to NHB donors. Moreover, liver-derived cells with a distinct hematopoietic phenotype give rise to substantial numbers of colony-forming units *in vitro*, confirming that HSPCs are present in adult human liver and mobilize during liver perfusion. Mobilized liver HSPCs may contribute to hematopoietic chimerism and allohyporesponsiveness after liver transplantation.

LAPTOP PRESENTATIES

Epitope hunting in HLA-DQ: modeling, predicting and chasing

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Because of the limited number of HLA-DQ alleles in the human population, antibody responses against HLA-DQ have a considerable impact on the transplantation chance of retransplant candidates. However, at present there is limited insight in which epitopes of the HLA-DQ molecules are recognized by antibodies and few studies addressed the question which epitopes are immunodominant. Factors complicating this epitope hunt are that both subunits of HLA-DQ show diversity and antibodies can recognize a single subunit or an epitope formed by a combination of both subunits. In the current study we aim to identify the immunodominant epitopes of the HLA-DQ molecule. Therefore, we selected patients showing DQ antibodies in the single antigen bead assays and made use of the bead reactivities of the single antigen test to identify the epitope. To this end we generated a simplified table of the polymorphic residues (in both DQA and DQB simultaneously) that are present on the individual beads and deducted epitopes by filling in the single antigen reaction profile. Out of 32 patients there were 16 in which the bead reactivity pattern could be coupled to a single epitope, 9 in which we cannot discriminate between multiple epitopes and 7 in which we could not identify the epitope. In all 16 patients, we identified the epitope in the DQB subunit and we did not identify a serum in which the entire bead reactivity is explained by reactivity against DQA. Notably, we only observed 3 epitopes in these 16 patients. The epitope generated by amino acid 52-57 was dominant as in 12 out of 16 sera the bead reactivity could be coupled to this epitope. Of the remaining 4 patients, 3 displayed the epitope formed by amino acid 84-90 and 1 patient had an antibody against AA 37-38. Our data clearly indicate that epitopes in DQB are more immunogenic as compared to DQA epitopes. The epitope formed by amino acid 52-57 in the DQB molecule is immunodominant, but the distinct reaction patterns in our patient sera suggests that this region habours multiple epitopes. At present we are performing in sillico modeling to explain the immunodominance of specific epitopes and we are studying how antibodies against these specific epitopes impact the retransplantation chance.

Rare HLA alleles within the CWD groups redefined by a new SBT typing strategy

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The common and well defined (CWD) HLA alleles define alleles that should be considered in resolving ambiguous typing results to prevent laborious and expensive testing. Limited full length sequence data hamper the assignment of infrequent alleles and their allele frequencies. Since alleles with silent nucleotide differences or differences outside the peptide binding groove have not been typed extensively, many of them have been grouped in the CWD group 3 (alleles under consideration) or 4 (excluded alleles). We developed a new sequence based typing strategy that enables easy resolution of ambiguities and elucidation of all nucleotide differences, including silent polymorphism located in exons and intervening introns and polymorphism located outside the peptide binding groove. The new SBT approach uses group specific amplification of exons 1 through 8 for class I and exons 2 through 4 for class II. In our database 92 class I and 61 class II alleles, belonging to cwd groups 3 or 4, were identified in 158 and 203 individuals respectively. Among those were 20 silent class I alleles, 13 silent class II alleles and 8 class I and 6 class II alleles with differences beyond the peptide binding groove. Nine class I silent alleles were observed in > 1 individual and sequencing with the new typing strategy revealed that in 5 cases the allele assigned to the CWD group3/4 was more frequent than the allele in the CWD group1. Seven class II silent alleles were present in > 1 individual and additional sequencing showed that in 1 case the allele assigned to the CWD group3/4 was more prevalent than the allele in the CWD group1.
In conclusion, the use of our new extended SBT typing strategy redefined the rarity of HLA alleles in CWD groups.

Predicted Indirectly Recognizable HLA epitopes presented by HLA-DRB1 correlate with the de novo development of donor-specific HLA IgG antibodies after kidney transplantation

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Background. HLA class-I mismatches selectively induce antibody formation after kidney transplantation. The de novo development of donor-specific IgG HLA class-I antibodies may be dependent on the HLA class-II background of the patient by presenting T-helper epitopes within the recognized HLA class-I antigens. Methods. The correlation between antibody production against mismatched donor human leukocyte antigens (HLA) class I and the number of HLA class II-restricted predicted indirectly recognizable HLA epitopes (PIRCHE-IIs) in the respective HLA class-I mismatches was investigated. To this end, we analyzed sera taken after nefrectomy from a cohort of 21 non-immunized individuals that received and rejected a renal transplant Results. Fourty-nine HLA class-I mismatches were found which all contained immunogenic eplets according to HLA-Matchmaker. Donor specific HLA antibody responses were detected against 38 HLA class-I mismatches after nefrectomy. These mismatches were found to contain a larger number of PIRCHE-IIs when compared to mismatches which did not induce donor specific HLA antibodies. Most PIRCHE-IIs (more than 60%) were not part of an eplet as defined by HLA-Matchmaker.
Conclusions. Our data suggest that presentation of donor-derived HLA class-I peptides by recipient HLA class-II molecules plays a significant role in de novo development of donor-specific IgG HLA antibodies.

A validated rapid LC-MS/MS method for the determination of free fraction of tacrolimus

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Therapeutic drug monitoring of tacrolimus (TAC) is commonly based on whole blood concentrations. Within the therapeutic range TAC proves to be effective and prevents organ rejection, but adverse effects, especially neuro- and nephrotoxicity, are frequently observed in transplant recipients. In previous studies of Zahir et al. the fraction unbound (Fu) of TAC in patients experiencing TAC-related side effects was found to be significantly higher while whole blood concentrations of TAC were not different. The Fu of TAC is thought to correlate with nephrotoxicity and, seems more predictable for monitoring of toxicity than whole blood concentration. To date, few attempts were made to determine the Fu of TAC. Methods applied were based on the in vitro incubation of transplant recipient blood with ^{3}H-dihydro-TAC and measurement of the labeled compound estimating the actual Fu. To our knowledge, no method that measures the Fu of TAC has been published to date. Therefore, we developed an assay for direct determination of Fu of TAC in plasma. 4 mL plasma was used for equilibrium dialysis with phosphate-buffered saline (pH 7.4) at 22 °C to isolate the Fu. Following dialysis, a liquid-liquid extraction in acidic medium (4M HCL) with 5 mL dichloromethane was performed with ascomycin as internal standard. After evaporation of the organic layer, the residue was reconstituted in 150 µl methanol/water (50/50, vol/vol). Samples were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Linearity was shown in a range of 0.005-0.2 ng/ml (r^2 > 0.98). Quality samples of low, mid and high levels showed coefficients of variation < 15%. As a result, the relationship between Fu of TAC and nephro- and neurotoxicity can now extensively be studied in various organ transplant recipient populations.

MnTMPyp, a selective superoxide dismutase mimetic, reduces oxidative stress in brain dead donors

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Renal allografts retrieved from brain dead donors show inferior transplant outcomes compared to living donor grafts. Brain death (BD) leads to immunological activation and hemodynamic instability. Reactive oxygen species (ROS) are implicated in BD-induced organ damage. This study investigated whether pre-treatment with the free radical scavenger MnTMPyP reduces inflammation and renal injury in brain-dead organ donors. BD was induced in male F344 rats (275-300g, n=7) by inflating a subdurally placed balloon catheter. Rats were treated with saline or MnTMPyP (5mg/kg) one hour before BD. After 4 hours of BD, serum and kidneys were collected. Sham-operated rats treated with saline or MnTMPyP served as controls. Malondialdehyde (MDA) levels, indicative for oxidative stress, were measured with the thiobarbituric acid (TBA) assay. Tissue gene expression was measured by Real Time qPCR. Tissue protein expression was detected by immunohistochemical analyses. Pre-treatment of brain-dead donor organs with MnTMPyP significantly reduced serum MDA levels and renal expression of the stress protein HO-1. Interestingly, renal mRNA levels of HO-1 were increased. Furthermore, renal mRNA levels of TNF- α and E-selectin were decreased but not significantly.
This study shows that treatment of brain-dead donors with MnTMPyP is effective in reducing systemic oxidative stress.

Hypothermic machine perfusion of the pancreas for islet transplantation

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Introduction: Because of the shortage of donor pancreas for pancreatic islet transplantation we aim to enlarge the donor pool by using extended criteria donors. Organs from these donors are associated with high susceptibility to hypoxic damage. Optimization of organ preservation methods may ameliorate hypoxic damage. The traditional cold storage (CS) method of pancreas preservation lead to hypoxia of the pancreatic tissue. It is hypothesized that oxygenated hypothermic machine perfusion (HMP) of the pancreas results in less hypoxia, less hypoxic damage resulting in a better quality of the isolated pancreatic islets. Methods: In a pilot study 5 extended criteria human donor pancreas were procured and transported using HTK/UW CS (8.3 +/- 3.10 hrs CIT). The pancreases were splitted transversally at the neck, resulting in a head and tail section. From each organ, one section was preserved on ice (CS). The other section was subjected to 3 hours arterio-venous HMP (UW-MP, 100% pO2, 20mmHg). After prolonged CS and HMP islets were isolated separately. Pair wise, HMP (2 head, 3 tail) sections were compared with the CS (3 head, 2 tail) sections on organ physiology (edema, oxygenation, amylase, lipase, LDH), isolation parameters (tissue retrieval, gene expression, ATP content, islet yield and islet purity) and islet quality (gene expression, islet survival, induction ratio) parameters. Results: No significant changes were observed in islet yield, islet survival or islet function. In the HMP perfusate increasing concentrations of amylase, lipase and LDH were detected, in line with the expected wash-out. Interstitial pO2 increased strongly from non-detectable levels to 80-90% pO2. In general, mRNA expression was elevated in HMP tissue as compared to CS. The change in tissue ATP content after three hours HMP increased significantly. Conclusion: Three hours of oxygenated machine perfusion of the pancreas does not increase islet yield, function or viability. However, it does lead to a better oxygenation status of the tissue, as implied by the increased pO2, mRNA levels and ATP content in the pancreatic tissue. We hypothesize that increasing duration of HMP will translate augmented organ parameters to better islet preparations.

Preoperative fasting protects aged obese male and female mice against renal ischemia-reperfusion injury

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Introduction: Renal ischemia-reperfusion injury (IRI) is inevitable during kidney transplantation and leads to oxidative stress, tissue injury and inflammation. IRI is a risk factor for delayed graft function, acute rejection and long-term transplant loss. We have previously reported that preoperative fasting in young-lean C57BL/6 male mice offers robust protection against renal IRI. Since patients are heterogeneous, generally of older age and suffer from (co)morbidities, we investigated the effects of preoperative fasting on renal IRI in mice with a different age, gender, bodyweight and genetic background. Methods: Male and female F1-FVB/C57BL6-hybrid mice with an average age of 73 weeks and a weight of 47.4 respectively 47.1 grams were randomized to either preoperative ad libitum feeding or fasting for 72 hours, followed by renal IRI. Based on previous results, renal IRI in males was induced by clamping both renal pedicles for 37 minutes. In females, known to be more resistant to ischemic renal damage, 60 minutes of ischemia was applied. Wellbeing, bodyweight, kidney function and survival of the animals were monitored until day 28 postoperatively. Histopathological analysis of the kidneys was done in a blinded manner by a pathologist. Results: Preoperative fasting significantly improved survival after renal IRI in both sexes compared with ad libitum fed controls. All ad libitum fed males (n=8) died or were sacrificed as a result of morbidity indicative of irreversible kidney failure, whereas 7 of 8 of the fasted males survived in good health (p=0.0171). Nine of 11 ad libitum fed females died or had to be sacrificed, whereas 7 of 10 fasted mice survived (p=0.0040). Fasted mice of both sexes had a better kidney function as shown by lower serum urea levels after IRI. Histopathological analysis showed significantly less acute tubular necrosis and increased regeneration in kidneys from fasted mice. In the surviving mice, bodyweight gradually decreased in the first two postoperative weeks but slowly increased in the weeks thereafter.
Conclusions: Similar to young healthy male mice, preoperative fasting protects against renal IRI in both male and female aged obese mice. These findings suggest a general protective response of dietary restriction against renal IRI regardless of age, sex, bodyweight and genetic background. Therefore, dietary restriction could be a non-invasive intervention inducing increased oxidative stress resistance in older and obese patients as well.

Oxidative damage in clinical ischemia/reperfusion injury: a reappraisal

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Aims: Ischemia/reperfusion (I/R) injury is a common clinical problem. Although the pathophysiological mechanisms underlying I/R injury are unclear, oxidative damage is considered a key factor in the initiation of I/R injury. Findings from preclinical studies consistently show that quenching reactive oxygen and nitrogen species (RONS), thus limiting oxidative damage alleviates I/R injury. Results from clinical intervention studies on the other hand are largely inconclusive. In this clinical study, we systematically evaluated release of established biomarkers of oxidative and nitrosative damage during planned I/R of kidney and heart in a wide range of clinical conditions. Results: Sequential arteriovenous concentration differences allowed specific measurements over the reperfused organ in time. None of the biomarkers of oxidative and nitrosative damage (i.e. malondialdehyde, 15(S)-8-iso-prostaglandin F2α, nitrite, nitrate and nitrotyrosine) were released upon reperfusion. Cumulative urinary measurements confirmed plasma findings. As of these negative findings we tested for oxidative stress during I/R and found activation of Nrf2, the master regulator of oxidative stress signaling, but no upregulation of the antioxidant response genes (i.e. Nfr2, HMOX1, NQO1 and GSTA2 expression) was observed upon reperfusion. Innovation: This comprehensive, clinical study evaluates the role of RONS in I/R injury in two different human organs (kidney and heart). Results show oxidative stress but do not provide evidence for oxidative damage during early reperfusion, thereby challenging the prevailing paradigm on RONS-mediated I/R injury.
Conclusion: Findings from this study suggest that the contribution of oxidative damage in human I/R may be less than commonly thought and propose a re-evaluation of the mechanism of I/R.

Angiopoietin-1 administration in a brain death rat model

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Kidneys derived from deceased brain dead (DBD) donors have inferior outcomes after transplantation compared to kidneys from living donors. Also, DBD donors suffer from bacterial translocation and endotoxemia. A link between Angiopoietin 1 (Ang1), Angiopoietin 2 (Ang2) and endotoxemia has been established. Ang1 and Ang2 are antagonictic ligands that bind to the Tie2 receptor. We aimed to modify the Ang1 levels in our brain death rat model to clarify whether exogenous administration of Ang1 has a protective role and may be of therapeutical value to improve outcome after DBD transplantation. We administrated 0,1µg/kg Ang1 or 0,9% saline 30 min before brain death (BD) induction. BD was induced by inflating a subdurally placed balloon catheter in rats. Two groups of sham operated animals were injected with either Ang1 or 0,9% saline (n = 7 for all groups). The animals were monitored for 4 hrs. Just before sacrificing the animals blood was collected and kidneys were harvested for histology and PCR. Plasma levels of ALT, AST, creatinine, LDH and urea increased in the BD groups compared to sham operated groups ($p<0.05$). The 2fold induction of E-selectin, P-selectin, VCAM-1, ICAM-1, IL-6, KIM-1 and HO-1 in the kidney is elevated in the BD groups compared to the sham operated groups ($p<0.05$). The renal TNF-α fold induction of the BD+Ang1 group is increased compared to both sham groups ($p<0.05$). Tie2 fold induction in the kidney is not influenced by Ang1 administration and decreased significantly in the BD groups (mean fold induction 0.45 BD+saline and 0.38 BD+Ang1) compared to the sham groups (mean fold induction 2.5 sham+saline and 2.6 sham+Ang1).
Functional and inflammatory markers were increased in the BD groups and not affected by this dosage of Ang1. These results show a remarkable effect of brain death on Tie2 fold induction. This reduction suggests a functional role for Tie2 in BD which could not be compensated by administrating Ang1.

Five year graft survival and delayed graft function predicted by pre-existent renal damage

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Organs derived from deceased brain dead (DBD) donors show worse function and more acute rejection episodes than those from living donors (LD). Brain death induces a progressive inflammatory response in potential donor organs. Minor morphological renal damage is present in healthy potential donors. We hypothesize that pre-existent morphological renal damage is more prominent in kidneys from DBD donors compared to LD and might predict graft failure after kidney transplantation. We used renal biopsies of 125 living and 73 DBD donors. Associations with donor characteristics and recipient outcome were determined. The degree of FGS, interstitial fibrosis (IF), intima thickness and vascular hyalinosis were scored. Sections were scored for macrophages, neutrophilic granulocytes and pre-fibrosis (α-SMA). Vascular hyalinosis, FGS, macrophages and granulocytes are markedly increased in DBD compared to LD ($p<0.05$). Arterial intima thickness and IF are not significantly different between the donor types. The degree of FGS and IF are associated with donor age ($\rho = 0.265$, $\rho = 0.315$ resp; both $p<0.01$). IF correlates with diastolic blood pressure and effective renal plasma flow (ERPF) prior to donation ($\rho = 0.144$, $\rho = -0.197$ resp; both $p<0.05$). Macrophages and arterial intima thickness correlated univariate with delayed graft function (DGF) (Exp(B)=261.1 and Exp(B)=1.021 resp; both $p<0.05$). In multivariate analysis, 5 year graft survival was best predicted by neutrophilic granulocytes ($p<0.05$). Separately analyzed, 1 year graft survival in DBD donor is best predicted by IF ($p<0.05$) and in LD by the degree of arterial intima thickness. Five year graft survival in DBD donor is best predicted by IF and in LD by the degree of vascular hyalinosis.
Pre-existent renal damage is more prominent in kidneys of DBD donors than in kidneys from LD. Therefore, therapeutic interventions in the DBD donor could be a tool to reduce the inflammatory damage of the graft-to-be and thereby improve organ quality and graft survival.

Donor pre-treatment with the heat shock protein-inducer geranylgeranylacetone reduces brain death-associated inflammation in the kidney at organ retrieval

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Brain dead-derived kidney grafts have inferior transplantation outcomes compared to living donated kidneys. The protective heat shock proteins (HSP) are known to be up-regulated, but at the end of the brain death period. To reduce the brain death-related kidney injury, we want to increase the Hsp72 expression at the start of brain death. We investigated whether geranylgeranylacetone (GGA), an Hsp72 inducer, can reduce the pro-inflammatory changes and improve kidney donor quality in an *in vivo* brain death rat model. Male F344 rats (275-300g, n=15) underwent slow induction of brain death and were kept brain dead for 4 hours. We administered GGA (400 mg/kg orally) or a saline vehicle 20h and 1h prior to brain death induction. Sham-operated animals (n=14) received the same treatment. At the moment of organ retrieval, the expression of Hsp72 is not increased by GGA. However, kidney interleukin-6 mRNA levels in GGA pre-treated brain dead rats were lower compared to saline-treated controls. Systemic ASAT levels were also reduced by GGA, indicating decreased inflammation.
These results suggest that GGA reduces pro-inflammation during the brain death period, despite the unchanged expression of Hsp72. To further explore the effects of GGA on HSP expression, we want to assess expression of HSF-1 and other stress-induced HSP: Hsp90, Hsp32.

Opposite effects of prednisolone treatment on liver and kidney graft from brain dead rat donors

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Introduction: Contradictory evidence has been published about utility of steroids in organ retrieval from brain dead (BD) donors. Therefore, this study investigated the effect of steroid treatment in the livers and kidneys of brain death rats. Methods: BD was induced in rats by inflating a subdurally placed balloon catheter. Animals were treated with saline or prednisolone (22.5 mg/kg) one hour before BD. After 4 hours of BD, serum, kidneys and livers were collected. Sham-operated rats treated with saline or prednisolone served as controls. Tissue gene expression was measured by Real Time qPCR. Tissue protein expression was detected by immunohistochemical analyses. Results: After the BD period we found a significant reduction in plasma levels of IL-6 and creatinine, but not in AST, ALT and LDH after prednisolone treatment. Interestingly, polymorphonuclear influx was reduced in liver tissue but not in kidney samples from treated animals. Relative expression of inflammatory genes were significant down-regulated in liver and kidney (IL-6, TNF-a, IL-1b and MCP-1). Relative Complement (C3) expression was decreased in kidney but increased in liver after prednisolone treatment. The Bcl2/BAX ratio of the kidney and liver in BD animals was lower when compared to sham animals. Prednisolone treatment did not affect this ratio in BD animals. Curiously, a protective cellular gene like HSP-70 was down-regulated in the liver due to prednisolone treatment.
Discussion: This study shows that pre-treatment of brain dead donors with prednisolone significantly reduces inflammation. This treatment improves renal function but does not reduce liver injury. This could be explained by the persistence of complement activation and a decrease of protective cellular mechanisms in the liver due to steroid treatment or it could be attributed to timing or dosing.

Anti-inflammatory effects of prednisolone treatment did not improve organ quality in brain dead rats

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Introduction: Our previous results showed a protective effect in kidney function but not in liver injury with the pre-treatment of brain dead (BD) rat donors with a single high-dose of prednisolone. Therefore, this study investigated the effect on organ quality of the post-treatment BD rat donor using prednisolone. Methods: BD was induced in rats by inflating a subdurally placed balloon catheter. Animals were treated with saline or prednisolone (5, 12,5 or 22.5 mg/kg) one hour after BD induction. After 4 hours of BD serum, kidneys and livers were collected. Tissue gene expression was measured by Real Time qPCR. Tissue protein expression was detected by immunohistochemical analyses. Results: After BD period we found a significant reduction of IL-6 but not creatinine plasma levels. AST and LDH plasma levels were increased due to prednisolone treatment. Relative expression of inflammatory genes (IL-6, IL-1b and MCP-1) were significantly down-regulated in liver and kidney. Relative Complement (C3) expression wasn't decreased in kidney and liver after prednisolone treatment.
Discussion: These preliminary results confirm previously investigated anti-inflammatory effects of prednisolone in BD rats. However, this treatment did not improve kidney function and increased liver injury markers. Currently, more analyses are being performed to clarify these results.

The effect of the β-Human Chorionic Gonadotropin-Related Peptide (AQGV) on a Brain Death Induced Inflammation in Rats

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Introduction: AQGV previously described by Khan et al has been utilized as an anti-inflammatory molecule. This new molecule showed promising results in septic and shock inflammatory models. Brain death (BD) is an inflammatory condition, which is deleterious for organ quality in transplantation. Therefore, we aim to assess the effect of AQGV in a brain death rat model. Method: BD was induced in rats by inflating a subdurally placed balloon catheter. Animals were treated with PBS or AQGV (30 mg/kg) one hour before BD. After 4 hours of BD, serum, kidneys and livers were collected. Sham-operated rats treated with PBS or AQGV served as controls. Tissue gene expression was measured by Real Time qPCR. Tissue protein expression was detected by immunohistochemical analyses. Results: After the BD period, plasma levels of IL-6, creatinine, AST, ALT and LDH were not significantly reduced after AQGV treatment. Polymorphonuclear influx in liver and kidney tissue were not reduced. Relative expression of inflammatory genes (IL-6, TNF-a, MCP-1 and C3) were not significantly down-regulated in liver and kidney.
Discussion: Pre-treatment with AQGV in this model did not show any anti-inflammatory effects. However, this could be attributed to timing or dosing. We postulate that AQGV could exert anti-inflammatory effects when administered after brain death induction based on the chemical properties.

Duplicated ureters and renal transplantation: a single-center experience and review of the literature

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Introduction: Complications of the transplant ureter are the most important cause of surgical morbidity after renal transplantation. The presence of ureteral duplication in the renal graft might result in an increased complication rate when compared with single ureter renal grafts. We analyzed our data of double ureter renal transplantations and performed a review of the existing literature. Methods: From January 1995 to April 2012, 1588 kidney transplantations were performed at the Academic Medical Center Amsterdam. Patient charts and surgical reports were reviewed retrospectively. Results: Twelve patients received a donor kidney with a double ureter (0.8%). In seven patients both ureters were separately anastomosed to the bladder, using two submucosal tunnels. In four patients a common ostium was created at the distal end of the double ureter and subsequently one anastomosis was performed to the bladder. In one patient one of the two ureters was ligated. No postoperative urological complications occured among the patients receiving a kidney with a double ureter. In the group who received a kidney with a single ureter, the urological complication rate was 14% (P=0.17). Mean creatinine levels at one week, one month and three months after kidney transplantation were comparable between both groups. Discussion: A double-ureter donor kidney is not associated with an increased complication rate after renal transplantation and yields equal outcomes as compared to single-ureter donor kidneys.
We conclude that a duplicated ureter does not have to be a contraindication for renal transplantation.

Risk factors for wound-related complications after renal transplantation

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Background: Wound-related complications like surgical site infection (SSI) and lymphocele are accountable for significant morbidity after renal transplantation. The aim of our study was to investigate risk factors for wound-related complications after renal transplantation and the effect of wound-related complications on outcome of renal transplantation. Methods: This retrospective analysis includes 108 consecutive adult patients who underwent a kidney transplantation between January 2010 and December 2010 at our center. Wound-related complications were defined as an SSI or a symptomatic lymphocele. Patient and donor characteristics were investigated. Results: We observed wound-related complications in twelve patients (11%; eight lymphoceles and five SSI's). Independent risk factors for wound related complications that were identified by multivariate analysis were recipient age > 70 years (odds ratio (OR) 16.0; P = 0.015), BMI > 30 kg/m2 (OR 15,8; P = 0.007), hypertensive nephropathy as cause of end-stage renal disease (OR 15,0; P = 0.009), urinary tract infection (OR 7.3; P = 0.042) and prolonged post-operative wound drainage (OR 15.3; P = 0.028). Wound-related complications lead to a prolonged hospital stay (22.8 ± 14.2 days versus 12.2 ± 5.1 days; P = 0,009), but did not result in increased incidence of delayed graft function, acute cellular rejection, graft failure or patient mortality.
Conclusions: Obesity, high recipient age, hypertensive nephropathy, urinary tract infections and prolonged wound drainage are independent risk factors for wound-related complications. Additionally, wound-related complications are associated with impaired renal function and prolonged hospital stay. Graft and patient survival are comparable in patients with and without wound-related complications.

Late fulminant antibody mediated rejection of a blood group ABO-incompatible kidney transplant during Serratia marcescens urosepsis

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Introduction: The major challenge in blood group ABO-incompatible (ABOi) transplantation is to minimize antibody-mediated rejection (AMR). Recently developed protocols aim for effective reduction of the ABO antibodies at the time of transplantation and allow for accommodation of the immune response afterwards. ABOi-related AMR occurs almost exclusively in the first weeks after transplantation. ABO antibodies result from contact with A- and B-like antigens in the intestines via nutrients and gut-associated bacteria. Theoretically, boosting of the anti-ABO immune response by any of these bacteria is potentially harmful as it may trigger AMR of ABOi kidney grafts. We demonstrate a patient with fulminant antibody-mediated rejection after ABOi kidney transplantation, whose anti-A IgM titers rose dramatically following *Serratia marcescens* (*S. marcescens*) sepsis. Case report: A 58-year-old woman underwent an ABOi kidney transplantation (A to O donation) for end-stage renal disease secondary to autosomal dominant polycystic kidney disease. Pre-desensitization titers of anti-A were 64 for IgM and 32 for IgG. These were successfully reduced to <1:8 after immunoadsorption. Immune suppressive treatment consisted of rituximab, tacrolimus, mycophenolate mofetil, prednisone and immunoglobulines. She was discharged with a serum creatinine of 113 umol/L. She was readmitted to our hospital 12 weeks after transplantation for *S. marcescens* urosepsis. Her anti-A IgM titer rose to >5000 and a fulminant biopsy proven AMR followed, necessitating transplantectomy. We hypothesized that the *S. marcescens* sepsis had stimulated anti-A antibody formation. Bacteria known to have cross reactivity with ABO antigens, can decrease erythrocyte agglutination by absorption of anti-ABO immunoglobulins. Therefore, we conducted a number of experiments to show that pre-incubation of anti-A sera with *S. marcescens* bacteria was able to block haemagglutination of blood group A erythrocytes. However, none of these experiments indicated the ability of *S. marcescens* to bind anti-A antibodies.
Conclusion: This case illustrates that antibody mediated rejection may occur exceptionally late after kidney transplantation. The very high anti-A titers (IgM >5000) suggest a recent challenge of the immune system with blood group A antigen. The concurrent urosepsis with *S. marcescens* is highly suggestive for a pathological role of this microorganism but we were unable to verify this with in vitro experimental evidence.

Nierfunctie na een longtransplantatie

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Het klinische beloop na een longtransplantatie (LoTR) kan worden beïnvloed door acute of chronische rejectie, opportunistische infecties en toxische bijwerkingen van de immunosuppressieve therapie. Een van de belangrijkste bijwerkingen van de immunosuppressie met calcineurine remmers is het optreden van verlies van nierfunctie. Welke patiënt na een LoTR ernstig verlies van nierfunctie zal oplopen is lastig te voorspellen. In dit onderzoek stond de vraag centraal door welke factoren de nierfunctie na een LoTR wordt beïnvloed. Om hierop antwoord te geven is er gekeken naar de relaties tussen de post-LoTR nierfunctie enerzijds en anderzijds de longfunctie, de leeftijd, de aanwezigheid van diabetes mellitus (DM) en de expositie aan tacrolimus. Het betreft een retrospectieve studie waarbij de patiënten uit één Nederlands centrum werden bestudeerd. Het elektronisch patiëntendossier werd gebruik om de nierfunctie, longfunctie en de dalspiegels van tacrolimus te verzamelen. Tevens kon via dit dossier de leeftijd en de aanwezigheid van DM worden vastgesteld. Van respectievelijk 68, 51, 38, 31 en 26 patiënten konden de nierfunctie, longfunctie en tacrolimus-dalspiegels worden verzameld na respectievelijk 1, 2, 3, 4 en 5 jaar na LoTR. Patiënten van >50 jaar hebben een significant slechtere nierfunctie post-LoTR dan patiënten <50 jaar ($p<0,023$). Patiënten met een gemiddeld hogere tacrolimusspiegel over het eerste jaar post-LoTR hebben een significant betere nierfunctie ($p<0,05$). Patiënten die een betere longfunctie hebben aan het einde van het eerste jaar post-LoTR hebben een significant slechtere nierfunctie na zowel 1 als 3 jaar ($p<0,05$). Patiënten die voor de LoTR al DM hebben, laten een significant slechtere nierfunctie zien post-LoTR in vergelijking met patiënten zonder DM.

Een Buschke Lowenstein tumor bij een transplantatiepatiënt: is interferon de oplossing?

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We presenteren U de ziektegeschiedenis van een 24-jarige vrouw met een Buschke Löwensteintumor. Sinds 10 jaar heeft zij een goed functionerend niertransplantaat. Onder immunosuppressieve therapie ontwikkelen zich uitgebreide condylomata acuminata in het ano-genitaal gebied, welke invaliderende pijn en abcesvorming veroorzaken. Aanvankelijk worden er veelvuldig resecties verricht d.m.v cryo- en coagulatiechirurgie, waarbij HPV type 16 en 18 worden gedetecteerd. De condylomata groeien echter het kleine bekken in en ontwikkelen zich tot een Buschke Löwensteintumor. Een volledige chirurgische resectie kan adequate curatie geven, doch de patiënte is ernstig cachectisch. Bovendien groeit de tumor infiltratief in het kleine bekken, waarbij er geen scheidingsvlak tussen tumor en overige weefsels zichtbaar is op de MRI. In het gebiopteerde weefsel wordt geen maligniteit gezien en derhalve is radiotherapie ook geen optie. Om eigen afweer van patiënte te stimuleren wordt haar immunosuppressieve therapie gereduceerd tot 1 dd 5 mg prednison. Het is opvallend dat de nierfunctie goed blijft. Dit leidt echter niet tot het beoogde effect van tumorreductie door versterking van de eigen afweer. In de literatuur worden enkele casus beschreven van patiënten met een gestoorde afweer obv HIV of immunosuppresiva met een Buschke Löwensteintumor, die succesvol behandeld worden met interferon. De meest geschikte behandeling lijkt het lokaal toedienen van de interferon. Aangezien deze tumor zich echter in het kleine bekken bevindt en zeer uitgebreid is besluiten we een systemische behandeling met peginterferon alfa-2b toe te passen. Bij een controle MRI na enkele maanden blijkt er afname te zijn van de tumormassa. De algehele toestand van de patiënte verslechtert echter en uiteindelijk overlijdt zij aan een sepsis, meest waarschijnlijk obv infectie in het kleine bekken. Dit is een zeldzaam voorkomend ziektebeeld bij onze transplantatiepopulatie. Een inventarisatie van deze problematiek op Nederlands en op Europees niveau zal ons meer inzicht geven in het voorkomen en de behandeling. De behandeling met interferon lijkt hoopgevend, maar dmv een grote studie zal de juiste dosering en de juiste toedieningsweg gevonden moeten worden.

Chronic hepatitis E in a renal transplant recipient

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Introductie: Hepatitis E virus (HEV) werd geacht uitsluitend een ziekte te zijn van ontwikkelingslanden. Echter, als gedemonstreerd in de volgende casus dient er ook in de Westerse wereld rekening mee te worden gehouden.
Casus: Een inmiddels 66-jarige vrouw wordt vervolgd op de transplantatie-polikliniek. In 2007 onderging zij een pre-emptieve niertransplantatie in verband met progressieve nierfunctiestoornissen bij glomerulonefritis e.c.i. Vanaf 2009 ontwikkelt zij levertestafwijkingen, malaise en arthralgieën. Analyse naar de levertestafwijkingen (standaard virologie, auto-immuun serologie, echo abdomen en leverbiopsie) levert geen diagnose op. Vanaf 2012 krijgt patiënte progressieve neurologische verschijnselen, waarbij het neurologisch beeld zeer aspecifiek was en niet paste bij enig bekend syndroom. Patiënte ontwikkelde een encephalopathie, een atactisch looppatroon, symptoom van Lhermitte, blaasledigingsstoornissen en perifere gevoelsstoornissen. Uitgebreide neurologische analyse middels liquorpunctie, virologisch onderzoek middels serologie en PCR-technieken, EEG, EMG en MRI levert geen diagnose op. Recente publicaties over HEV richtten de aandacht op dit virus. Het bleek dat zowel positieve serologie (IgM en IgG) als viraal RNA (genotype 3) konden worden aangetoond in bloed en liquor, als ook op een gearchiveerd monster, waarmee de diagnose chronische hepatitis E werd gesteld. Behandeling bestond uit verlaging van immunosuppressie die op dat moment nog bestond uit mycophenolaat-mofetil en prednisolon en start antivirale therapie middels ribavirine. Hiermee trad een geleidelijke daling op van de viral load en verbeterden haar encephalopathie en leverfunctiestoornissen. Vooralsnog persisteren haar loop- en blaasledigingsstoornissen.
Conclusie: HEV genotype 3 is niet zeldzaam en kan bij immuungecompromitteerde patiënten een chronische hepatitis veroorzaken, die kan leiden tot onbegrepen leverfunctiestoornissen, malaise en ernstige moeilijk te classificeren neurologische verschijnselen. Meer bekendheid met dit virus kan het stellen van de diagnose versnellen en de pathologie beperken. Serologische screening dient te worden toegevoegd aan het pretransplantatie virologisch onderzoek, waarbij patiënten zonder antistoffen in de toekomst gevaccineerd zouden kunnen worden.

Vitamin D status in children pre- and post renal transplantation: hyperparathyroidism and interaction with calcineurin inhibitors

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Objective: Supplementation of activated vitamin D analogues has been postulated in paediatric renal transplant recipients, but there is no evidence that 1,25(OH)2D deficiency contributes to hyperparathyroidism in children with GFR >50ml/min/1.73m². The aim of the study was to investigate the prevalence of 25OHD and 1,25(OH)2D deficiency in stable paediatric kidney transplant patients as compared with pre-transplant children and search for the evidence of interaction with calcineurin inhibitors. Patients and methods: We included 62 renal transplant recipients and compared them with 32 pre-transplant children in this retrospective cross-sectional study. Vitamin D supplementation (either alfacalcidol or cholecalciferol) and CKD stages 4 and 5 were exclusion criteria. The participants' median age was 12 years and they were 2- 133 (median 16.5) months post transplant at baseline. The median eGFR in the transplant group was 67 ml/min/1.73m². Sixty children were on calcineurin inhibitors. Results: In the transplant group at baseline 56% children had 25OHD deficiency or insufficiency and 45% hyperparathyroidism defined as PTH > 7.4 pmol/l. The 1,25(OH)2D levels were higher than 150 pmol/l in 50% children. PTH inversely correlated with 25OHD levels (r=-0.29, P=0.04), but not with 1,25(OH)2D. Renal transplant recipients had higher 1,25(OH)2D levels than pre-transplant children with CKD 1-3 (P=0.02) and the daily dose of tacrolimus and cyclosporine correlated with 1,25(OH)2D levels (r 0.39, P=0.038 and r 0.6 and P= 0.039, respectively).
Conclusions: In contrast with 25OHD, 1,25(OH)2 levels in post-transplant children were normal to high. 25OHD deficiency was widely prevalent among paediatric renal transplant recipients and correlated with hyperparathyroidism. This implicates the use of ergo-or cholecalciferol as first-line therapy of secondary hyperparathyroidism. The high 1,25(OH)2D levels in post transplant children can result from the interaction with calcineurin inhibitors.

Uitkomsten van niertransplantatie in de diverse bevolkingsgroepen van de regio Groot Amsterdam

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Zowel transplantaat- als patiëntoverleving na transplantatie zijn lager onder Amerikaanse Afrikanen in vergelijking tot Kaukasische Amerikanen. Aard van het grondlijden, het percentage levende donoren, duur van dialyse en sociaaleconomische factoren hebben daarin een rol. Wij onderzochten de uitkomsten van transplantatie in de etnische bevolkingsgroepen van de regio Amsterdam. Een retrospectieve cohort studie werd verricht onder alle niergetransplanteerden in de periode 2000-2011. Patiënten (n = 1064) werden gecategoriseerd volgens een onderverdeling, die de gemeente Amsterdam hanteert: "Nederlandse" achtergrond, Antilliaans, Surinaams, Turks, sub-Saharisch Afrikaans en Marokkaans. Een categorie "divers" werd toegevoegd. Transplantaat - en patiënt overleving werden geanalyseerd, en de volgende factoren: type donor, grondlijden, rejectie, het ontstaan van New Onset of Diabetes After Transplantation (NODAT) en het aantal No Shows op de polikliniek. 37% van alle getransplanteerden was van Niet Westerse (NW) achtergrond, waarvan 8.9%, 9.6%, 4.0%, 4.0% en 4.4% respectievelijk Antilliaans, Surinaams, Turks, sub-Saharisch Afrikaans en Marokkaans. De categorie divers betrof 5.5% van het totaal. Mean follow up was 55 ± 40 maanden. Het grondlijden in de NW groep was vaker dan in de westerse (W) groep Diabetes Mellitus II en hypertensie (15 vs. 4% en 17 vs. 22%). APKD kwam significant meer voor in de W groep. NW patiënten waren jonger ten tijde van transplantatie (47 ± 13 vs. 50 ± 13) en dialyseerden langer. Het percentage niertransplantatie met een levende donor (LD) nier betrof 17% in de NW vs. 41% in de W groep. Binnen de LD transplantaties kreeg de NW groep significant vaker een nier van familie. 5 Jaar transplantaatoverleving was 80% in de W en 73% in de NW groep. Transplantaatoverleving van alleen de Marokkaanse en de sub-saharische Afrikaanse groep verschilde signficant van de W groep. Het rejectie percentage was 24% in de W groep en 29% in de NW groep en 44% in de sub-saharische Afrikanen. De incidentie van NODAT verschilde niet tussen de groepen. Het aantal No Shows was significant hoger in de sub-saharische Afrikaanse en Surinaamse groep. Patiënten met een NW achtergrond dialyseren langer, hebben minder vaak een levende donor, hebben meer rejectie en kortere transplantaatoverleving. Stimulatie van het LD niertransplantatie programma i.c.m. patiëntvoorlichting en intensivering van de immunosuppressieve therapie zal de resultaten in deze groep kunnen verbeteren.

Is dysfunction of the HNF-1 beta gene involved in the pathogenesis of NODAT?

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Background: A dysfunction of the HNF-1beta gene can lead to renal loss of magnesium, hyperuricemia and diabetes mellitus. There is a striking similarity between this clinical phenotype and the frequent occurrence of new-onset diabetes after transplantation (NODAT), hypomagnesaemia and hyperuricemia in organ transplant patients treated with tacrolimus. We therefore hypothesized that dysfunction of the HNF-1beta gene might be involved in the pathogenesis of NODAT in tacrolimus treated renal transplant patients. Aim: To investigate whether NODAT, hypomagnesaemia and hyperuricemia are mutually associated in tacrolimus-treated renal transplant patients. Patients and methods: We conducted a retrospective study in adult patients who received a living donor kidney between January 2006 and December 2011, and were treated with tacrolimus. NODAT was defined as a HbA1c level of > 7.0% or the use of hypoglycemic drugs. We excluded patients with a prior history of diabetes mellitus. Magnesium and urate levels were measured immediately before transplantation and 1 week post transplantation. Results: Of the 263 patients, 53 (20.2%) developed NODAT within the first three months after transplantation. Patients without NODAT (n=210) served as controls. Both groups had a comparable dose of prednisone and tacrolimus at 3 months after transplantation. The BMI at the time of transplantation was significantly higher in the NODAT group (25.1±3.1 kg/m^2 versus 23.9±3.6 kg/m^2; p< 0.05). Prior to transplantation, the magnesium and urate levels did not differ between the NODAT and the control group: 0.94±0.17 mmol/L versus 0.96±0.18 mmol/L and 0.34±0.13 mmol/L versus 0.33±0.10 mmol/L (NS). Although both the magnesium and urate levels were lower post transplantation, there was again no difference between the NODAT and control group: 0.77±0.16 mmol/L versus 0.77±0.14 mmol/L and 0.31±0.10 mmol/L versus 0.31±0.35 mmol/L. Logistic regression analysis did not reveal a relation between NODAT and magnesium or urate levels.
Conclusion: We found no evidence for a clustering of NODAT, hyperuricemia and hypomagnesaemia in tacrolimus treated renal transplant patients. Involvement of the HNF-1beta gene in the pathogenesis of NODAT is therefore unlikely.

Soluble CD30 levels do not predict acute rejection after withdrawal of tacrolimus in renal transplant patients

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Reliable biomarkers predicting the safe reduction of immunosuppression after renal transplantation are not available. Soluble CD30 (sCD30) levels have been demonstrated to be associated with graft survival and possibly also with the incidence of acute rejection after renal transplantation. We investigated if sCD30 can be used as a marker for safe withdrawal of tacrolimus at six months after renal transplantation. We prospectively studied a cohort of 82 patients with stable graft function who were treated with the combination of tacrolimus, azathioprine or mycophenolate mofetil, and prednisone. From six months after transplantation, tacrolimus was tapered and discontinued in a period of 4 weeks under close monitoring for acute rejection. Signs of acute rejection within 6 months after withdrawal of tacrolimus occurred in 30 of the 82 patients. In 24 rejectors and 44 non-rejectors the sCD30 concentration was determined in serum collected before transplantation and immediately before withdrawal of tacrolimus. The sCD30 concentration decreased significantly after transplantation ($p<0.0001$). There was no difference in sCD30 levels between rejectors and non-rejectors, neither pre transplantation (134,8 ± 66,2 ng/mL versus 149,8 ± 78,0 ng/mL; NS) nor pre withdrawal (63,4 ± 33,5 ng/mL versus 78,0 ± 73,9 ng/mL; NS).
We conclude that soluble CD30 levels cannot be used to guide safe withdrawal of tacrolimus at 6 months after renal transplantation.

Novel biomarkers for the prediction of acute kidney injury in patients undergoing liver transplantation

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Introduction: Acute kidney injury (AKI) after liver transplantation (LT) is an important and common complication. Because of the delayed rise of serum creatinine (SCr) concentrations novel biomarkers such as neutrophil gelatinase-associated lipocalin (NGAL), glutathione S-transferase (GST), kidney injury molecule-1 (KIM-1) are proposed to detect AKI. Methods: We enrolled 26 consecutive adult liver transplant patients without pre-existent kidney disease to evaluate the diagnostic value of NGAL, GST and KIM-1 for the development of AKI. AKI was defined according to the Acute Kidney Injury Network (AKIN) criteria. Markers were measured at 4 time points after LT; immediately after admission to the intensive care unit (ICU) (T = 0) and 4, 8 and 24 hours thereafter (T = 4, T = 8, T = 24 respectively). NGAL was measured in both plasma and urine (pNGAL and uNGAL respectively) and 2 subsets of GST were measured in the urine (α-GST and pi-GST). Furthermore we evaluated plasma Cystatin C (pCyC) concentrations as an alternative measure for GFR. Results: 9 of the 26 patients developed AKI according to the AKIN criteria. pNGAL and uNGAL detected the presence of AKI at various time points with an optimal area under the curve (AUC) at T = 8 for pNGAL (0.86; p = 0.004) and an optimal AUC at T = 4 for uNGAL (0.80; p = 0.012). Both α-GST and pi-GST did not show a significant difference between the two groups. KIM-1 failed to detect AKI, but it rose significantly over time in both groups. pCyC could also differentiate between AKI and no-AKI at various time points with an optimal AUC at T = 8 of 0.85 (95% CI = 0.66 – 1.00; p= 0.005).
Conclusions: These findings suggest that especially PNGAL and UNGAL are promising biomarkers for the early detection of renal injury in patients after LT. Furthermore pCyC could be used as an alternative measure for GFR since it performed slightly better in the discrimination of AKI compared to SCr in this study.

MMP-2 CT/TT genotype is a risk factor for mortality or liver transplantation in primary sclerosing cholangitis

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Background: Primary sclerosing cholangitis (PSC) is a chronic inflammatory disease of the bile ducts, resulting in fibrotic strictures frequently necessitating orthotopic liver transplantation (OLT), often accompanied by ulcerative colitis (UC) Matrix metalloproteinases (MMPs) play a role in many fibrotic diseases due to their involvement in connective tissue remodeling related to cancer, inflammatory diseases and complications after OLT. We previously found the MMP-2 or -9 genotype not to be indicative for PSC in 314 OLT patients. Aim of the present study was to assess MMP-2 and MMP-9 gene polymorphisms within a group of PSC patients in relation to disease severity as evaluated by death or need for OLT. Methods: For this study, 132 PSC patients were included from two liver transplantation centers. Follow-up was from initial onset of PSC until OLT, death or end of follow-up. From these patients genomic DNA was extracted routinely from peripheral blood and/or tissue samples. MMP-2 (-1306 C/T) and MMP-9 (-1562 C/T) gene promoter polymorphisms were analyzed using high-resolution DNA melting analysis (HRMA) or by PCR followed by restriction length polymorphisms (RFLP) respectively. Demographical and clinicopathological variables such as OLT, time of OLT, age, gender, type of IBD and survival were analyzed. Results: Of the PSC patients 88 (66.7%) were male. Sixty (45.5%) PSC patients underwent OLT with a mean follow-up from PSC onset to OLT of 7.2 years (range 0.4 – 21 years). Mean age at OLT was 46 years (range 18.3 – 67.7 years). Twenty-years cumulative incidence of death or OLT in the CT/TT group was 92.7% compared to 53.0% in the wild-type group (CC) ($p=0.02$) and reached 93.2% when MMP-2 CT/TT genotype was accompanied by ulcerative colitis (UC) compared to 51.2% with UC and wild-type MMP-2 ($p<0.01$). Age at onset of PSC (aHR=1.03; 95% CI 1.01-1.05) and MMP-2 CT/TT genotype were independent risk factors for OLT or death (aHR=1.97; 95% CI 1.20 - 3.23), both $p<0.01$). In contrast, no signifycant association was found between MMP-9 genotype and the risk of OLT in PSC patients.
Conclusion: MMP-2 CT/TT genotype in PSC is a significant independent risk factor for disease severity as reflected by patient mortality or need for OLT and identifies, therefore, this polymorphism as a disease modifying gene.

Psychological factors associated with medication adherence among young adult kidney transplant recipients

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Background: There is much evidence for elevated levels of medication nonadherence among patients who undergo transplantation at a young age. The aim of this study was to investigate how coping and satisfaction of psychological needs (autonomy, competence and relatedness) are related to medication adherence among young transplant recipients. Method: We conducted a cross-sectional, face-to-face interview study among kidney transplant recipients aged 21-30 years currently enrolled at out our out-patient clinic. Adherence was measured using the Basel Assessment of Adherence to Immunosuppressive Medication Scale (BAASIS©). Independent variables were: age at transplantation (Group 1 < 18; Group 2 > 18); socio-demographic characteristics, psychological needs (Basic Psychological Needs Scale); coping strategies [COPE-easy]; and mood (Positive And Negative Affect Schedule). Results: Of the 93 invited, 66 (71%) patients participated (67% male; median age 25; 47% in Group 1 and 53% in Group 2). Sixty-four percent of patients were classified nonadherent in past 4 weeks. Twenty participants (30%) reported nonadherence on the Taking dimension (missing a dose at least once) while 34 (51.5%) reported nonadherence on the Timing dimension (doses taken >2 hrs before or after prescribed time). Age at first transplantation, socio-demographic characteristics, satisfaction of psychological needs and coping were not significantly related to adherence classification. However, greater satisfaction of autonomy and relatedness needs was related to higher self-rated overall adherence. Nonadherent recipients also scored significantly lower on negative affect.
Conclusion: A high level of nonadherence was found among young transplant recipients. Promoting autonomy and relatedness may offer a way of increasing medication adherence in young patients. Contrary to the literature, adherent patients were found to have *higher* negative affect than nonadherent patients. The strict medication regime may be experienced as limiting and thus influence mood among some young patients, or conversely, fear of rejection may also generate negative mood but promote adherence.

Psychological processes that contribute to psychological well-being and social participation among young adult kidney transplant recipients

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Background: Kidney disease and transplantation can have a significant impact on the development and well-being of young individuals. The aim of this study was to investigate how coping and satisfaction of psychological needs (autonomy, competence and relatedness) are related to the outcomes of psychological well-being and social participation. Method: We conducted a cross-sectional, face-to-face interview study among kidney transplant recipients aged 21-30 currently enrolled at out our out-patient clinic. Exclusion criteria included having undergone a transplant in the past year, not speaking sufficient Dutch and cognitive limitations. The main outcomes were measured using the Positive And Negative Affect Schedule (PANAS), the Satisfaction With Life Scale (SWLS)] and the Course of Life Questionnaire (CLQ). Independent variables were: age at transplantation (Group 1 < 18 years; Group 2 > 18 years); psychological needs [Basic Psychological Needs Scale (BPNS)]; and coping strategies [COPE-easy]. Results: Of the 93 invited, 66 (71%) patients participated (67% male, median age 25, 47% were in Group1 and 53% in Group 2). Group 1 reported significantly lower achievement of autonomy development milestones than Group 2 but no other differences in other outcomes. Satisfaction of all psychological needs, lower avoidance, greater active coping and lower substance use as a coping strategy were significantly related to higher psychological well-being. Satisfaction of autonomy and relatedness needs was related to greater achievement of social development milestones.
Conclusion: Greater satisfaction of psychological needs, in particular autonomy and relatedness, was related to greater psychological well-being and social participation. These modifiable factors may be amenable to intervention for those who report reduced well-being or social participation, particularly those transplanted in childhood.

JAK inhibitor tofacitinib interferes with interferon alpha mediated inhibition of hepatitis C replication

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Background: End stage liver disease caused by chronic hepatitis C infection is a leading indication for liver transplantation. After transplantation however, reinfection of the graft occurs universally, leading to accelerated fibrosis and early development of cirrhosis. The use of immunosuppressive medication after transplantation may contribute to the aggravated course of infection and increased resistance to antiviral therapy. Therefore, there is a need for new immunosuppressive agents. Tofacitinib is a new immunosuppressant that was developed as a selective inhibitor of the Janus kinase 3 (JAK3) but may inhibit other members of the JAK family. Therefore, the aim of our research is to investigate the effect of tofacitinib on HCV replication and JAK1 mediated interferon-α (IFN-α) signalling. Methods: As a model for HCV replication we used a Huh7 hepatoma cell line, stably transfected with the non-structural coding sequence of HCV coupled to a luciferase reporter (Huh7-ET). The amount of luciferase in these cells is a direct representation of HCV replication. A Huh7 cell line stably transfected with a luciferase gene controlled by an interferon response element (Huh7-ISRE-luc) was used to investigate effects of tofacitinib on IFN-α signal transduction. Results: In Huh7-ISRE-luc cells, tofacitinib inhibited IFN-α stimulated gene expression in a dose dependent manner. The highest dose of tofacitinib (1000 ng/ml) completely inhibited IFN-α stimulated gene expression and 100 ng/ml tofacitinib reduced IFN-α activity by 50%. With 10 U/ml IFN-α a complete inhibition of HCV replication was observed. This IFN-α mediated inhibition of HCV was completely abrogated by tofacitinib in a dose dependent manner.
Conclusion: Although tofacitinib was developed as a specific inhibitor of JAK3, with a reported 100-fold less potency for JAK1, we found that tofacitinib effectively inhibits IFN-α regulated gene expression, and interferes with IFN-α mediated inhibition of HCV replication. This observation explains the higher incidence of viral infections found in patients that are treated with tofacitinib.

Dendritic cell-derived CXCL1: no role in T cell activation or differentiation

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Background: Tolerogenic dendritic cells (DC) have the potential to induce antigen-specific tolerance. We created a DC with a tolerogenic phenotype (low expression of co-stimulatory molecules and high IL10:IL12 production ratio) by blocking GSK3 with LiCl and stimulating TLR2 with PAM_3Cys ($LiClPAM_3$ DC). However, in a heart transplantation model in mice, pretreatment with $LiClPAM_3$ DC resulted in accelerated graft rejection compared to untreated DC. Further evaluation revealed a high CXCL1 production by these $LiClPAM_3$ DCs. The role of DC-derived CXCL1 within DC–T cell interaction has not been studied yet. We hypothesized that CXCL1 can activate naive T cells and contributes to the immunogenic character of the $LiClPAM_3$ DCs. Material and methods: Dendritic cells were cultured from C57Bl6 bone marrow with GM-CSF. To create $LiClPAM_3$ DCs, lithium chloride and PAM_3Cys were added during the last 24 hours of culture. Balb/c CFSE labeled splenocytes, or splenocytes depleted for MHC-II or CXCR2-positive cells, were used as responder cells in proliferation assays. In specific experiments, recombinant CXCL1 (rCXCL1), anti-CXCL1, or SB225002, a specific antagonist of CXCR2 (the receptor for CXCL1), was added. The proliferative response was evaluated by dilution of CFSE signal and cytokine levels were measured in culture supernatants. Results: Biological active rCXCL1 had no effect on the proliferation of T cells that were cultured in anti-CD3ε coated plates. Also, rCXCL1 did not affect the proliferation of T cells co-cultured with immature DC. Neutralizing $LiClPAM_3$ DC-derived CXCL1 by adding anti-CXCL1 to co-cultures of $LiClPAM_3$ DC and T cells had no influence on the proliferative response or on T cell activation. To test whether CXCL1 could have an indirect effect on T cells (eg via granulocytes), we depleted the responder cells for CXCR2-expressing cells or blocked CXCR2 on splenocytes by SB225002. CXCR2-depleted or CXCR2-blocked splenocytes stimulated by $LiClPAM_3$ DC showed a similar proliferative response and cytokine production compared to untreated splenocytes.
Discussion: $LiClPAM_3$ DCs produce large amounts of CXCL1 and are potent T cell stimulators. Nevertheless, DC- derived CXCL1 does not directly or indirectly influence T cell activation or proliferation. Possibly, a different cytokine, chemokine or membrane receptor is responsible for the unexpected immunogenic character of the $LiClPAM_3$ DCs.

hCMV-specific CD8+ T cells in lymph nodes from renal transplant recipients contain 'true' memory cells

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We have recently shown that lymph nodes (LN) contain hCMV-specific $CD8^+$ T cells that resemble central memory cells, a phenotype that is not found in peripheral blood (PB). We have also shown that the LN hCMV-pp65-specific $CD8^+$ T cell pool contained clones not found in PB. It is not known what the contribution of these LN hCMV-specific $CD8^+$ T cells is to the PB pool upon viral recall. We therefore studied the contribution of these clones to the PB pool during CMV reactivation. Two patients experiencing a hCMV reactivation after kidney transplantation were studied. hCMV-pp65-specific $CD8^+$ T cells of pre transplantation LN and paired PB, as well as PB obtained during reactivation and 1 year after transplantation were analyzed for clonal relationships by high-throughput-sequencing of the TCR-Vβ-CDR3 region. In the first patient the PB pool was restricted to one clone and the LN pool consisted of an identical clone and one extra unique clone. During two subsequent reactivations the CMV-specific pool remained restricted to the same dominant clone. Though the unique LN clone did not contribute to the PB pool, it could not be determined whether the vigorous expansion of the CMV-specific pool during both reactivations was caused by the LN derived clone or the PB derived clone, since both clones were identical. In the second patient both overlapping and unique clones were present in LNs and PB. The unique LN derived clones could be found to add substantially to the PB pool upon reactivation. In fact, at that time point the major clone was a LN derived one. However, also in this patient, not all unique LN clones added to the PB pool upon reactivation.
In conclusion, LNs seem to harbor a unique pool of 'true' memory hCMV-pp65-specific $CD8^+$ T cells that proliferate vigorously and contribute to the PB population upon antigenic recall.

Primary CMV infection has a limited effect on the immunological age of kidney transplant recipients

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Background: Immunological ageing of the T cell compartment is related to a decreased T cell immunity. Cytomegalovirus (CMV) infection in healthy individuals has been associated with an ageing effect on the T cell compartment and contributes to the immunedeficiency of the elderly. In this study, we investigated the effect of a primary CMV infection on T cell ageing in CMV- kidney transplant (KTx) recipients, who received a kidney from a CMV+ donor. Methods: The T cell receptor excision circle (TREC) content and % $CD31^+$ naïve T cells were measured as markers for thymic output. The relative telomere length (RTL) was determined as a measure for proliferative history and immunophenotyping was used to establish the differentiation status of circulating T cells. CMV- KTx recipients receiving a kidney from a CMV+ donor (D+/R-, n=31) were compared to those receiving one from a CMV- donor (D-/R-, n=47) matching for age and immunosuppressive medication. Patients were followed prior to and at 3, 6 and 12 months post transplantation. All patients received valganciclovir during the first 6 months after transplantation. Results: At 6 months, 30% of the D+/R- KTx recipients had detectable anti-CMV IgG titers and 100% at 12 months. Four recipients developed CMV disease and were excluded from analysis. Primary CMV infection did not affect the TREC content, % $CD31^+$ naïve T cells and RTL of CD4 and CD8 T cells. Twelve months following KTx, absolute numbers of $CD8^+$ memory T cells were increased ($p<0.05$) mainly as a result of a significant increase in terminally differentiated EMRA $CD8^+$ T cells ($p=0.03$). A significant increase in the % of memory $CD4^+$ as well as $CD8^+$ T cells lacking CD28 expression was noticed for the D+/R- KTx recipients when comparing percentages pre to 12 months following KTx, i.e. 5.68% vs. 19.83% ($p<0.05$) and 32.71% vs. 60.45% ($p<0.01$), respectively. This increase in number of differentiated T cells was not detected in the D-/R- KTx recipients.
Conclusion: Primary CMV infection in D+/R- KTx recipients does not affect thymic output or telomere length and therefore does not induce generalized immunological T cell ageing. However, CMV infection substantially increases the number of terminally differentiated CD8 and to a lesser extent CD4 T cells.
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Towards optimal monitoring of memory B cell responses in sensitized patients

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The presence of donor specific HLA antibodies (DSA) detectable in sensitive luminex assays is associated with graft rejection, but only in a proportion of the patients. Recently we have developed several in vitro assays to measure the number and/or reactivity of memory B cells, which may be of additional value for the risk estimation in patients with DSA. However, the current assays to measure immunoglobulin production require prolonged stimulation of B cells of typically 8 to 14 days, which makes them less useful for monitoring. Furthermore, such long B cell activation periods invariably lead to a high rate of cell death, and may therefore influence clonal distribution. To improve on the currently available B cell stimulation protocols, we have analysed the kinetics of total B cells and FACS sorted naive ($CD19^+IgD^+CD27^-$) and memory ($CD19^+IgD^-CD27^+$) B cells upon polyclonal activation. We analysed the dead/alive cell ratios (by 7AAD and eosin staining), proliferation capacity (by thymidine incorporation) and immunoglobulin production (by IgM/IgG ELISPOT) at several time points. For antigen specific responses, tetanus toxoid (TT) antigen-specific ELISPOT assays were performed. Upon B cell stimulation, we found low dead/alive ratios for the first 6 days of culture in all B cell fractions. Cell death gradually increased starting from day 6 up to day 10. The highest proliferation rate of memory B cells was at day 3 whereas for unsorted and naïve B cells the peak proliferation was at day 6. When we assessed immunoglobulin production by ELISPOT, we found that IgG spots were produced exclusively by memory cells and were detectable as early as day 2 of the culture period. To confirm early detection of antigen specific memory responses we performed TT antigen-specific ELISPOT assays at several time points after stimulation. In these preliminary experiments, we could readily detect TT antigen-specific memory IgG responses after 3 days of stimulation.
Our data showing low cell death up to 6 days of cell culture and the ability of detecting (antigen-specific) IgG spots produced by memory cells as early as day 2 of the culture suggest that short culture periods of 2-4 days might be sufficient to detect the memory B cell responses. We aim to extend our results to determine whether shorter culture periods may be used to detect antigen specific memory B cell responses.

Dietary restriction and fasting arrest B and T cell development and recruits mature B and T cells to the bone marrow

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Background**:** Seventy-two hours of preoperative fasting (F) or 2 weeks of 30% dietary restriction (DR) offers robust protection against renal ischemia-reperfusion injury (IRI) in mice. However, the mechanism remains to be elucidated. We hypothesize that immunomodulation plays a pivotal role. Adaptive immunity, especially the B and T lymphocytes, has been shown to play a crucial role in the pathophysiology of IRI. Therefore, we investigated the impact of fasting and dietary restriction on B and T cells in the various lymphoid organs. Materials and Methods: Male C57Bl/6 mice were fed ad libitum (AL) or underwent 72 hours F or 30% DR for 2 weeks (n=8/group). Consequently, the mice were sacrificed and bone marrow (BM), thymus, spleen and mesenteric lymph nodes (mLN) were harvested and processed into cell suspensions. These cells were stained with B and T cell markers for FACS analysis such as CD19, B220, CD3, CD4, CD8 etc. These stained cells were then phenotypically analysed on a LSRII flow cytometer. Results**:** Both DR and F cause significant ($p \leq 0.05$) depletion in the early immature B cell development and a significant increase in recirculating mature B cells ($p \leq 0.05$) in the BM as compared to that of the AL group. The splenic $CD19^{+}B220^{+}$ B cell population was significantly decreased ($p \leq 0.001$) in the F group while no changes were observed in the DR group when compared with AL. Other B cell subsets showed a decrease of all subtypes such as IgMlowIgDlow, $IgM^{+}IgD^{-}$ as well as $IgM^{+}IgD^{+}$ subsets in the F group ($p \leq 0.05$). Significant downregulation in marginal as well as follicular zone B cells was also observed in spleen with again a pronounced effect due to F ($p \leq 0.005$). The same trend was also observed in mLN. $CD3^{+}$T cells in BM, spleen and mLN were significantly increased in F group ($p \leq 0.05$) as compared to that of AL and DR group while the T cell development in thymus was arrested at the early DN2 stage and ISP stage ($p \leq 0.05$), hence causing confinement of the T cell development and maturation. Conclusion**:** Dietary interventions cause alternations in all the lymphoid compartments. Compared to DR, F has a more pronounced effect. We conclude that DR and F arrest B and T cell development and also cause recruitment of both recirculating mature B cells and T cells to the BM. These alterations in the lymphoid compartments may be one of the mechanisms by which dietary interventions protect against renal IRI, but further investigation into this is required.

Natural regulatory T-cells impair the donor-specific cytotoxic T-Cell response long after transplantation

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Introduction: $CD4^+CD25^{high}CD127^-$ are potent regulators of cytokine production and proliferation of alloreactive T-cells. Although, cytotoxic T-cells are the effector cells that destroy the allograft. The influence of regulatory T-cells (Tregs) on the donor-specific cytotoxic capacity of cytotoxic T-lymphocytes (CTL) is unknown. Therefore, we questioned whether the donor-specific CTL response is controlled by Tregs after renal transplantation. Material and methods: Blood was sampled from 12 patients after renal transplantation (mean 4.7 years ±1.2). We assessed the involvement of Tregs both by depleting them from patients' PBMCs as well as by reconstituting them to the PBMCs depleted for Tregs. Patients' cells were incubated with irradiated donor or 3rd-party cells in the presence of IL-2, IL-7 and IL-15 to obtain optimal numbers of donor or third-party reactive effector memory CTLs. After 7 days of culture, a cell mediated lympholysis assay (CML) was performed by adding effector cells to a fixed number of europium labelled target cells (effector:target ratio starting from 40:1 to 0.6:1). Results: Donor-specific hyporesponsiveness compared to 3rd-party response was found in all effector:target ratios ($p=0.003$). Depletion of Tregs from PBMC resulted in an increased donor-reactive CML response in 9/12 patients. Reconstitution of the Tregs to the $CD25^-$ negative effector T cells, inhibited the donor-reactive CML response again in 9/12 patients. The potency to inhibit 3rd-party reactivity was comparable: 11/12 were inhibited. When we characterised the Tregs by phenotypic analysis, we found that most of the $CD4^+CD127^-$ $FoxP3^+$ Tregs expressed Helios, the marker of natural Tregs (nTregs $Helios^+$: mean±SD; 68% ±4.1). Only 5% ±2.3 nTregs were capable to produce IFN-γ after stimulating the cells with PMA/ionomycin, while 28% ±13 of the iTregs ($Helios^-$ induced Tregs) produced IFN-γ.
Conclusion: Functional nTregs circulate long after renal transplantation, these cells inhibit the donor-specific CTL response.

A systematic review identifying psychosocial risk factors for living kidney donors

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Background: Living kidney donation is the preferred treatment for patients with end-stage renal disease, because of better long-term recipient and graft survival, shorter waiting list times, and better quality of life for the recipient than after postmortal donation. Although most donors recover well after surgery, about 10-25% of donors experience emotional or physical problems on the longer term. The current review provides an overview of psychosocial risk factors identified in the literature for longer term physical and emotional problems in living kidney donors. Methods: A literature search was conducted using PubMed, Embase, Web of Science and Psych INFO. Due to the limited number of pre-post donation prospective studies, cross-sectional and prospective quantitative studies were included. Results: Psychosocial factors including psychological distress and unrealistic expectations before the donation, as well as fatigue and perceived negative donor-recipient relationship changes after the donation have been reported as predictors for longer term physical and emotional problems in living kidney donors.
Discussion: The limited number of studies on this topic provides a preliminary indication of specific psychosocial risk factors for developing longer term emotional and physical problems in living kidney donors. More prospective research is needed to get insight in pre-donation predictors of these problems, in order to enable better screening of potential donors and to focus pre- or post-donation interventions.

Modifiable factors in Access to Living Donor Kidney Transplantation among Diverse Populations

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Objective: Despite living donor kidney transplantation (LDKT) being the optimal treatment option for patients with end-stage renal disease, we observed a significant inequality in the number of LDKT performed between patients of non-Western European and Western European origin. The aim of this study was to explore modifiable hurdles to LDKT that may help explain this inequality. Methods: A questionnaire on knowledge, risk perception, communication, subjective norm, and willingness towards LDKT was completed by 160 end-stage renal patients who were referred to the pre-transplantation outpatient clinic (participation rate 92%) prior to their consultation with the nephrologist. The questionnaire was available in 9 languages. Multivariate analyses of variance and binary regression analyses were conducted to explore and explain differences between patients with and without a living donor controlling for socio-demographic factors. Results: There were signifycantly fewer patients of non-Western descent (11/82) that brought a living donor to the outpatient clinic than patients of Western descent (38/78). Patients without a living donor were less likely to be employed than patients with a living donor (p<0.001). Furthermore, patients without a living donor were undergoing haemodialysis more often (p=0.003) and spent on average 15 to 23 months longer on dialysis (p=0.002) compared to those with a living donor. Non-Western descent, long duration of dialysis, low knowledge, little communication on kidney disease and low willingness to communicate with individuals from the social network were significantly related to the absence of a living donor.
Conclusions: After correcting for non-modifiable socio-demographic factors, knowledge, willingness to communicate and actual communication were identified as modifiable factors that are related to the likelihood that a patient brings a potential living donor to the first visit at the pre-transplantation clinic. This observation makes knowledge and communication strong candidates to address in interventions aiming to reduce the inequality in LDKT among potential transplant candidates.

Knowledge as a predictor for having a living kidney donor?

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Introduction**:** Living donor kidney transplantation (LDKT) is regarded as the optimal treatment option for patients with end-stage renal disease. The aim of this study was to investigate if knowledge about dialysis, transplantation and living donation differentiates between patients with and without a living donor at their first visit to the outpatient pre-transplantation clinic. Methods**:** We measured the knowledge regarding kidney diseases and renal replacement therapies of 78 kidney patients who were newly referred to the outpatient pre-transplantation clinic. All were asked to fill in the validated Rotterdam Renal Replacement Knowledge-Test (RRK-T) which is available in 9 languages. The R3K-T contains two subscales: 'Dialysis and Transplantation' (10 items) and 'Living Donation' (11 items). After the first visit all patients were provided with several educational materials: hospital education. The patients *without* a living donor were also asked to complete out the questionnaire again during a later visit to the outpatient clinic. Results**:** At the first visit 49/78 patients had a living donor. This group scored significantly higher on the total scale of the R3K-T (p=0.002) and on the two subscales (p=0.012 and p=0.005) compared the group of patients without a living donor. When the R3K-T was administered again to patients without a living donor after the hospital education, they had the same score on overall knowledge (p=0.104) and the same scores on both subscales (p=0.134 and p=0.190) as on the first examination.
Discussion**:** Greater knowledge of dialysis and renal replacement therapies appears to differentiate between patients with and without a living donor during their first visit to the outpatient pre-transplantation clinic. Moreover, those without a living donor do not appear to benefit in terms of knowledge from the standard educational efforts. Patients without a living donor may benefit from a more interactive and tailored educational programme in addition to the current education.

A Psychometric Analysis of the Rotterdam Renal Replacement Knowledge-Test (R3K-T) using Multidimensional Item Response Theory (MIRT)

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Objective: Knowledge is one of the strongest motivators for promoting well-informed (shared) decision making. Nevertheless, there is no validated and standardized test of the level of knowledge among renal patients regarding kidney disease and all treatment options. Therefore, the objective of this study was to investigate the psychometric properties of a questionnaire that assesses patients' knowledge on kidney disease and renal replacement therapies for use in research and practice. Methods: A 30-item list was validated in 187 patients on dialysis and in 83 patients who were undergoing living donor kidney transplantation the following day. Additionally, the test was administered to 2 representative reference groups from the general population of Dutch residents (n=515) and North American residents (n=550) using a web-based survey. The test is available in 9 languages. Firstly, using the 2PL model from Item Response Theory we assessed Differential Item Functioning (DIF) for all the groups. Secondly, we examined the difficulty and discriminative properties of the questionnaire by using Multidimensional IRT (MIRT). Thirdly, norm-references were calculated. Results: Almost all items showed good discrimination and threshold parameters based on the fitted 2PL model. DIF was found for 5 redundant items which would distort trait level estimates. MIRT analyses were subsequently employed for the remaining 25 items. Two stable dimensions with 21 items were retrieved for which norm-references for the dialysis and transplantation group were calculated. The first dimension 'Dialysis and Transplantation' contains 11 items and the second dimension 'Living Donation' contains 10 items.
Conclusions: This study resulted in a questionnaire, the R3K-T, which enables reliable testing of patient's knowledge on kidney disease and treatment options. Further validation of the R3K-T in more specific groups, such as living kidney donors, for which subscale scores may contain clinically relevant information would increase practical rigor of this test.

Public solicitation of organs from living donors – an ELPAT view

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The issue of public solicitation is amongst the most controversial in living organ donation. The objective of our paper is to critically assess the arguments concerning public solicitation and to offer recommendations. While the legal framework is not that different between the EU and US (both condemn financially-driven solicitation), the practices of both transplant centers and of individuals needing a transplant vary. The main difference can be observed between certain liberal practices in the US (where one can find commercially operated websites soliciting organs altruistically donated from living donors), and more conservative practices in the EU (where such websites are absent). This is an attempt to clarify the terminology concerning public solicitation, the different levels of public solicitation, and the motivations of recipients and donors. Firstly we elaborate an operational definition for public solicitation that is consistent with the ELPAT classification of living donors. Secondly we evaluate the various arguments from the literature, both in favor of public solicitation and against it. Although they look contradictory, in most cases the same arguments are used both to defend the legitimacy of public solicitation and to condemn it. The arguments are classified according to the manner in which they influence the actions of recipients and/or donors, and regarding the influence on the donation/transplantation process at individual and societal level. Finally, we offer a set of recommendations. While we do not recommend it as a general practice, in our opinion, the acceptability of public solicitation by the patient or medical team could be explored for special cases, e.g. highly sensitized individuals or other patients with little chance of receiving a transplant otherwise.

Psychosocial screening of the unspecified living kidney donors in The Netherlands

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Background: The first unspecified donation (formerly known as anonymous or altruistic donation) was performed in 2000 in Rotterdam. Since 2007 unspecified living kidney donors have been accepted in all 8 university centers in The Netherlands. However, little is known about the psychosocial screening of these donors. In this study we describe the psychosocial screening of unspecified living kidney donors in the 8 kidney transplant centers in The Netherlands. Methods: We collected data about which professionals conduct the screening, which validated instruments are used, and what the psychosocial contraindications are. From each center the living donor coordinator responded to this questionnaire. Results: In all centers a multidisciplinary team is involved in the screening of unspecified living kidney donors. Most donors are screened by a nephrologist, social worker and a nurse practitioner / living donor coordinator. A consultation is also included with a psychologist or psychiatrist. One center added an independent physician for the unspecified donor screening. Four centers use the SCL-90 (a clinical diagnostic self-report scale for psychological complaints). One center uses a NEO-Five Factor Inventory (NEO-FFI) assessment (a personality test). Three centers do not use validated questionnaires. In all centers the psychologist used the same topics during the interview: motivation for donation, reality awareness, realistic expectations of donation, and psychiatric illness in the past. Contraindications include active psychosis or addiction, personality disorder and psychosocial instability. In one center age is a contraindication, unspecified donors must be older than 25 years.
Conclusion: All centers screened the unspecified living kidney donors with a psychologist / psychiatrist using an in-depth clinical interview. Sixty-three percent of the team used additional validated questionnaires. Psychosocial screening of unspecified living kidney donors is based on the National and International guidelines, but there are variations in the contraindications used.

Altruistische donatie

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De afgelopen jaren zien we in NL een toename van het aantal transplantaties met altruïstische donoren. Altruïstische donoren zijn mensen die hun nier beschikbaar stellen zonder dat ze een nierpatiënt persoonlijk kennen. Zij willen anderen behulpzaam zijn met geen of nauwelijks eigenbelang. Het ontstaan van deze vorm van donatie is niet duidelijk aan te geven, mogelijk door mond op mond reclame en/of media-aandacht. In ons ziekenhuis hebben we de afgelopen jaren een stabiel aantal altruïstische donaties gedaan: 2008: 2 2009: 3 2010: 4 2011: 3 2012: 3. In totaal hebben we met deze 15 donoren 25 niertransplantaties kunnen doen. In totaal hebben 32 altruïsten een intakegesprek gehad, waarvan 15 getransplanteerd,2 gepland,6 afgehaakt na gesprek=22% (gerichte donatie 0,05%),5 afgekeurd = 16% (gerichte donatie 15%),1 afgehaakt na screening, 2 in voorbereiding. Bij de psychologische beoordeling is er niemand afgewezen, bij gerichte donatie wordt dit niet gedaan. De motivatie voor donatie was globaal:, bekend met nierpatiënt:8, geloofsovertuiging:3, iets goeds doen:12, n.a.v. Tv-uitzending: 7, waarvan 5 aanmeldingen in oktober/november 2012, deze mensen gaven aan dat zij door een tv-uitzending het laatste zetje kregen om met ons contact op te nemen. (donorweek+live-operatie). De vraag is hoe gaan we om met deze mogelijke toename.
Conclusie; altruïstische donoren in de intakefase haken vrij vaak af 22 % tegenover 0.05% in de gerichte groep, tijdens de screeningfase worden er gemiddeld een gelijk aantal afgekeurd. Concluderend lijkt een goede voorlichting van essentieel belang is om zo goed mogelijk gebruik te maken van de altruïstische donor.

Painscore and patients preferences in the repetitive use of the Dried Blood Spot method in comparison to venous puncture in renal transplant recipients

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Introductie*:* Het is gebruikelijk om de bloedconcentratie van immunosuppressiva te controleren om toxiciteit tgv overdosering en verhoogde kans op rejectie tgv onderdosering te vermijden. Gebruikelijk wordt dit gedaan dmv veneuze afname van een zogenaamde dalspiegel, waarvoor de patiënt 's ochtends naar het ziekenhuis moet komen. Dit is erg belastend voor de patiënt en geeft een piekbelasting bij de afdeling bloedafname en op de polikliniek. Daarom is door ons de bloedspotmethode ontwikkeld tbv het bepalen van de tacrolimus dalspiegel. Patiënten kunnen hierdoor thuis zelf het bloedmonster afnemen en per post op te sturen. De toepasbaarheid in de praktijk heeft zich bewezen, echter het oordeel van de patiënt in vergelijking met de veneuze afname was nog niet goed onderzocht. Methoden: In het kader van een studie waren 40 patiënten voor de DBS getraind (16 vrouwen en 24 mannen, gemiddeld 4.5 jaar na Tx). Deze groep heeft in totaal 136 vergelijkingen gedaan. Meteen na elkaar werd in het ziekenhuis een veneuze afname door de verpleegkundige en een vingerprik tbv de bloedspotmethode door de patiënt verricht. De patiënt noteerde hierna op een Visual Analogue Scale van 0 – 10 (VAS)zijn pijnbeleving en zijn perceptie van moeilijkheid om de bloodspot uit te voeren. Resultaten: De patiënten scoorden de DBS als duidelijk minder pijnlijk dan de veneuze afname, ongeacht de dikte van de benodigde naals bij de DBS methode. Zij ervaarden de methode van DBS als zeer gemakkelijk.
Conclusie*:* Patiënten geven de voorkeur aan bloedafname middels de DBS-methode tov de veneuze bloedafname t.a.v. pijnlijkheid. en gemak van afname.

Who has high expectations of donation? Exploring the psychological profile of living kidney donors

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Objective**:** High expectations regarding personal consequences of donation (e.g. personal growth) are suggested to be an important component in the psychosocial screening of potential living kidney donors. However, little is known about who has high expectations and what the potential consequences may be. In this study, we explored the relationship between the psychological profile of living kidney donors and their expectations before donation. Methods**:** A cohort of potential living kidney donors (N=137) completed the following questionnaires before donation: Living Donation Expectancies Questionnaire, Brief Symptom Inventory, Positive And Negative Affect Schedule, Satisfaction With Life Scale, Mental Health Continuum-Short Form, stress-subscale of the Depression Anxiety Stress Scale, Brief COPE, Social Support List Interactions, and the Social Support List Discrepancies. We obtained sociodemographic characteristics from medical records. Results**:** Using multiple linear regression analyses we found that higher expectations regarding Interpersonal Benefit were related to higher depression, higher negative affect, lower phobic anxiety, and higher experienced social support. Higher expectations regarding Personal Growth were related to higher negative affect and a lower level of education. Expectations regarding Spiritual Benefit were higher if the donor had a religious affiliation and among those with a lower education. Higher expectations regarding negative Health Consequences were related to older age, higher negative affect, less use of an active coping style, and a lower level of education.
Conclusions**:** Donors with higher negative affect and lower education had higher positive and negative expectations regarding the donation process. What is not yet clear from these data is the direction of causality between these factors. An important question is the extent to which high expectations of donation relate to subsequent mental health after donation. We are currently investigating this in a prospective cohort study among all our living donors.

MijnKinderNierNet

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MijnKinderNierNet is een onderdeel van MijnZorgnet (www.MijnZorgnet.nl). MijnZorgnet is het sociale digitale netwerk e zorg. Het is een beveiligde ontmoetingsplek waar patiënten elkaar en ook hun zorgverleners kunnen ontmoeten. Patiënten kunnen zich aansluiten bij (besloten) groepen die voor hen belangrijk zijn. Op MijnKinderNierNet zijn de groepen kinderen in predialyse, dialyse en niertransplantatie vertegenwoordigd. Patiënten en ouders kunnen na aanmelding ervaringen met elkaar uitwisselen, hulpverleners vragen stellen en informatie vinden m.b.t. de diverse nierfunctievervangende therapieën en andere wetenswaardigheden m.b.t. ziekte, therapie, vergoedingen en regelgeving. Tevens heeft men de mogelijkheid om een eigen persoonlijk dossier bij te houden. In MijnKinderNierNet komen alle aspecten bij elkaar en kan de patiënt de juiste informatie vinden op het door hem gewenste tijdstip. tijdstip. Voor de zorgverleners betekent MijnKinderNierNet een forum waarop we direct met de patiënten in contact kunnen komen en waarop we hen snel kunnen informeren over nieuwsfeiten e.d. Zodra een bericht op MijnKinderNierNet wordt geplaatst ontvangen de leden automatisch een email. De functie van "chatten met de dokter" zal begin 2013 worden geïmplementeerd. Hierbij is er sprake van een virtueel spreekuur waarbij een van de zorgverleners vragen van de patiënten direct kan beantwoorden. MijnKinderNierNet brengt patiënten onderling, maar ook patiënten en zorgverleners dichter bij elkaar. In mijn presentatie zal ik ingaan op ervaringen vanuit patiënten- en zorgverlenerperspectief.

Aanmeldingsformulier lidmaatschap

naam en voorletters			m / v
voornaam			geb. datum:
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specialisme / functie			
doctoraal examen	neen / ja d.d.	zo ja, studierichting:	
arts examen	n.v.t. / ja d.d.		
inschrijving MSRC	neen / ja d.d.	BIG registratie nr.	
huisadres			
postcode en plaats			
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