Proyecto Prometeo II

Monitorización del trasplante renal: ¿tiene impacto clínico?

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Grupo III | Monitorización histológica

Portavoz | **Francesc Moreso**
Hospital Vall d'Hebrón, Barcelona

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GRUPO 3-Monitorización Histológica (Dr. Francesc Moreso)

	Apellidos	Nombre	Hospital	Ciudad	Artículos asignados	Nº Art.
1	Cañas	Laura	Hospital Univ. Germans Trias i Pujol	Badalona (Barcelona)	**1. Chronic Renal Allograft Damage: Existing Challenges.** Transplantation 2011; 91 (suppl 9): S10-S15. Arias M, Seron D, Moreso F, Bestard O, Praga M. **2. Antibody-mediated vascular rejection of kidney allografts: a population-based study.** The Lancet 2013; 381: 313-319. Lefaucher C, Loupy A, Vernery D et al. **3. Specificity of Histological Markers of Long-Term CNI Nephrotoxicity in Kidney-Transplant Recipients Under Low-Dose Cyclosporine Therapy** American Journal of Transplantation. 2011;11:2635–46 Snanoudj R, Royal V, Elie C, Rabant M, Girardin C, Morelon E, Kreis H, Fournet JC, Noël LH, Legendre C. **4. ABO blood group-incompatible living donor kidney transplantation: a prospective, single-centre analysis including serial protocol biopsies.** Nephrol Dial Transplant 2009; 24: 298-303. Oettl T, Halter J, Bachmann A et al. **5. IgA nephropathy recurs early in the graft when assessed by protocol biopsy.** Nephrol Dial Transplant 2011; 0: 1–6 Ortiz F, Gelpi R, Koskinen P.	**10** **20** **30** **41** **50**
2	Amenábar	Juan José	Hospital de Cruces	Bilbao	**1. Do protocol transplant biopsies improve kidney transplant outcomes?** Curr Opin Nephrol Hypertens. 2012;21:580–86 Chapman JR **2. Early subclinical rejection as a risk factor for late chronic humoral rejection.** Transplantation 2012; 93:41–46. Moreso F, Carrera M, Goma M, et al. **3. Tacrolimus exposure and evolution of renal allograft histology in the first year after transplantation.** Am J Transplant 2007; 7:2114–2123. Naesens M, Lerut E, Damme BV, et al. **4. Analysis of Renal Transplant Protocol Biopsies in ABO-Incompatible Kidney Transplantation** American Journal of Transplantation. 2008;8:86–94 Setoguchi K, Ishida H, Shimmura H, Shimizu T, Shirakawa H, Omoto K, Toki D, Iida S, Setoguchi S, Tokumoto T, Horita S, Nakayama H, Yamaguchi Y, Tanabe K. **5. Fibrosis progression according to epithelial-mesenchymal transition profile: a randomized trial of everolimus versus CsA.** Am J Transplant. 2015 May;15(5):1303-12. doi: 10.1111/ajt.13132. Epub 2015 Mar 23. Rostaing L, Hertig A, Albano L, Anglicheau D, Durrbach A, Vuiblet V, Moulin B, Merville P, Hazzan M, Lang P, Touchard G, Hurault deLigny B, Quéré S, Di Giambattista F, Dubois YC, Rondeau E; CERTITEM Study Group.	**9** **19** **29** **40** **49**

3	Vilalta	Ramón	Hospital Vall d'Hebrón	Barcelona	**1. Protocol biopsies in renal transplantation: Prognostic value of structural monitoring.** Kidney Int 2007; 72: 690-697. Seron D, Moreso F. **2. Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant.** Am J Transplant 2012; 12:1157–1167. Wiebe C, Gibson IW, Blydt-Hansen TD et al. **3. No Difference in Degree of Interstitial Sirius Red–Stained Area in Serial Biopsies from Area under Concentration-over-Time Curves–Guided Cyclosporine versus Tacrolimus-Treated Renal Transplant Recipients at One Year.** J Am Soc Nephrol 2006; 17: 305-312. Rowsahni AT, Scholten EM, Bemelman F et al. **4. C4d and C3d staining in biopsies of ABO- and HLA-incompatible renal allografts: correlation with histologic findings.** Am J Transplant 2006; 6: 1829-1840. Haas M, Rahman RH, Racusen LC et al. **5. Adverse Outcomes of Tacrolimus Withdrawal in Immune–Quiescent Kidney Transplant Recipients.** J Am Soc Nephrol 2015; doi: 10.1681/ASN.2014121234. Hricik DE, Formica RN, Nickerson P et al.	**8** **18** **28** **39** **48**
4	Bernis Carro	Carmen	Hospital Universitario de la Princesa	Madrid	**1. Long-term results of biopsy-guided selection and allocation of kidneys from older donors in older recipients.** Am J Transplant 2012; 12: 2781-2788. Fernandez-Lorente L, Riera L, Bestard O et al. **2. The histology of solitary renal allografts at 1 and 5 years after transplantation.** Am J Transplant 2011; 11: 698-707. Stegall MD, Park WD, Larson TS et al. **3. Complete Avoidance of Calcineurin Inhibitors in Renal Transplantation: A Randomized Trial Comparing Sirolimus and Tacrolimus.** Am J Tranplant 2006; 6: 514-522 Larson TS, Dean PG, Stegall MD et al. **4. Subclinical Rejection in Stable Positive Crossmatch Kidney Transplant Patients: Incidence and Correlations** American Journal of Transplantation 2009;9:1826–34 Kraus ES, Parekh RS, Oberai P, Lepley D, Segev DL, Bagnasco S, Collins V, Leffell M, Lucas D, Rabb H, Racusen LC, Singer AL, Stewart ZA, Warren DS, Zachary AA, Haas M, Montgomery RA. **5. Angiotensin II blockade in kidney transplant recipients.** J Am Soc Nephrol 2013; 24: 320–327 Ibrahim HN, Jackson S, Connaire J et al.	**7** **17** **27** **38** **53**
5	Calvo	Natividad	Hospital Clínico San Carlos	Madrid	**1. Long-term outcome of renal transplantation from older donors.** N Engl J Med 2006; 354: 343–352. Remuzzi G, Cravedi P, Perna A et al. **2. Inflammation in Areas of Tubular Atrophy in Kidney Allograft Biopsies: A Potent Predictor of Allograft Failure.** Am J Transplant 2010; 10: 2066-2073. Mannon RB, Matas AJ, Grande JP et al. **3. Costimulation blockade with belatacept in renal transplantation.** N Eng J Med 2005; 335:770-781. Vincenti F, Larsen C, Durrbach A et al. **4. Subclinical Acute Antibody-Mediated Rejection in Positive Crossmatch Renal Allografts.** Am J Transplant 2006; 6: 1-10. Haas M, Montgomery RA, Segev DL et al. **5. The impact of surveillance and rapid reduction in immunosuppression to control BK virus-related graft injury in kidney transplantation** Transplant International. 2013;26(8):822-32. ISSN 0934-0874 Elfadawy N, Flechner SM, Liu X, Schold J, Tian D, Srinivas TR, Poggio E, Fatica R, Avery R, Mossad SB.	**6** **16** **26** **37** **52**

6	Crespo Albiach	José Francisco	Hospital Universitario Doctor Peset	Valencia	**1. The reproducibility and predictive value on outcome of renal biopsies from expanded criteria donors.** Kidney Int 2014; 85 (5): 1161-8. Azancot MA, Moreso F, Salcedo M et al. **2. Presence of FoxP3+ regulatory T cells predicts outcome of subclinical rejection of renal allografts.** J Am Soc Nephrol 2008; 19: 2020-2026. Bestard O, Cruzado JM, Rama I et al. **3. De novo kidney transplantation without use of calcineurin inhibirtors preserves renal structure and function at two years.** Am J Transplant 2004; 4: 1776-1785. Flechner SM, Kurian SM, Solez K et al. **4. Histologic findings one year after positive crossmatch or ABO blood group incompatible living donor kidney transplantation.** Am J Transplant 2006; 6: 1841–1847. Gloor JM, Cosio FG, Rea DJ et al. **5. Impact of Early Conversion From Tacrolimus to Sirolimus on Chronic Allograft Changes in Kidney Recipients on Rapid Steroid Withdrawal** Transplantation. 2012;93: 47–53 Heilman RL, Cortese C, Geiger XJ, Younan K, Wadei HM, Mai ML, Reddy KS, Gonwa TA.	**5** **15** **25** **36** **47**
7	Delgado Mallen	Patricia	Hospital Universitario de Canarias	La Laguna - Tenerife	**1. The predictive value of kidney allograft baseline biopsies for long-term graft survival.** J Am Soc Nephrol 2013; 24: 1913-1923. De Vusser K, Lerut E, Kuypers D et al. **2. Subclinical rejection associated with chronic allograft nephropathy in protocol biopsies as a risk factor for late graft loss.** Am J Transplant 2006; 6: 747-752. Moreso F, Ibernon M, Gomà M et al. **3. Increased C4d in post-reperfusion biopsies and increased donor specific antibodies at one-week posttransplant are risk factors for acute rejection in mild to moderately sensitized kidney transplant recipients** Kidney International. 2013;83:1185–92 Djamali A, Muth BL, Ellis TM, Mohamed M, Fernandez LA, Miller KM, Bellingham JM, Odorico JS, Mezrich JD, Pirsch JD, D'Alessandro TM, Vidyasagar V, Hofmann RM, Torrealba JR, Kaufman DB, Foley DP. 4**5. Assessment of the risk of chronic allograft dysfunction after renal transplantation in a randomized cyclosporine withdrawal trial.** Transplantation 2006; 82: 657-662. Hazzan M, Buob D, Lavalette M et al.	**4** **14** **35** **46**
8	Gallego Samper	Roberto	Hospital Dr. Negrín	Las Palmas	**1. The Maryland aggregate pathology index: a deceased donor kidney biopsy scoring system for predicting graft failure.** Am J Transplant 2008; 8: 2316–2324. Munivenkatappa RB, Schweitzer EJ, Papadimitriou JC et al. **2. The natural history of chronic allograft nephropathy.** N Eng J Med 2003; 349: 2326-2333. Nankivell BJ, Borrows RJ, Chang CLS et al. **3. Sirolimus-based therapy following early cyclosporine withdrawal provides significantly improved renal histology and function at 3 years.** Am J Transplant 2004; 4: 953-961, 2004. Mota A, Arias M, Taskinen EI y cols. **4. Transplant glomerulopathy: subclinical incidence and association with alloantibody.** Am J Transplant 2007; 7: 2124-2132. Gloor J, Sethi S, Stegall MD et al. **5. Renal transplantation: can we reduce calcineurin inhibitor/stop steroids? Evidence based on protocol biopsy findings.** J Am Soc Nephrol. 2003; 14 (3): 755-66. Gotti E, Perico N, Perna A et al.	**3** **13** **24** **34** **45**

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9	Suárez Fernández	Mª Luisa	Hospital Central de Asturias	Oviedo	**1. A simple clinic-histopathological composite scoring system is highly predictive of graft outcomes in marginal donors.** Am J Transplant 2008; 8: 2325–2334. Anglicheau D, Loupy A, Lefaucheur C et al. **2. Seguridad y eficacia de la biopsia ambulatoria en trasplante renal.** Nefrología 2014; 34 (6): 749-755. Torres-Rodriguez IB, Castella-Fierro E, Serres-Creixell X et al. **3. Comparison of four different immunosuppression protocols without long-term steroid therapy in kidney recipients monitored by surveillance biopsy: five-year outcomes.** Transpl Immunol 2008; 20:32–42. Anil Kumar MS, Irfan Saeed M, Ranganna K, et al. **4. Donor-specific antibodies accelerate arteriosclerosis after kidney transplantation.** J Am Soc Nephrol 2011; 22: 975-983. Hill GS, Nochy D, Bruneval P et al. **5. Kidney Allograft Survival After Acute Rejection, the Value of Follow-Up Biopsies** American Journal of Transplantation. 2013;13:2334–41 El Ters M, Grande JP, Keddis MT, Rodrigo E, Chopra B, Dean PG, Stegall MD, Cosio FG	**2** **12** **23** **33** **44**
10	**Moreso**	**Francesc**	**Hospital Vall d'Hebrón**	**Barcelona**		
11	Rodríguez	Manuel Ángel	Hospital Torrecárdenas	Almería	**1. Glomerular function, structure, and number in renal allografts from older deceased donors.** J Am Soc Nephrol 2009; 20: 181-188. Tan JC, Workeneh B, Busque S, Blouch K, Derby G, Myers BD. **2. Protocol biopsy of the stable renal transplant: A multicenter study of methods and complication rates.** 2003; Transplantation 76: 969–973, 2003. Furness PN, Philpott CM, Chorbadjian MT et al. **3. Capillary C4d and Kidney Allograft Outcome in Relation to Morphologic Lesions Suggestive of Antibody-Mediated Rejection.** Clin J Am Soc Nephrol. 2015 Jun 12. pii: CJN.09901014. [Epub ahead of print] Kikić Ž, Kainz A, Kozakowski N, Oberbauer R, Regele H, Bond G, Böhmig GA. **4. Significance of C4d Banff Scores in Early Protocol Biopsies of Kidney Transplant Recipients with Preformed Donor-Specific Antibodies (DSA)** American Journal of Transplantation 2011;11: 56–65 Loupy A, Hill GS, Suberbielle C, Charron D, Anglicheau D, Zuber J, Timsit MO, Duong JP, Bruneval P, Vernerey D, Empana JP, Jouven X, Nochy D, Legendre CH. **5. Repeat True Surveillance Biopsies in Kidney Transplantation** Transplantation 2012;93:908-13 Buchmann TN, Wolff T, Bachmann A, Guerke L, Steiger J, Mihatsch MJ, Dickenmann M.	**1** **11** **22** **32** **43**

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11	Rodrigo	Emilio	Hospital Marqués de Valdecilla	Santander	**1. Subclinical Rejection Phenotypes at 1 Year Post-Transplant and Outcome of Kidney Allografts.** J Am Soc Nephrol. 2015 Jan 2. pii: ASN.2014040399. [Epub ahead of print] Loupy A, Vernerey D, Tinel C, Aubert O, Duong van Huyen JP, Rabant M, Verine J, Nochy D, Empana JP, Martinez F, Glotz D, Jouven X, Legendre C, Lefaucheur C. **2. Early corticosteroid withdrawal in recipients of renal allografts: a single-center report of ethnically diverse recipients and recipients of marginal deceased-donor kidneys.** Transplantation 2012; 94 (8): 837-44. Aull MJ, Dadhania D, Afaneh C et al. **3. Lack of benefit of early protocol biopsies in renal transplant patients receiving TAC and MMF: a randomized study.** Am J Transplant 2007; 7:2538–2545. Rush D, Arlen D, Boucher A et al. **4. Recurrent idiopathic membranous nephropathy: early diagnosis by protocol biopsies and treatment with anti-CD20 monoclonal antibodies.** Am J Transplant 2009; 9: 2800-2807. El Zoghby ZM, Grande JP, Fraile MG et al.	**21** **31** **42** **51**

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1. Glomerular function, structure, and number in renal allografts from older deceased donors. J Am Soc Nephrol 2009; 20: 181-188.

Tan JC, Workeneh B, Busque S, Blouch K, Derby G, Myers BD.

ABSTRACT:

The 5-yr survival rate of renal allografts is significantly lower for grafts from older deceased donors than from younger deceased donors. For evaluation of the potential contribution of renal senescence in this shortened graft survival, glomerular function and structure were analyzed in allografts from deceased donors older than 55 yr ("aging") or younger than 40 yr ("youthful"). Aging donors had a significantly higher prevalence of sclerotic glomeruli ($P < 0.002$), and their nonsclerotic glomeruli tended to be larger, had a larger filtration surface area ($P = 0.02$), and had a higher single-nephron ultrafiltration coefficient (K_f; $P = 0.07$), suggesting a compensatory response to functional loss of glomeruli. After serum creatinine reached a stable nadir in the transplant recipients, GFR and its hemodynamic determinants were evaluated and the whole allograft Kf was computed. Compared with the allografts from youthful donors, allografts from aging donors exhibited a 32% lower GFR, which was exclusively attributable to a 45% reduction in allograft Kf (both $P < 0.001$). In addition, the number of functioning glomeruli per allograft was profoundly lower in grafts from aging donors than from youthful donors ($3.6 \pm 2.1 \times 10^5$ versus $8.5 \pm 3.4 \times 10^5$; $P < 0.01$), and this could not be explained by the relatively modest 17%prevalence of global glomerulosclerosis in the aging group. The marked reduction in overall glomerular number in many aging donors may lead to a "remnant kidney" phenomenon, potentially explaining the shorter mean survival of these allografts.

2. A simple clinic-histopathological composite scoring system is highly predictive of graft outcomes in marginal donors.

Am J Transplant 2008; 8: 2325–2334.

Anglicheau D, Loupy A, Lefaucheur C et al.

The predictive value of pre-implantation biopsies versus clinical scores has not been studied extensively in marginal donors. Pre-implantation biopsies were performed in 313 kidneys from donors that were ≥ 50 years of age (training set, n = 191; validation set, n = 122). The value of the donor clinical parameters and histological results in predicting 1-year estimated glomerular filtration rate (eGFR) <25 mL/min/1.73 m2 was retrospectively evaluated. In multivariate analysis, the only clinical parameters associated with low eGFR were donor hypertension and a serum creatinine level ≥150 lmol/L before organ recovery. Clinical scores (Nyberg and Pessione) were not significantly associated with graft function. Regarding histological parameters, univariate analysis showed that glomerulosclerosis (GS) (p = 0.02), arteriolar hyalinosis (p = 0.03) and the Pirani (p = 0.02) and chronic allograft damage index (CADI) (p = 0.04) histological scores were associated with low eGFR. The highest performance in predicting low eGFR was achieved using a composite score that included donor serum creatinine (≥150 lmol/L or <150 lmol/L), donor hypertension and GS (≥10% or <10%). The validation set confirmed the critical importance of taking into account biopsy and clinical parameters during marginal donor evaluation. In conclusion, clinical scores are weak predictors of graft outcomes with marginal donors. Instead, a simple and convenient composite score strongly predicts graft function and survival and may facilitate optimal allocation of marginal donors.

3. The Maryland aggregate pathology index: a deceased donor kidney biopsy scoring system for predicting graft failure.

Am J Transplant 2008; 8: 2316–2324.

Munivenkatappa RB, Schweitzer EJ, Papadimitriou JC et al.

Despite the common use of diagnostic pretransplant deceased donor kidney biopsy, there is no consensus on the prognostic significance of the pathologic findings. In order to assist clinicians with interpretation we analyzed 371 pretransplant biopsies and correlated the findings with graft failure. Glomerular pathology was assessed with percent glomerulosclerosis (GS), glomerular size and periglomerular fibrosis (PGF); vascular pathology with arterial wall-to-lumen ratio (WLR) and arteriolar hyalinosis and interstitial pathology with measurement of cumulative fibrosis and presence of scar. Using two-thirds of the study population as a model-development cohort, we found that biopsy features independently associated with an increased risk of graft failure were GS ≥15%, interlobular arterial WLR ≥0.5 and the presence of PGF, arteriolar hyalinosis or scar. The Maryland Aggregate Pathology Index (MAPI), was developed from these parameters and validated on the remaining one-third of the population. Five-year actuarial graft survival was 90% for kidneys with MAPI scores between 0 and 7, 63% for scores from 8 to 11 and 53% forscoresfrom12to15($p < 0.001$). We conclude MAPI may help transplant physicians estimate graft survival from the preimplantation biopsy findings, in clinical situations similar to this study population (cold ischemia over 24 h, GS < 25%).

4. The predictive value of kidney allograft baseline biopsies for long-term graft survival.
J Am Soc Nephrol 2013; 24: 1913-1923.
De Vusser K, Lerut E, Kuypers D et al.

ABSTRACT:
The effect of baseline histology and individual histologic lesions at the time of transplantation on long-term graft survival has been evaluated using different scoring systems, but the predictive capacity of these systems has not been adequately validated. All kidney recipients transplanted in a single institution between 1991 and 2009 who underwent a baseline kidney allograft biopsy at transplantation were included in this prospective study (N=548). All baseline biopsies were rescored according to the updated Banff classification, and the relationship between the individual histologic lesions and donor demographics was assessed using hierarchical clustering and principal component analysis. Survival analysis was performed using Cox proportional hazards analysis and log-rank testing. Mean follow-up time was 6.7 years after transplantation. Interstitial fibrosis, tubular atrophy, and glomerulosclerosis associated significantly with death-censored graft survival, whereas arteriolar hyalinosis and vascular intimal thickening did not. Notably, donor age correlated significantly with interstitial fibrosis, tubular atrophy, and glomerulo-sclerosis and associated independently with graft survival. On the basis of these findings, a novel scoring system for prediction of 5-year graft survival was constructed by logistic regression analysis. Although the predictive performance of previously published histologic scoring systems was insufficient to guide kidney allocation in our cohort (receiver operating characteristic area under the curve #0.62 for each system), the new system based on histologic data and donor age was satisfactory for prediction of allograft loss (receiver operating characteristic area under the curve = 0.81) and may be valuable in the assessment of kidney quality before transplantation.

5. The reproducibility and predictive value on outcome of renal biopsies from expanded criteria donors.

Kidney Int 2014; 85 (5): 1161-8.

Azancot MA, Moreso F, Salcedo M et al.

Reproducibility and predictive value on outcome are the main criteria to evaluate the utility of histological scores. Here we analyze the reproducibility of donor biopsy assessment by different on-call pathologists and the retrospective evaluation by a single renal pathologist blinded to clinical outcomes. We also evaluate the predictive value on graft outcome of both evaluations. A biopsy was performed in donors with any of the following: age X55 years, hypertension, diabetes, creatinine 41.5 mg/dl, or stroke. Glomerulosclerosis, interstitial fibrosis, tubular atrophy, intimal thickening, and arteriolar hyalinosis evaluated according to the Banff criteria were added to obtain a chronic score. Biopsies were classified as mild (X3), intermediate (4–5), or advanced (6–7) damage, and unacceptable (X8) for transplantation of 127 kidneys biopsied. Weighted j value between both readings was 0.41 (95% CI: 0.28–0.54). Evaluation of biopsies by the renal pathologist was significantly and independently associated with estimated 12-month glomerular filtration rate and a significant composite outcome variable, including death-censored graft survival and time to reach an estimated glomerular filtration rate o30 ml/min per 1.73 m2. Thus, there was no association between readings of on-call pathologists and outcome. The lack of association between histological scores obtained by the on-call pathologists and graft outcome suggests that a specific training on renal pathology is recommended to optimize the use of kidneys retrieved from expanded criteria donors.

6. Long-term outcome of renal transplantation from older donors.
N Engl J Med 2006; 354: 343–352.
Remuzzi G, Cravedi P, Perna A et al.

Background: Long-term survival of kidney grafts from older donors is inferior to that of grafts from younger donors. We sought to determine whether selecting older kidneys according to their histologic characteristics before implantation would positively influence long-term outcome.
Methods: In a prospective cohort study, we assessed outcomes among 62 patients who received one or two histologically evaluated kidneys from donors older than 60 years of age. These outcomes were compared with outcomes among 248 matched recipients of single kidney grafts that had not been histologically evaluated and were either from donors 60 years of age or younger (124 positive-reference recipients who, according to available data, were expected to have an optimal outcome) or from those older than 60 years (124 negative-reference recipients, expected to have a worse outcome). The primary end point was graft survival.
Results: During a median period of 23 months, 4 recipients (6 percent) of histologically evaluated kidneys progressed to dialysis, as compared with 7 positive-reference recipients (6 percent) and 29 negative-reference recipients (23 percent). Graft survival in recipients of histologically evaluated kidneys did not differ significantly from that of grafts in positive-reference recipients but was superior to that of grafts in negative-reference recipients (hazard ratio for graft failure in the negative-reference recipients relative to the recipients of histologically evaluated kidneys, 3.68; 95 percent confidence interval, 1.29 to 10.52; P = 0.02). The performance of preimplantation histo-logic evaluation predicted better survival both in the whole study group (P = 0.02) and among recipients of kidneys from older donors (P = 0.01).
Conclusions: The long-term survival of single or dual kidney grafts from donors older than 60 years of age is excellent, provided that the grafts are evaluated histologically before im-plantation. This approach may help to expand the donor-organ pool for kidney trans-plantation.

7. Long-term results of biopsy-guided selection and allocation of kidneys from older donors in older recipients.

Am J Transplant 2012; 12: 2781-2788.

Fernandez-Lorente L, Riera L, Bestard O et al.

ABSTRACT:

In our old-for-old program, we discard or allocate older extended criteria donor kidneys to single (SKT) or dual kidney transplantation (DKT) depending on histological Remuzzi's score in recipients older than 60 years. Here, we analyze the long-term results of this program and try to identify independent predictors of patient and graft survival. Between December 1996 and January 2008, we performed 115 SKT and 88 DKT. Discard rate was 15%. Acute rejection incidence was higher in SKT than in DKT (22.6% vs. 11.4%, p = 0.04). Renal function was better in DKT than in SKT up to 5 years after transplantation. Surgical complications were frequent in DKT. Ten-year cumulative graft survival was significantly lower in the SKT group (31% vs. 53%, p = 0.03). In SKT, histological score 4 provided similar graft survival than 3 or less, whereas in DKT score 4, 5 or 6 displayed similar outcome. Finally, independent predictors of graft survival were history of major adverse cardiac event and 1-year serum creatinine, rather than SKT or DKT. In conclusion, this biopsy-guided old-for-old strategy resulted in acceptable long-term graft survival. Our results suggest that DKT should be considered for scores of 5 or 6 only.

8. Protocol biopsies in renal transplantation: Prognostic value of structural monitoring.
Kidney Int 2007; 72: 690-697.
Seron D, Moreso F.

ABSTRACT:
the natural history of renal allograft damage has been characterized in serial protocol biopsies. The prevalence of subclinical rejection (SCR) is maximal during the first months and it is associated with the progression of interstitial fibrosis/tubular atrophy (IF/TA) and a decreased graft survival. IF/TA rapidly progress during the first months and constitutes an independent predictor of graft survival. IF/TA associated with transplant vasculopathy, SCR, or transplant glomerulopathy implies a poorer prognosis than IF/TA without additional lesions. These observations suggest that protocol biopsies could be considered a surrogate of graft survival. Preliminary data suggest that the predictive value of protocol biopsies is not inferior to acute rejection or renal function. Additionally, protocol biopsies have been employed as a secondary efficacy variable in clinical trials. This strategy has been useful to demonstrate a decrease in the progression of IF/TA in some calcineurin-free regimens. Quantification of renal damage is associated with graft survival suggesting that quantitative parameters might improve the predictive value of protocol biopsies. Validation of protocol biopsies as a surrogate of graft survival is actively pursued, as the utility of classical surrogates of graft outcome such as acute rejection has become less useful because of its decreased prevalence with actual immunosuppression.

9. Do protocol transplant biopsies improve kidney transplant outcomes?

Curr Opin Nephrol Hypertens. 2012;21:580–86

Chapman JR

ABSTRACT:

Purpose of review: The research undertaken on 'protocol' renal transplant biopsies has provided a rich, if not the richest, approach to better understanding of the immune and nonimmune impacts upon the transplant. The purpose of this review is to detail how the direct benefit to the patient also lies in these renamed 'surveillance' biopsies.
Recent findings: Undertaken at fixed time points after transplantation, biopsy provides individual diagnoses with which the clinician can vary immunosuppression both in intensity and in the type of agent used to modify pathological processes early in their course. Initial nonfunction from acute tubular necrosis, subclinical cellular and humoral rejection, calcineurin inhibitor nephrotoxicity, BK virus nephropathy and recurrent glomerulonephritis are all important diagnoses for which early intervention provides better therapeutic outcomes than delaying until they are clinically evident.
Summary: This review provides the recent evidence that has convinced many transplant units to embark upon surveillance programmes for their patients in order to individualize their immunosuppression and thus gain better outcomes.

10. Chronic Renal Allograft Damage: Existing Challenges.
Transplantation 2011; 91 (suppl 9): S10-S15.
Arias M, Seron D, Moreso F, Bestard O, Praga M.

11. Protocol biopsy of the stable renal transplant: A multicenter study of methods and complication rates.

2003; Transplantation 76: 969–973, 2003.

Furness PN, Philpott CM, Chorbadjian MT et al.

Background: Clinical trials in renal transplantation must use surrogate markers of long-term graft survival if conclusions are to be drawn at acceptable speed and cost. Morphologic changes in transplant biopsies provide the earliest available evidence of damage, and "protocol" biopsies from stable grafts can be used to reduce the number of patients needed in clinical trials. This approach has been inhibited by concerns over safety, but the risk of biopsy of a stable kidney, with no active inflammation or acute functional impairment, has never been formally estimated.
Methods: In accordance with a predefined set of questions, a retrospective audit of a sequential series of protocol biopsies was performed in four major transplant centers.
Results: A total of 2,127 biopsy events were assessed for major complications, and 1,486 were assessed for minor ones. There were no deaths. One graft was lost, under circumstances indicating that the loss should have been prevented. Three episodes of hemorrhage required direct intervention. Three further patients required transfusion. There were two episodes of peritonitis, but one was arguably an unrelated event. All serious complications presented within 4 hr of biopsy.
Conclusions: The incidence of clinically significant complications after protocol biopsy of a stable renal transplant is low. Direct benefits to the patients concerned (irrespective of the benefit that may accrue in clinical trials) were not formally assessed but seem likely to outweigh the risk of the procedure. We believe that it is ethically justifiable to ask renal trans-plant recipients to undergo protocol biopsies in clinical trials and routine care.

12. Seguridad y eficacia de la biopsia ambulatoria en trasplante renal.
Nefrología 2014; 34 (6): 749-755.
Torres-Rodriguez IB, Castella-Fierro E, Serres-Creixell X et al.

Antecedentes: La biopsia del aloinjerto renal se realiza habitualmente con el paciente hospitalizado.
Objetivo: Evaluar la seguridad y eficacia de un programa de biopsias ambulatorio en receptores de trasplante renal.
Métodos: En diciembre de 2011 se inició un programa ambulatorio de biopsias en trasplante renal. Se contraindica la biopsia ambulatoria en los casos siguientes: 1) tratamiento anticoagulante, 2) Trombocitopenia < 50 000/mm3, 3) índice de masa corporal > 35 kg/m2, 4) hipertensión arterial no controlada. Se compara la seguridad y eficacia de las biopsias realizadas bajo hospitalización en el período 2007-2011 (n = 124) con las biopsias ambulatorias realizadas durante el período 2011-2013 (n = 219) y las realizadas en este mismo período bajo hospitalización (n = 42).
Resultados: Entre diciembre de 2011 y diciembre de 2013 se han indicado 230 biopsias desde la consulta externa y se han realizado 219 (95 %) en régimen ambulatorio. La incidencia de complicaciones mayores (necesidad de transfusión y/o embolización) ha sido de 0,8 % para el período 2007-2011 y de 2,4 % para las realizadas bajo hospitalización del período 2011-2013 (p = 0,475). No se observaron complicaciones mayores en el grupo de pacientes con biopsias realizadas de forma ambulatoria. La tasa de complicaciones menores (hematuria macroscópica, hematoma o fístula que no requirieron transfusión ni embolización) no ha sido distinta entre los grupos (3,2 %, 7,1 % y 2,7 %, respectivamente). La adecuación de la muestra obtenida según los criterios de Banff no ha sido distinta entre los grupos (p = 0,052).
Conclusión: La realización ambulatoria de la biopsia de injerto renal es un procedimiento seguro y eficaz.

13. The natural history of chronic allograft nephropathy.

N Eng J Med 2003; 349: 2326-2333.

Nankivell BJ, Borrows RJ, Chang CLS et al.

Background: With improved immunosuppression and early allograft survival, chronic allograft nephropathy has become the dominant cause of kidney-transplant failure.
Methods: We evaluated the natural history of chronic allograft nephropathy in a prospective study of 120 recipients with type 1 diabetes, all but 1 of whom had received kidney–pancreas transplants. We obtained 961 kidney-transplant–biopsy specimens taken regularly from the time of transplantation to 10 years thereafter.
Results: Two distinctive phases of injury were evident as chronic allograft nephropathy evolved. An initial phase of early tubulointerstitial damage from ischemic injury ($P<0.05$), prior severe rejection ($P<0.01$), and subclinical rejection ($P<0.01$) predicted mild disease by one year, which was present in 94.2 percent of patients. Early subclinical rejection was common (affecting 45.7 percent of biopsy specimens at three months), and the risk was increased by the occurrence of a prior episode of severe rejection and reduced by tacrolimus and mycophenolate therapy (both $P<0.05$) and gradually abated after one year. Both subclinical rejection and chronic rejection were associated with increased tubulointerstitial damage ($P<0.01$). Beyond one year, a later phase of chronic allograft nephropathy was characterized by microvascular and glomerular injury. Chronic rejection (defined as persistent subclinical rejection for two years or longer) was uncommon (5.8 percent). Progressive high-grade arteriolar hyalinosis with luminal narrowing, in-creasing glomerulosclerosis, and additional tubulointerstitial damage was accompanied by the use of calcineurin inhibitors. Nephrotoxicity, implicated in late ongoing injury, was almost universal at 10 years, even in grafts with excellent early histologic findings. By 10 years, severe chronic allograft nephropathy was present in 58.4 percent of patients, with sclerosis in 37.3 percent of glomeruli. Tubulointerstitial and glomerular damage, once established, was irreversible, resulting in declining renal function and graft failure.
Conclusions: Chronic allograft nephropathy represents cumulative and incremental damage to nephrons from time-dependent immunologic and nonimmunologic causes.

14. Subclinical rejection associated with chronic allograft nephropathy in protocol biopsies as a risk factor for late graft loss.

Am J Transplant 2006; 6: 747-752.

Moreso F, Ibernon M, Gomà M et al.

Chronic allograft nephropathy (CAN) in protocol biop-sies is associated with graft loss while the association between subclinical rejection (SCR) and outcome has yielded contradictory results. We analyze the predictive value of SCR and/or CAN in protocol biopsies on death-censored graft survival. Since 1988, a protocol biopsy was done during the first 6 months in stable grafts with serum creatinine <300 lmol/L and protein-uria <1 g/day. Biopsies were evaluated according to Banff criteria. Borderline changes and acute rejection were grouped as SCR. CAN was defined as presence of interstitial fibrosis and tubular atrophy. Mean follow-up was 91 ± 46 months. Sufficient tissue was obtained in 435 transplants. Biopsies were classified as normal (n = 186), SCR (n = 74), CAN (n = 110) and SCR with CAN (n = 65). Presence of SCR with CAN was associated with old donors, percentage of panel reactive antibodies and presence of acute rejection before pro-tocol biopsy. Cox regression analysis showed that SCR with CAN (relative risk [RR]: 1.86, 95% confidence in-terval [CI]: 1.11–3.12; p = 0.02) and hepatitis C virus (RR: 2.27, 95% CI: 1.38–3.75; p = 0.01) were indepen-dent predictors of graft survival. In protocol biopsies, the detrimental effect of interstitial fibrosis/tubular atrophy on long-term graft survival is modulated by SCR.

15. Presence of FoxP3+ regulatory T cells predicts outcome of subclinical rejection of renal allografts.

J Am Soc Nephrol 2008; 19: 2020-2026.

Bestard O, Cruzado JM, Rama I et al.

Subclinical rejection (SCR) of renal allografts refers to histologic patterns of acute rejection despite stable renal function. The clinical approach to SCR is controversial; it would be helpful to identify biomarkers that could determine whether the identified cellular infiltrates were detrimental. For inves-tigation of whether the presence of $FoxP3^+$ regulatory T cells (Treg) could help determine the functional importance of tubulointerstitial infiltrates observed in 6-mo protocol biopsies, 37 cases of SCR were evaluated. The presence of $FoxP3^+$ Treg discriminated harmless from injurious infiltrates, evidenced by independently predicting better graft function 2 and 3 yr after transplantation. Furthermore, the FoxP3 Treg/$CD3^+$ T cell ratio positively correlated with graft function at 2 yr after transplantation, suggesting that an increasing proportion of Treg within the global T cell infiltrate may facilitate renal engraftment; therefore, immunostaining for $FoxP3^+$ Treg in patients with SCR on protocol biopsies may ultimately be useful to identify patients who may require alterations in their immunosuppressive regimens.

16. Inflammation in Areas of Tubular Atrophy in Kidney Allograft Biopsies: A Potent Predictor of Allograft Failure.

Am J Transplant 2010; 10: 2066-2073.

Mannon RB, Matas AJ, Grande JP et al.

The Banff scoring schema provides a common ground to analyze kidney transplant biopsies. Interstitial inflammation (i) and tubulitis (t) in areas of viable tissue are features in scoring acute rejection, but are excluded in areas of tubular atrophy (TA). We studied inflammation and tubulitis in a cohort of kidney transplant recipients undergoing allograft biopsy for new-onset late graft dysfunction (N = 337). We found inflammation ('iatr ') and tubulitis ('tatr ') in regions of fibrosis and atrophy to be strongly correlated with each other ($p < 0.0001$). Moreover, iatr was strongly associated with death-censored graft failure when compared to recipients whose biopsies had no inflammation, even after adjusting for the presence of interstitial fibrosis (Hazard Ratio = 2.31, [1.10–4.83]; $p = 0.0262$) or TA (hazard ratio = 2.42, [1.16–5.08]; $p = 0.191$), serum creatinine at the time of biopsy, time to biopsy and i score. Further, these results did not qualitatively change after additional adjustments for C4d staining or donor specific antibody. Stepwise regression identified the most significant markers of graft failure which include iatr score. We propose that a more global assessment of inflammation in kidney allograft biopsies to include inflammation in atrophic areas may provide better prognostic information. Phenotypic characterization of these inflammatory cells and appropriate treatment may ameliorate late allograft failure.

17. The histology of solitary renal allografts at 1 and 5 years after transplantation.
Am J Transplant 2011; 11: 698-707.
Stegall MD, Park WD, Larson TS et al.

Previous studies suggest that the majority of renal allografts are affected by progressive, severe chronic histologic injury, yet studies using current protocols are lacking. The goal of this study was to examine the prevalence and progression of histologic changes using protocol allograft biopsies at 1 and 5 years after solitary kidney transplantation in patients trans-planted between 1998 and 2004. Chronic histologic changes generally were mild at both 1 and 5 years and were similar in deceased and living donor kidneys. The overall prevalence of moderate or severe fibrosis was 13% (60/447) at 1 year and 17% (60/343) at 5 years. In a subgroup of 296 patients who underwent both 1- and 5-year biopsies, mild fibrosis present at 1 year progressed to more severe forms at 5 years in 23% of allografts. The prevalence of moderate or severe arteriolar hyalinosis was similar in tacrolimus and calcineurin inhibitor-free immunosuppression. These results in the recent era of transplantation demonstrate fewer, less severe and less progressive chronic histologic changes in the first 5 years after transplantation than previously reported.

18. Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant.

Am J Transplant 2012; 12:1157–1167.

Wiebe C, Gibson IW, Blydt-Hansen TD et al.

The natural history for patients with de novo donor-specific antibodies (dnDSA) and the risk factors for its development have not been well defined. Furthermore, clinical and histologic correlation with serologic data is limited. We studied 315 consecutive renal transplants without pretransplant DSA, with a mean follow-up of 6.2 ± 2.9 years. Protocol (n = 215) and for cause (n = 163) biopsies were analyzed. Solid phase assays were used to screen for dnDSA posttransplant. A total of 47 out of 315 (15%) patients developed dnDSA at a mean of 4.6 ± 3.0 years posttransplant. Independent predictors of dnDSA were HLA-DRb 1MM > 0 (OR 5.66, $p < 0.006$); and nonadherence (OR 8.75, $p < 0.001$); with a strong trend toward clinical rejection episodes preceding dnDSA (OR 1.57 per rejection episode, $p = 0.061$). The median 10-year graft survival for those with dnDSA was lower than the No dnDSA group (57% vs. 96%, $p < 0.0001$). Pathology consistent with antibody-mediated injury can occur and progress in patients with dnDSA in the absence of graft dysfunction and furthermore, nonadherence and cellular rejection con-tribute to dnDSA development and progression to graft loss.

19. Early subclinical rejection as a risk factor for late chronic humoral rejection.
Transplantation 2012; 93:41–46.
Moreso F, Carrera M, Goma M, et al.

Background: Subclinical rejection and interstitial fibrosis and tubular atrophy (IF/TA) in protocol biopsies are associated with outcome. We study the relationship between histologic lesions in early protocol biopsies and histologic diagnoses in late biopsies for cause.
Materials and Methods: Renal transplants with a protocol biopsy performed within the first 6 months posttransplant between 1988 and 2006 were reviewed. Biopsies were evaluated according to Banff criteria, and C4d staining was available in biopsies for cause.
Results: Of the 517 renal transplants with a protocol biopsy, 109 had a subsequent biopsy for cause which showed the following histological diagnoses: chronic humoral rejection (CHR) (n=44), IF/TA (n=42), recurrence of the primary disease (n=11), de novo glomerulonephritis (n=7), T-cell-mediated rejection (n=4), and polyoma virus nephropathy (n=1). The proportion of retransplants (15.9% vs. 2.3%, P=0.058) and the prevalence of subclinical rejection were higher in patients with CHR than in patients with IF/TA (52.3% vs. 28.6%, P=0.0253). Demographic donor and recipient characteristics and clinical data at the time of protocol biopsy were not different between groups. Logistic regression analysis showed that subclinical rejection (relative risk, 2.52; 95% confidence interval, 1.1– 6.3; P=0.047) but not retransplantation (relative risk, 6.7; 95% confidence interval, 0.8 –58.8; P=0.085) was associated with CHR. Conclusion. Subclinical rejection in early protocol biopsies is associated with late appearance of CHR.

20. Antibody-mediated vascular rejection of kidney allografts: a population-based study.
The Lancet 2013; 381: 313-319.
Lefaucher C, Loupy A, Vernery D et al.

Summary:
Background: Rejection of allografts has always been the major obstacle to transplantation success. We aimed to improve characterisation of different kidney-allograft rejection phenotypes, identify how each one is associated with anti-HLA antibodies, and investigate their distinct prognoses.
Methods: Patients who underwent ABO-compatible kidney transplantations in Necker Hospital and Saint-Louis Hospital (Paris, France) between Jan 1, 1998, and Dec 31, 2008, were included in our population-based study. We assessed patients who provided biopsy samples for acute allograft rejection, which was defined as the association of deterioration in function and histopathological lesions. The main outcome was kidney allograft loss—ie, return to dialysis. To investigate distinct rejection patterns, we retrospectively assessed rejection episodes with review of graft histology, C4d in allograft biopsies, and donor-specific anti-HLA antibodies.
Findings 2079 patients were included in the main analyses, of whom 302 (15%) had acute biopsy-proven rejection. We identified four distinct patterns of kidney allograft rejection: T cell-mediated vascular rejection (26 patients [9%]), antibody-mediated vascular rejection (64 [21%]), T cell-mediated rejection without vasculitis (139 [46%]), and antibody-mediated rejection without vasculitis (73 [24%]). Risk of graft loss was 9·07 times (95 CI 3·62–19·7) higher in antibody-mediated vascular rejection than in T cell-mediated rejection without vasculitis (p<0·0001), compared with an increase of 2·93 times (1·1–7·9; P=0·0237) in antibody-mediated rejection without vasculitis and no significant rise in T cell-mediated vascular rejection (hazard ratio [HR] 1·5, 95% CI 0·33–7·6; p=0·60).
Interpretation: We have identified a type of kidney rejection not presently included in classifications: antibody-mediated vascular rejection. Recognition of this distinct phenotype could lead to the development of new treatment strategies that could salvage many kidney allografts.

21. Subclinical Rejection Phenotypes at 1 Year Post-Transplant and Outcome of Kidney Allografts.

J Am Soc Nephrol. 2015 Jan 2. pii: ASN.2014040399. [Epub ahead of print]

Loupy A, Vernerey D, Tinel C, Aubert O, Duong van Huyen JP, Rabant M, Verine J, Nochy D, Empana JP, Martinez F, Glotz D, Jouven X, **Legendre** C, Lefaucheur C.

ABSTRACT:

Kidney allograft rejection can occur in clinically stable patients, but long-term significance is unknown. We determined whether early recognition of subclinical rejection has long-term consequences for kidney allograft survival in an observational prospective cohort study of 1307 consecutive nonselected patients who underwent ABO-compatible, complement-dependent cytotoxicity-negative crossmatch kidney transplantation in Paris (2000-2010). Participants underwent prospective screening biopsies at 1 year post-transplant, with concurrent evaluations of graft complement deposition and circulating anti-HLA antibodies. The main analysis included 1001 patients. Three distinct groups of patients were identified at the 1-year screening: 727 (73%) patients without rejection, 132 (13%) patients with subclinical T cell-mediated rejection (TCMR), and 142 (14%) patients with subclinical antibody-mediated rejection (ABMR). Patients with subclinical ABMR had the poorest graft survival at 8 years post-transplant (56%) compared with subclinical TCMR (88%) and nonrejection (90%) groups (P<0.001). In a multivariate Cox model, subclinical ABMR at 1 year was independently associated with a 3.5-fold increase in graft loss (95% confidence interval, 2.1 to 5.7) along with eGFR and proteinuria (P<0.001). Subclinical ABMR was associated with more rapid progression to transplant glomerulopathy. Of patients with subclinical TCMR at 1 year, only those who further developed de novo donor-specific antibodies and transplant glomerulopathy showed higher risk of graft loss compared with patients without rejection. Our findings suggest that subclinical TCMR and subclinical ABMR have distinct effects on long-term graft loss. Subclinical ABMR detected at the 1-year screening biopsy carries a prognostic value independent of initial donor-specific antibody status, previous immunologic events, current eGFR, and proteinuria.

22. Capillary C4d and Kidney Allograft Outcome in Relation to Morphologic Lesions Suggestive of Antibody-Mediated Rejection.

Clin J Am Soc Nephrol. 2015 Jun 12. pii: CJN.09901014. [Epub ahead of print]

Kikić Ž, Kainz A, Kozakowski N, Oberbauer R, Regele H, Bond G, Böhmig GA.

ABSTRACT:

Background and objectives: Recent studies highlighting a role of C4d- antibody-mediated rejection (ABMR) have debated whether C4d staining has independent value as a rejection marker. Considering the presumed role of complement as an important effector of graft injury, this study hypothesized that capillary C4d, a footprint of antibody-triggered complement activation, indicates a particularly severe manifestation of ABMR.
Design, setting, participants, & measurements: This large retrospective clinicopathologic study sought to assess the clinical predictive value of C4d staining in relation to ABMR morphology. Overall, 885 renal allograft recipients who underwent transplantation between 1999 and 2006 (median duration of follow-up, 63.3 [interquartile range, 40.6-93.5] months; 206 graft losses) were included if they had had one or more indication biopsies. A total of 1976 biopsy specimens were reevaluated for capillary C4d staining (C4d data were available for 825 patients) and distinct morphologic lesions suggestive of ABMR, including glomerulitis, peritubular capillaritis, capillary microthrombi, transplant glomerulopathy, and severe intimal arteritis.
Results: C4d+ patients, with or without ABMR features, had worse death-censored 8-year graft survival (53% or 67%) than C4d- patients (66% or 81%; P<0.001). In Cox regression analysis, C4d was associated with a risk of graft loss independently of baseline confounders and ABMR morphology (hazard ratio, 1.85 [95% confidence interval, 1.34 to 2.57]; P<0.001). The risk was higher than that observed for C4d- patients, a finding that reached statistical significance in patients showing fewer than two different ABMR lesions. Moreover, in a mixed model, C4d was independently associated with a steeper decline of eGFR (slope per year, -8.23±3.97 ml/min per 1.73 m^2; P<0.001).
Conclusions: These results suggest that detection of intragraft complement activation has strong independent value as an additional indicator of ABMR associated with adverse kidney transplant outcomes.

23. Comparison of four different immunosuppression protocols without long-term steroid therapy in kidney recipients monitored by surveillance biopsy: five-year outcomes.
Transpl Immunol 2008; 20:32–42.
Anil Kumar MS, Irfan Saeed M, Ranganna K, et al.

ABSTRACT:
Induction and maintenance immunosuppression protocols with or without long-term steroid therapy in kidney transplant recipients are variable and are transplant center-specific. The aim of this prospective randomized pilot study was to compare 5-year outcomes in kidney recipients maintained on 4 different calcineurin inhibitor (CNI)-based immunosuppression protocols without long-term steroid therapy. Two hundred consenting patients who received kidney transplants between June 2000 and October 2004 were enrolled in 4 immunosuppression protocol groups, with 50 patients in each group: cyclosporine (CSA)/mycophenolate mofetil (MMF), CSA/sirolimus (SRL), tacrolimus (TAC)/MMF, and TAC/SRL. Induction therapy was done with basiliximab and methylprednisolone. Steroids were withdrawn on post-transplant day 2, and long-term steroid therapy was not used. Demographic characteristics among the four groups were comparable; approximately 50% of the recipients were African American and ≥80% of the kidneys transplanted were from deceased donors. Clinical acute rejection (CAR) was confirmed by biopsy and treated with intravenous pulse steroid therapy. Steroid-unresponsive CAR was treated with Thymoglobulin. Surveillance biopsies were performed at 1, 6, 12, 24, 36, 48, and 60 months to evaluate subclinical acute rejection (SCAR), chronic allograft injury (CAI), and other pathological changes per the Banff 2005 schema. The primary end point was CAR, and secondary end points were 5-year patient and graft survival rates, renal function, SCAR, CAI, and adverse events. In the first year post-transplant, the incidence of CAR was 18% in the CSA/MMF group, 8% in the CSA/SRL group,14% in the TAC/MMF group, and 4% in the TAC/SRL group (CSA/MMF vs. TAC/SRL; p=0.05). The incidence of SCAR was 22% in the CSA/MMF group, 8% in the CSA/SRL group, 16% in the TAC/MMF group, and 6% in the TAC/SRL group (CSA/MMF vs. CSA/SRL and TAC/SRL; p=0.05). After the first year, the incidences of CAR and SCAR decreased and were comparable in all 4 groups. At 5 years post-transplant, cumulative CAI due to interstitial fibrosis/tubular atrophy (IF/TA), hypertension (HTN), and chronic calcineurin inhibitor (CNI) toxicity was observed in 54%, 48%, and 8% of the CSA/MMF group vs. 16%, 36%, and 12%of the CSA/SRL group vs. 38%, 24% and 6% of the TAC/MMF group vs.14%, 25% and 12% of the TAC/SLR group (IF/TA: CSA/MMF vs. CSA/SRL and TAC/SRL; p= 0.04, HTN: CSA/MMF vs. TAC/MMF and TAC/SRL; p=0.05, CNI toxicity: TAC/SRL and CSA/SRL vs. TAC/MMF; p=0.05). Five-year patient and graft survival rates were 82% and 60% in the CSA/MMF group, 82% and 60% in the CSA/SRL group, 84% and 62% in the TAC/MMF group, and 82% and 64% in the TAC/SRL group (p=0.9). Serum creatinine levels and creatinine clearances at 5 years were comparable among the groups. Our data show that the rates of CAR and SCAR in the first year post-transplant were significantly lower in the CSA/SRL and TAC/SRL groups and that cumulative CAI rates due to IF/TA and HTN at 5 years were significantly lower in the TAC/MMF, TAC/SRL, and CSA/SRL groups than in the CSA/MMF group. Despite significant differences in the incidences of CAR and SCAR and prevalence of different types of CAI at 5 years, renal function and patient and graft survival rates at 5 years were comparable among kidney recipients maintained on 4 different immunosuppression protocols without long-term steroid therapy.

24. Sirolimus-based therapy following early cyclosporine withdrawal provides significantly improved renal histology and function at 3 years.
Am J Transplant 2004; 4: 953-961, 2004.
Mota A, Arias M, Taskinen EI y cols.

Graft function and histology are predictive of renal transplant survival. The Rapamune Maintenance Regi-men study demonstrated that early cyclosporine (CsA) withdrawal from a sirolimus (SRL)-CsA-steroid (ST) regimen improved renal function and blood pressure. We report the protocol-mandated biopsy findings from that study. Renal transplant patients (n == 430) receiving SRL-CsA-ST were randomized at 3 months after transplantation to remain on SRL-CsA-ST, or to have CsA withdrawn (SRL-ST group). Protocol-mandated biopsies were performed at engraftment and at 12 and 36 months. Two pathologists blindly evaluated 484 biopsies to obtain the Chronic Allograft Damage Index (CADI) scores. At 36 months among patients with se-rial biopsies (n == 63), the mean CADI score was significantly lower with SRL-ST(4.70 vs. 3.20, p == 0.003), as was the mean tubular atrophy score (0.77 vs. 0.32, p << 0.001). All six components of the CADI score were nu-merically lower in SRL-ST group; moreover, inflamma-tion and the tubular atrophy scores decreased signifi-cantly in the SRL-ST group between 12 and 36 months. The calculated glomerular filtration rate at 36 months was significantly better in the CsA-withdrawal group (54.8 vs. 68.2 mL/min, p == 0.009). In conclusion, with- drawing CsA from the SRL-CsA-ST regimen resulted in improved renal histology and function.

25. De novo kidney transplantation without use of calcineurin inhibirtors preserves renal structure and function at two years.
Am J Transplant 2004; 4: 1776-1785.
Flechner SM, Kurian SM, Solez K et al.

26. Costimulation blockade with belatacept in renal transplantation.
N Eng J Med 2005; 335:770-781.
Vincenti F, Larsen C, Durrbach A et al.

Background: Renal transplantation is the standard of care for patients with end-stage renal disease. Although maintenance immunosuppression with calcineurin inhibitors yields excellent one-year survival, it is associated over the long term with high rates of death and graft loss, owing in part to the adverse renal, cardiovascular, and metabolic effects of these agents. The use of potentially less toxic agents, such as belatacept, a selective blocker of T-cell activation, may improve outcomes.
Methods: We randomly assigned renal-transplant recipients to receive an intensive or a less-intensive regimen of belatacept or cyclosporine. All patients received induction therapy with basiliximab, mycophenolate mofetil, and corticosteroids. The primary objective was to demonstrate the noninferiority of belatacept over cyclosporine in the incidence of acute rejection at six months (with an upper bound of the 95 percent confidence interval around the treatment difference of less than 20 percent).
Results: At six months, the incidence of acute rejection was similar among the groups: 7 per-cent for intensive belatacept, 6 percent for less-intensive belatacept, and 8 percent for cyclosporine. At 12 months, the glomerular filtration rate was significantly higher with both intensive and less-intensive belatacept than it was with cyclosporine (66.3, 62.1, and 53.5 ml per minute per 1.73 m2, respectively), and chronic allograft nephropathy was less common with both regimens of belatacept than with cyclosporine (29 per-cent, 20 percent, and 44 percent, respectively). Lipid levels and blood-pressure values were similar or slightly lower in the belatacept groups, despite the greater use of lipid-lowering and antihypertensive medications in the cyclosporine group.
Conclusions: Belatacept, an investigational selective costimulation blocker, did not appear to be inferior to cyclosporine as a means of preventing acute rejection after renal transplantation. Belatacept may preserve the glomerular filtration rate and reduce the rate of chronic allograft nephropathy.

27. Complete Avoidance of Calcineurin Inhibitors in Renal Transplantation: A Randomized Trial Comparing Sirolimus and Tacrolimus.

Am J Tranplant 2006; 6: 514-522

Larson TS, Dean PG, Stegall MD et al.

Calcineurin inhibitors have decreased acute rejection and improved early renal allograft survival, but their use has been implicated in the development of chronic nephrotoxicity. We performed a prospective, randomized trial in kidney transplantation com-paring sirolimus-MMF-prednisone to tacrolimus-MMF-prednisone. Eighty-one patients in the sirolimus group and 84 patients in the tacrolimus group were enrolled (mean follow-up = 33 months; range 13–47 months). At 1 year, patient survival was similar in the groups (98% with sirolimus, 96% with tacrolimus; p = 0.42) as was graft survival (94% sirolimus vs. 92% tacrolimus, p = 0.95). The incidence of clinical acute rejection was 10% in the tacrolimus group and 13% in the sirolimus group (p = 0.58). There was no difference in mean GFR measured by iothalamate clearance be-tween the tacrolimus and sirolimus groups at 1 year (61 ± 19 mL/min vs. 63 ± 18 mL/min, p = 0.57) or 2 years (61 ± 17 mL/min vs. 61 ± 19 mL/min, p = 0.84). At 1 year, chronicity using the Banff schema showed no difference in interstitial, tubular or glomerular changes, but fewer chronic vascular changes in the sirolimus group. This study shows that a CNI-free regimen using sirolimus-MMF-prednisone produces similar acute rejection rates, graft survival and renal function 1–2 years after transplantation compared to tacrolimus-MMF-prednisone.

28. No Difference in Degree of Interstitial Sirius Red–Stained Area in Serial Biopsies from Area under Concentration-over-Time Curves–Guided Cyclosporine versus Tacrolimus-Treated Renal Transplant Recipients at One Year.

J Am Soc Nephrol 2006; 17: 305-312.

Rowsahni AT, Scholten EM, Bemelman F et al.

Interstitial fibrosis is the main characteristic of chronic allograft nephropathy and long-term graft failure. Cyclosporin (CsA) is thought to be more fibrogenic than tacrolimus. In a prospective, randomized, multicenter trial using a calcineurin-sparing regimen, renal interstitial volume was compared in CsA- and tacrolimus-treated renal transplant recipients by image analysis of Sirius red (SR)-stained cortical areas in protocol biopsies obtained at 6 mo (n = 94) and 12 mo (n = 97) after transplantation. Immunosuppression consisted of CsA or tacrolimus, CD25 mAb, mycophenolate mofetil, and prednisolone. CsA therapy increased the 6-mo risk for subclinical rejection. The prevalence of subclinical rejection was 38.8% in the CsA-treated and 15.2% in the tacrolimus-treated patient group (P = 0.012). Strikingly, no difference in the degree of interstitial SR–stained area was detectable between the two treatment groups. In particular, previous subclinical rejection episodes did not influence the degree of interstitial volume. Also, no difference in GFR occurred at 1 yr, when the mean GFR mounted 63 ml/min. No significant differences in the degree of interstitial SR–stained area could be observed at 6 and 12 mo between CsA- and tacrolimus-treated renal transplant recipients. Although CsA-treated patients developed significantly more subclinical rejections at 6 mo, this did not influence the degree of SR staining or the change in renal function at 1 yr.

29. Tacrolimus exposure and evolution of renal allograft histology in the first year after transplantation.

Am J Transplant 2007; 7:2114–2123.

Naesens M, Lerut E, Damme BV, et al.

Tacrolimus has a narrow therapeutic window and is characterized by a large inter-individual variability in bioavailability. The impact of tacrolimus exposure on subclinical evolution of graft histology has not been studied in renal recipients. This analysis included 239 protocol biopsies (obtained at implantation, 3 and 12 months) of 120 consecutive kidney recipients treated with tacrolimus, mycophenolate mofetil (MMF) and corticosteroids. Biopsies were scored according to the Banff 2001 criteria and a chronicity score was calculated. Prospective pharmacokinetic data were included in the analysis (5544 tacrolimus predose blood concentrations and tacrolimus AUC0−12 at 3 and 12 months). Higher donor age and higher number of human leukocyte antigen-DR (HLA-DR) mismatches were independent predictors of subclinical acute rejection at 3 months, present in 8.7% of patients. The number of HLA-DR mismatches was independently associated with biopsy-proven clinical acute rejection. Biopsy-proven acute rejection episodes and low mean tacrolimus exposure were independently associated with higher increase in chronicity scores between 3 and 12 months after transplantation. This observational study suggests that rejection phenomena and immune-mediated mechanisms remain important in the early progression of chronic allograft pathology. Tacrolimus doses or systemic exposure were not associated with lesions of calcineurin inhibitor nephrotoxicity, suggesting that other factors determine susceptibility to tacrolimus nephrotoxicity.

30. Specificity of Histological Markers of Long-Term CNI Nephrotoxicity in Kidney-Transplant Recipients Under Low-Dose Cyclosporine Therapy

American Journal of Transplantation. 2011;11:2635–46

Snanoudj R, Royal V, Elie C, Rabant M, Girardin C, Morelon E, Kreis H, Fournet JC, Noël LH, Legendre C.

ABSTRACT:

The specificity of chronic histological lesions induced by calcineurin inhibitors (CNI) is often questioned, but few studies have directly compared long-term lesions in renal-transplant patients who received this treatment and those who did not. We therefore conducted a retrospective study of 141 kidney-transplant recipients treated with (n = 48) or without (n = 93) cyclosporine (CsA) to compare the histological lesions observed at 3-month, 24-month and 10-year protocol biopsies. All of the chronic elementary lesions (glomerulosclerosis, interstitial fibrosis, tubular atrophy, arteriolar hyalinosis, fibrointimal thickening) progressed in frequency and severity in both groups, although significantly more in the CsA group. Ten-year biopsy results showed that 92% of patients in the CsA-treated group and 65% in the control group had arteriolar hyalinosis lesions. When we focused on muscular arteriolar hyaline deposits more specific to CsA arteriolopathy, we observed these lesions in 68% of CsA patients and 28% of patients who had never received CsA. CsA was not the sole factor involved in the development of arteriolar hyalinosis and was independently associated with an increased risk of graft loss. In summary, we observed that histological lesions commonly attributed to CsA nephrotoxicity were not sufficiently specific to definitively diagnose CNI nephrotoxicity.

31. Early corticosteroid withdrawal in recipients of renal allografts: a single-center report of ethnically diverse recipients and recipients of marginal deceased-donor kidneys.
Transplantation 2012; 94 (8): 837-44.
Aull MJ, Dadhania D, Afaneh C et al.

Background: Candidacy for kidney transplantation is being progressively liberalized, and the safety and efficacy of early withdrawal of corticosteroids in high-risk patients have not been fully characterized.
Methods: We analyzed the safety and efficacy of an early corticosteroid withdrawal regimen of rabbit antithymocyte globulin induction, tacrolimus, mycophenolate mofetil, and steroid withdrawal by day 5 after transplantation in our study cohort of 634 kidney transplant recipients that included 27% African American and 18% Hispanic recipients. Fifty-five percent of the recipients were recipients of deceased-donor kidneys, and 46% of deceased-donor kidneys were kidneys from expanded criteria donors.
Results: Kaplan-Meier patient survival at 1, 3, and 5 years after transplantation was 98.6%, 94.6%, and 90.2%, and death-censored graft survival was 96.2%, 91.9%, and 87.6%, respectively. During a mean follow-up of 57 months, 89.3% of patients remained off of corticosteroids, and the incidence of acute rejection including subclinical rejection identified by protocol biopsy was 12.0%. Multivariable analysis identified age older than 60 years as protective against (P=0.01) and the African American ethnicity as a risk factor for (P=0.03) rejection. Delayed graft function (PG0.0001), rejection (PG0.0001), and transplant panel reactive antibody 20% or more (P=0.03) were risk factors for graft loss. Opportunistic infections included viral in 15.3%, fungal in 1.6%, and parasitic in 0.6% of the patients. Posttransplantation malignancy occurred in 9.1% of patients.
Conclusions: An early corticosteroid withdrawal regimen of rabbit antithymocyte globulin induction, tacrolimus, and mycophenolate mofetil is associated with excellent patient and kidney graft survival in an ethnically diverse population with risk factors for poor outcomes.

32. Significance of C4d Banff Scores in Early Protocol Biopsies of Kidney Transplant Recipients with Preformed Donor-Specific Antibodies (DSA)

American Journal of Transplantation 2011;11: 56–65

Loupy A, Hill GS, Suberbielle C, Charron D, Anglicheau D, Zuber J, Timsit MO, Duong JP, Bruneval P, Vernerey D, Empana JP, Jouven X, Nochy D, Legendre CH.

ABSTRACT:

The significance of C4d-Banff scores in protocol biopsies of kidney transplant recipients with preformed donor-specific antibodies (DSA) has not been determined. We reviewed 157 protocol biopsies from 80 DSA+ patients obtained at 3 months and 1 year post-transplant. The C4d Banff scores (1,2,3) were associated with significant increments of microcirculation inflammation (MI) at both 3 months and 1 year post-transplant, worse transplant glomerulopathy and higher class II DSA-MFI ($p < 0.01$). Minimal-C4d had injury intermediate between negative and focal, while focal and diffuse-C4d had the same degree of microvascular injury. A total of 54% of patients had variation of C4d score between 3 months and 1 year posttransplant.

Cumulative (3 month + 1 year) C4d scores correlated with long-term renal function worsening ($p = 0.006$). However, C4d staining was not a sensitive indicator of parenchymal disease, 55% of C4d-negative biopsies having evidence of concomitant MI. Multivariate analysis demonstrated that the presence of MI and class II DSA at 3 months were associated with a fourfold increased risk of progression to chronic antibody mediated rejection independently of C4d ($p < 0.05$). In conclusion, the substantial fluctuation of C4d status in the first year post-transplant reflects a dynamic humoral process. However, C4d may not be a sufficiently sensitive indicator of activity, MI and DSA being more robust predictors of bad outcome.

33. Donor-specific antibodies accelerate arteriosclerosis after kidney transplantation.
J Am Soc Nephrol 2011; 22: 975-983.
Hill GS, Nochy D, Bruneval P et al.

ABSTRACT:
In biopsies of renal allografts, arteriosclerosis is often more severe than expected based on the age of the donor, even without a history of rejection vasculitis. To determine whether preformed donor-specific antibodies (DSAs) may contribute to the severity of arteriosclerosis, we examined protocol biopsies from patients with (n = 40) or without (n = 59) DSA after excluding those with any evidence of vasculitis. Among DSA-positive patients, arteriosclerosis significantly progressed between month 3 and month 12 after transplant (mean Banff cv score 0.65 ± 0.11 to 1.12 ± 0.10, P = 0.014); in contrast, among DSA-negative patients, we did not detect a statistically significant progression during the same time-frame (mean Banff cv score 0.65 ± 0.11 to 0.81 ± 0.10, P = not significant). Available biopsies at later time points supported a rate of progression of arteriosclerosis in DSA-negative patients that was approximately one third that in DSA-positive patients. Accelerated arteriosclerosis was significantly associated with peritubular capillary leukocytic infiltration, glomerulitis, subclinical antibody-mediated rejection, and interstitial inflammation. In conclusion, these data support the hypothesis that donor-specific antibodies dramatically accelerate post-transplant progression of arteriosclerosis.

34. Transplant glomerulopathy: subclinical incidence and association with alloantibody.
Am J Transplant 2007; 7: 2124-2132.
Gloor J, Sethi S, Stegall MD et al.

Transplant glomerulopathy (TG) usually has been de-scribed as part of a constellation of late chronic histologic abnormalities associated with proteinuria and declining function. The current study used both protocol and clinically-indicated biopsies to investigate clinical and subclinical TG, their prognosis and possible association with alloantibody. We retrospectively studied 582 renal transplants with a negative pre-transplant T-cell complement dependent cytotoxicity crossmatch. TG was diagnosed in 55 patients, 27 (49%) based on protocol biopsy in well-functioning grafts. The cumulative incidence of TG increased over time to 20% at 5 years. The prognosis of subclinical TG was equally as poor as TG diagnosed with graft dysfunction, with progressive worsening of histopathologic changes and function. Although TG was associated with both acute and chronic histologic abnormalities, 14.5% of TG biopsies showed no interstitial fibrosis or tubular atrophy, while 58% (7/12) of biopsies with severe TG showed only minimal abnormalities. TG was associated with acute rejection, pretransplant hepatitis C antibody positivity and anti-HLA antibodies (especially anti-Class II), with the risk increasing if the antibodies were donor specific. We suggest that subclinical TG is an under-recognized cause of antibody-mediated, chronic renal allograft in-jury which may be mechanistically distinct from other causes of nephropathy.

35. Increased C4d in post-reperfusion biopsies and increased donor specific antibodies at one-week posttransplant are risk factors for acute rejection in mild to moderately sensitized kidney transplant recipients

Kidney International. 2013;83:1185–92

Djamali A, Muth BL, Ellis TM, Mohamed M, Fernandez LA, Miller KM, Bellingham JM, Odorico JS, Mezrich JD, Pirsch JD, D'Alessandro TM, Vidyasagar V, Hofmann RM, Torrealba JR, Kaufman DB, Foley DP.

ABSTRACT:

In order to define the intensity of immunosuppression, we examined risk factors for acute rejection in desensitization protocols that use baseline donor-specific antibody levels measured as mean fluorescence intensity (MFI_{max}). The study included 146 patients transplanted with a negative flow crossmatch and a mean follow-up of 18 months with the majority (83%) followed for at least 1 year. At the time of transplant, mean-calculated panel-reactive antibody and MFI_{max} ranged from 10.3–57.2% and 262–1691, respectively, between low- and high-risk protocols. Mean MFI_{max} increased significantly from transplant to 1 week and 1 year. The incidence of acute rejection (mean 1.65 months) as a combination of clinical and subclinical rejection was 32%, including 14% cellular, 12% antibody-mediated, and 6% mixed rejection. In regression analyses, only C4d staining in post-reperfusion biopsies (hazard ratio 3.3, confidence interval 1.71–6.45) and increased specific antibodies at 1-week post transplant were significant predictors of rejection. A rise in MFI_{max} by 500 was associated with a 2.8-fold risk of rejection. Thus, C4d staining in postreperfusion biopsies and an early rise in donor specific antibodies after transplantation are risk factors for rejection in moderately sensitized patients.

36. Histologic findings one year after positive crossmatch or ABO blood group incompatible living donor kidney transplantation.
Am J Transplant 2006; 6: 1841–1847.
Gloor JM, Cosio FG, Rea DJ et al.

Recent protocols have allowed successful positive crossmatch (+XM) and ABO incompatible (ABOI) kidney transplantation, although their long-term out-come is not clear. To begin to assess this issue we compared protocol biopsies performed 12 months posttransplant in 37 +XM, 24 ABOI and 198 conventional allografts. Although the majority in all three groups had only minimal histologic changes, transplant glomerulopathy (TG) was significantly increased in +XM (22% vs. 13% ABOI vs. 8% conventional, $p = 0.015$), and correlated with prior humoral rejection (HR) by multivariate analysis (odds ratio 17.5, $p \leq 0.0001$). Patients with a prior history of HR also had a significant increase in interstitial fibrosis (No HR 54% vs. HR 86%, $p = 0.045$). In the absence of HR no difference in histologic changes was seen between groups, although all three groups had a demonstrable mild increase in iNterstitial fibrosis from biopsies performed at the time of transplant. Thus, although HR is associated with an increase in TG, in its absence allograft histology is similar in +XM, ABOI and conventional allografts 1 year posttransplant.

37. Subclinical Acute Antibody-Mediated Rejection in Positive Crossmatch Renal Allografts.
Am J Transplant 2006; 6: 1-10.
Haas M, Montgomery RA, Segev DL et al.

Subclinical antibody-mediated rejection (AMR) has been described in renal allograft recipients with stable serum creatinine (SCr), however whether this leads to development of chronic allograft nephropathy (CAN) remains unknown.

We retrospectively reviewed data from 83 patients who received HLA-incompatible renal allografts following desensitization to remove donor-specific antibodies (DSA). Ten patients had an allograft biopsy showing subclinical AMR [stable SCr, neutrophil margination in peritubular capillaries (PTC), diffuse PTC C4d, positive DSA] during the first year post-transplantation; 3 patients were treated with plasmapheresis and intravenous immunoglobulin. Three patients had a subsequent rise in SCr and an associated biopsy with AMR; 5 others showed diagnostic or possible subclinical AMR on a later protocol biopsy. One graft was lost, while remaining patients have normal or mildly elevated SCr 8–45 months post-transplantation. However, the mean increase in CAN score (cg + ci + ct + cv) from those biopsies showing subclinical AMR to follow-up biopsies 335 ± 248 (SD) days later was significantly greater (3.5 ± 2.5 versus 1.0 ± 2.0, p = 0.01) than that in 24 recipients of HLA-incompatible grafts with no AMR over a similar interval (360 ± 117 days), suggesting that subclinical AMR may contribute to development of CAN.

38. Subclinical Rejection in Stable Positive Crossmatch Kidney Transplant Patients: Incidence and Correlations

American Journal of Transplantation 2009;9:1826–34

Kraus ES, Parekh RS, Oberai P, Lepley D, Segev DL, Bagnasco S, Collins V, Leffell M, Lucas D, Rabb H, Racusen LC, Singer AL, Stewart ZA, Warren DS, Zachary AA, Haas M, Montgomery RA.

ABSTRACT:

We reviewed 116 surveillance biopsies obtained approximately 1, 3, 6 and 12 months posttransplantation from 50 +XM live donor kidney transplant recipients to determine the frequency of subclinical cell-mediated rejection (CMR) and antibody-mediated rejection (AMR). Subclinical CMR was present in 39.7% of the biopsies at 1 month and >20% at all other time points. The presence of diffuse C4d on biopsies obtained at each time interval ranged from 20 to 30%. In every case, where histological and immunohistological findings were diagnostic for AMR, donor-specific antibody was found in the blood, challenging the longheld belief that low-level antibody could evade detection due to absorption on the graft. Among clinical factors, only recipient age was associated with subclinical CMR. Clinical factors associated with subclinical AMR were recipient age, positive cytotoxic crossmatch prior to desensitization and two mismatches of HLA DR 51, 52 and 53 alleles. Surveillance biopsies during the first year post-transplantation for these high-risk patients uncover clinically occult processes and phenotypes, which without intervention diminish allograft survival and function.

39. C4d and C3d staining in biopsies of ABO- and HLA-incompatible renal allografts: correlation with histologic findings.

Am J Transplant 2006; 6: 1829-1840.

Haas M, Rahman RH, Racusen LC et al.

Biopsies of ABO-incompatible and positive crossmatch (HLA-incompatible) renal allografts were retrospec-tively examined to compare results of C4d and C3d staining, and the correlation between such staining and histologic findings suggestive of antibody-mediated rejection (AMR). A total of 75 biopsies (55 protocol, 17 for graft dysfunction, 3 for other indications) of 24 ABO-incompatible grafts and 244 biopsies (103 protocol, 129 for graft dysfunction, 12 for other indications) of 66 HLA-incompatible grafts were examined; all were stained for C4d and ~40% for C3d.In ABO-incompatible grafts, 80% of protocol biopsies and 59% performed for graft dysfunction showed C4d staining in peritubular capillaries (PTC); this staining was not correlated with neutrophil margination in PTC. In HLA-incompatible grafts, PTC C4d was present in 26% of protocol biopsies and 60% of biopsies for graft dysfunction; 92% of biopsies with >1+ (0–4+ scale), diffuse PTC C4d had ≥1+ margination and/or thrombotic microangiopathy (TMA), compared with 12% of C4d-negative biopsies. C3d was somewhat more pre-dictive of margination than C4d in ABO-incompatible, but not HLA-incompatible, grafts. In summary, while PTC C4d deposition indicates probable AMR in biopsies of HLA-incompatible grafts, including protocol biopsies, there is no histologic evidence that C4d deposition is correlated with injury in most ABO-incompatible grafts.

40. Analysis of Renal Transplant Protocol Biopsies in ABO-Incompatible Kidney Transplantation

American Journal of Transplantation. 2008;8:86–94

Setoguchi K, Ishida H, Shimmura H, Shimizu T, Shirakawa H, Omoto K, Toki D, Iida S, Setoguchi S, Tokumoto T, Horita S, Nakayama H, Yamaguchi Y, Tanabe K.

ABSTRACT:

Numerous studies have shown that protocol biopsies have predictive power. We retrospectively examined the histologic findings and C4d staining in 89 protocol biopsies from 48 ABO-incompatible (ABO-I) transplant recipients, and compared the results with those of 250 controls from 133 ABO-compatible (ABO-C) transplant recipients given equivalent maintenance immunosuppression.

Others have shown that subclinical rejection (borderline and grade I) in ABO-C grafts decreased gradually after transplantation. In our study, however, subclinical rejection in the ABO-I grafts was detected in 10%, 14% and 28% at 1, 3 and 6–12 months, respectively.

At 6–12 months, mild tubular atrophy was more common in the ABO-C grafts whereas the incidence of transplant glomerulopathy did not differ between the two groups (ABO-C: 7%; ABO-I: 15%; $p = 0.57$). In the ABO-I transplants, risk factors for transplant glomerulopathy in univariate analysis were positive panel reactivity (relative risk, 45.0; $p < 0.01$) and a prior history of antibody-mediated rejection (relative risk, 17.9; $p = 0.01$). Furthermore, C4d deposition in the peritubular capillaries was detected in 94%, with diffuse staining in 66%. This deposition, however, was not linked to antibody-mediated rejection. We conclude that, in the ABO-I kidney transplantation setting, detection of C4d alone in protocol biopsies might not have any diagnostic or therapeutic relevance.

41. ABO blood group-incompatible living donor kidney transplantation: a prospective, single-centre analysis including serial protocol biopsies.

Nephrol Dial Transplant 2009; 24: 298-303.

Oettl T, Halter J, Bachmann A et al.

Background: ABO incompatible kidney transplantation using antigen-specific immunoadsorption is increasingly performed but data on outcome, complications and proto-col biopsies are still scarce. The present prospective single-centre study was aimed at these issues.
Methods: This was a prospective single-centre cohort study of 10 successive ABO incompatible living donor kidney transplantations at the University Hospital Basel from September 2005 to October 2007. The following parameters were closely monitored during the whole follow-up: graft function, albuminuria, blood group antibody titres, CD19+ cell count, total IgG and IgG subclasses, CMV antigenaemia, decoy cells in the urine, EBV and polyoma BK virus PCR in the blood. Protocol biopsies were per-formed on Days 0 and 7 after 3, 6, 12 and 18 months.
Results: Patient and graft survival is 100% after a median follow-up of 489 days (range 183–916 days). Median serum creatinine is 137 µmol/l (range 70–215 µmol/l), and median urine albumin–creatinine ratio (UACR) is 3.1 mg/mmol (range 0.6–7.8 mg/mmol) at the time of the last follow-up. All patients had sustained diminished CD19+ cell count and/or total IgG concentrations. Neither CMV antigenaemia nor EBV replication in the blood was observed. Seven patients had positive polyoma BK virus replication in the blood but none developed polyoma virus-associated nephropathy (PVAN). Protocol biopsies revealed rejection Banff IIa in three patients on Day 7, and in one patient after 3 and 6 months. Banff Ia rejection was found in five patients. All rejection episodes resolved. Mild signs of chronic antibody-mediated rejection were observed in five patients.
Conclusions: ABO-incompatible kidney transplantation seems to be successful and safe. Modifications of the cur-rent protocol may be possible and may further reduce potential side effects and costs.

42. Lack of benefit of early protocol biopsies in renal transplant patients receiving TAC and MMF: a randomized study.

Am J Transplant 2007; 7:2538–2545.

Rush D, Arlen D, Boucher A et al.

We conducted a randomized, multicenter study to determine whether treatment of subclinical rejection with increased corticosteroids resulted in beneficial outcomes in renal transplant patients receiving tacrolimus (TAC), mycophenolate mofetil (MMF) and prednisone. One hundred and twenty-one patients were randomized to biopsies at 0,1,2,3 and 6 months (Biopsy arm), and 119 to biopsies at 0 and 6 months only (Control arm). The primary endpoint of the study was the prevalence of the sum of the interstitial and tubular scores (ci + ct) > 2 (Banff) at 6 months. Secondary endpoints included clinical and subclinical rejection and renal function. At 6 months, 34.8% of the Biopsy and 20.5% of the Control arm patients had a ci + ct score ≥ 2($p = 0.07$). Between months 0 and 6, clinical rejection episodes were 12 in 10 Biopsy arm patients and 8 in 8 Control arm patients ($p = 0.44$). Over-all prevalence of subclinical rejection in the Biopsy arm was 4.6%. Creatinine clearance at 6 months was 72.9 ± 21.7 in the Biopsy and 68.90 mL/min ± 18.35 mL/min in the Control arm patients ($p = 0.18$). In conclusion, we found no benefit to the procurement of early protocol biopsies in renal transplant patients receiving TAC, MMF and prednisone, at least in the short term. This is likely due to their low prevalence of subclinical rejection.

43. Repeat True Surveillance Biopsies in Kidney Transplantation

Transplantation 2012;93:908-13

Buchmann TN, Wolff T, Bachmann A, Guerke L, Steiger J, Mihatsch MJ, Dickenmann M.

ABSTRACT:

Background: Protocol biopsies are assigned to fixed points in time after transplantation irrespective of renal function.
Usually, it is not known whether there is graft dysfunction at the time of biopsy. This study analyzes repeat protocol biopsies in the absence of any clinical signs of graft dysfunction at the time of biopsy (i.e., ‘‘true surveillance biopsy’’).
Methods: Observational single center study. Kidney transplant recipients with protocol biopsies after 3 and 6 months were analyzed.
Results: Three hundred seventy patients had protocol biopsies after 3 and 6 months. One hundred forty-eight patients (40%; 296 biopsies) with a median follow-up of 3.4 years (range, 0.95-7.7 years), fulfilled the criteria of repeat true surveillance biopsies. Graft survival censored for death was 100% at 1 year, 96% at the end of follow-up.
One hundred eighty-four biopsies (62%) revealed pathological findings, mainly subclinical rejection (3/6 months: 41% vs. 45%; P = 0.2) and chronic lesions (3/6 months: 22% vs. 44%; P<0.001). Grafts with repeat pathological findings at 3 and 6 months had a significant decline in graft function at end of follow-up compared with grafts with no or only singular pathology (median delta estimated glomerular filtration rate: -10.24 vs. -0.19; P = 0.005).
Ninety-three of 148 patients (63%) had a therapeutic intervention as a consequence of the biopsy.
Conclusions: Less than 50% of protocol biopsies were performed in the absence of any clinical signs of graft dysfunction. A high proportion of these biopsies revealed pathological findings that were associated with a significant decrease in long-term graft function.

44. Kidney Allograft Survival After Acute Rejection, the Value of Follow-Up Biopsies

American Journal of Transplantation. 2013;13:2334–41

El Ters M, Grande JP, Keddis MT, Rodrigo E, Chopra B, Dean PG, Stegall MD, Cosio FG

ABSTRACT:

Kidney allografts are frequently lost due to alloimmunity. Still, the impact of early acute rejection (AR) on long-term graft survival is debated. We examined this relationship focusing on graft histology post-AR and assessing specific causes of graft loss. Included are 797 recipients without anti-donor antibodies (DSA) at transplant who had 1 year protocol biopsies. 15.2% of recipients had AR diagnosed by protocol or clinical biopsies. Compared to no-AR, all histologic types of AR led to abnormal histology in 1 and 2 years protocol biopsies, including more fibrosis + inflammation (6.3% vs. 21.9%), moderate/severe fibrosis (7.7% vs. 13.5%) and transplant glomerulopathy (1.4% vs. 8.3%, all $p < 0.0001$). AR were associated with reduced graft survival (HR = 3.07 (1.92–4.94), $p < 0.0001$). However, only those AR episodes followed by abnormal histology led to reduced graft survival. Early AR related to more late alloimmune-mediated graft losses, particularly transplant glomerulopathy (31% of losses). Related to this outcome, recipients with AR were more likely to have new DSA class II 1 year posttransplant (no-AR, 11.1%; AR, 21.2%, $p = 0.039$). In DSA negative recipients, early AR often leads to persistent graft inflammation and increases the risk of new DSA II production. Both of these post-AR events are associated with increased risk of graft loss.

45. Renal transplantation: can we reduce calcineurin inhibitor/stop steroids? Evidence based on protocol biopsy findings.

J Am Soc Nephrol. 2003; 14 (3): 755-66.

Gotti E, Perico N, Perna A et al.

Abstract:

How to combine antirejection drugs and which is the optimal dose of steroids and calcineurin inhibitors beyond the first year after kidney transplantation to maintain adequate immuno-suppression without major side effects are far from clear. Kidney transplant patients on steroid, cyclosporine (CsA), and azathioprine were randomized to per-protocol biopsy (n = 30) or no-biopsy (n = 29) 1 to 2 yr posttransplant. Steroid or CsA were discontinued or reduced on the basis of biopsy to establish effects on drug-related complications, acute rejection, and graft function over 3 yr of follow-up. Serum creatinine, GFR (plasma clearance of iohexol), RPF (renal clearance of paminohippurate), CsA pharmacokinetics, and adverse events were monitored yearly. At the end, patients underwent a second biopsy. Per-protocol biopsy histology revealed no lesions (n = 5, steroid withdrawal), CsA nephropathy (n = 13, CsA discontinuation/reduction), or chronic rejection (n = 12, standard therapy). Reducing the drug régimen led to overall fewer side effects related to immunosuppression as compared with standard therapy or no-biopsy. Steroids were safely stopped with no acute rejection or graft loss. Complete CsA discontinuation was associated with acute rejection in the first four patients. Lowering CsA to low target CsA trough (30 to 70 ng/ml) never led to acute rejection or major renal function deterioration. Biopsy patients on conventional regimen had no acute rejection, one graft loss, no significant change in GFR, and significant RPF decline. No-biopsy controls: no acute rejection, one graft loss, significant decline of GFR and RPF. By serial biopsy analysis, severe lesions did not develop in patients with steroid discontinuation in contrast to patients on standard therapy over follow-up. CsA reduction did not adversely affect histology. Per-protocol biopsy more than 1 yr after kidney transplantation is a safe procedure to guide change of drug regimen and to lower the risk of major side effects.

46. Assessment of the risk of chronic allograft dysfunction after renal transplantation in a randomized cyclosporine withdrawal trial.

Transplantation 2006; 82: 657-662.

Hazzan M, Buob D, Lavalette M et al.

Background: We report the two-year follow-up of a trial comparing the three-month postgraft discontinuation of either cyclosporine (CsA) or mycophenolate mofetil (MMF) from a triple-drug regimen after de novo renal transplantation. Methods. One hundred and eight patients were enrolled in this study and randomized to be withdrawn from CsA (MMF group, n=54) or MMF (CsA group, n=54).
Results: Despite an increased risk of acute rejection and a lower, but nonsignificant, two-year graft survival, CsA withdrawal induced a sustained improvement of the renal function. At one year, the chronic allograft damage index was similar in both the MMF and CsA groups. However, CsA elimination resulted in a higher incidence of C4d deposits, irrespective of the occurrence of a prior acute rejection. While this finding could suggest a risk of chronic rejection in the MMF group, the outcome did not appear to be related to the C4d status. Moreover, logistic regression analysis showed that only two factors, acute rejection and the one-year glomerular filtration rate level, were predictive of a significant decline of the renal function at two years.
Conclusions: These results point out the need to secure the minimization of the calcineurin inhibitors after renal transplantation, in order to reduce the risk of acute rejection in these patients, because this strategy allows the improvement of the one-year renal function which is predictive of a chronic allograft dysfunction.

47. Impact of Early Conversion From Tacrolimus to Sirolimus on Chronic Allograft Changes in Kidney Recipients on Rapid Steroid Withdrawal

Transplantation. 2012;93: 47–53

Heilman RL, Cortese C, Geiger XJ, Younan K, Wadei HM, Mai ML, Reddy KS, Gonwa TA.

ABSTRACT:

Background: Calcineurin-inhibitor therapy is a contributing factor to the origin of interstitial fibrosis and tubular atrophy (IFTA).
Methods: We conducted a prospective randomized trial of conversion of tacrolimus to sirolimus at 1-month posttransplant in kidney transplant recipients on rapid steroid withdrawal. We compared the chronic changes (IFTA and sum of Banff chronic scores—Total Score) on protocol biopsies at 1 month, 1 year, and 2 years in all randomized patients. We compared the outcomes between treatment groups and analyzed the impact of previous rejection on the chronic changes.
Results: We randomized 122 patients, 62 to sirolimus and 60 to tacrolimus. The 1-year biopsy was performed in 54 patients (90%) of the tacrolimus group and 56 patients (90%) of the sirolimus group. The proportion of biopsies with IFTA more than or equal to 2 and the Total Score more than 2 increased over the 2 years but were not different between the study groups at any time point. On the 1-year biopsy, there was more IFTA, and the fraction with Total Score more than 2 was higher in the tacrolimus group with previous rejection. In the cohort without rejection, there was a significant progression of the IFTA and Total Score between 1 and 2 years in both the sirolimus and tacrolimus groups.
Conclusion: Conversion from tacrolimus to sirolimus at 1-month posttransplant in kidney transplant recipients on rapid steroid withdrawal does not decrease the progression of chronic changes on protocol biopsies during the first 2 years even in those patients without previous acute rejection.

48. Adverse Outcomes of Tacrolimus Withdrawal in Immune–Quiescent Kidney Transplant Recipients.

J Am Soc Nephrol 2015; doi: 10.1681/ASN.2014121234.

Hricik DE, Formica RN, Nickerson P et al.

ABSTRACT:

Concerns about adverse effects of calcineurin inhibitors (CNIs) have prompted development of protocols that minimize their use. Whereas previous CNI withdrawal trials in heterogeneous cohorts showed unacceptable rates of acute rejection (AR), we hypothesized that we could identify individuals capable of tolerating CNI withdrawal by targeting immunologically quiescent kidney transplant recipients. The Clinical Trials in Organ Transplantation-09 Trial was a randomized, prospective study of nonsensitized primary recipients of living donor kidney transplants. Subjects received rabbit antithymocyte globulin, tacrolimus, mycophenolate mofetil, and prednisone. Six months post-transplantation, subjects without de novo donor-specific antibodies (DSAs), AR, or inflammation at protocol biopsy were randomized to wean off or remain on tacrolimus. The intended primary end point was the change in interstitial fibrosis/tubular atrophy score between implantation and 24-month protocol biopsies. Serially collected urine CXCL9 ELISA results were correlated with outcomes. The study was terminated prematurely because of unacceptable rates of AR (4 of 14) and/or de novo DSAs (5 of 14) in the tacrolimus withdrawal arm. Positive urinary CXCL9 predated clinical detection of AR by a median of 15 days. Analyses showed that .16 HLA-DQ epitope mismatches and pretransplant, peripheral blood, donor–reactive IFN-g ELISPOT assay results correlated with development of DSAs and/or AR on tacrolimus withdrawal. Although data indicate that urinary CXCL9 monitoring, epitope mismatches, and ELISPOT assays are potentially informative, complete CNI withdrawal must be strongly discouraged in kidney transplant recipients who are receiving standard-of-care immunosuppression, including those who are deemed to be immunologically quiescent on the basis of current clinical and laboratory criteria.

49. Fibrosis progression according to epithelial-mesenchymal transition profile: a randomized trial of everolimus versus CsA.

Am J Transplant. 2015 May;15(5):1303-12. doi: 10.1111/ajt.13132. Epub 2015 Mar 23.

Rostaing L, Hertig A, Albano L, Anglicheau D, Durrbach A, Vuiblet V, Moulin B, Merville P, Hazzan M, Lang P, Touchard G, Hurault deLigny B, Quéré S, Di Giambattista F, Dubois YC, Rondeau E; CERTITEM Study Group.

ABSTRACT:

Markers of epithelial-mesenchymal transition (EMT) may identify patients at high risk of graft fibrogenesis who could benefit from early calcineurin inhibitor (CNI) withdrawal. In a randomized, open-label, 12-month trial, de novo kidney transplant patients received cyclosporine, enteric-coated mycophenolate sodium (EC-MPS) and steroids to month 3. Patients were stratified as EMT+ or EMT- based on month 3 biopsy, then randomized to start everolimus with half-dose EC-MPS (720 mg/day) and cyclosporine withdrawal (CNI-free) or continue cyclosporine with standard EC-MPS (CNI). The primary endpoint was progression of graft fibrosis (interstitial fibrosis/tubular atrophy [IF/TA] grade increase ≥1 between months 3-12) in EMT+ patients. 194 patients were randomized (96 CNI-free, 98 CNI); 153 (69 CNI-free, 84 CNI) were included in histological analyses. Fibrosis progression occurred in 46.2% (12/26) CNI-free EMT+ patients versus 51.6% (16/31) CNI EMT+ patients ($p = 0.68$). Biopsy-proven acute rejection (BPAR, including subclinical events) occurred in 25.0% and 5.1% of CNI-free and CNI patients, respectively ($p < 0.001$). In conclusion, early CNI withdrawal with everolimus initiation does not prevent interstitial fibrosis. Using this CNI-free protocol, in which everolimus exposure was relatively low and administered with half-dose EC-MPS, CNI-free patients were overwhelmingly under-immunosuppressed and experienced an increased risk of BPAR.

50. IgA nephropathy recurs early in the graft when assessed by protocol biopsy.
Nephrol Dial Transplant 2011; 0: 1–6
Ortiz F, Gelpi R, Koskinen P.

ABSTRACT:

Background: The recurrence of IgA nephropathy (IgAN) in the allograft is common. Factors related to IgA recur-rence are unclear. The aims of this study were to deter-mine the incidence of IgAN recurrence as assessed by protocol biopsies and to identify predictive factors for recurrence.
Methods: We identified 65 protocol biopsies taken before the second year post-transplantation in patients with IgAN as primary renal disease. Diagnosis of recurrence of IgA was based on the detection of at least 11 mesangial deposits of IgA. Pathological findings and clinical characteristics were retrospectively compared between recurrent and non-recurrent cases.
Results: IgAN recurrence rate was 32%. Mesangial C3 was detected in 83% of recurrent cases versus 17% in non-recur-rent patients ($P < 0.001$). Normal urinalysis was observed in 52%. Non-recurrent patients had arteriolar hyalinosis in 31%of the cases versus none in IgAN recurrence (P ¼ 0.006). Seventy-nine per cent of cyclosporine users were free of recurrence, whereas 45% of the patients without cyclosporine experienced recurrence (P ¼ 0.03). The odds ratio (OR) for IgAN recurrence in patients using cyclosporine was 0.3 (confidence interval 0.1–0.9). Zero HLA-DR mismatch was associated with non-recurrence ($P < 0.01$). The OR for IgA recurrence was 6.7 if any degree of DR mismatch was present. IgAN recurrent patients had better glomerular filtration rate, but after censoring delayed graft function, the differences disappeared. Graft loss due to IgA recurrence was only 3%.
Conclusions: IgAN recurrence rate was 32%. The histological diagnosis was not accompanied by abnormalities in the urinalysis in one-half of the patients. Full DR match and cyclosporine were associated with non-recurrence.

51. Recurrent idiopathic membranous nephropathy: early diagnosis by protocol biopsies and treatment with anti-CD20 monoclonal antibodies.

Am J Transplant 2009; 9: 2800-2807.

El Zoghby ZM, Grande JP, Fraile MG et al.

Membranous nephropathy (MN) recurs posttransplant in 42% of patients. We compared MN recurrence rates in a historical cohort transplanted between 1990 and 1999 and in a current cohort diagnosed by protocol biopsies, we analyzed the progression of the disease and we assessed the effects of anti-CD20 antibodies (Rituximab) on recurrent MN. The incidence of recur-rent MN was similar in the historical (53%) and the cur-rent cohorts (41%), although in the later the diagnosis was made earlier (median, 4[2–21] months vs. 83[6–149], p = 0.002) and the disease was clinically milder. Twelve out of 14 patients (86%) with recurrent MN in the current cohort had progressive increases in proteinuria. Eight recipients were treated with Rituximab after their proteinuria increased from median, 211 mg/day (64–4898) at diagnosis to 4489 (898–13 855) (p = 0.038). Twelve months post-Rituximab, 75% of patients had either partial (PR) or complete remission (CR). After 24 months 6/7 (86%) had PR/CR and one patient relapsed. Posttreatment biopsies showed resorption of electron dense immune deposits in 6/7 cases and were negative for C3 (4/7) and IgG (3/7). Protocol biopsies al-low early diagnosis of subclinical recurrent MN, which is often progressive. Treatment of recurrent MN with Rituximab is promising and should be evaluated in a prospective randomized controlled trial.

52. The impact of surveillance and rapid reduction in immunosuppression to control BK virus-related graft injury in kidney transplantation

Transplant International. 2013;26(8):822-32. ISSN 0934-0874

Elfadawy N, Flechner SM, Liu X, Schold J, Tian D, Srinivas TR, Poggio E, Fatica R, Avery R, Mossad SB.

ABSTRACT:

We prospectively screened 609 consecutive kidney (538) and kidney-pancreas (71) transplant recipients for BK viremia over a 4-year interval using polymerase chain reaction viral load detection and protocol kidney biopsies. We found that BK viremia is common at our center: total cases 26.7%, cases during first year 21.3% (mean 4 months), and recipients with ≥10 000 copies/ml 12.3%.

We found few predictive clinical or demographic risk factors for any BK viremia or viral loads ≥10,000 copies/ml, other than prior treatment of biopsy confirmed acute rejection and/or higher immunosuppressive blood levels of tacrolimus (P = 0.001) or mycophenolate mofetil (P = 0.007). Viral loads at diagnosis (<10 000 copies/ml) demonstrated little impact on graft function or survival. However, rising copy numbers demand early reductions in immunosuppressive drug doses of at least 30–50%. Viral loads >185 000 copies/ml at diagnosis were predictive of BK virus-associated nephropathy (BKVAN; OR: 113.25, 95% CI: 17.22–744.6, P < 0.001). Surveillance for BK viremia and rapid reduction of immunosuppression limited the incidence of BKVAN to 1.3%. The addition of leflunomide or ciprofloxacin to immunosuppressive dose reduction did not result in greater rates of viral clearance. These data support the role of early surveillance for BK viremia to limit the impact on transplant outcome, although the most effective schedule for screening awaits further investigation.

53. Angiotensin II blockade in kidney transplant recipients.

J Am Soc Nephrol 2013; 24: 320–327

Ibrahim HN, Jackson S, Connaire J et al.

ABSTRACT:

Interstitial fibrosis/tubular atrophy (IF/TA) contributes to the loss of kidney allografts, and treatment or preventive options are lacking. We conducted a double-blind, randomized, placebo-controlled trial to determine whether angiotensin II blockade prevents the expansion of the cortical interstitial compartment, the precursor of fibrosis. We randomly assigned 153 transplant recipients to receive losartan, 100 mg (n=77), or matching placebo (n=76) within 3 months of transplantation, continuing treatment for 5 years. The primary outcome was a composite of doubling of the fraction of renal cortical volume occupied by interstitium from baseline to 5 years or ESRD from IF/TA. In the intention-to-treat analysis, using only patients with adequate structural data, the primary endpoint occurred in 6 of 47 patients who received losartan and 12 of 44 who received placebo (odds ratio [OR], 0.39; 95% confidence interval [CI], 0.13–1.15; P=0.08). We found no significant effect of losartan on time to a composite of ESRD, death, or doubling of creatinine level. In a secondary analysis, losartan seemed to reduce the risk of a composite of doubling of interstitial volume or all-cause ESRD (OR, 0.36; 95% CI, 0.13–0.99; P=0.05), but this finding requires validation. In conclusion, treatment with losartan did not lead to a statistically significant reductionina composite of interstitial expansion or ESRD from IF/TA in kidney transplant recipients.

Proyecto Prometeo II

Grupo I | Monitorización inmunológica

Referencias Bibliográficas

Organizado por

SET
SOCIEDAD ESPAÑOLA DE TRASPLANTE

Con la colaboración de

NOVARTIS

1. Summary of FDA Antibody-Mediated Rejection Workshop

American Journal of Transplantation. 2011; 11: 896–906

Archdeacon P, Chan M, Neuland C, Velidedeoglu E, Meyer J, Tracy L, Cavaille-Coll M, Bala S, Hernandez A, Albrecht R.

The Food and Drug Administration (FDA) held an open public workshop in June 2010 to discuss the current state of science related to antibody-mediated rejection (AMR) in kidney transplantation. Desensitization, acute AMR and chronic AMR (CAMR) were considered in the context of clinical trial design. Participants discussed experiences with HLA antibody detection and quantitation and the utility of monitoring donor specific antibodies (DSAs) to inform the management of patients with AMR. The role for animal models was discussed. Diagnostic and prognostic features of histology were presented, followed by discussion of sensitivity and specificity of various criteria. The published literature on treatment of acute AMR was summarized, which consisted of case series and limited data from controlled clinical trials. Considerations for future clinical trials were presented, including endpoints and statistical evaluations of outcome. Although many issues need further consideration, the meeting enabled an important exchange of ideas between experts in the field.

2. The Role of Immunoglobulin-G Subclasses and C1q in De Novo HLA-DQ Donor-Specific antibody Kidney Transplantation Outcomes

Transplantation. 2013;95: 1113-9

Freitas MC, Rebellato LM, Ozawa M, Nguyen A, Sasaki N, Everly M, Briley KP, Haisch CE, Bolin P, Parker K, Kendrick WT, Kendrick SA, Harland RC, Terasaki PI.

Background: AntiYHLA-DQ antibodies are the predominant HLA class II donor-specific antibodies (DSAs) after transplantation. Recently, de novo DQ DSA has been associated with worse allograft outcomes. The aim of this study was to determine the further complement-binding characteristics of the most harmful DQ DSA.
Methods: Single-antigen bead technology was used to screen 284 primary kidney transplant recipients for the presence of posttransplantation DQ DSA. Peak DSA sera of 34 recipients with only de novo DQ DSA and of 20 recipients with de novo DQ plus other DSAs were further analyzed by a modified single-antigen bead assay using immunoglobulin (Ig)-G subclass-specific reporter antibodies and a C1q-binding assay.
Results: Compared with recipients who did not have DSA, those with de novo persistent DQ-only DSA and with de novo DQ plus other DSAs had more acute rejection (AR) episodes (22%, P=0.005; and 36%, P=0.0009), increased risk of allograft loss (hazards ratio, 3.7, P=0.03; and hazards ratio, 11.4, P=0.001), and a lower 5-year allograft survival.
De novo DQ-only recipients with AR had more IgG1/IgG3 combination and C1q-binding antibodies (51%, P=0.01; and 63%, P=0.001) than patients with no AR. Furthermore, the presence of C1q-binding de novo DQ DSA was associated with a 30% lower 5-year allograft survival (P=0.003).
Conclusions: The presence of de novo persistent, complement-binding DQ DSA negatively impacts kidney allograft outcomes. Therefore, early posttransplantation detection, monitoring, and removal of complement-binding DQ might be crucial for improving long-term kidney transplantation outcomes.

3. Complement-Binding Anti-HLA Antibodies and Kidney-Allograft Survival

N Engl J Med. 2013;369:1215-26.

Loupy A, Lefaucheur C, Vernerey D, Prugger C, Duong van Huyen JP, Mooney N, Suberbielle C, Frémeaux-Bacchi V, Méjean A, Desgrandchamps F, Anglicheau D, Nochy D, Charron D, Empana JP, Delahousse M, Legendre C, Glotz D, Hill GS, Zeevi A, Jouven

Background: Anti-HLA antibodies hamper successful transplantation, and activation of the complement cascade is involved in antibody-mediated rejection. We investigated whether the complement-binding capacity of anti-HLA antibodies plays a role in kidney-allograft failure.
Methods: We enrolled patients who received kidney allografts at two transplantation centers in Paris between January 1, 2005, and January 1, 2011, in a population-based study.
Patients were screened for the presence of circulating donor-specific anti-HLA antibodies and their complement-binding capacity. Graft injury phenotype and the time to kidney-allograft loss were assessed.
Results: The primary analysis included 1016 patients. Patients with complement-binding donor-specific anti-HLA antibodies after transplantation had the lowest 5-year rate of graft survival (54%), as compared with patients with non–complement-binding donor-specific anti-HLA antibodies (93%) and patients without donor-specific anti-HLA antibodies (94%) (P<0.001 for both comparisons). The presence of complement-binding donor-specific anti-HLA antibodies after transplantation was associated with a risk of graft loss that was more than quadrupled (hazard ratio, 4.78; 95% confidence interval [CI], 2.69 to 8.49) when adjusted for clinical, functional, histologic, and immunologic factors. These antibodies were also associated with an increased rate of antibody-mediated rejection, a more severe graft injury phenotype with more extensive microvascular inflammation, and increased deposition of complement fraction C4d within graft capillaries. Adding complement-binding donor specific anti-HLA antibodies to a traditional risk model improved the stratification of patients at risk for graft failure (continuous net reclassification improvement, 0.75; 95% CI, 0.54 to 0.97).
Conclusions: Assessment of the complement-binding capacity of donor-specific anti-HLA antibodies appears to be useful in identifying patients at high risk for kidney-allograft loss.

4. REVISIÓN AC no HLA

AJT 2013;13:831

Nickerson PW

5. Posttransplant monitoring of de novo human leukocyte antigen donor-specific antibodies in kidney transplantation.

Current Opinion Organ Transplantation. 2013;18(4):470-7.

Wiebe C, Nickerson P.

ABSTRACT:

Purpose of review: To summarize the evidence supporting the negative impact of de novo donor-specific antibodies (dnDSA) in renal transplantation and to describe the natural history associated with the development of dnDSA.
Recent findings: Recent studies have increased our appreciation of the risk factors that predispose to dnDSA while illuminating how these risk factors may relate to the pathophysiology underlying its development. In addition, details regarding the natural history of dnDSA are now available in the context of the different clinical pathologic phenotypes that occur in the patients in whom it develops. Common pitfalls in defining and monitoring dnDSA, when understood, may provide some explanation for the heterogeneity in published studies.
Summary: Recognizing that dnDSA is a major cause of late graft loss, and, more importantly, is detectable in many cases long before dysfunction or graft loss occurs, identifies an opportunity to intervene and change the outcome for the patient.

6. Humoral Immune Response and Allograft Function in Kidney Transplantation

Am J Kidney Dis. 2015 May

Filippone EJ, Farber JL

ABSTRACT:

HLA antibodies can damage a kidney transplant. In January 2013, consensus guidelines from The Transplantation Society were published regarding technical aspects of HLA antibody determination, as well as their potential significance in the pre- and posttransplantation periods. During the past 2 years, new studies have been reported, but controversies remain. In this article, these new data related to HLA antibodies in kidney transplantation are reviewed and compared to relevant prior research. Pretransplantation sensitization issues are discussed, including the new more sensitive assays (flow cytometry and solid-phase immunoassays such as Luminex single-antigen bead assays). A positive complement-dependent cytotoxicity crossmatch remains an absolute contraindication to transplantation, although a positive flow cytometry crossmatch is only a relative contraindication. Positivity only by solid-phase assays increases the risk for acute rejection and transplant loss, but acceptable cutoffs are not defined. The sensitizing effect of red blood cell transfusions is substantiated. Following allograft failure, continued immunosuppression decreases the risk of sensitization, whereas overall, the effect of nephrectomy remains uncertain. Regarding the posttransplantation period, new data are available concerning the timing and significance of donor-specific antibodies (DSA). Whereas some centers report DSA appearance after years, others detect DSA within months. The prominence of class II DSA, especially DQ, in the posttransplantation period is noted. The relevance of non-HLA antibodies is discussed, including anti-endothelial cell antibodies, major histocompatibility complex class I chain-related protein A antibodies, and angiotensin II type 1 receptor autoantibodies.

7. Subclinical Lesions and Donor-Specific Antibodies in Kidney Transplant Recipients Receiving Tacrolimus-Based Immunosuppressive Regimen Followed by Early Conversion to Sirolimus. Transplantation. 2015 Apr 29. [Epub ahead of print]

de Sandes-Freitas TV, Felipe CR, Campos ÉF, de Lima MG, Soares MF, de Franco MF, Aguiar WF, Tedesco-Silva H, Medina-Pestana JO.

ABSTRACT:

Background: There is no evidence on the incidence of subclinical inflammation and scaring lesions in patients receiving tacrolimus (TAC) minimization and elimination immunosuppressive regimens.
Methods: This study analyzed preimplantation, 3 and 24 months protocol biopsies and anti-HLA donor-specific antibodies (DSA) in 140 low immunological risk kidney transplant recipients receiving reduced TAC exposure, prednisone, and mycophenolate, randomized at 3 months to be converted or not to sirolimus (SRL).
Results: Mean TAC concentrations were 6.0 ± 2.4 ng/mL and 5.8 ± 2.2 ng/mL at 3 and 24 months. The incidence of subclinical inflammation lesions at 3 months was 9.3%. The incidence of (interstitial fibrosis) IF/(tubular atrophy) TA at month 24 was 57.6%, higher in SRL compared to TAC group (68.8 vs 44.4%; P = 0.022). Patients converted to SRL showed higher incidence of acute rejection (7.3% vs 0%), proteinuria (59.6% vs 25%; P = 0.001), and DSA (17.8% vs 7.3%; P = 0.201), respectively. Biopsy-proven acute rejection (odds ratio [OR] 2.32, 95% confidence interval [95% CI], 0.979-5.518, P = 0.056), subclinical inflammation lesions at 3 months (OR, 11.75; 95% CI, 1.286-107.474; P = 0.029) and conversion to SRL (OR, 2.72; 95% CI, 1.155-6.383; P = 0.022) were associated with IF/TA at month 24. Black ethnicity (OR, 0.22; 95% CI, 0.058-0.873; P = 0.031), donor age (OR, 2.74; 95% CI, 1.329-5.649; P = 0.006), and conversion to SRL (OR, 2.34; 95% CI, 1.043-5.267; P = 0.039) were associated with inferior renal function at 24 months.
Conclusions: In kidney transplant recipients receiving reduced TAC exposure, subclinical inflammation lesions at 3 months were associated with IF/TA at 24 months. Conversion from TAC to SRL was associated with inferior renal function, higher incidence of IF/TA, and trends to higher incidence of DSA at 24 months.

8. Adverse Outcomes of Tacrolimus Withdrawal in Immune-Quiescent Kidney Transplant Recipients.

J Am Soc Nephrol. 2015 Apr 29.

Hricik DE, Formica RN, Nickerson P, Rush D, Fairchild RL, Poggio ED, Gibson IW, Wiebe C, Tinckam K, Bunnapradist S, Samaniego-Picota M, Brennan DC, Schröppel B, Gaber O, Armstrong B, Ikle D, Diop H, Bridges ND, Heeger PS; Clinical Trials in Organ Transplantation-09 Consortium.

ABSTRACT:

Concerns about adverse effects of calcineurin inhibitors (CNIs) have prompted development of protocols that minimize their use. Whereas previous CNI withdrawal trials in heterogeneous cohorts showed unacceptable rates of acute rejection (AR), we hypothesized that we could identify individuals capable of tolerating CNI withdrawal by targeting immunologically quiescent kidney transplant recipients. The Clinical Trials in Organ Transplantation-09 Trial was a randomized, prospective study of nonsensitized primary recipients of living donor kidney transplants. Subjects received rabbit antithymocyte globulin, tacrolimus, mycophenolate mofetil, and prednisone. Six months post-transplantation, subjects without de novo donor-specific antibodies (DSAs), AR, or inflammation at protocol biopsy were randomized to wean off or remain on tacrolimus. The intended primary end point was the change in interstitial fibrosis/tubular atrophy score between implantation and 24-month protocol biopsies. Serially collected urine CXCL9 ELISA results were correlated with outcomes. The study was terminated prematurely because of unacceptable rates of AR (4 of 14) and/or de novo DSAs (5 of 14) in the tacrolimus withdrawal arm. Positive urinary CXCL9 predated clinical detection of AR by a median of 15 days. Analyses showed that >16 HLA-DQ epitope mismatches and pretransplant, peripheral blood, donor-reactive IFN-γ ELISPOT assay results correlated with development of DSAs and/or AR on tacrolimus withdrawal. Although data indicate that urinary CXCL9 monitoring, epitope mismatches, and ELISPOT assays are potentially informative, complete CNI withdrawal must be strongly discouraged in kidney transplant recipients who are receiving standard-of-care immunosuppression, including those who are deemed to be immunologically quiescent on the basis of current clinical and laboratory criteria.

9. C1q Binding Activity of De Novo Donor-specific HLA Antibodies in Renal Transplant Recipients With and Without Antibody-mediated Rejection

Transplantation 2015;99: 1151–1155.

Yell M

Background: Complement fixation by donor-specific HLA antibodies (DSA) is a primary mechanism for antibody-mediated damage of organ allografts. Using a recently developed kit that measures C1q binding to distinguish complement fixing and nonfixing antibodies, studies showed that C1q + DSAs have a higher risk of rejection and graft loss compared to C1q-DSA. The objective of this study was to assess the ability of the C1q-binding assay to identify clinically significant de novo DSA in renal transplant recipients and to define the properties of DSA that confer C1q binding ability.

Methods: The DSA-positive sera from 34 kidney recipients, 19 with biopsy-proven antibody-mediated rejection (AMR) + and 15 who were AMR−, were assayed in C1q-binding assays (C1q Screen; One Lambda, Inc. Canoga Park, CA). The correlation between C1q-binding activity, presence of AMR, DSA mean fluorescence intensity (MFI) values, and immunoglobulin G isotype was determined.

Results: Fifty-three per-cent (10/19) of sera from AMR+ patients had C1q + DSA, whereas only 13% (2/15) of sera from AMR− patients contained C1q + DSA. C1q + DSA exhibited significantly higher MFI values regardless of whether they were from AMR+ or AMR− patients (16,118 ± 6698 vs 6429 ± 4003; $P < 0.0001$). C1q + DSA converted to C1q − when diluted to a comparable MFI level as the C1q − DSA from AMR− patients, and some C1q − antibodies converted to C1q + when concentrated to MFI levels comparable to those observed for AMR+/C1q + sera.

Conclusions: The C1q binding activity by de novo DSA in patients with AMR largely reflects differences in antibody strength. The C1q assay does not appear to distinguish functionally distinct DSA with clinical significance.

10. Utility of HLA Antibody Testing in Kidney Transplantation.

J Am Soc Nephrol. 2015

Konvalinka A, Tinckam K.

ABSTRACT:

HLA antigens are polymorphic proteins expressed on donor kidney allograft endothelium and are critical targets for recipient immune recognition. HLA antibodies are risk factors for acute and chronic rejection and allograft loss. Solid-phase immunoassays for HLA antibody detection represent a major advance in sensitivity and specificity over cell-based methods and are widely used in organ allocation and pretransplant risk assessment. Post-transplant, development of de novo donor-specific HLA antibodies and/or increase in donor-specific antibodies from pretransplant levels are associated with adverse outcomes. Although single antigen bead assays have allowed sensitive detection of recipient HLA antibodies and their specificities, a number of interpretive considerations must be appreciated to understand test results in clinical and research contexts. This review, which is especially relevant for clinicians caring for transplant patients, discusses the technical aspects of single antigen bead assays, emphasizes their quantitative limitations, and explores the utility of HLA antibody testing in identifying and managing important pre- and post-transplant clinical outcomes.

11. Influence of de novo donor-specific antibody on early renal allograft function recovery

Ren Fail. 2015 Apr;37(3):462-8

Zheng J, Xue W, Jing X, Hou J, Tian X, Tian P, Ding X, Pan X, Yan H, Feng X, Xiang H, Li Y, Ding C

ABSTRACT:

Background: The aim of the present study is to investigate the impact of de novo donor-specific antibodies (dnDSA) on early graft function, to provide objective reference for early clinical diagnosis and reasonable individualized treatment.
Methods: 305 cases of renal transplant patients for the first time were observed in this study. Follow-up time for all recipients was 6 months after operation. HLA antibody, DSA, renal function were monitored after transplant.
Results: In total of 305 cases, 66 cases (21.64%) were HLA antibody positive and 21 cases (6.89%) showed acute rejection (AR) in 6 months after transplant. The HLA antibody-positive patients included six cases of dnDSA-positive and 60 cases of dnDSA-negative. The incidence of AR was 2.09% (5/239) in HLA antibody-negative patients, 18.33% (11/60) in HLA antibody positive with DSA-negative patients, and 83.33% (5/6) in HLA antibody-positive patients with DSA-positive. There was a big difference between DSA-negative and DSA-positive patients ($p < 0.01$). The recovery time of AR patients with DSA-positive were longer than DSA-negative patients, and the recovery graft function of AR patient with DSA-positive were not as good as those with DSA-negative.
Conclusions: The appearance of dnDSA in the early stage of kidney transplantation is a warning sign of AR occurrence. Dynamic monitoring of HLA antibody and DSA could predict the state of graft function, and play an important role in the prevention of AR, timely and effectively.

12. Non-HLA antibodies: angiotensin II type 1 receptor (anti-AT1R) and endothelin-1 type A receptor (anti-ETAR) are associated with renal allograft injury and graft loss.

Transplant Proc. 2014 Oct;46(8):2618-21

Banasik M, Boratyńska M, Kościelska-Kasprzak K, Kamińska D, Zmonarski S, Mazanowska O, Krajewska M, Bartoszek D, Zabińska M, Myszka M, Kamińska M, Hałoń A, Dawiskiba T, Szyber P, Sas A, Klinger M.

ABSTRACT:

Introduction: Non-HLA antibodies specific for angiotensin II type 1 receptor (anti-AT1R) and endothelin-1 type A receptor (anti-ETAR) of vascular cells activate signaling pathways leading to cell proliferation and vascular injury. The aim of this study was to evaluate the impact of non-HLA antibodies on kidney allograft morphology and function in patients who underwent a kidney biopsy due to renal function impairment.
Patients and methods: The study included 65 consecutive renal transplant patients who were evaluated for the presence of non-HLA and anti-HLA antibodies at the time of transplant biopsy. Results of pre-transplant CDC cross-match were negative. A kidney allograft biopsy was performed between 6 days and 13 years (42 ± 49 months) after transplantation, and the diagnosis was made on the basis of the Banff criteria. The level >9 U/L of anti-AT1R and anti-ETAR antibodies was considered high.
Results: A high level of non-HLA antibodies (anti-AT1R and/or anti-ETAR) was found in 7 (10.7%) of 65 patients at the time of biopsy. Graft loss in the non-HLA-positive patients was significantly higher (71% in non-HLA-positive cases after 7.8 ± 2.6 months vs 11% after 6 months in non-HLA-negative cases [P = .00099]). In these non-HLA-positive patients, the mean anti-AT1R level was 15.3 ± 9.4 U/L and the mean anti-ETAR level was 13.8 ± 8.6 U/L. In only 2 of these patients were anti-HLA antibodies additionally detected: anti-class I in 1 and anti-class II in both patients. The mean serum creatinine level was 2.34 ± 0.6 mg/dL at the time of biopsy. Results of an early biopsy revealed acute vascular rejection (Banff grade IIB). Chronic allograft injury was found (grading cg1-3, cv1-2, ci1-2, ct1-2) in the remaining 6 patients. C4d was present in 3 of 7 patients.
Conclusions: High levels of anti-AT1R and/or anti-ETAR antibodies were associated with morphological and functional allograft injury and graft loss in these study patients. Non-HLA antibodies can be helpful in assessing the risk of graft failure.

13. The clinical spectrum of de novo donor-specific antibodies in pediatric renal transplant recipients.

Am J Transplant. 2014 Oct;14(10):2350-8

Kim JJ, Balasubramanian R, Michaelides G, Wittenhagen P, Sebire NJ, Mamode N, Shaw O, Vaughan R, Marks SD.

ABSTRACT:

The development of donor-specific HLA antibodies (DSA) is associated with worse renal allograft survival in adult patients. This study assessed the natural history of de novo DSA, and its impact on renal function in pediatric renal transplant recipients (RTR). HLA antibodies were measured prospectively using single-antigen-bead assays at 1, 3, 6 and 12 months posttransplant followed by 12-monthly intervals and during episodes of allograft dysfunction. Of 215 patients with HLA antibody monitoring, 75 (35%) developed DSA at median of 0.25 years posttransplant with a high prevalence of Class II (70%) and HLA-DQ (45%) DSA. DSA resolved in 35 (47%) patients and was associated with earlier detection (median, inter-quartile range 0.14, 0.09-0.33 vs. 0.84, 0.15-2.37 years) and lower mean fluorescence intensity (MFI) (2658, 1573-3819 vs. 7820, 5166-11 990). Overall, DSA positive patients had more rapid GFR decline with a 50% reduction in GFR at mean 5.3 (CI: 4.7-5.8) years versus 6.1 (5.7-6.4) years in DSA negative patients ($p = 0.02$). GFR decreased by a magnitude of 1 mL/min/1.73 m(2) per log10 increase in Class II DSA MFI ($p < 0.01$). Using Cox regression, independent factors predicting poorer renal allograft outcome were older age at transplant (hazard ratio 1.1, CI: 1.0-1.2 per year), tubulitis (1.5, 1.3-1.8) and microvasculature injury (2.9, 1.4-5.7). In conclusion, pediatric RTR with de novo DSA and microvasculature injury were at risk of allograft failure.

14. Detection of C3d-binding donor-specific anti-HLA antibodies at diagnosis of humoral rejection predicts renal graft loss.

J Am Soc Nephrol. 2015 Feb;26(2):457-67

Sicard A, Ducreux S, Rabeyrin M, Couzi L, McGregor B, Badet L, Scoazec JY, Bachelet T, Lepreux S, Visentin J, Merville P, Fremeaux-Bacchi V, Morelon E, Taupin JL, Dubois V, Thaunat O.

ABSTRACT:

Antibody-mediated rejection (AMR) is a major cause of kidney graft loss, yet assessment of individual risk at diagnosis is impeded by the lack of a reliable prognosis assay. Here, we tested whether the capacity of anti-HLA antibodies to bind complement components allows accurate risk stratification at the time of AMR diagnosis. Among 938 kidney transplant recipients for whom a graft biopsy was performed between 2004 and 2012 at the Lyon University Hospitals, 69 fulfilled the diagnosis criteria for AMR and were enrolled. Sera banked at the time of the biopsy were screened for the presence of donor-specific anti-HLA antibodies (DSAs) and their ability to bind C1q and C3d using flow bead assays. In contrast with C4d graft deposition, the presence of C3d-binding DSA was associated with a higher risk of graft loss (P<0.001). Despite similar trend, the difference did not reach significance with a C1q-binding assay (P=0.06). The prognostic value of a C3d-binding assay was further confirmed in an independent cohort of 39 patients with AMR (P=0.04). Patients with C3d-binding antibodies had worse eGFR and higher DSA mean fluorescence intensity. In a multivariate analysis, only eGFR <30 ml/min per 1.73 m(2) (hazard ratio [HR], 3.56; 95% confidence interval [CI], 1.46 to 8.70; P=0.005) and the presence of circulating C3d-binding DSA (HR, 2.80; 95% CI, 1.12 to 6.95; P=0.03) were independent predictors for allograft loss at AMR diagnosis. We conclude that assessment of the C3d-binding capacity of DSA at the time of AMR diagnosis allows for identification of patients at risk for allograft loss.

15. Evolution of donor-specific antibodies (DSA) and incidence of de novo DSA in solid organ transplant recipients after switch to everolimus alone or associated with low dose of calcineurin inhibitors.

Clin Transplant. 2014 Sep;28(9):1054-60

Perbos E, Juinier E, Guidicelli G, Dromer C, Merville P, Billes MA, Taupin JL, Neau-Cransac M.

ABSTRACT:

Background: Everolimus (EVR) is used in organ transplantation to minimize calcineurin inhibitors (CNI). Some studies pointed out an increase in rejection and de novo donor-specific antibodies (DSA) incidence in kidney transplant patients after switch to EVR and CNI withdrawal. The aims of our study were to determine the evolution of anti-HLA antibodies and the incidence of de novo DSA in transplant recipients after conversion to EVR.
Methods: Heart, lung, kidney, and liver transplant recipients were included in a retrospective, monocentric case-control study. Anti-HLA antibodies were identified at transplantation, pre-switch, and at three, six, and 12 months post-switch.
Results: Conversion to EVR was performed about six yr after the transplant, and low-dose CNI was maintained in 60% of patients. We found no statistical difference for rejection, evolution of preformed anti-HLA antibodies or de novo DSA, after conversion to EVR or not. Incidence of anti-class II DSA tended to increase at month 12 whatever the immunosuppressive regimen.
Conclusions: Late conversion to EVR appears to be safe and to not modify the natural evolution of anti-HLA antibodies in organ transplantation. As 60% of patients received EVR and low doses of CNI, it seems that such combinations could be used with a good outcome.

16. Changes in successive measures of de novo donor-specific anti-human leukocyte antigen antibodies intensity and the development of allograft dysfunction

Transplantation. 2014 Nov 27;98(10):1097-104

Dieplinger G, Everly MJ, Rebellato LM, Haisch CE, Briley KP, Bolin P, Kendrick WT, Kendrick SA, Morgan C, Harland RC, Terasaki PI.

ABSTRACT:

Background: Many patients develop de novo donor-specific anti-human leukocyte antigen antibodies (dnDSA) after transplantation. Despite development of dnDSA, not all patients will immediately fail. This study analyzes dnDSA intensity and longitudinal trends as prospective clinical parameters to assess subsequent allograft function.
Methods: Twenty-four patients with dnDSA onset in the first 2 years after transplantation received antibody monitoring by LABScreen single antigen beads. Estimated glomerular filtration rate (eGFR) was recorded at time of dnDSA onset and up to 24 months thereafter. The dnDSA mean fluorescence intensity (MFI) of the stable function patient group (n=8; eGFR decline ≤ 25%) was compared with the impaired function patient group (n=16; eGFR decline>25%) using first year peak MFI (pMFI), eight month MFI change (ΔMFI), and eighteen month MFI trend (MFI slope).
Results: Both groups showed similar dnDSA characteristics (time to onset after transplantation, class I/II distribution, and initial MFI). Between groups, MFI trends were analyzed. Impaired patients showed a higher pMFI during the first year (median pMFI, 13,055 vs. 2,397; P=0.007). Longitudinal analysis revealed that ΔMFI was strongly associated with dysfunction. Both a ΔMFI increase greater than 20% as well as a stronger increase (ΔMFI>50%) were followed by graft dysfunction in almost all patients and could significantly differentiate between stable and impaired function patients (P=0.001 and P=0.04, respectively).
Conclusion: Our study suggests that tracking dnDSA intensity, particularly in the early period after onset, is important to estimate the impact of dnDSA on the allograft and could, therefore, determine help on how best to monitor patients with dnDSA.

17. De novo donor-specific human leukocyte antigen antibodies early after kidney transplantation

Transplantation. 2014 Dec 27;98(12):1310-5

Heilman RL, Nijim A, Desmarteau YM, Khamash H, Pando MJ, Smith ML, Chakkera HA, Huskey J, Valdez R, Reddy KS

ABSTRACT:

Background: Our aim was to determine the incidence of de novo donor-specific human leukocyte antigen (HLA) antibody (dnDSA) during the first year after kidney transplantation and the impact of early dnDSA on acute rejection and protocol biopsy findings.
Methods: We selected all patients who received a kidney transplant at our center between July 2010 and March 2012. Single antigen bead assay was performed at 1, 4 and 12 months after transplantation. Only DSAs with a mean fluorescence intensity (MFI) of greater 999 were included.
Results: We included 245 kidney transplant recipients who did not have a DSA before transplantation. At 12 months, 8.2% of the patients developed dnDSA; 2.4% of them were to HLA class I and 6.5% to HLA class II. Of the 32 patients with a dnDSA at 1 or 4 months, only 8 (25%) persisted at 12 months. The risk of antibody-mediated rejection (AMR) was higher in the dnDSA group. For the dnDSA group with MFI of 3,000 or greater (compared with the group with MFI<3,000), the hazard ratio for AMR was 10.6 (95% confidence interval, 2.27-49.5). The cumulative incidence of AMR or mixed rejection at 1 year was 30% in the group with dnDSA MFI level of 3,000 or greater but only 4% for the group with dnDSA with MFI less than 3,000. On 1-year protocol biopsies, the dnDSA group showed more interstitial inflammation, tubulitis, and glomerulitis.
Conclusion: We conclude that dnDSA occurring during the first posttransplantation year may be transient, and the risk of AMR is higher in patients with a dnDSA MFI level that is greater than 3,000.

18. Transplantation: CNIs to mTOR inhibitors--effects on allosensitization?
Nat Rev Nephrol. 2014 Aug;10(8):425-6
Gupta A, Kaplan B.

19. Intermediate-term graft loss after renal transplantation is associated with both donor-specific antibody and acute rejection.

Transplantation. 2014 Mar 15;97(5):534-40

Devos JM, Gaber AO, Teeter LD, Graviss EA, Patel SJ, Land GA, Moore LW, Knight RJ.

ABSTRACT:

Background: Renal transplant recipients with de novo DSA (dDSA) experience higher rates of rejection and worse graft survival than dDSA-free recipients. This study presents a single-center review of dDSA monitoring in a large, multi-ethnic cohort of renal transplant recipients.
Methods: The authors performed a nested case-control study of adult kidney and kidney-pancreas recipients from July 2007 through July 2011. Cases were defined as dDSA-positive whereas controls were all DSA-negative transplant recipients. DSA were determined at 1, 3, 6, 9, and 12 months posttransplant, and every 6 months thereafter.
Results: Of 503 recipients in the analysis, 24% developed a dDSA, of whom 73% had dDSA against DQ antigen. Median time to dDSA was 6.1 months (range 0.2-44.6 months). After multivariate analysis, African American race, kidney-pancreas recipient, and increasing numbers of human leukocyte antigen mismatches were independent risk factors for dDSA. Recipients with dDSA were more likely to suffer an acute rejection (AR) (35% vs. 10%, P<0.001), an antibody-mediated AR (16% vs. 0.3%, P<0.001), an AR ascribed to noncompliance (8% vs. 2%, P=0.001), and a recurrent AR (6% vs. 1%, P=0.002) than dDSA-negative recipients. At a median follow-up of 31 months, the death-censored actuarial graft survival of dDSA recipients was worse than the DSA-free cohort (P=0.002). Yet, for AR-free recipients, there was no difference in graft survival between cohorts (P=0.66).
Conclusions: Development of dDSA was associated with an increased incidence of graft loss, yet the detrimental effect of dDSA was limited in the intermediate term to recipients with AR.

20. Impact of IgM and IgG3 anti-HLA alloantibodies in primary renal allograft recipients.
Transplantation. 2014 Mar 15;97(5):494-501.
Everly MJ, Rebellato LM, Haisch CE, Briley KP, Bolin P, Kendrick WT, Kendrick SA, Morgan C, Maldonado AQ, Harland RC, Terasaki PI.

ABSTRACT:

Background: With standard IgG donor-specific anti-HLA antibody (DSA) testing, it is unclear which immunoglobulin-G (IgG) DSA positive patients will fail. We looked further into the immune response by studying immunoglobulin-M (IgM) and IgG subclass 3 (IgG3) DSA to determine if these identify the IgG DSA patients at highest risk for allograft loss.
Methods: In 189 consecutively transplanted primary renal allograft recipients, sera were collected sequentially pre- and posttransplant. Of the 189, 179 patients had sera available to retrospectively test for anti-HLA IgG, IgM, and IgG3 antibodies via LABScreen single-antigen bead assay and were included in the study. All patients had a negative crossmatch. Per patient, all DSA (IgM, IgG3, and IgG) refers to the same serologic specificity.
Results: Overall, 100 (56%) patients developed an alloimmune response (IgM or IgG DSA positive, or both). Ninety-five patients developed IgM DSA and 47 patients developed IgG DSA. IgM DSA was detected in 42 of 47 patients with IgG DSA. IgM DSA alone did not increase the allograft loss risk, whereas IgG DSA did (P=0.002). Once IgG DSA appeared, IgM DSA persisted in 33 patients and an isotype switch to IgG3 positive DSA occurred in 25 patients. Patients with IgM persistent IgG3 positive DSA (n=19) were more likely to have allograft failure than those without (P=0.02).
Conclusion: This study shows the evolution of the humoral immune response from IgM to IgG DSA posttransplant. We found that development of IgM persistent IgG3 positive DSA identifies the most dangerous IgG DSA subpopulation.

21. Is there a role for detection of complement-binding antibodies in kidney transplantation?
Am J Kidney Dis. 2014 Apr;63(4):558-60.
Locke JE, Segev DL.

22. Higher risk of kidney graft failure in the presence of anti-angiotensin II type-1 receptor antibodies.

Am J Transplant. 2013 Oct;13(10):2577-89.

Taniguchi M, Rebellato LM, Cai J, Hopfield J, Briley KP, Haisch CE, Catrou PG, Bolin P, Parker K, Kendrick WT, Kendrick SA, Harland RC, Terasaki PI.

ABSTRACT:

Reports have associated non-HLA antibodies, specifically those against angiotensin II type-1 receptor (AT1R), with antibody-mediated kidney graft rejection. However, association of anti-AT1R with graft failure had not been demonstrated. We tested anti-AT1R and donor-specific HLA antibodies (DSA) in pre- and posttransplant sera from 351 consecutive kidney recipients: 134 with biopsy-proven rejection and/or lesions (abnormal biopsy group [ABG]) and 217 control group (CG) patients. The ABG's rate of anti-AT1R was significantly higher than the CG's (18% vs. 6%, $p < 0.001$). Moreover, 79% of ABG patients with anti-AT1R lost their grafts (vs. 0%, CG), anti-AT1R levels in 58% of those failed grafts increasing posttransplant. With anti-AT1R detectable before DSA, time to graft failure was 31 months-but 63 months with DSA detectable before anti-AT1R. Patients with both anti-AT1R and DSA had lower graft survival than those with DSA alone (log-rank $p = 0.007$). Multivariate analysis showed that de novo anti-AT1R was an independent predictor of graft failure in the ABG, alone (HR: 6.6), and in the entire population (HR: 5.4). In conclusion, this study found significant association of anti-AT1R with graft failure. Further study is needed to establish causality between anti-AT1R and graft failure and, thus, the importance of routine anti-AT1R monitoring and therapeutic targeting.

23. Consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplantation.

Transplantation. 2013 Jan 15;95(1):19-47

Tait BD, Süsal C, Gebel HM, Nickerson PW, Zachary AA, Claas FH, Reed EF, Bray RA, Campbell P, Chapman JR, Coates PT, Colvin RB, Cozzi E, Doxiadis II, Fuggle SV, Gill J, Glotz D, Lachmann N, Mohanakumar T, Suciu-Foca N, Sumitran-Holgersson S, Tanabe K, Taylor CJ, Tyan DB, Webster A, Zeevi A, Opelz G

ABSTRACT:

Background: The introduction of solid-phase immunoassay (SPI) technology for the detection and characterization of human leukocyte antigen (HLA) antibodies in transplantation while providing greater sensitivity than was obtainable by complement-dependent lymphocytotoxicity (CDC) assays has resulted in a new paradigm with respect to the interpretation of donor-specific antibodies (DSA). Although the SPI assay performed on the Luminex instrument (hereafter referred to as the Luminex assay), in particular, has permitted the detection of antibodies not detectable by CDC, the clinical significance of these antibodies is incompletely understood. Nevertheless, the detection of these antibodies has led to changes in the clinical management of sensitized patients. In addition, SPI testing raises technical issues that require resolution and careful consideration when interpreting antibody results.
Methods: With this background, The Transplantation Society convened a group of laboratory and clinical experts in the field of transplantation to prepare a consensus report and make recommendations on the use of this new technology based on both published evidence and expert opinion. Three working groups were formed to address (a) the technical issues with respect to the use of this technology, (b) the interpretation of pretransplantation antibody testing in the context of various clinical settings and organ transplant types (kidney, heart, lung, liver, pancreas, intestinal, and islet cells), and (c) the application of antibody testing in the posttransplantation setting. The three groups were established in November 2011 and convened for a "Consensus Conference on Antibodies in Transplantation" in Rome, Italy, in May 2012. The deliberations of the three groups meeting independently and then together are the bases for this report.
Results: A comprehensive list of recommendations was prepared by each group. A summary of the key recommendations follows. Technical Group: (a) SPI must be used for the detection of pretransplantation HLA antibodies in solid organ transplant recipients and, in particular, the use of the single-antigen bead assay to detect antibodies to HLA loci, such as Cw, DQA, DPA, and DPB, which are not readily detected by other methods. (b) The use of SPI for antibody detection should be supplemented with cell-based assays to examine the correlations between the two types of assays and to establish the likelihood of a positive crossmatch (XM). (c) There must be an awareness of the technical factors that can influence the results and their clinical interpretation when using the Luminex bead technology, such as variation in antigen density and the presence of denatured antigen on the beads. Pretransplantation Group: (a) Risk categories should be established based on the antibody and the XM results obtained. (b) DSA detected by CDC and a positive XM should be avoided due to their strong association with antibody-mediated rejection and graft loss. (c) A renal transplantation can be performed in the absence of a prospective XM if single-antigen bead screening for antibodies to all class I and II HLA loci is negative. This decision, however, needs to be taken in agreement with local clinical programs and the relevant regulatory bodies. (d) The presence of DSA HLA antibodies should be avoided in heart and lung transplantation and considered a risk factor for liver, intestinal, and islet cell transplantation. Posttransplantation Group: (a) High-risk patients (i.e., desensitized or DSA positive/XM negative)

should be monitored by measurement of DSA and protocol biopsies in the first 3 months after transplantation. (b) Intermediate-risk patients (history of DSA but currently negative) should be monitored for DSA within the first month. If DSA is present, a biopsy should be performed. (c) Low-risk patients (nonsensitized first transplantation) should be screened for DSA at least once 3 to 12 months after transplantation. If DSA is detected, a biopsy should be performed. In all three categories, the recommendations for subsequent treatment are based on the biopsy results. **Conclusions:** A comprehensive list of recommendations is provided covering the technical and pretransplantation and posttransplantation monitoring of HLA antibodies in solid organ transplantation. The recommendations are intended to provide state-of-the-art guidance in the use and clinical application of recently developed methods for HLA antibody detection when used in conjunction with traditional methods.

24. A Novel ELISPOT Assay to Quantify HLA-Specific B Cells in HLA-Immunized Individuals
American Journal of Transplantation. 2012; 12: 1469–78
Heidt S, Roelen DL, de Vaal YJ, Kester MG, Eijsink C, Thomas S, van Besouw NM, Volk HD, Weimar W, Claas FH, Mulder A.

Quantification of the humoral allow immune response is generally achieved by measuring serum HLA antibodies, which provides no information about the cells involved in the humoral immune response. Therefore, we have developed an HLA-specific B-cell ELISPOT assay allowing for quantification of B cells producing HLA antibodies. We used recombinant HLA monomers as target in the ELISPOT assay. Validation was performed with human B-cell hybridomas producing HLA antibodies.
Subsequently, we quantified B cells producing HLA antibodies in HLA-immunized individuals, non-HLA immunized individuals and transplant patients with serum HLA antibodies. B-cell hybridomas exclusively formed spots against HLA molecules of corresponding specificity with the sensitivity similar to that found in total IgG ELISPOT assays. HLA-immunized healthy individuals showed up to 182 HLA-specific B cells per million total B cells while non immunized individuals had none. Patients who were immunized by an HLAA2- mismatched graft had up to 143 HLA-A2-specific B cells per million total B cells. In conclusion, we have developed and validated a highly specific and sensitive
HLA-specific B-cell ELISPOT assay, which needs further validation in a larger series of transplant patients. This technique constitutes a new tool for quantifying humoral immune responses.

25. Quantification of HLA class II-specific memory B cells in HLA-sensitized Individuals

Human Immunology. 76 (2015) 129–36

Karahan GE, de Vaal YJ, Roelen DL, Buchli R, Claas FH, Heidt S.

ABSTRACT:

For the quantification of HLA-specific memory B cells from peripheral blood of sensitized individuals, a limited number of methods are available. However, none of these are capable of detecting memory B cells directed at HLA class II molecules. Since the majority of antibodies that occur after transplantation appear to be specific for HLA class II, our aim was to develop an assay to detect and quantify HLA class II-specific memory B cells from peripheral blood.

By using biotinylated soluble HLA class II molecules as detection agent, we were able to develop an HLA class II-specific memory B cell ELISPOT assay. The assay was validated using B cell-derived hybridom as that produce human monoclonal antibodies directed at specific HLA class II molecules. In pregnancy immunized females, we found memory B cell frequencies ranging from 25 to 756 spots per 106 B cells specific for the immunizing paternal HLA class II molecules, whereas in non-immunized males no significant spot formation was detected.

Here, we present a novel ELISPOT assay for quantifying HLA class II-specific memory B cells from peripheral blood. This technique provides a unique tool for monitoring the HLA class II-specific memory B cell pool in sensitized transplant recipients.

26. Monitoring B cell subsets and alloreactivity in kidney transplantation.

Transplant Rev (Orlando). 2015 Apr;29(2):45-52.

Crespo M, Heidt S, Redondo D, Pascual J.

ABSTRACT:

B cells are the precursors of antibody producing plasma cells that can give rise to the formation of donor-specific antibodies. However, recent data suggest that besides their role in antibody production, B cells participate in antibody-independent responses, potentially leading to allograft rejection or allograft tolerance. The presence of CD20(+) B cells in kidney graft biopsies has been shown during severe acute rejection episodes and during chronic rejection. Furthermore, operationally tolerant kidney transplant recipients showed a clear B cell dominated fingerprint of tolerance. Several techniques exist to study B cells on different levels. Numerous classification schemes allow for the distinction of many different B cell subsets using flow cytometry. Regardless, data on B cell subsets during stable graft function, rejection or tolerance remain scarce. To obtain a complete picture of the role of B cells during transplantation, antigen specific B cell assays may be required. Therefore, techniques have now been developed that allow for studying the specificity and frequency of HLA specific B cells. Here, we present an overview of the existent assays, panels and techniques intended to characterize peripheral B cells, and the currently available HLA specific B cell functional assays that may allow for monitoring the humoral alloimmune response in transplant recipients.

27. Circulating Alloreactive T Cells Correlate with Graft Function in Longstanding Renal Transplant Recipients

J Am Soc Nephrol. 2008;19(7):1419-29. doi: 10.1681/ASN.2007050539.

Bestard O, Nickel P, Cruzado JM, Schoenemann C, Boenisch O, Sefrin A, Grinyó JM, Volk HD, Reinke P.

ABSTRACT:

Monitoring for alloreactive memory T cells after organ transplantation may allow individualization of immunosuppression. Two pathways of T cell allorecognition have been implicated in chronic graft dysfunction: Direct (recipient T cells respond to donor peptides presented by donor antigen-presenting cells) and indirect (donor peptides are processed and presented by recipient antigen-presenting cells).

Previous studies have assessed these alloresponses only during the first 2 yr after kidney transplantation, so this study correlated the presence of circulating donor-reactive memory/effector T cells, primed by both pathways, in 34 longstanding living-donor renal transplant recipients using the highly sensitive IFN-γ Elispot assay. Remarkably, 59% of patients had directly primed donor-reactive T cells, and their presence correlated directly with serum creatinine (P _ 0.001) and inversely with estimated GFR (P _ 0.042). Multivariate analysis revealed that hyporesponsiveness of direct, donor-specific T cells was the only variable that significantly correlated with graft function and that antidonor indirect alloreactivity was the only variable that significantly correlated with proteinuria. Interestingly, when both allorecognition pathways were considered together, patients with undetectable direct alloreactivity had better longterm graft function, independent of allosensitization by the indirect pathway. In conclusion, circulating donor-specific alloreactive T cells primed by both pathways are detectable long after transplantation and are associated with graft injury. Assessment of alloreactive memory/effector T cells might be helpful to tailor individual immunosuppression regimens for transplant recipients in the future.

28. Prospective assessment of antidonor cellular alloreactivity is a tool for guidance of immunosuppression in kidney transplantation

Kidney International. 2013; 84:1226–36

Bestard O, Cruzado JM, Lucia M, Crespo E, Casis L, Sawitzki B, Vogt K, Cantarell C, Torras J, Melilli E, Mast R, Martinez-Castelao A, Gomà M, Reinke P, Volk HD, Grinyó JM.

ABSTRACT:

Current characterization of the immune risk in renal transplant patients is only focused on the assessment of preformed circulating alloantibodies; however, alloreactive memory T cells are key players in mediating allograft rejection. Immune monitoring of antidonor alloreactive memory/effector T cells using an IFN-c Elispot has been shown to distinguish patients at risk for immune-mediated graft dysfunction, suggesting a potential tool for immunosuppression individualization. In this nonrandomized study, we prospectively assessed donor and nondonor T-cell alloreactivity in 60 highly alloreactive patients receiving calcineurin inhibitor-based immunosuppression and in non-T-cell alloreactive transplant recipients treated with a calcineurin inhibitor-free regimen. The impact was evaluated using 1-year allograft outcome. We found a strong association between ongoing antidonor T-cell alloreactivity and histological lesions of acute T cell–mediated rejection in 6-month protocol biopsies, distinguishing those patients with better 1-year graft function, regardless of immunosuppression regimen. Interestingly, evidence for enhanced immune regulation, driven by circulating Foxp3-demethylated regulatory T cells, was only observed among patients achieving antidonor T-cell hyporesponsiveness. Thus, prospective evaluation of donor-specific T-cell sensitization may add crucial information on the alloimmune state of transplanted patients to be used in daily clinical practice.

29. Cross-Validation of IFN-g Elispot Assay for Measuring Alloreactive Memory/Effector T Cell Responses in Renal Transplant Recipients

American Journal of Transplantation. 2013; 13: 1880–90

Bestard O, Crespo E, Stein M, Lúcia M, Roelen DL, de Vaal YJ, Hernandez-Fuentes MP, Chatenoud L, Wood KJ, Claas FH, Cruzado JM, Grinyó JM, Volk HD, Reinke P.

ABSTRACT:

Assessment of donor-specific alloreactive memory/effector T cell responses using an IFN-g Elispot assay has been suggested to be a novel immune-monitoring tool for evaluating the cellular immune risk in renal transplantation. Here, we report the cross-validation data of the IFN-g Elispot assay performed within different European laboratories taking part of the EU RISET consortium. For this purpose, development of a standard operating procedure (SOP), comparisons of lectures of IFN-g plates assessing intra- and interlaboratory assay variability of allogeneic or peptide stimuli in both healthy and kidney transplant individuals have been the main objectives. We show that the use of a same SOP and count-settings of the Elispot bioreader allow low coefficient variation between laboratories.

Frozen and shipped samples display slightly lower detectable IFN-g frequencies than fresh samples.

Importantly, a close correlation between different laboratories is obtained when measuring high frequencies of antigen-specific primed/memory T cell alloresponses.

Interestingly, significant high donor-specific alloreactive T cell responses can be similarly detected among different laboratories in kidney transplant patients displaying histological patterns of acute T cell mediated rejection. In conclusion, assessment of circulating alloreactive memory/effector T cells using an INF-γ Elispot assay can be accurately achieved using the same SOP, Elispot bioreader and experienced technicians in kidney transplantation.

30. Enzyme Linked Immunosorbent Spot (ELISPOT) Assay for Interferon-Gamma Independently Predicts Renal Function in Kidney Transplant Recipients

American Journal of Transplantation. 2003; 3: 878–84

Hricik DE, Rodriguez V, Riley J, Bryan K, Tary-Lehmann M, Greenspan N, Dejelo C, Schulak JA, Heeger PS.

ABSTRACT:

Post-transplant monitoring of cellular immunity might be useful in predicting long-term outcomes of kidney transplant recipients. We used an enzyme linked immune absorbent spot (ELISPOT) assay
to serially measure the frequency of peripheral blood lymphocytes producing interferon-gamma in response to stimulator cells from donors or third parties in 55 primary kidney transplant recipients.

Mean frequencies measured during the first 6 months after transplantation correlated significantly with the serum creatinine concentration at both 6 and 12months following transplantation. The mean frequencies were higher in patients with acute rejection than in those without acute rejection. Multiple regression analyses indicated that the correlations between the early ELISPOT measurements of interferon-gamma and serum creatinine were independent of acute rejection, delayed graft function, or the presence of panel reactive antibodies before transplantation. Patients with low mean frequencies of interferon-producing cells in the early post-transplant period were generally free from acute rejection and exhibited excellent renal function at 6 and 12months post-transplant. In conclusion, using the ELISPOT assay, we show an independent correlation between early cellular alloreactivity and long-term renal function. Increased levels of early alloreactivity measured with this assay may serve as a surrogate for chronic allograft dysfunction.

31. Modified ELISPOT technique — Highly significant inverse correlation of post-Tx donor-reactive IFNγ-producing cell frequencies with 6 and 12 months graft function in kidney transplant recipients

Transplant Immunology. 2006;16: 232–7

Näther BJ, Nickel P, Bold G, Presber F, Schönemann C, Pratschke J, Volk HD, Reinke P.

ABSTRACT:

Background: Upcoming trials for immunosuppression minimization and tolerance induction require the development of reliable in vitro assays for monitoring cellular alloimmunity in transplant patients. The IFN-γ ELISPOT assay represents a promising tool for monitoring alloreactive memory/effector T cells. As T lymphopenia is a common finding during the early post-transplant (post-Tx) period, the IFN-γ ELISPOT technique was here modified by using ELISPOT responder cells with enhanced percentage and standardized number of 200,000 T cells per well.
Methods: Peripheral blood mononuclear cells (PBMNC) of kidney transplant recipients were depleted of CD14+ and CD15+ cells to increase the percentage of T cells from average 47.8% to 71.5% before transplantation (pre-Tx) and from 39.7% to 74.9% post-Tx. The assay was tested in a population of 23 de novo renal transplant patients for clinical relevance. Before and at 2–3 times during the first 6 months post-Tx, IFN-γ- producing donor-reactive as well as recall antigen-reactive cell frequencies (Candida, tuberculin, tetanus) were determined and correlated with outcome.
Results: Pre-Tx donor-reactive ELISPOT frequencies were enhanced in patients with acute rejection compared to non-rejectors. Moreover, mean post-Tx donor-reactive ELISPOT frequencies showed a highly significant inverse correlation with renal function at 6 and 12 months. In contrast, recall antigen-reactive ELISPOT frequencies did not correlate with outcome.
Conclusion: Our results suggest that the modified donor-reactive ELISPOT approach might provide a useful surrogate marker for renal transplant outcome with possible utility especially in T-lymphopenic patients.

32. Monitoring T cell alloreactivity.

Transplant Rev (Orlando). 2015 Apr;29(2):53-9.

Mehrotra A, Leventhal J, Purroy C, Cravedi P.

ABSTRACT:

Currently, immunosuppressive therapy in kidney transplant recipients is center-specific, protocol-driven, and adjusted according to functional or histological evaluation of the allograft and/or signs of drug toxicity or infection. As a result, a large fraction of patients receive too much or too little immunosuppression, exposing them to higher rates of infection, malignancy and drug toxicity, or increased risk of acute and chronic graft injury from rejection, respectively. The individualization of immunosuppression requires the development of assays able to reliably quantify and/or predict the magnitude of the recipient's immune response toward the allograft. As alloreactive T cells are central mediators of allograft rejection, monitoring T cell alloreactivity has become a priority for the transplant community. Among available assays, flow cytometry based phenotyping, T cell proliferation, T cell cytokine secretion, and ATP release (ImmuKnow), have been the most thoroughly tested. While numerous cross-sectional studies have found associations between the results of these assays and the presence of clinically relevant post-transplantation outcomes, data from prospective studies are still scanty, thereby preventing widespread implementation in the clinic. Future studies are required to test the hypothesis that tailoring immunosuppression on the basis of results offered by these biomarkers leads to better outcomes than current standard clinical practice.

33. Novel Strategies for Immunological Monitoring of Kidney Transplant Recipients: From MicroRNA to Alloantibodies.

Clinical Transplants. 2013: 257-67.

Heidt S, Eikmans M, Roelen DL, Claas FH.

ABSTRACT:

Predicting and diagnosing acute kidney allograft rejection by non-invasive biomarkers is a major goal in clinical transplantation research. Such biomarkers can be reduced to the various stages of the alloimmune response, from transcriptional regulation to immunological effector mechanisms. Here, we describe novel insights into exciting areas of transplantation-related biomarker research that may be translated to non-invasive monitoring strategies. First, we will elaborate on microRNAs, which represent stable, small non-coding ribonucleic acid molecules that can specifically regulate gene expression. Secondly, we will discuss novel methods to monitor human leukocyte antigen (HLA)-specific B cells, as well as the anti-HLA antibodies they produce. Incorporation of these new biomarkers and methodologies into cross-platform biomarker panels may help to improve non-invasive prediction and detection of allograft rejection.

34. Differential effects of donor-specific alloantibody

Transplantation Reviews (Orlando). 2009;23:25–33

Turgeon NA1, Kirk AD, Iwakoshi NN.

ABSTRACT:

Alloantigen exposure typically provokes an adaptive immune response that can foster rejection of transplanted organs, and these responses present the most formidable biological barrier to kidney transplantation. Although most cellular alloimmune responses can be therapeutically controlled with T-cell–specific immunosuppressants, humoral alloimmune responses remain relatively untamed. Importantly, humoral immunity, typically manifesting as allospecific antibody production, is increasingly recognized for its variable appearance after kidney transplantation. Indeed, the appearance of alloantibody can herald the onset of rapid and destructive antibody-mediated rejection or have no demonstrable acute effects. The factors determining the end result of alloantibody formation remain poorly understood. This review will discuss the breadth of alloantibody responses seen in clinical kidney transplantation and provide an overview of potential factors explaining the phenotypic variability associated with humoral alloimmunity. We propose several avenues ripe for future investigation including the influence of innate immune components and the potential influence of heterologous immune responses in determining the ultimate clinical import of an alloantibody response.

35. Immune function assay (ImmuKnow) as a predictor of allograft rejection and infection in kidney transplantation

Clin Transplant 2013; 27: E351–8. doi: 10.1111/ctr.12134

He J, Li Y, Zhang H, Wei X, Zheng H, Xu C, Bao X, Yuan X, Hou J.

ABSTRACT:

Background: The Cylex ImmuKnow (IK) assay provides a rapid and quantitative assessment of T-cell-mediated immune function. Studies have shown correlations between ImmuKnow assay and adverse events, such as immunosuppression and low or high calcineurin inhibitor trough levels. We investigated the correlation between IK changes and rejection or infection in kidney transplant patients and studied the potential application of the IK assays in optimizing individual immunosuppressive therapy.
Methods: ImmuKnow assay was used to determine dynamic intracellular ATP changes in CD4 cells in 193 samples from 42 kidney transplant patients and 25 healthy subjects. Patients were categorized into rejection, infection, and event-free groups. The IK values were assayed and analyzed between kidney transplant patients and healthy controls.
Results: Most IK values fell between 200 and 599 ng/mL from pretransplantation to 30 months post-transplantation. The mean IK values continuously increased throughout 30 months. Incidental allograft rejection patients had significantly higher IK values compared with the event-free patients and controls. However, infection patients had significantly lower IK values. Seven days after treatment, IK values in rejection/infection patients were different compared with the values in autograft patients, and there was a significant correlation between calcineurin inhibitor (FK506) trough levels and IK values in rejection/ infection patients. Serum creatinine levels in the rejection patients were significantly higher than those in the event-free patients, and C-reactive protein levels were significantly higher in the infection patients compared with the event-free patients.
Conclusions: The IK assay combined with other biomarkers can be used to identify kidney transplant patients at high risk of rejection and infection.

36. Preoperative Cylex assay predicts rejection risk in patients with kidney transplant

Clin Transplant 2014: 28: 606–10. doi: 10.1111/ctr.12359

Myslik F, House AA, Yanko D, Warren J, Caumartin Y, Rehman F, Jevnikar AM, Stitt L, Luke PP.

ABSTRACT:

Introduction and Objectives: The ImmuKnow assay measures cell-mediated immunity by quantifying ATP release from CD4+ T-cells in peripheral blood. Herein, we hypothesized that this assay could predict complications associated with over-/under-immunosuppression in patients with kidney transplant (KT).
Methods: Sixty-seven patients undergoing KT were recruited prospectively and had ATP levels measured preoperatively, and at specified intervals over two months. Clinicians were blinded to ATP levels. Clinical events including rejection and infection/cancer were documented with a median follow-up of 21 months. Parameters including absolute ATP levels and changes in ATP patterns (slopes, delta) were analyzed. Association between ATP parameters and clinical outcomes was compared using the likelihood-ratio test and Kaplan– Meier curves.
Results: Absolute ATP values postoperatively had poor predictive value with regard to rejection or infection/malignancy. As well, changes in ATP values were poorly associated with complications. Importantly, patients with pre-transplant ATP values <300 ng/mL had significantly less rejection episodes vs. those with ATP values >300 ng/Ml ($p < 0.0001$).
Conclusions: For the first time, we have evidence that a preoperative ImmuKnow level can stratify patients with KT into low/high risk groups for rejection. Future studies used to assess the utility of this assay to design individualized immunosuppressive regimens are required.

37. Trends in immune function assay (ImmuKnow; Cylex_) results in the first year post-transplant and relationship to BK virus infection

Nephrol Dial Transplant 2012;27: 2565–70

Gralla J, Huskey J, Wiseman AC.

ABSTRACT:

Background: The ImmuKnow assay is a functional Tcell assay (TCA) that may quantify cellular immune responsiveness following renal transplantation. Using a standard protocol of TCA sampling in the first year post-transplant, we examined changes in TCA values over time and tested for an association between TCA and BK virus (BKV) infection as a marker of over immunosuppression.
Methods: We performed a single-center retrospective analysis of 897 TCA results in 414 renal transplant recipients obtained at 0 (N = 122), 1 (N = 316), 6 (N = 258) and 12 (N = 201) months post-transplant from May 2005 to July 2009 with concurrent urine and blood BKV polymerase chain reaction measurements.
Results: Nearly 40% of patients experienced a decrease in TCA of >150 ng/mL from 1 to 6 months (mean 466–356 ng/mL, $P < 0.0001$) and remained stable from 6 to 12 months (mean 357 versus 370 ng/mL, $P = 0.33$). Neither a change in TCA of >150 ng/mL nor a TCA value of $\leq$ 225 ng/mL were associated with a diagnosis of BKV infection at 1 or 6 months, while TCA $\leq$ 225 ng/mL was associated with BKV infection at 12 months ($P = 0.005$).
Conclusions: A reduction in TCA from 1 to 6 months posttransplant is common and is not associated with conditions of over-immunosuppression, rendering the interpretation of changes in TCA during this time period difficult. BKV infection is associated with low TCA values at 12 months, suggesting that patients with low TCA values after 6 months may benefit from potential tailoring of immunosuppression or more aggressive monitoring to prevent subsequent BKV infection.

38. Lack of Association of Immune Cell Function Test With Rejection in Kidney Transplantation

Transplantation Proceedings. 2011;43:2168–70

Torío A, Fernández EJ, Montes-Ares O, Guerra RM, Pérez MA, Checa MD.

ABSTRACT:

Background: The Cylex Immuknow assay provides a rapid assessment of global immune function in immunocompromised patients by measuring the global immune responses of CD4 T cells from a whole-blood sample. It may help to monitor the immune status of immunosuppressed transplant patients. However, earlier studies have shown that there is no consensus on the utility of the Immuknow assay in renal transplant rejection.
Methods: T-cell activation was determined by measuring an increase of intracellular adenosine triphosphate (iATP) from CD4 cells in 227 samples from 116 kidney transplant patients. The results were analyzed regarding patient clinical status, namely, rejection, infection, or stability. In addition, we measured the immunologic response of 108 healthy control subjects.
Results. There were 24 infectious and 36 rejection episodes. iATP concentrations differed significantly between stable and infected patients (180.5 ± 55.2 vs 375.3 ± 140.1 ng/mL; $P < .001$) and between infected patients and control subjects (180.5 ± 55.2 vs 436.5 ± 112 ng/mL; $P < .001$). No correlation was observed between patients suffering an acute rejection episode with this response.
Conclusions: Our results confirmed that the Immuknow assay identified transplant patients at risk for infection. It may provide information to guide immunosuppressive therapy, but the assay did not seem to have the potential to differentiate subjects experiencing rejection.

39. Increased intracellular adenosine triphosphate level as an index to predict acute rejection in kidney transplant recipients

Transplant Immunology 2014;30:18–23

Wang XZ, Jin ZK, Tian XH, Xue WJ, Tian PX, Ding XM, Zheng J, Li Y, Jing X, Luo ZZ.

ABSTRACT:

Background: Peripheral blood CD4+ T cell adenosine triphosphate (ATP) release has been reported to be an adjunct tool to evaluate global cellular immune response in solid-organ transplant recipients. However, the correlation between the ATP level and rejection was controversial. The aim of this prospective clinical study was to explore the association between the intracellular ATP level and the occurrence, progression, and treatment of acute rejection (AR) episodes, determine the predicting value of intracellular ATP level for AR in kidney transplant (KT) recipients.
Patients and methods: In the period of October 2011 to October 2012, 140 KT recipients were recruited and followed for six months after transplantation. Patients were categorized into stable group and AR group according to their clinical course. Whole blood samples were collected pretransplantation, and at 7, 14, 21, and 28 days, and at 2, 3, 4, 5 and 6 months post-transplantation. Additional blood samples were obtained from AR patients on the day AR occurred, on the day before and 3 and 7 days after intravenous anti-rejection therapy started, and on the day when AR reversed. The intracellular ATP in CD4+ T cells was detected by ImmuKnow Immune Cell Function Assay according to the manufacturer's instruction.
The absolute number of CD4+T cells and the trough levels of tacrolimus and cyclosporine were also measured.
Results: The ATP level detected on the day AR occurred (627.07 ± 149.85 ng/ml) was obviously higher than that of the stable group (320.48 ± 149.11 ng/ml, P b 0.05). ATP value decreased to 265.35 ± 84.33 ng/m at the end of anti-rejection therapy, which was obviously lower than that measured on the day before the anti-rejection therapy started (665.87 ± 162.85 ng/ml, $P < 0.05$). ROC analysis revealed that increased intracellular adenosine triphosphate level showed better sensitivity and specificity than those obtained using single time point detection (89.5% vs 85.0%;95.0% vs 88.9%). The best cutoff value was 172.55 ng/ml. A positive correlation between the intracellular ATP level and absolute CD4+ T cell number ($r = 0.656$, $P < 0.001$) was found in the patients with CD4+ T cell counts <200/μl.

40. A Peripheral Blood Diagnostic Test for Acute Rejection in Renal Transplantation

American Journal of Transplantation 2012; 12: 2710–8

Li L, Khatri P, Sigdel TK, Tran T, Ying L, Vitalone MJ, Chen A, Hsieh S, Dai H, Zhang M, Naesens M, Zarkhin V, Sansanwal P, Chen R, Mindrinos M, Xiao W, Benfield M, Ettenger RB, Dharnidharka V, Mathias R, Portale A, McDonald R, Harmon W, Kershaw D, Vehaskari VM, Kamil E, Baluarte HJ, Warady B, Davis R, Butte AJ, Salvatierra O, Sarwal MM.

ABSTRACT:

Monitoring of renal graft status through peripheral blood (PB) rather than invasive biopsy is important as it will lessen the risk of infection and other stresses, while reducing the costs of rejection diagnosis. Blood gene biomarker panels were discovered by microarrays at a single center and subsequently validated and cross-validated by QPCR in the NIH SNSO1 randomized study from 12 US pediatric transplant programs. A total of 367 unique human PB samples, each paired with a graft biopsy for centralized, blinded phenotype classification, were analyzed (115 acute rejection (AR), 180 stable and 72 other causes of graft injury). Of the differentially expressed genes by microarray, Q-PCR analysis of a five gene-set (*DUSP1, PBEF1, PSEN1, MAPK9* and *NKTR*) classified AR with high accuracy. A logistic regression model was built on independent training set (n = 47) and validated on independent test-set (n = 198)samples, discriminating AR from STA with 91% sensitivity and 94% specificity and AR from all other non-AR phenotypes with 91% sensitivity and 90% specificity. The 5-gene set can diagnose AR potentially avoiding the need for invasive renal biopsy. These data support the conduct of a prospective study to validate the clinical predictive utility of this diagnostic tool.

41. A Three-Gene Assay for Monitoring Immune Quiescence in Kidney Transplantation

J Am Soc Nephrol. 2014 Nov 26. pii: ASN.2013111239. [Epub ahead of print]

Roedder S, Li L, Alonso MN, Hsieh SC, Vu MT, Dai H, Sigdel TK, Bostock I, Macedo C, Metes D, Zeevi A, Shapiro R, Salvatierra O, Scandling J, Alberu J, Engleman E, Sarwal MM.

ABSTRACT:

Organ transplant recipients face life-long immunosuppression and consequently are at high risk of comorbidities. Occasionally, kidney transplant recipients develop a state of targeted immune quiescence (operational tolerance) against an HLA-mismatched graft, allowing them to withdraw all immunosuppression and retain stable graft function while resuming immune responses to third-party antigens. Methods to better understand and monitor this state of alloimmune quiescence by transcriptional profiling may reveal a gene signature that identifies patients for whom immunosuppression could be titrated to reduce patient and graft morbidities. Therefore, we investigated 571 unique peripheral blood samples from348HLA-mismatched renal transplant recipients and 101 non transplant controls in a four-stage study including microarray, quantitative PCR, and flow cytometry analyses. We report a refined and highly validated (area under the curve, 0.95; 95% confidence interval, 0.92 to 0.97) peripheral blood three-gene assay (KLF6, BNC2, CYP1B1) to detect the state of operational tolerance by quantitative PCR. The frequency of predicted alloimmune quiescence in stable renal transplant patients receiving long-term immunosuppression (n=150) was 7.3% by the three-gene assay.

Targeted cell sorting of peripheral blood from operationally tolerant patients showed a significant shift in the ratio of circulating monocyte-derived dendritic cells, with significantly different expression of the genes constituting the three-gene assay. Our results suggest that incorporation of patient screening by specific cellular and gene expression assays may support the safety of drug minimization trials and protocols.

42. The kSORT assay to detect renal transplant patients at high risk for acute rejection: results of the multicenter AART study.

Plos One nov 2014

Roedder S, Sigdel T, Salomonis N, Hsieh S, Dai H, Bestard O, Metes D, Zeevi A, Gritsch A, Cheeseman J, Macedo C, Peddy R, Medeiros M, Vincenti F, Asher N, Salvatierra O, Shapiro R, Kirk A, Reed E, Sarwal MM.

ABSTRACT:

BACKGROUND: Development of noninvasive molecular assays to improve disease diagnosis and patient monitoring is a critical need. In renal transplantation, acute rejection (AR) increases the risk for chronic graft injury and failure. Noninvasive diagnostic assays to improve current late and nonspecific diagnosis of rejection are needed. We sought to develop a test using a simple blood gene expression assay to detect patients at high risk for AR.

METHODS AND FINDINGS: We developed a novel correlation-based algorithm by step-wise analysis of gene expression data in 558 blood samples from 436 renal transplant patients collected across eight transplant centers in the US, Mexico, and Spain between 5 February 2005 and 15 December 2012 in the Assessment of Acute Rejection in Renal Transplantation (AART) study. Gene expression was assessed by quantitative real-time PCR (QPCR) in one center. A 17-gene set--the Kidney Solid Organ Response Test (kSORT)--was selected in 143 samples for AR classification using discriminant analysis (area under the receiver operating characteristic curve [AUC] = 0.94; 95% CI 0.91-0.98), validated in 124 independent samples (AUC = 0.95; 95% CI 0.88-1.0) and evaluated for AR prediction in 191 serial samples, where it predicted AR up to 3 mo prior to detection by the current gold standard (biopsy). A novel reference-based algorithm (using 13 12-gene models) was developed in 100 independent samples to provide a numerical AR risk score, to classify patients as high risk versus low risk for AR. kSORT was able to detect AR in blood independent of age, time post-transplantation, and sample source without additional data normalization; AUC = 0.93 (95% CI 0.86-0.99). Further validation of kSORT is planned in prospective clinical observational and interventional trials.

CONCLUSIONS: The kSORT blood QPCR assay is a noninvasive tool to detect high risk of AR of renal transplants. Please see later in the article for the Editors' Summary.

43. Differentially expressed gene transcripts using RNA sequencing from the blood of immunosuppressed kidney allograft recipients.

PLoS One. 2015 May 6;10(5):e0125045.

Dorr C, Wu B, Guan W, Muthusamy A, Sanghavi K, Schladt DP, Maltzman JS, Scherer SE, Brott MJ, Matas AJ, Jacobson PA, Oetting WS, Israni AK

ABSTRACT:

We performed RNA sequencing (RNAseq) on peripheral blood mononuclear cells (PBMCs) to identify differentially expressed gene transcripts (DEGs) after kidney transplantation and after the start of immunosuppressive drugs. RNAseq is superior to microarray to determine DEGs because it's not limited to available probes, has increased sensitivity, and detects alternative and previously unknown transcripts. DEGs were determined in 32 adult kidney recipients, without clinical acute rejection (AR), treated with antibody induction, calcineurin inhibitor, mycophenolate, with and without steroids. Blood was obtained pre-transplant (baseline), week 1, months 3 and 6 post-transplant. PBMCs were isolated, RNA extracted and gene expression measured using RNAseq. Principal components (PCs) were computed using a surrogate variable approach. DEGs post-transplant were identified by controlling false discovery rate (FDR) at < 0.01 with at least a 2 fold change in expression from pre-transplant. The top 5 DEGs with higher levels of transcripts in blood at week 1 were TOMM40L, TMEM205, OLFM4, MMP8, and OSBPL9 compared to baseline. The top 5 DEGs with lower levels at week 1 post-transplant were IL7R, KLRC3, CD3E, CD3D, and KLRC2 (Striking Image) compared to baseline. The top pathways from genes with lower levels at 1 week post-transplant compared to baseline, were T cell receptor signaling and iCOS-iCOSL signaling while the top pathways from genes with higher levels than baseline were axonal guidance signaling and LXR/RXR activation. Gene expression signatures at month 3 were similar to week 1. DEGs at 6 months post-transplant create a different gene signature than week 1 or month 3 post-transplant. RNAseq analysis identified more DEGs with lower than higher levels in blood compared to baseline at week 1 and month 3. The number of DEGs decreased with time post-transplant. Further investigations to determine the specific lymphocyte(s) responsible for differential gene expression may be important in selecting and personalizing immune suppressant drugs and may lead to targeted therapies.

44. Post-Transplant Anti-HLA Class II Antibodies as Risk Factor for Late Kidney Allograft Failure

American Journal of Transplantation 2006;6:2316–20

Campos EF, Tedesco-Silva H, Machado PG, Franco M, Medina-Pestana JO, Gerbase-DeLima M.

ABSTRACT:

The purpose of this study was to prospectively analyze the relationship between the post-transplant anti- HLA class I and/or class II panel reactive antibodies and graft failure due to chronic allograft nephropathy (CAN). We studied 512 first kidney recipients transplanted at a single center, with a graft functioning for at least 3 years. A single blood sample was collected from each patient for antibody evaluation. The median posttransplant time after blood collection was 4.4 years and did not differ between patients with (n = 91) or without anti-HLA antibodies (n=421). Female gender, pregnancies and blood transfusions were associated with the presence of anti-HLA class I antibodies. Graft function deterioration was associated with anti-HLA class II antibodies. Multivariate analysis showed independent association for creatinine levels (RR = 7.5), acute rejection (RR=2.6), recipient male gender (RR= 3.6) and anti-HLA class II antibodies (RR = 2.9) and CAN-associated graft loss. In conclusion, the presence of anti-HLA class II antibodies conferred a risk for graft loss before a decline in renal function and increased the risk of graft failure in patients who already had a decline in graft function. Thus, anti-HLA class II antibody monitoring is a useful tool for the management of long-term kidney recipients.

45. Interpretation of HLA single antigen bead assays

Transplantation Reviews (Orlando) 2013;27:108–11

Ellis TM

ABSTRACT:

The introduction of single antigen bead (SAB) assays for detection and quantitation of HLA antibodies has improved our ability to identify and manage allosensitized transplant candidates and recipients and to improve organ allocation, and was critical to the creation of national paired kidney exchanges. The principal limitations of the technology have been detailed in the literature and include artifacts resulting in non-specific background, variability, lack of standardization, and interpretive challenges. Accurate interpretation of SAB assays requires consideration of a number of factors, including identification of epitope reactivity patterns, mean fluorescence intensity (MFI) values, patient history, and appreciation of individual bead and assay nuances. The MFI value provides an estimate of relative HLA antibody levels although limited by saturation and epitope distribution effects. A better understanding of SAB assays and MFI values will be necessary to ensure appropriate application of these assays clinically and a higher quality of antibody data used in support of published clinical studies.

46. B Cell Markers of Operational Tolerance Can Discriminate Acute Kidney Allograft Rejection From Stable Graft Function

Transplantation 2015;99: 1058–1064

Sebastiaan Heidt, Manon Vergunst, Jacqueline D.H. Anholts, Marlies E.J. Reinders, Johan W. de Fijter, Michael Eikmans, and Frans H.J. Claas

Background: Recently, several B cell–related markers have been described to be upregulated during operational tolerance in kidney allograft recipients. Little data exist on these markers during allograft rejection.
Methods: In this study, we investigated regulation-associated B-cell phenotypes in peripheral blood mononuclear cells (PBMCs) of kidney transplant recipients with (n = 21) and without (n = 22) acute rejection (AR). We also determined expression levels of the B cell–related genes, MS4A1, TCL1A, and CD79B, in PBMCs and isolated B cells. Patient samples were analyzed before transplantation at discharge and at time of AR before initiation of antirejection therapy or at matching time points in patients with stable graft function.
Results: On transplantation, the peripheral CD19+CD24hiCD38hi transitional B cell subset strongly declined, regardless of the subsequent occurrence of AR. In contrast, the CD19+CD27+CD24hi subset remained stable after transplantation in both patients groups. MS4A1 gene expression levels in PBMC were comparable between patient groups at all time points. In contrast, TCL1A expression levels increased in stable patients, but decreased in patients at the time of AR in both PBMC and isolated B cells. CD79B expression levels in stable patients were unaltered after transplantation in PBMC but showed an increase in the B cell fraction at discharge. At the time of AR, CD79B gene expression was significantly lower compared to stable patients, being most apparent in the B-cell fraction.
Conclusion: These results suggest that, in addition to being markers for immunologic unresponsiveness, gene expression levels of TCL1A and CD79B may also identify immune activation in the setting of kidney transplantation.

47. Tracking donor-reactive T cells: evidence for clonal deletion in tolerant kidney transplant patients

Sci Transl Med. 2015 January 28; 7(272): 272ra10.

Heather Morris, Susan DeWolf, Harlan Robins, Ben Sprangers, Samuel A. LoCascio, Brittany A. Shonts, Tatsuo Kawai, Waichi Wong, Suxiao Yang, Julien Zuber, Yufeng Shen, and Megan Sykes

ABSTRACT:

T cell responses to allogeneic major histocompatibility (MHC) antigens present a formidable barrier to organ transplantation, necessitating long-term immunosuppression to minimize rejection. Chronic rejection and drug-induced morbidities are major limitations that could be overcome by allograft tolerance induction. Tolerance was first intentionally induced in humans via combined kidney and bone marrow transplantation (CKBMT), but the mechanisms of tolerance in these patients are incompletely understood. We now establish an assay to identify donor-reactive T cells and test the role of deletion in tolerance after CKBMT. Using high-throughput sequencing of the TCRB chain CDR3 region, we define a fingerprint of the donor-reactive T cell repertoire prior to transplantation and track those clones post-transplant. We observed post-transplant reductions in donor-reactive T cell clones in three tolerant CKBMT patients; such reductions were not observed in a fourth, non-tolerant, CKBMT patient or in two conventional kidney transplant recipients on standard immunosuppressive regimens. T cell repertoire turnover due to lymphocyte-depleting conditioning only partially accounted for the observed reductions in tolerant patients; in fact, conventional transplant recipients showed expansion of circulating donor-reactive clones, despite extensive repertoire turnover. Moreover, loss of donor-reactive T cell clones more closely associated with tolerance induction than in vitro functional assays. Our analysis supports clonal deletion as a mechanism of allograft tolerance in CKBMT patients. The results validate the significance of donor-reactive T cell clones identified pre-transplant by our method, supporting further exploration as a potential biomarker of transplant outcomes.

48. High Proportion of Pretransplantation Activated Regulatory T cells (CD4+CD25highCD62L+CD45RO+) Predicts Acute Rejection in Kidney Transplantation: Results of a Multicenter Study

Transplantation 2014;98: 1213Y1218

David San Segundo, Olga Millán, Pedro Muñoz-Cacho, Francisco Boix, Estela Paz-Artal, Paloma Talayero, José Mª Morales, Manuel Muro, Mª Ángeles De Cos, Lluis Guirado, Santiago Llorente, Julio Pascual,11 Manuel Arias, Mercé Brunet, and Marcos López-Hoyos

Background: Prognostic biomarkers of acute rejection (AR) in solid organ transplantation have been addressed in multiple small retrospective studies, and there is a critical need for multicenter studies. Because of their tolerogenic properties, regulatory T cells (Tregs) play an important role in transplant outcome.
Methods: In the present multicenter study, we have retrospectively examined different Treg subpopulations in an independent cohort of kidney transplant patients within first year after kidney transplantation. All participating centers used identical flow cytometry standard operating procedures.
Results: Seventy-five renal transplant patients were included, and six of them experienced an AR episode. The activated Treg (aTreg) subpopulation (CD4+CD25highCD62L+CD45RO+) was increased in the AR group before trans-plantation, and an aTreg percentage higher than 1.46% before kidney transplantation conferred an increased risk of AR. The univariate logistic regression model achieved an area under the curve of 81.6%. By including recipient age and thymoglobulin induction as variables in a multivariate logistic regression model, the prediction of AR improved to 92.4%.

49. Should IFN-γ, IL-17 and IL-2 be considered predictive biomarkers of acute rejection in liver and kidney transplant? Results of a multicentric study

Clinical Immunology (2014) 154, 141–154

O. Millán, L. Rafael-Valdivia, D. San Segundo, F. Boix, M.J. Castro-Panete, M. López-Hoyos, M. Muro, D. Valero-Hervás, A. Rimola, M. Navasa, P. Muñoz, M. Miras, A. Andrés, L. Guirado, J. Pascual, M. Brunet

ABSTRACT:

Acute rejection (AR) remains a major challenge in organ transplantation, and there is a need for predictive biomarkers. In the present multicenter study, we prospectively examined a series of biomarkers in liver and kidney recipients. Intracellular expression of IFN-γ, IL-17 and IL-2 and IL-17 soluble production were evaluated both pre-transplantation and post-transplantation (1st and 2nd week, 1st, 2nd and 3rd month). 142 transplant patients (63 liver/79 kidney) were included in the study. Twenty-eight recipients (14 liver/14 kidney) developed AR. Pre- and post-transplantation intracellular expression of %IFN-γ+ in CD4+CD69+ and in CD8+CD69+ and soluble IL17 identified liver and kidney transplant patients at high risk of AR. Pre-transplantation, %IL-2+ in CD8+CD69+ also identified kidney patients at high risk. We constructed pre- and post-transplantation risk prediction models, based on a composite panel of biomarkers, which could provide the basis for future studies and will be a useful tool for the selection and adjustment of immunosuppressive treatments.

50. Preformed circulating HLA-specific memory B cells predict high risk of humoral rejection in kidney transplantation

Kidney International , (15 July 2015) | doi:10.1038/ki2015.205

Marc Lúcia, Sergi Luque, Elena Crespo, Edoardo Melilli, Josep M Cruzado, Jaume Martorell, Marta Jarque, Salvador Gil-Vernet, Anna Manonelles, Josep M Grinyó and Oriol Bestard

The accurate evaluation of donor-specific antibodies (DSAs) has allowed a precise identification of sensitized patients at risk of antibody-mediated rejection (ABMR). However, the scale of the humoral response is not always fully addressed, as it excludes the complete memory B-cell (mBC) pool such as that caused by antigen-specific mBC. Using a novel B-cell ELISpot assay approach, we assessed circulating mBC frequencies against class I and II HLA antigens in highly sensitized and nonsensitized patients in the waiting list for kidney transplantation. Also, kidney transplant patients undergoing ABMR were evaluated for the presence of donor-specific mBCs both at the time of rejection and before transplantation. For this purpose, 278 target HLA-sp antigens from 70 patients were studied and compared to circulating HLA-sp antibodies. Both class I and II HLA-sp mBC frequencies were identified in highly sensitized individuals but not in nonsensitized and healthy individuals, many years after first sensitization. Also, high donor-specific mBC responses were clearly found both during ABMR and before transplantation, regardless of circulating DSA. The higher the donor-specific mBC response, the more aggressive the allograft rejection. Thus, assessing donor-specific mBC frequencies may be relevant to better refine patient alloimmune-risk stratification, and provides new insight into the mechanisms of the adaptive humoral alloimmune response taking place in kidney transplantation.

Proyecto Prometeo II

Grupo II | Monitorización farmacológica

Referencias Bibliográficas

Organizado por

SET
SOCIEDAD ESPAÑOLA DE TRASPLANTE

Con la colaboración de

NOVARTIS

6. New developments in the immunosuppressive drug monitoring of cyclosporine, tacrolimus, and azathioprine

Clinical Biochemistry 34 (2001) 9–16

Victor W. Armstrong, Michael Oellerich

ABSTRACT:
The calcineurin inhibitors cyclosporine and tacrolimus form the cornerstones of most immunosuppression protocols. Because of their variable pharmacokinetics, and their narrow therapeutic indices, post-transplant immunosuppressive drug monitoring is an essential part of patient care to minimize the risks of toxicity or acute rejection. Furthermore, a reduction in the rate of acute rejection has been shown to result in a lower rate of graft loss due to chronic rejection. The introduction of the microemulsion formulation of cyclosporine with its more consistent bioavailability has renewed interest in the use of alternative sampling strategies to the trough cyclosporine concentration. Both pharmacokinetic and pharmacodynamic considerations support the concept that determination of cyclosporine during the absorption phase (0–4 h) might offer a better prediction of cyclosporine immunosuppressive efficacy. Initial investigations suggest that monitoring a 2-h postdose concentration C2 may provide a more efficacious alternative to trough monitoring for optimizing therapy with Neoral. Tacrolimus has a 10- to 100-fold greater *in vitro* immunosuppressive activity compared with cyclosporine. Consistent with its greater potency, therapeutic whole blood trough concentrations for tacrolimus are around 20-fold lower than the corresponding cyclosporine concentrations.
The correlation between toxicity and tacrolimus trough concentrations appears to be stronger than that for acute rejection. The results from a concentration-ranging trial in primary kidney-transplantation and liver-transplantation trials all found a significant relationship between toxicity and tacrolimus trough levels. Azathioprine is converted *in vivo* to 6-mercaptopurine, which is subsequently metabolized to the pharmacologically active 6-thioguanine nucleotides. The latter are also responsible for the cytotoxic side effects. Reliance on blood counts to monitor azathioprine therapy can be misleading, and they do not provide information on immunosuppresive efficacy. More pertinent information can be obtained through the measurement of thiopurine S-methyltransferase activity and the quantification of intracellular 6-thioguanine nucleotides concentrations in red blood cells. Prospective studies have demonstrated the clinical utility of determining 6-thioguanine nucleotides to individualise immunosuppressive therapy with azathioprine not only in the field of transplantation, but also in inflammatory bowel disease.

7. Monitoring of azathioprine treatment by determination of 6-thioguanine nucleotide concentrations in erythrocytes.

Transplantation. 1994 Oct 15;58(7):803-8.

Bergan S, Rugstad HE, Bentdal O, Stokke O.

ABSTRACT:

Thioguanine nucleotides (6-TGN) are intracellular metabolites that may contribute to the antiproliferative effects of AZA. The objectives of our study were to describe the variability of 6-TGN concentrations during AZA therapy and to investigate possible correlations between 6-TGN levels and subsequent myelosuppression. We measured 6-TGN concentrations in RBC of 65 renal transplant recipients from day 0 until 11-64 days after transplantation. High 6-TGN concentrations were observed in relation to elevated S-creatinine. In 15 patients, 6-TGN concentrations above 200 pmol/8 x 10(8) RBCs were measured (high 6-TGN group: mean maximal 6-TGN = 552 pmol/8 x 10(8) RBCs, SE = 91). In the remaining 50 patients, mean maximal 6-TGN was 82 pmol/8 x 10(8) RBCs, SE = 6.1 (low t-TGN group). In the former group, mean S-creatinine measured on the day of maximal 6-TGN was 466 mumol/L (SE = 62.3), while in the latter it was 190 (SE = 14.7). In the high 6-TGN group, we observed a lower mean nadir neutrophil count than in the low 6-TGN group (3.4 vs. 5.1 x 10(9) neutrophils/L). The nadir neutrophil count occurred, on the average, 12.7 days after maximal 6-TGN in the high 6-TGN group, with no such delay in the low 6-TGN group. This study demonstrates for the first time that 6-TGN in RBCs may rise to very high levels during impaired renal function. Furthermore, the results support the hypothesis that myelosuppressive side effects of AZA therapy correlate with 6-TGN concentrations. Renal transplant recipients may benefit from the monitoring of AZA through RBC 6-TGN measurements.

8. Possibilities for Therapeutic Drug Monitoring of Azathioprine: 6-Thioguanine Nucleotide Concentrations and Thiopurine Methyltransferase Activity in Red Blood Cells

Ther Drug Monit. 1997 Jun;19(3):318-26.

S. Bergan, H. E. Rugstad, B. Klemetsdal, T. Giverhaug, 0. Bentdal, G. S0dal, A. Hartmann, tI. Aarbakkc, and O. Stokke

Summary: The objectives of this study were to establish monitoring of azathioprine (AZA) treatment in renal allograft recipients by red blood cell (RBC) 6- thioguanine nucleotide (6-TGN) measurements and to characterize the variability of RBC thiopurine methyltransferase (TPMT) activity and the effects on 6- TGN levels and the incidence of rejection episodes. In 82 renal allograft recipients, the effect of standard AZA dosage (3 mg/kg tapered to 1 mg/kg) was compared with higher dosages (3 mg/kg for several days) under 6-TON monitoring, The authors measured TPMT in these patients and in a group not receiving AZA, The authors did not find an inverse correlation between RBC TPMT activity and 6-TGN concentrations, and baseline TPMT activity did not prediet the incidence of rejection episodes. The slight increase in RBC TPMT activity after transplant was associated with the use of furosemide rather than AZA; in the five patients receiving furosemide for less than 10 days, TPMT activity declined. The higher AZA dosage in the 6-TGN monitored group was not sufficient to increase RBC 6-TGN to target levels (100 to 200 pmol/8 X 10(8) RBC); median 6-TGN levels were similar in the two groups, as was the incidence of rejection episodes. Based on these findings, the authors suggest that higher dosages be studied in conjunction with 6-TGN monitoring, to explore the possibilities for therapeutic improvements.

9. Monitored high-dose azathioprine treatment reduces acute rejection episodes after renal transplantation.

Transplantation. 1998 Aug 15;66(3):334-9.

Bergan S, Rugstad HE, Bentdal O, Sødal G, Hartmann A, Leivestad T, Stokke O.

ABSTRACT:

Background: Azathioprine (AZA) is widely used in organ transplantation. Common practice is to adjust dose according to body weight only, despite documented pharmacokinetic variability. The purpose of this study was to investigate whether high-dose AZA treatment monitored by 6-thioguanine nucleotides (6-TGN) levels reduces the incidence of rejection episodes in renal transplantation without a corresponding increase in myelotoxicity.
Methods: Patients receiving cyclosporine, steroids, and AZA were randomized into either the low-dose AZA group (3 mg/kg on day 0, then 2 mg/kg/day the first week and 1 mg/kg/day thereafter) or the high-dose AZA group. In the latter, AZA was started at 5 mg/kg/day and then adjusted to keep 6-TGN concentrations (measured twice weekly) between 100 and 200 pmol/8 x 10(8) RBCs.
Results: A total of 360 transplant recipients were included in the final analysis. The cumulative incidence of first rejection episodes was reduced by 21%, from 62.8% in the low-dose group to 49.4% in the high-dose group (difference: 13.3%; 95% confidence interval: 3.2-23.5). Similar results were found in subgroups according to HLA-DR match. The 6-TGN concentration was significantly higher in the high-dose AZA group during the first month, and the reduction in rejection episodes was achieved in the same period. A larger proportion of patients in the high-dose group had nadir white blood cell count below 2.0 x 10(9) leukocytes/L (13.3% vs. 4.4%; difference: 8.9%; confidence interval: 3.1-14.7).
Conclusions: High-dose AZA therapy in a triple-drug regimen, monitored by 6-TGN, will keep myelotoxicity within acceptable limits with the benefit of a reduction in acute rejection episodes.

11. Monitoring Azathioprine Therapy in Pediatric Renal Transplant Patients With Red Blood Cell Thiopurine Methyltransferase

Transplantation Proceedings, 32, 361–363 (2000)

T. Dervieux, Y. Médard, V. Baudouin, A. Maisin, D. Zhang, C. Loirat, and E. Jacqz-Aigrain

AZATHIOPRINE is a prodrug commonly used in immunosuppressive regimen after renal transplantation.
After conversion to 6-mercaptopurine, the drug is metabolized into the active 6-thiopurine nucleotides and is catabolized to methyl 6-mercaptopurine by the thiopurine methyltransferase (TPMT), an enzyme under genetic control. In the present study, the inter- and intraindividual variations of red blood cell TPMT activity and relationship with the effects of azathioprine were evaluated during the first year postrenal transplantation in 22 paediatric patients. A significant correlation between the initial pretransplant TPMT activity and its subsequent change after 1 month was detected ($P = .01$). The initial TPMT activity was not related to the occurrence of rejection episodes during the period of the study. In contrast, TPMT activity and the percentage of variation of TPMT activity from baseline determined at month 1 were found higher in the patients who rejected by comparison with those who did not reject during the first 3 months or during the first year following transplantation ($P < .005$). Our preliminary results suggest a link between high TPMT activity, leading to an increase of azathioprine catabolism and high occurrence of rejection.
Azathioprine is commonly administered in association with cyclosporine and corticosteroids to prevent allograft rejection after renal transplantation. Azathioprine is rapidly converted into 6-mercaptopurine and then either catabolized into inactive metabolites by xanthine oxydase (6-thiouric acid) and thiopurine methyltransferase (methyl 6-mercaptopurine) or metabolized by hypoxanthine guanine phosphoribosyl transferase into active 6-thioguanine nucleotides.[1]
Thiopurine methytransferase (TPMT; E.C. 2.1.1.67) is a cytosolic enzyme that catalyses the thiol methylation of 6-mercaptopurine and related compounds.
Red blood cell TPMT activity is polymorphic and under genetic control. Approximately 89% of Caucasians are homozygous for the high activity allele, 11% are heterozygous, and about 1 subject in 300 individuals inherits TPMT-deficiency as an autosomal recessive trait.[2] Patients being homozygous for recessive alleles encoding low TPMT activity accumulate high concentrations of 6-TGN and are at high risk for severe myelosuppression under azathioprine.[3] Red blood cell TPMT activity is correlated with the activity in the liver[4], and an increase of the activity during the first 3 months postrenal transplantation under azathioprine therapy was reported previously.[5] In the present study, red blood cell TPMT were measured repeatedly during the first year post-transplantation in paediatric renal transplant recipients to evaluate the inter- and intraindividual variations and the relationship with the pharmacologic properties of azathioprine.

17. Cyclosporine A-Based Immunotherapy in Adult Living Donor Liver Transplantation: Accurate and Improved Therapeutic Drug Monitoring by 4-hr Intravenous Infusion

Transplantation 2011;92: 100–105)

Taizo Hibi, Minoru Tanabe, Ken Hoshino, Yasushi Fuchimoto, Shigeyuki Kawachi, Osamu Itano, Hideaki Obara, Masahiro Shinoda, Naoki Shimojima, Kentaro Matsubara, Yasuhide Morikawa, and Yuko Kitagawa

Background: A paucity of data exists for evaluating therapeutic drug monitoring in association with clinical outcomes of cyclosporine A (CYA) treatment in living donor liver transplantation (LDLT).
Methods: A retrospective cohort analysis was conducted on 50 consecutive adult patients who underwent LDLT between 2001 and 2009 to investigate the feasibility and efficacy of 4-hr continuous intravenous infusion of CYA-based immunotherapy (4-hr CYA-IV, n=27) and compare the pharmacokinetic profile and short-term prognoses with an oral microemulsion formulation of CYA (CYA-ME, n=23).
Results: All patients in the 4-hr CYA-IV group reached target CYA peak by day 3 compared with only 22% in the CYA-ME group (P<0.001). Adjustability to achieve the target range was easier in the 4-hr CYA-IV group compared with the CYA-ME group (P=0.017). Acute cellular rejection rate was lower in the 4-hr CYA-IV group (0%) compared with the CYA-ME group (17%, P=0.038). A subset analysis of the CYA-ME group revealed that CYA exposure was affected by external bile output (P=0.006). Patients in the CYA-ME group showed increased risk of switch to tacrolimus (35%) compared with the 4-hr CYA-IV group (7%, P=0.030). Toxicities and mortality rates were equivalent. The optimal initial dose of oral CYA at conversion from the 4-hr CYA-IV was considered to be 3-fold greater than that of the intravenous dose.
Conclusions: In LDLT, our 4-hr CYA-IV immunosuppression protocol was superior to CYA-ME oral dosing and allowed accurate therapeutic drug monitoring with excellent patient compliance.

18. Optimization of Cyclosporine Therapy With New Therapeutic Drug Monitoring Strategies: Report From the International NeoralT TDM Advisory Consensus Meeting (Vancouver, November 1997)

Transplantation Proceedings, 30, 1645–1649 (1998)

P. Keown, B.D. Kahan, A. Johnston, G. Levy, S.P. Dunn, F. Cittero, J.M. Grino, P.F. Hoyer, P. Wolf, and P.F. Halloran

CYCLOSPORINE (CsA) has a very narrow therapeutic range because of the fine line between adequate immunosuppression and the risk of drug-induced side effects.

Therapeutic drug monitoring (TDM) of CsA is an essential component of the patient's long-term management plan and involves the use of blood concentrations of the drug to individualize dosing regimens based on pharmacokinetic principles.[1] The traditional method of optimizing a CsA dose regimen in a patient is by titrating the predose blood concentration of CsA ("trough" level) to a designated range that is considered therapeutic and nontoxic.

The designated range of CsA blood concentrations has evolved from previous TDM consensus discussions that have contributed to a standardized CsA monitoring strategy that has been adopted by the majority of transplantation programs.[2]

Although CsA trough-level monitoring has developed as the standard of practice for patient management, more accurate pharmacokinetic predictors of clinical outcomes for patients receiving CsA have been identified. The area under the time-blood CsA concentration curve (AUC) was found to be the most sensitive predictor of outcomes such as acute rejection episodes and graft loss at 1 year posttransplant in adult renal transplant recipients in two independent studies.[3,4] A subsequent re-evaluation of the CsA pharmacokinetic database from University of Texas[5] identified intrapatient variability in AUCs as a significant risk factor in development of chronic rejection. There has been little movement toward the adoption of full AUC monitoring because of the impracticality, cost of multiple samples for analysis, and possibly by a resistance in the transplant community to change their standards of practice without clear evidence of benefit.

The introduction of Neoralt as a superior formulation of CsA has provided us with the stimulus to reevaluate the traditional approach to therapeutic monitoring of the drug.

The improved and more consistent AUCs from the Neoralt formulation compared with Sandimmunet have been demonstrated in several studies in stable and de novo renal transplant patients.[6–9] Superior CsA pharmacokinetics with the Neoralt formulation compared to Sandimmunet have also been documented in liver,[10] lung,[11] and cardiac transplant patients.[12]

Several studies have documented a significant reduction in the incidence of acute rejection rates in Neoralt-treated versus Sandimmunet-treated de novo renal[13] and liver [14,15] transplant recipients which confirms the important influence of consistent and enhanced bioavailability of CsA on clinical outcomes in the transplant recipient.

The focus on CsA pharmacokinetics and clinical outcomes has resulted in a much more extensive pharmacokinetic database from Neoralt clinical trials than all the Sandimmunet data combined over the past 15 years. This wealth of pharmacokinetic data on Neoralt has re-awakened interest in the utility of monitoring blood concentrations of cyclosporine, and subsequently has stimulated exploration of other therapeutic monitoring strategies. Examples of TDM strategies for Neoralt are listed in Table 1.

The goals of this meeting were to discuss, explore, and re-evaluate therapeutic monitoring standards for Neoralt with a focus on sparse-sampling-derived AUCs and singlepoint sampling methods and to provide the transplant community with suggestions concerning first steps on a pathway toward optimizing clinical outcomes with Neoralt.

19. Patient management by Neoral C2 monitoring: an international consensus statement1

Transplantation Vol. 73, S12–S18, No. 9, May 15, 2002

Gary Levy, Eric Thervet, John Lake, and Kazuharu Uchida On Behalf Of The Concert Group6

ABSTRACT:

The Consensus on Neoral C_2: Expert Review in Transplantation (CONCERT) attendees achieved consensus on the following points, based on currently available data from independent clinical studies using Neoral (cyclosporine microemulsion):

1. Scientific validation of Neoral C_2 monitoring

1.1 An understanding of cyclosporine pharmacokinetics is critical for designing a monitoring strategy that maximizes clinical outcome following organ transplantation

1.2 Neoral absorption during the first 4 hours postdose ($AUC_{0–4}$) represents the period of greatest variability among patients. Adequate CsA absorption during this period is important for effective rejection prophylaxis in the early posttransplant phase

1.3 C_0 does not correlate well with $AUC_{0–4}$ in patients receiving Neoral

1.4 C_2 is the best single time-point predictor of $AUC_{0–4}$ in all types of Neoral-treated transplant patient populations that have been studied (adult renal, liver, heart and lung patients, and pediatric renal and liver transplant recipients)

1.5 Additional CsA concentration sampling beyond the C_2 time point, such as C_6, may be required in low absorbers of CsA

2. Clinical data in de novo adult renal transplant patients

2.1 C_0 monitoring in patients receiving Neoral distinguishes poorly between those who will experience acute rejection and those who will remain rejection-free

2.2 Existing data indicate that higher C_2 levels are highly correlated with a lower risk of acute rejection during the early posttransplant period in patients receiving Neoral. Data on outcomes beyond 1 year are awaited

2.3 The association between C_2 and risk of acute rejection is maintained in Neoral-treated patients who receive an antibody induction agent or concomitant mycophenolate mofetil (MMF) therapy

2.4 Achieving an adequate C_2 level early after transplantation is associated with reduced risk of acute rejection in adult renal transplant recipients

2.5 Adjustment of Neoral dose based on C_2 monitoring does not appear to result in impaired early renal function in the short-term, if slow absorbers are identified and managed appropriately. Long-term data are pending

3. Clinical data in de novo adult liver transplant patients

3.1 Patient management with Neoral C_2 monitoring reduces the incidence and severity of acute rejection compared to C_0 monitoring

3.2 Renal function is not compromised by C_2 monitoring in adult liver transplant patients receiving Neoral

4. Clinical outcomes in maintenance renal and liver transplant patients

4.1 Patient management with Neoral C_2 monitoring can improve renal function in maintenance patients by identifying patients who are receiving excessive CsA

4.2 Using C_2 monitoring in maintenance patients can also reduce the incidence and severity of hypertension by identifying patients who are receiving excessive CsA

4.3 Current data suggest that there is an association between C_2 level and risk of chronic allograft nephropathy, in adult renal transplant patients receiving Neoral

5. C_2 targets for adult renal transplant patients

5.1 Guideline C_2 targets have been proposed for the first month posttransplant in adult renal transplant patients receiving Neoral, with subsequent step-wise reductions in C_2 target over time

5.2 Long-term target C_2 levels for prevention of chronic allograft nephropathy remain to be confirmed

6. C_2 targets for adult liver transplant patients

6.1 Guideline C_2 targets have been proposed for adult liver transplant recipients receiving Neoral

7. C_2 targets for other transplant patient types

7.1 C_2 target levels have not yet been established for cardiothoracic organ recipients, living-related liver transplant recipients, kidney-pancreas transplant patients, or pediatric transplant patients receiving Neoral

8. Accuracy of C_2 sampling

8.1 There is a 15-minute “window of opportunity” before and after the 2-hour time point during which the C_2 sample can be taken in order to remain within a 10% margin of error

8.2 In most instances, C_2 targets do not require adjustment for different immunoassay types currently available

9. Pharmacoeconomics

9.1 Neoral C_2 monitoring is at least cost neutral compared to C_0 monitoring and may generate cost savings in adult renal transplant patients

10. Conclusions of the CONCERT conference

10.1 C_2 monitoring is the optimal method to monitor Neoral in adult de novo renal and liver transplant patients (2)

10.2 Preliminary data indicate that Neoral C_2 monitoring may have clinical benefits in maintenance adult renal and liver transplant recipients who are receiving excessive CsA and in whom the Neoral dose is reduced

10.3 Maintenance patients who are experiencing suspected CsA-related toxicity may benefit from use of Neoral C_2 monitoring to detect CsA overexposure

10.4 Further data sre required from prospective, randomized multicenter trials to evaluate the possible long-term clinical benefits of adopting Neoral C_2 monitoring in de novo and maintenance transplant patients, both in terms of rejection prophylaxis and overall safety profile

20. Use of Neoral C2 monitoring: a European consensus

Transplant International 18 (2005) 768–778

Bjorn Nashan, Andreas Bock, Jean-Louis Bosmans, Klemens Budde, Hans de Fijter, Bryon Jaques, Atholl Johnston, Rainer Luck, Karsten Midtvedt, Luis M Pallardó , Andrew Ready, Ephrem Salamé , Mauro Salizzoni, Francisco Suarez and Eric Thervet

Summary: Large-scale clinical trials using C2 monitoring of cyclosporine (CsA) microemulsion (Neoral) in renal transplant recipients have demonstrated low acute rejection rates and good tolerability with a low adverse event profile in a variety of settings: with or without routine induction therapy; in combination with mycophenolate mofetil; with standard-exposure or low-exposure Neoral; and in patients with immediate or delayed graft function. In liver transplantation, C2 monitoring significantly reduces the severity and incidence of acute rejection compared with C0 monitoring, without adverse consequences in terms of renal function or tolerability.

Different C2 targets are appropriate depending on adjunctive immune suppression, level of immunologic risk, CsA tolerability, risk of renal toxicity and time since transplantation. CsA absorption may increase substantially in most patients during the first 1–2 weeks post-transplant, and this should be taken into account to avoid overshooting C2 target range. A patient with a low C2 value may be either a low or a delayed absorber of CsA, or be a normal absorber who is receiving too low a dose of Neoral. C2 monitoring alone is insufficient to differentiate between these types of patients, and measurement of additional time points is recommended. Adopting C2 monitoring in maintenance transplant patients identifies those who are overexposed to CsA. In summary, randomized, prospective, multicenter studies and single-center trials have evaluated Neoral C2 monitoring within a range of regimens in different organ types, providing a robust evidence base for the benefits of this sensitive monitoring technique.

21. Monitoring of cyclosporine levels in transplant recipients using self-administered fingerprick sampling

Clin Transplant 2006: 20: 221–225

Yonan N, Martyszczuk R, Machaal A, Baynes A, Keevil BG.

ABSTRACT:

Use of C2 monitoring for cyclosporineA(CsA) microemulsion results in improved clinical outcomes vs. trough (C0) monitoring.

Logistical issues include accurate timing of the C2 sample; requirement for sample dilution with most standard assay techniques; and inconvenience for patients. Recently, it has been shown that CsA concentrations in capillary blood correlate closely with levels in venepuncture samples, and that liquid chromatography tandem mass spectrometry (LC-MS/MS) can analyse CsA concentration using undiluted capillary blood from fingerprick samples. In a study to assess the feasibility of CsA monitoring, 52 stable heart transplant patients were provided with kits to take fingerprick trough and C2 blood samples at home, returning them to the laboratory by post for LC-MS/MS analysis. In total, 225 samples were provided, of which 14 (6%) were unsuitable for analysis because of clotting (n510) or insufficient volume (n54). Discomfort was not a problem and initial difficulties that some patients reported in taking the samples resolved with experience. All samples were returned by the postal system in a timely manner. Use of fingerprick assays could allow transplant physicians to have access to C2 levels when patients visit the clinic for review, and avoids the need for patients to attend the clinic or local healthcare centre solely for venepuncture. A barrier to more widespread introduction of fingerprick testing is likely to be lack of suitable MS facilities and trained personnel. In conclusion, self-administered fingerprick testing for CsA blood levels is practical to implement and highly convenient for patients and offers advantages for the transplant team.

22. Liquid Chromatography–Tandem Mass Spectrometry Outperforms Fluorescence Polarization Immunoassay in Monitoring Everolimus Therapy in Renal Transplantation

Ther Drug Monit 2010;32:413–419

Dirk Jan A. R. Moes, PharmD, Rogier R. Press, PharmD, Johan W. de Fijter, MD, PhD, Henk-Jan Guchelaar, PharmD, PhD, and Jan den Hartigh, PharmD, PhD

Background: There is a need to monitor everolimus blood concentrations in renal transplant recipients as a result of its high pharmacokinetic variability and narrow therapeutic window. However, analytical methods to determine blood concentrations often differ in performance. Therefore, we investigated whether two commonly used therapeutic drug monitoring methods for everolimus were in agreement and to what extent their differences could lead to differences in dosage advice.
Design and Methods: Six hundred twelve whole blood samples were obtained from 28 adult renal transplant recipients receiving everolimus and prednisolone therapy. These samples included 286 everolimus trough concentrations. The remaining samples were obtained up to 6 hours post everolimus intake and allowed calculation of 84 AUCs0–12h. All samples were analyzed with fluorescence polarization immunoassay (FPIA) on an Abbott TDxFLx analyzer and liquid chromatography–tandem mass spectrometry (LC-MS/MS).
Results: Everolimus blood concentrations measured with FPIA and LC-MS/MS were not in agreement. Concentrations determined by FPIA were, on average, 23% higher than concentrations quantified by LC-MS/MS. Moreover, concentrations lower than 15 mg/L or AUC0–12h determined with FPIA could be twofold higher than with LC-MS/MS. This variability can lead to clinically relevant differences in dose adjustment of up to 1.25 mg everolimus despite using a correction factor of 23%. Finally, when trough concentrations were measured with FPIA, higher intrapatient variability was observed compared with the use of LC-MS/MS.
Conclusion: LC-MS/MS outperforms FPIA for clinical drug monitoring and intervention of everolimus therapy in adult renal transplant recipients on dual therapy with prednisolone. Specifically, the use of FPIA can lead to clinically relevant differences in everolimus dosage advice and higher intrapatient variability.

24. Determination of blood everolimus concentrations in kidney and liver transplant recipients using the sirolimus antibody conjugated magnetic immunoassay (ACMIA)

Clin Lab. 2011;57(5-6):403-6.

Bouzas L, Tutor JC.

ABSTRACT:

Background: The aim of our study was to evaluate the possible determination of everolimus concentrations using the newly-introduced sirolimus antibody conjugated magnetic immunoassay (ACMIA).
Methods: Everolimus concentrations were determined in 100 blood samples from kidney (n = 47) and liver (n = 53) transplant recipients using the IMx sirolimus microparticle enzyme immunoassay (MEIA) from Abbott as previously described (Clin Biochem 2007;40:132-36) and sirolimus ACMIA from Siemens Healthcare Diagnostics Ltd.
Results: The ACMIA everolimus values were significantly higher than those of MEIA ($p < 0.001$). Analogous slope and intercept values were obtained in the linear regression between the ACMIA and MEIA results when compared to the Seradyn Certican everolimus controls or the blood samples from transplant recipients. Correction of the ACMIA values using the regression equation obtained for the control material (ACMIAcorrected = 0.55 ACMIA + 1.14) led to a satisfactory relationship with the results provided by the MEIA for the patients' samples (MEIA = 1.00 ACMIA corrected + 0.30, $r = 0.905$, $p < 0.001$).
Conclusions: The sirolimus ACMIA on the Dimension platform, which does not require manual pre-treatment of the blood samples, may be an acceptable option for therapeutic everolimus monitoring, significantly reducing technician time in comparison to other widely-used immunoassays.

27. Everolimus Therapeutic Concentration Range Defined from a Prospective Trial with Reduced-Exposure Cyclosporine in De Novo Kidney Transplantation

Ther Drug Monit 2004;26:499–505

John M. Kovarik, Helio Tedesco, Julio Pascual, Giovanni Civati, Marie-Noelle Bizot, Johanna Geissler and Heinz Schmidli

ABSTRACT:

Prospective therapeutic drug monitoring of everolimus was performed in a 1-year multicenter trial in 237 de novo kidney transplant patients. Trough blood levels, rejection episodes, and safety parameters were evaluated to define an appropriate therapeutic concentration range for everolimus in this setting. Patients were randomized to everolimus starting doses of 0.75 mg bid (n = 112) or 1.5 mg bid (n = 125). Doses were then individualized based on everolimus trough blood levels (C0) in an attempt to maintain troughs ≥3 ng/mL; no upper limit was specified. The regimen also contained corticosteroids and cyclosporine with an early dose reduction in months 2–3 posttransplant based on concentrations 2 hours postdose (C2). Cyclosporine C0 levels were also collected. Prospective therapeutic drug monitoring of everolimus C0 in patients starting at 0.75 mg bid led to dose adjustments in 52% of patients to an average long-term dose of 0.93 ± 0.36 mg bid. This gave median (10th to 90th percentile) C0 levels of 5.3 (3.4–7.9) ng/mL. In patients starting at 1.5 mg bid, 55% had dose adjustments leading to an average long-term dose of 1.24 ± 0.35 mg bid. This yielded C0 levels of 7.2 (4.4–11.6) ng/mL. Cyclosporine dosing began on average at 274 ± 78 mg bid, was down titrated in months 2–3 from 181 ± 80 mg to 81 ± 33 mg bid, and stabilized at 70 ± 26 mg bid thereafter. This yielded median C2 levels of 1165 ng/mL in month 1, a down-titration with levels of 853 and 630 ng/mL in months 2 and 3, and a posttitration level of 472 ng/mL. The corresponding median cyclosporine C0 was 242 ng/mL initially and 70 ng/mL in the posttitration phase. In patients starting at 0.75 mg bid everolimus and an early down-titration of cyclosporine, everolimus C0 between 3 and 8 ng/mL was an effective and safe concentration range. Concentrations up to 12 ng/mL were tolerated over the first year posttransplant. This trial demonstrated that therapeutic monitoring of everolimus can be prospectively performed for dose individualization. Maintaining everolimus troughs in the range 3 to 8 ng/mL in the first posttransplant year with reduced-exposure cyclosporine is associated with good efficacy and safety profiles.

28. Therapeutic drug monitoring for everolimus in kidney transplantation using 12-month exposure, efficacy, and safety data

Clin Transplant 2005: 19: 145–152. ª Blackwell Munksgaard, 2005

Lorber MI, Ponticelli C, Whelchel J, Mayer HW, Kovarik J, Li Y, Schmidli H.

ABSTRACT:

The aims of the current study were to determine whether therapeutic drug monitoring (TDM) might benefit kidney transplant recipients receiving everolimus, and to establish dosage recommendations when everolimus is used in combination with cyclosporine and corticosteroids. The analysis was based on data from 779 patients enrolled in two 12-month trials. Everolimus trough concentrations ≥3 ng/mL were associated with a reduced incidence in biopsy-proven acute rejection (BPAR) in the first month (p = 0.0001) and the first 6 months (p =0.0001), and reduced graft loss compared with lower concentrations (4% vs. 20%, respectively). By contrast, cyclosporine in the standard concentration range had no impact on BPAR within the same timeframes. Most patients receiving everolimus 1.5 or 3 mg/d achieved trough concentrations above the therapeutic threshold of 3 ng/mL, regardless of reductions in cyclosporine dose. TDM simulation showed that just two dose adjustments would achieve median everolimus trough values ≥3 ng/mL in 95% of patients during the first 6 months.This investigation indicates that improved efficacy is likely when TDM is considered as an integral component of the immunosuppressive strategy of everolimus.

34. Focus on mTOR inhibitors and tacrolimus in renal transplantation: Pharmacokinetics, exposure–response relationships, and clinical outcomes

Transplant Immunology 31 (2014) 22–32

Fuad Shiha, Uwe Christians, Lonnie Smith, Jason R.Wellen, Bruce Kaplan

ABSTRACT:

Mammalian target of rapamycin (mTOR)-inhibitor-containing immunosuppressive regimens have been developed as part of calcineurin inhibitor (CNI) minimization/withdrawal strategies for renal transplant recipients, with the goal of avoiding CNI-associated nephrotoxicity. This review focuses on the pharmacokinetic interactions and exposure–response relationships of mTOR inhibitors and tacrolimus (TAC), the most widely used CNI. We also discuss key randomized clinical studies that have evaluated use of this combination in renal transplantation.

Pharmacokinetic studies have shown that mTOR inhibitors, everolimus (EVR) and sirolimus (SRL), have a large intra- and inter-patient variability in drug exposure, and narrow therapeutic windows (trough levels [C0] 3–8 ng/mL and 5–15 ng/mL, respectively). Consequently, routine therapeutic drug monitoring of EVR and SRL is recommended to optimize efficacy and minimize toxicity in individual patients. As there is a good correlation between C0 and area under the curve (AUC), C0 can be used as a convenient and reliable measure of mTOR drug exposure. Clinical data on the use of EVR or SRL in TAC minimization strategies in renal transplantation are limited.

Available evidence suggests that treatment with EVR allows early and substantial TAC minimization when used with basiliximab induction and corticosteroids, to achieve good renal function without compromising efficacy or safety. However, data comparing this combination with other regimens are lacking. Results with SRL are more mixed. SRL in combination with reduced TAC has been shown to provide less nephrotoxicity than the SRL/standard TAC combination, with comparable efficacy and safety. However, this approach has been shown to be inferior to other regimens in terms of patient/graft survival and biopsy-proven acute rejection (vs MMF/TAC) as well as renal function (vs MMF/TAC and SRL/MMF). Further studies are needed to define the therapeuticwindowfor TAC when used in combination with mTOR inhibitors, evaluate VR/reduced TAC versus other regimens, assess long-term outcomes, and determine efficacy and safety in high-risk patients.

35. Therapeutic drug monitoring

Nephrology 2007; 12, S57–S65

Trevillian P.

Background: The introduction of CSA in the early 1980s was immediately associated with an enhanced 1 year renal allograft survival. Subsequently, there has been a protracted learning curve on how to optimally use the drug in renal transplant recipients to further enhance outcomes. Over the past two decades, there have been changes to recommended CSA dosing, changes in concomitant medications, and one major change to the oral drug formulation. Lately, there has also been the introduction of generic formulations of CSA. In 1988, Kasiske *et al*. showed in a prospective study that although C0 levels of CSA correlated poorly with dose, Cmax was significantly correlated with dose, AUC and elimination half-life (T1/2). Those who suffered acute rejection had a significantly lower Cmax by 15–31%.1 Since then, there have been many pharmacokinetic (PK) studies confirming other time point estimates (generally C2 or C3) or abbreviated AUC as better predictors of CSA exposure (as measured by full AUC 0–12) than C0 levels.

Most of the inter-individual PK variability occurs in the first 4 h post dose and more recently, studies have concentrated on predicting the AUC 0–4 in what is now called 'absorption profiling'. Poor or variable absorbers of CSA have a worse outcome than good absorbers of the drug.2 Thus, attempts have been made to set early and late target ranges for C2 and AUC by comparing receiver operating characteristic curves of these PK parameters with observed rejection rates and toxicity parameters. Tacrolimus was introduced into clinical practice in the mid-1990s and has a similar absorption profile to CSA but with a lower peak to trough ratio. Consequently, there has been an accepted dogma that trough monitoring reliably reflects TAC exposure and unlike CSA, there has been little pressure to adopt more precise methods of TDM.

Intuitively, one might expect that monitoring patients by these more precise measurements of CNI exposure would translate to better clinical outcomes. On the other hand, it can be argued that the ultimate guide to CNI efficacy is to measure calcineurin inhibition in recipient immune effector cells. The likely best predictor of nephrotoxicity is the drug uptake by the renal allograft itself. These assays are available but remain laboratory research tools. The main aim in researching this guideline was to review the quality of evidence to date that TDM of CNI blood levels by any other method than trough monitoring is advantageous.

37. Therapeutic drug monitoring in pediatric renal transplantation

Pediatr Nephrol (2015) 30:253–265

Lutz T. Weber

Abstract Finding the balance between clinical efficacy and toxicity of immunosuppressive drugs is a challenge in renal transplantation (RTx), but especially in pediatric RTx patients. Due to the expected longer life-span of pediatric transplant patients and the long-term consequences of drug-induced infectious, malignant and cardiovascular adverse effects, protocols which minimize immunosuppressive therapy make conceptual sense. In this context, therapeutic drug monitoring is a tool which provides support for the individualization of therapy. It has, however, limitations, and specific data in the pediatric cohort are comparatively sparse. There is large heterogeneity among the studies conducted to date in terms of methods, follow-up, endpoints, immunosuppressive regimens and patients. In addition, data from adult studies are not readily transferrable to the pediatric situation. This educational review gives a concise overview on aspects of therapeutic drug monitoring in pediatric RTx.

38. Therapeutic drug monitoring for immunosuppressants

Clinica Chimica Acta 313 Ž2001. 241–253

Steven H.Y. Wong

ABSTRACT:

Background: Immunosuppressants have significantly increased patient survival, e.g. in renal transplant up to 90% for the first year. *Methods*: Four immunosuppressants are used for clinical applications in the United States: cyclosporine (CsA).
(Sandimmune and Neoral), FK 506–tacrolimus (ProGraf), mycophenolic mofetil (CellCept)—the prodrug for the mycophenolic acid (MPA), and rapamycin (RAPA) (Sirolimus) For CsA and FK 506, the rationale for monitoring is due to the variable pharmacokinetics, acute infection, dosage adjustment, non-compliance check, and for long-term maintenance therapy. Targeted whole blood concentrations ranges are: for CsA, 100–400 ng/ml depending on the methods, therapy and organs; and for FK 506, 5–20 ng/ml. For MPA, drug bioavailability—the plasma area-under-curve up to 12 h of 32.2–60.6 mg h/l was correlated to the biopsy-proven rejection rate of -10%. Monitoring is advocated for liver and renal transplants, for pediatrics, and for checking for non-compliance. RAPA monitoring is useful to check for variable pharmacokinetics, for non-compliance and others. The therapeutic range is tentatively targeted for 5–15 ng/ml. Monitoring ethodologies are: for CsA, immunoassays such as fluorescence polarization immunoassay, and liquid chromatography (LC); for FK 506, microparticle enzyme immunoassay (MEIA); for MPA, enzyme multiplied immunoassay and LC; and for RAPA, MEIA, LC and LC-mass spectrometry. Proficiency survey programs for CsA and FK 506 are available from the US and Europe.
Conclusions: Monitoring of immunosuppressants has become an essential adjunct to the drug therapy for organ transplant patients.

40. Inosine monophosphate dehydrogenase variability in renal transplant patients on long-term mycophenolate mofetil therapy

Br J Clin Pharmacol / 69:1 / 38–50

Laurent R. Chiarelli, Mariadelfina Molinaro, Carmelo Libetta, Carmine Tinelli, Laura Cosmai, Giovanna Valentini, Antonio Dal Canton & Mario Regazzi

AIMS: Long-term mycophenolate mofetil (MMF) therapy may induce inosine 5'-monophosphate dehydrogenase (IMPDH) activity in peripheral blood mononuclear cells (PBMCs), thus decreasing MMF immunosuppressive properties. Pharmacodynamic monitoring was used to investigate whether biological activity is altered after long-term therapy.
Methods: IMPDH activity was measured in PBMC samples from 54 stable kidney transplant patients, already on MMF (for at least 3 months), before (*t*0) and 2 h after (*t*2) MMF morning dose administration; levels were monitored for up to 15 months, together with total mycophenolic acid (MPA) and free MPA concentrations.
Results: During the 15 months' monitoring, *t*0 IMPDH activity in transplant recipients increased from 5.9 ± 3.7 nmol h^{-1} mg^{-1} [95% confidence interval (CI) 4.9, 6.9] to 9.0 _ 3.9 nmol h-1 mg-1 (95% CI 7.2, 10.8), with an intra- and interpatient variability of 28% and 42%. Five patients experienced acute rejection during the follow-up: *t*0 IMPDH activity was increased during rejection *vs.* non rejection, and the trend was significantly higher in rejecting than in non rejecting subjects for the whole monitoring period.
Conclusions: Even though a correlation has been found between IMPDH activity and rejection, its efficacy as a predictive tool in long-term transplant outcomes may be affected by high interpatient variability; on the other hand, continuous monitoring of the IMPDH trend could make an effective prognostic parameter of rejection. Other trials also including pre-transplant data on both IMPDH expression and activity are warranted to better assess their role as biomarkers for MPA effect in clinical practice.

43. Monitoring of inosine monophosphate dehydrogenase activity and expression during the early period of mycophenolate mofetil therapy in de novo renal transplant patients.

Drug Metab Pharmacokinet. 2013;28(2):109-17. Epub 2012 Aug 14.

Molinaro M, Chiarelli LR, Biancone L, Castagneto M, Boschiero L, Pisani F, Sabbatini M, Sandrini S, Arbustini E, Tinelli C, Regazzi M, Schena FP, Segoloni GP.

ABSTRACT:

Measurement of inosine-monophosphate dehydrogenase (IMPDH) activity or gene expression was used as a further approach in pharmacokinetics (PK)/pharmacodynamic (PD)-guided mycophenolate mofetil (MMF) therapy. Forty-four de novo kidney transplant patients were enrolled; 35 of these completed the study, and were followed for 24 weeks for clinical status, PK parameters, IMPDH activity and IMPDH1/2 gene expression. IMPDH activity and expression were measured in peripheral blood mononuclear cells before transplant and at week 2,4,12 and 24, drawn before (t0) and 2 h (t2 h) after MMF administration. No significant correlation was found between IMPDH activity/expression and PK parameters. For both genes, significant enhancement in t2 h expression was observed, then decreases towards week 24 with a trend following steroid dosages. Seven patients experienced acute rejection (AR) and exhibited significantly higher pre-transplant expression of both IMPDH1 (median 3.42 vs. 0.84; p=0.0025), and IMPDH2 genes (135 vs. 104; p=0.0218) with respect to non-rejecting patients. A significant association was also found between pre-transplant IMPDH1 mRNA and haematological complications (p=0.032). This study suggests that high steroid dosages may influence IMPDH1/2 expression, hampering their use as a PD biomarker, particularly during the early post-transplant period. The measurement of pre-transplant levels of IMPDH1/2 may contribute to prediction of individual drug responsiveness to improve the clinical management of patients in MMF therapy.

44. Customized Mycophenolate Dosing Based on Measuring Inosine-Monophosphate Dehydrogenase Activity Significantly Improves Patients' Outcomes After Renal Transplantation

Transplantation 2010;90: 1536–1541

Matthias C. Raggi, Stephanie B. Siebert, Werner Steimer, Tibor Schuster, Manfred J. Stangl, and Dietmar K. Abendroth

Background: Significant relationships have been reported between the uptake of mycophenolic acid (MPA) and the risk of acute rejection. In a prospective study after renal transplantation, we assessed the value of measuring inosinemonophosphate dehydrogenase (IMPDH) activity as a predictive indicator of an acute rejection episode in the initial postoperative period.
Patients and Methods: Fifty-two patients received 360 mg enteric-coated mycophenolate-sodium two times per day with concomitant tacrolimus/cyclosporine A, providing a total of 122 pharmacodynamic profiles. IMPDH activity was measured by a validated high-performance liquid chromatography method in four plasma samples collected at predose, 30 and 60 min, 2 and 4 hr, and preoperative, during weeks 1 and 2 and 3 months after transplantation. MPA concentrations were measured by mass spectrometry. Inhibition of IMPDH was correlated to the MPA values, MPA area under the curves, and predose levels of the different calcineurin inhibitors.
Results: Comparing the two groups (group I: rejection; n=17; mean age 51±15 years vs. group II: no rejection; n=35; mean age 51±14 years), we found a significantly (P<0.001) lower inhibition of IMPDH in group I (26.5%±11% vs. 56.7%±18%) already in the first week after transplantation. There was no correlation of MPA values (6.85±4 vs. 4.1±3 mg/L; first week) nor with the calcineurin inhibitor trough blood levels. Area under the curves for MPA did not differ significantly. Furthermore, IMPDH activity was a reliable predictor of rejection episodes and inflammation.
Conclusion: The data suggest that measuring biologic response may be a more valuable indicator than traditional therapeutic drug monitoring of MPA. Patients at risk for rejection could be earlier identified, and the therapeutic potential of MPA will be optimized.

46. Inosine Monophosphate Dehydrogenase MessengerRNA Expression Is Correlated to Clinical Outcomes in Mycophenolate Mofetil–Treated Kidney Transplant Patients, Whereas Inosine Monophosphate Dehydrogenase Activity Is Not

Ther Drug Monit 2009;31:549–556

Ferdi Sombogaard, PharmD, Annemiek M. A. Peeters, BSc, Carla C. Baan, PhD, Ron A. A. Mathot, PhD, Monique E. Quaedackers, PhD, Arnold G. Vulto, PhD, Willem Weimar, PhD, and Teun van Gelder, PhD

ABSTRACT:

Measurement of the pharmacodynamic biomarker inosine monophosphate dehydrogenase (IMPDH) activity in renal transplant recipients has been proposed to reflect the biological effect better than using pharmacokinetic parameters to monitor mycophenolate mofetil therapy. The IMPDH assays are however labor intensive and this complicates implementation into patient care. Quantification of IMPDH messenger RNA (mRNA) could form an attractive alternative. This study was designed to correlate IMPDH mRNA levels with IMPDH activity and clinical outcome in renal transplant recipients. From a cohort of 101 renal transplant patients, blood samples were drawn pre transplantation and at 4 times after transplantation. IMPDH activity, IMPDH type 1 and type 2 mRNA levels, and mycophenolic acid concentrations were measured and correlated to clinical outcomes. No correlation was found between IMPDH type 1 and type 2 mRNA levels and IMPDH activity in preand posttransplant samples. A significant increase in IMPDH mRNA levels was found between day 6 and day 140 after transplantation.

IMPDH type 1 and type 2 mRNA levels before transplant showed a trend toward statistically significant higher levels in patients with an acute rejection ($P = 0.052$ and $P = 0.058$). After transplant, the IMPDH type 1 and type 2 mRNA levels were significantly lower in patients with an acute rejection ($P = 0.026$ and $P = 0.007$). We conclude that IMPDH mRNA levels do not correlate with IMPDH activity but are nevertheless correlated with acute rejections. Furthermore, although the regulation of the expression of the 2 isoforms is presumed to be different, in this study, the changes in the expression of type 1 mRNA closely paralleled those of type 2.

47. Mycophenolate Blood Level Monitoring: Recent Progress

American Journal of Transplantation 2009; 9: 1495–1499

T. van Gelder

The concentration–effect relationship for mycophenolic acid (MPA), and the high variability in MPA concentrations in patients on standard dose mycophenolate mofetil (MMF) therapy, for some centers has provided enough evidence to implement therapeutic drug monitoring (TDM) for MMF in daily practice. Two randomized trials Adaption de Posologie du MMF en Greffe Renale (APOMYGRE) and fixed-dose versus concentration controlled (FDCC) investigated the added benefit of TDM for MMF in renal transplant recipients. The APOMYGRE study showed a significant reduction in the incidence of acute rejection in concentration controlled patients, while the FDCC study had a negative outcome, despite a similar study design. Although it was expected that these prospective trials would give the final answer to the question of whether or not TDM for MMF would be of benefit, it seems that the studies have not had much impact on patient management. Several trials have shown the importance of early adequate exposure to MPA in the first week after transplantation. As it will be hard to improve MPA exposure with TDM, this early, ongoing study now investigates the use of an increased starting dose. The increased starting dose will avoid underexposure to MPA in higher proportions of patients shortly after transplantation but may result in more toxicity in patients with MPA exposures exceeding the upper threshold of the therapeutic window.

50. Monitoring of mycophenolic acid and kidney function during combined immunosuppressive therapy

Clin Chem Lab Med 2011;49(11):1849–1853

Anna V. Oláh, László Asztalos, Gergely Ivády, Éva Varga, Ágota M. Kovács, János Kappelmayer and József Varga

ABSTRACT:

Background: Mycophenolic acid (MPA), a selective inhibitor of lymphocyte proliferation, has lately been used to improve renal function and prolong graft survival in renal transplanted patients. Still, there is no consensus considering the recommended dosing and the therapeutic range of MPA.
Methods: To estimate the safe therapeutic range of MPA, its plasma level and indicators of kidney function were measured in 216 patients (138 male, 78 female, age 46±12 years) 67±46 months after transplantation. Besides MPA, patients received cyclosporine (Group A, n=122) or tacrolimus (Group B, n=77). Seventeen patients (Group C) were treated with MPA in combination with everolimus or sirolimus. Plasma MPA was measured by enzyme inhibition assay.
Results: In the whole study group MPA level increased with the dose of MPA (p=0.013). MPA level was below the therapeutic range in 40% (Group A) and 45% (Group B) of patients, respectively. MPA was 1.9±1.56 mg/L in Group A, 2.4±1.69 mg/L in Group B. In Group A MPA level increased and cyclosporine decreased with the progress of renal disease.
Conclusions: Increasing MPA/cyclosporine ratio at more severe stages of chronic kidney disease was tolerable for the patients and rejection could be avoided. Tubular damage detected by urinary N-acetyl-b-D-glucosaminidase did not correlate with the MPA level.

51. Pharmacology and toxicology of mycophenolate in organ transplant recipients: an update

Arch Toxicol (2014) 88:1351–1389

Christine E. Staatz · Susan E. Tett

ABSTRACT:

This review aims to provide an update of the literature on the pharmacology and toxicology of mycophenolate in solid organ transplant recipients. Mycophenolate is now the antimetabolite of choice in immunosuppressant regimens in transplant recipients. The active drug moiety mycophenolic acid (MPA) is available as an ester pro-drug and an enteric-coated sodium salt. MPA is a competitive, selective and reversible inhibitor of inosine- 5'-monophosphate dehydrogenase (IMPDH), an important rate-limiting enzyme in purine synthesis. MPA suppresses T and B lymphocyte proliferation; it also decreases expression of glycoproteins and adhesion molecules responsible for recruiting monocytes and lymphocytes to sites of inflammation and graft rejection; and may destroy activated lymphocytes by induction of a necrotic signal. Improved long-term allograft survival has been demonstrated for MPA and may be due to inhibition of monocyte chemoattractant protein 1 or fibroblast proliferation. Recent research also suggested a differential effect of mycophenolate on the regulatory T cell/helper T cell balance which could potentially encourage immune tolerance. Lower exposure to calcineurin inhibitors (renal sparing) appears to be possible with concomitant use of MPA in renal transplant recipients without undue risk of rejection. MPA displays large between- and within-subject pharmacokinetic variability. At least three studies have now reported that MPA exhibits nonlinear pharmacokinetics, with bioavailability decreasing significantly with increasing doses, perhaps due to saturable absorption processes or saturable enterohepatic recirculation. The role of therapeutic drug monitoring (TDM) is still controversial and the ability of routine MPA TDM to improve long-term graft survival and patient outcomes is largely unknown. MPA monitoring may be more important in high-immunological recipients, those on calcineurin-inhibitor-sparing regimens and in whom unexpected rejection or infections have occurred.

The majority of pharmacodynamic data on MPA has been obtained in patients receiving MMF therapy in the first year after kidney transplantation. Low MPA area under the concentration time from 0 to 12 h post-dose (AUC0–12) is associated with increased incidence of biopsy-proven acute rejection although AUC0–12 optimal cut-off values vary across study populations. IMPDH monitoring to identify individuals at increased risk of rejection shows some promise but is still in the experimental stage. A relationship between MPA exposure and adverse events was identified in some but not all studies. Genetic variants within genes involved in MPA metabolism (UGT1A9, UGT1A8, UGT2B7), cellular transportation (SLCOB1, SLCO1B3, ABCC2) and targets (IMPDH) have been reported to effect MPA pharmacokinetics and/or response in some studies; however, larger studies across different ethnic groups that take into account genetic linkage and drug interactions that can alter a patient's phenotype are needed before any clinical recommendations based on patient genotype can be formulated.

There is little data on the pharmacology and toxicology of MPA in older and paediatric transplant recipients.

53. Tacrolimus and sirolimus in capillary dried blood spots allows for remote monitoring
Pediatr Transplantation 2015: 19: 101–106
Dickerson JA, Sinkey M, Jacot K, Stack J, Sadilkova K, Law YM, Jack RM.

ABSTRACT:
Therapeutic drug monitoring of tacrolimus and sirolimus plays a significant role in the clinical follow-up of transplant patients receiving IMS therapy. Success of transplant and favorable patient outcome relies on maintaining adequate therapeutic drug levels. The purpose of this research is to assess the clinical utility of remote collection of DBS for immunosuppressant monitoring and compare the IMS level in paired collections of venous whole blood and DBS.
Sirolimus and tacrolimus levels were clinically correlated in capillary blood collected from a finger poke with venous whole blood from pediatric, post-transplant patients. The participants took the dried blood spot card home with them with a pre-addressed, postage-paid envelope and mailed it back to the laboratory. Overall, a small but statistically significant negative bias was observed (-0.6 ng/mL, p = 0.0011). A chart review was performed to assess whether clinical management would have changed, and none of the cases revealed a clinically significant change. Sirolimus in DBS also correlated with venous levels. Overall, a small but statistically negative bias was observed (-0.8 ng/mL, p = 0.029). In summary, analysis of IMS levels in DBS is possible, and the difference noted between capillary and venous blood is within the clinically acceptable limits.

54. Long-term evaluation of analytical methods used in sirolimus therapeutic drug monitoring
Clin Transplant 2014: 28: 243–251
Holt DW, Mandelbrot DA, Tortorici MA, Korth-Bradley JM, Sierka D, Levy DI, See Tai S, Horowitz GL.

ABSTRACT:
Results of therapeutic monitoring of sirolimus blood concentrations are assay and laboratory dependent. This study compared performance over time of the IMx microparticle enzyme immunoassay (MEIA), Architect chemiluminescent microparticle immunoassay (CMIA), and liquid chromatography with mass spectrometric detection (LC/MS/MS) as part of a proficiency testing scheme. Pooled samples from sirolimus-treated patients and whole-blood samples spiked with known quantities of sirolimus were assayed monthly between 2004 and 2012. When results of pooled patient samples were compared with LC/ MS/MS, the MEIA assay showed an overall mean percent bias of -2.3% ± 11.2% that, although initially positive, became increasingly negative from 2007 through 2009. The CMIA, which replaced the MEIA assay, had a mean percent bias of 21.9% ± 12.3%, remaining stable from 2007 through 2012. Similarly, for spiked samples, the MEIA showed an increasingly negative bias over time vs. LC/MS/MS, whereas CMIA maintained a stable positive bias. Based on comparison of immunoassay measurements on individual patient samples, CMIA values were more than 25% higher than MEIA values. These results highlight the importance of continued proficiency testing and regular monitoring of sirolimus assay performance. Clinicians must be aware of the methodology used and adjust target levels accordingly to avoid potential effects on efficacy and toxicity.

59. Time-dependent variability in tacrolimus trough blood levels is a risk factor for late kidney transplant failure

Kidney International (2014) 85, 1404–1411

Ruth Sapir-Pichhadze, Yao Wang, Olusegun Famure, Yanhong Li and S. Joseph Kim

Wide variations in tacrolimus levels have been identified as a risk factor for inferior kidney allograft survival but past studies have not properly accounted for the dynamic nature of drug exposure over time. Here we evaluated whether time-varying exposure to tacrolimus increases the risk of long-term adverse outcomes in a retrospective cohort study in adult kidney transplant recipients on tacrolimus-based immunosuppression. Time-dependent Cox proportional hazards models were used to examine the association between the standard deviation of tacrolimus levels (TacSD) starting at 1-year post-transplant and the composite end point of late allograft rejection, transplant glomerulopathy, or total graft loss (including death). Among 356 patients, there was a significant 27% increase in the adjusted hazard of the composite end point for every 1-unit increase in TacSD (hazard ratio 1.27 (95% confidence interval 1.03, 1.56)). There was also a graded increase in the relative hazard for the composite end point by TacSD threshold (hazard ratios 1.33, 1.50, 1.84, and 2.56 for TacSD 1.5, 2, 2.5, and 3, respectively).
The results were similar for total graft loss and the composite end point excluding death. Thus, increased time-dependent TacSD may be an independent risk factor for adverse kidney transplant outcomes. TacSD may serve as a monitoring tool to identify high-risk patients. Whether interventions to decrease TacSD will improve outcomes requires further study.

61. Intra-patient variability in tacrolimus exposure: Causes, consequences for clinical management.

Transplant Rev (Orlando2015 Apr;29(2):78-84. doi: 10.1016/j.trre.2015.01.002. Epub 2015 Jan 14.

Shuker N, van Gelder T, Hesselink DA.

ABSTRACT:

Tacrolimus (Tac) is widely used for the prevention of rejection after solid organ transplantation. Finding the optimal balance between effective Tac concentrations and toxicity is a challenge and requires therapeutic drug monitoring. In addition to the well-known inter-patient variability, the clinical use of Tac is also complicated by considerable intra-patient variability (IPV) in Tac exposure. Tac IPV is defined as the amount of fluctuation of whole-blood concentrations over a certain period of time during which the Tac dose remains unchanged. A high IPV in Tac exposure has recently been recognized as a strong risk factor for acute rejection and poor long-term kidney transplantation outcome. In addition to non-adherence, several other factors determine the magnitude of the IPV in Tac exposure. Quantification of IPV is easy and can be easily incorporated into everyday clinical practice as a tool for optimizing transplantation outcomes.

62. Development of limited sampling strategies for the estimation of tacrolimus area under the curve in adult kidney transplant recipients according to the post transplantation time.

Ther Drug Monit. 2015 Jan 26. [Epub ahead of print]

Karim A, Zohra C, Mouna H, Nadia BF, Sabra A, Mezri EM, Naceur B, Habib S, Amel C.

ABSTRACT:

Background: Limited sampling strategies (LSS), using few sampling times after dosing, have been used to reliably predict tacrolimus area under the 12-hour concentration-time curve (AUC). As the pharmacokinetics of tacrolimus is subject to significant changes over the exposure time to this drug, it can be hypothesized that the reliability of the LSS would also change. This study aimed to develop a reliable and practical LSS allowing the estimation of tacrolimus AUC in Tunisian kidney transplant recipients taking into account the post transplantation time.
Methods: Thirty Tunisian patients were enrolled into 3 groups (10 in each group) according to the post-transplantation period: Period 1: between 1 day and 3 months, Period 2: between 3 and 12 months and Period 3 over 12 months, as defined by the European consensus conference on therapeutic drug monitoring of tacrolimus. Samples were collected just before and 0.5, 1, 2, 4, 6, 8, and 12 hours after tacrolimus administration. The full pharmacokinetic profiles obtained from these timed concentration data were used to choose the best sampling times. Error indices (mean absolute prediction error (MAE) and the root mean squared prediction error (RMSE)) were used to evaluate the predictive performance.
Results: Among the one-point estimations, the C4-predicted AUC showed the highest correlation with the measured one during Period 1 and Period 2 (r= 0.94 and 0.91 respectively) but not Period 3 (r = 0.76). The C0-predicted and the measured AUC become less and less correlated from Period 1 to Period 3 (r = 0.81, 0.75 and 0.66) respectively. Only the model including the C0/C2 provided a high correlation between predicted and measured tacrolimus AUC regardless of the post-transplant period (r = 0.95, 0.96, 0.98 and RMSE = 4.1, 5.8, 4.2 during Period 1, 2 and 3 respectively).
Conclusions: Our data clearly indicate that the predictive performance of LSS is prone to change according to the post transplantation time. A two-time points LSS was found to be sufficient to predict tacrolimus AUC. The LSS using C0 and C2 is reliable, accurate and practical to estimate the AUC of tacrolimus regardless of the post transplantation time.

64. Chronopharmacokinetics of ciclosporin and tacrolimus

Clin Pharmacokinet. 2006;45(8):775-88.

M. Baraldo, M. Furlanut

ABSTRACT:

The correct use of immunosuppressive drugs has a considerable influence on the prognosis of patients with organ transplants. The appropriate utilisation of the drugs involves the administration of an adequate dosage to reach the blood concentrations that will suppress the alloimmune response, while avoiding secondary toxicities. However, transplanted patients exhibit heterogeneous immunological responses and high inter- and intraindividual pharmacokinetic variabilities. One cause of these variabilities that is rarely considered is circadian rhythms. In vitro and in vivo experiments have clearly demonstrated that all organisms are highly organised according to an internal biological clock that influences various physiological functions. Considering that the absorption, distribution, metabolism and elimination of drugs is influenced by the physiological functions of the body, it is not surprising that the pharmacokinetic, and consequently the pharmacodynamic, profiles of drugs can be influenced by circadian rhythms. Ciclosporin, a mainstay immunosuppressive drug used following organ transplantation, displays minimum blood concentration (C(min)), maximum blood concentration (C(max)) and area under the blood concentration-time curve (AUC) in the morning that are generally higher than the corresponding parameters in the evening. These observations are supported by the ciclosporin total body clearance and elimination half-life in the morning, which are, on average, higher and shorter, respectively, than those in the evening. In addition, the disposition of tacrolimus is determined by the time of administration. The tacrolimus C(max) and AUC after the morning dose are significantly higher than those after the evening dose. Finally, the results reported in this review suggest considering more carefully the chronopharmacokinetics of tacrolimus and ciclosporin in order to obtain better results with fewer adverse effects. Significantly, the morning appears to be the best time for therapeutic monitoring using the C(min), C(max), concentration at 2 hours after dosing and AUC to modify dosages of tacrolimus and ciclosporin. Less certain are any conclusions about whether, in order to obtain better immunosuppressive control, higher doses must be administered when these drugs are given in the evening to compensate for the higher levels of interleukin-2.

65. Development of a population PK model of tacrolimus for adaptive dosage control in stable kidney transplant patients.

Ther Drug Monit. 2015 Apr;37(2):246-55. doi: 10.1097/FTD.0000000000000134.

Andreu F, Colom H, Grinyó JM, Torras J, Cruzado JM, Lloberas N

ABSTRACT:

Background: Tacrolimus pharmacokinetics (PK) presents a high variability that hampers its therapeutic use. The aims of this study are to: (1) develop a population pharmacokinetic (PPK) model for tacrolimus and to identify the factors that contribute to the variability of tacrolimus PK in renal transplant patients; and (2) to establish a new Bayesian estimator that can easily and routinely be applied in the hospital. A new PPK model may allow efficacy to be optimized, improve dose regimens, minimize side effects, and decrease the cost of extensive area under the curve (AUC) monitoring.
Methods: PPK analysis of the full PK profiles of 16 patients on 5 occasions was performed with NONMEM 7.2. Biochemical variables (hematocrit, hemoglobin, aspartate aminotransferase, and others) were analyzed.
Results: A 2-open-compartment model with interoccasion variability best described the PK of tacrolimus. Three transit compartments provided the best description of the absorption process. The hematocrit, aspartate aminotransferase, and alanine aminotransferase were not significant in the covariate analysis. External validation with 91 patients proved the good predictability of the model with a bias and precision of 0.37 mcg/L (CI 95%, -0.11 to 1.20 mcg/L) and 0.38 mcg/L (CI 95%, 0.02 to 1.21 mcg/L), respectively. A limited sampling strategy using 1 sampling point at predose (trough concentrations) showed a good performance in AUC0-12h estimation with a correlation between AUCfull and AUCLSS, bias and imprecision of r = 0.75, 6.78% (range, -16.26% to 30.06%) and 1.42% (IC 95%, 0.14%-3.61%), respectively.
Conclusions: The PPK model developed provides reliable prior information for Bayesian adaptive control of dosage regimens of tacrolimus to achieve the desired AUC goals in stable renal transplant patients.

66. Which Genetic Determinants Should be Considered for Tacrolimus Dose Optimization in Kidney Transplantation? A Combined Analysis of Genes Affecting the CYP3A Locus.

Ther Drug Monit. 2015 Jun;37(3):288-95. doi: 10.1097/FTD.0000000000000142.

Bruckmueller H, Werk AN, Renders L, Feldkamp T, Tepel M, Borst C, Caliebe A, Kunzendorf U, Cascorbi I

ABSTRACT:

Background: Tacrolimus is established as immunosuppressant after kidney transplantation. Polymorphism of the cytochrome P450 3A5 (CYP3A5) gene contributes significantly to tacrolimus dose requirements. Recently, CYP3A4*22 was reported to additionally affect tacrolimus pharmacokinetics (PK). In addition, there are further polymorphic genes, possibly influencing CYP3A activity [pregnane x receptor NR1I2, P450 oxidoreductase (POR), and peroxisome proliferator-activator receptor alpha (PPARA)]. We aimed to investigate combined effects of these gene variants on tacrolimus maintenance dose and PK in patients with stable kidney transplantation of 2 study centers.
Methods: A total of 223 white patients (German cohort, 136; Danish cohort, 87) was included and genotyped for CYP3A5 (rs776746), CYP3A4 (rs35599367), NR1I2 (rs2276707), POR (rs1057868), and PPARA (rs4253728). Dosage and trough concentration/dose ratios were considered separately. A subset was investigated for comprehensive PK parameters.
Results: Tacrolimus dose, trough concentration, and trough concentration/dose ratio did not differ between the German and Danish cohort. CYP3A5*3 and CYP3A4*22 contributed to dose requirements only in the German and in the total cohort. Homozygous carriers of both variants required 4.8 ± 3.1 mg, whereas carriers of the wild types required 165% higher mean tacrolimus doses (12.5 ± 7.7 mg, P = 1.4 × 10). The PK investigation revealed only nonsignificant impact of CYP3A4 genotypes on AUC12h in CYP3A5 nonexpressers (P = 0.079, power = 57%). For the entire sample, the final multiple linear regression model for trough concentration/dose ratio included CYP3A5, CYP3A4, and age. It explained 18.3% of the interindividual variability of tacrolimus trough concentration/dose ratios (P = 8.8 × 10).
Conclusions: Therapeutic drug monitoring remains essential in clinical care of patients with kidney transplantation. Genotyping of CYP3A5 and CYP3A4, however, could facilitate rapid dose finding to adapt the appropriate immunosuppressant dose, whereas other genetic factors had only little or no effect.

67. Lessons from routine dose adjustment of tacrolimus in renal transplant patients based on global exposure.

Ther Drug Monit. 2013 Jun;35(3):322-7. doi: 10.1097/FTD.0b013e318285e779.

Saint-Marcoux F, Woillard JB, Jurado C, Marquet P.

ABSTRACT:

Objectives: Since 2007, a number of transplantation centers have been routinely using an expert system for tacrolimus (TAC) dose adjustment in kidney allograft recipients, based on PK modeling and Bayesian estimation for area-under-the-curve (AUC) determination. This has allowed the setting up of a large database of TAC pharmacokinetic profiles and AUC values, a part of which was analyzed here.
Methods: We retrospectively studied 2030 requests posted by 21 different centers for routine TAC dose adjustment in 1000 different adult renal transplant patients (not enrolled in any kind of concentration-controlled clinical trial). For each request, the following information was obtained: time elapsed since transplantation, TAC daily dose, calculated AUC, and trough concentration (C0).
Results: The dose-standardized exposure to TAC significantly and progressively increased in the months after transplantation: from month (M) 1 to M9 C0/dose increased from 2.33 to 3.44 mcg·L(1)·mg(1) and AUC/dose from 43.1 to 64.2 mcg·h(1)·L(1)·mg(1), respectively. On the contrary, in patients beyond the first year whose C0 or AUC was in the target range, the odds of remaining in this range were high for a long time period, suggesting a low intrapatient variability in the stable phase. Regression analyses showed that the correlation between C0 and AUC was better in the first 3-month period ($r(2) = 0.76$) than later on ($r(2) \leq 0.67$). Using the regression equations obtained, AUC ranges corresponding to different applicable C0 targets were calculated.
Conclusions: From a large number of kidney graft recipients, we have estimated the relationships between C0 and AUC, modeled the evolution of TAC exposure with time and defined AUC targets that could be useful to lead further controlled-concentration trials and improve routine TAC therapeutic drug monitoring.

68. Controlled-dose versus fixed-dose mycophenolate mofetil for kidney transplant recipients: a systematic review and meta-analysis of randomized controlled trials.

Transplantation. 2013 Aug 27;96(4):361-7. doi: 10.1097/TP.0b013e31828c6dc7.

Wang X, Qin X, Wang Y, Huang Z, Li X, Zeng Q, Zeng H, Lu Y, Wang L, Lin T.

ABSTRACT:

Background: Although mycophenolate mofetil (MMF) is recommended at a fixed dose, there is increasing interest in controlled-dose (CD) MMF based on therapeutic drug monitoring. We systematically evaluated published randomized controlled trials (RCTs) on the efficacy and safety of CD versus fixed-dose MMF for kidney transplant recipients.
Methods: The electronic databases Medline, Embase, and Cochrane Library (up to June 2012) were searched to identify relevant RCTs. Two reviewers independently applied the study selection criteria, examined the study quality, and extracted the data. Dichotomous measures were expressed as relative risk (RR) and continuous outcomes were expressed as weighted mean difference, both with 95% confidence intervals (CIs). All statistical analyses were performed using Review Manager 5.1.6.
Results: Four RCTs met our selection criteria and included 1755 de novo recipients. The differences between CD and fixed-dose MMF in treatment failure (RR, 0.95; 95% CI, 0.82-1.10; P=0.52), serum creatinine clearance (weighted mean difference, 2.46; 95% CI, -1.15 to 6.07; P=0.18), total gastrointestinal adverse events (RR, 1.23; 95% CI, 0.65-2.35; P=0.53), diarrhea (RR, 1.08; 95% CI, 0.92-1.25; P=0.35), anemia (RR, 1.24; 95% CI, 0.95-1.64; P=0.12), leukopenia (RR, 1.12; 95% CI, 0.93-1.35; P=0.25), thrombocytopenia (RR, 0.80; 95% CI, 0.47-1.36; P=0.41), and malignancy (RR, 0.61; 95% CI, 0.27-1.38; P=0.23) were not statistically significant. Furthermore, total infections were more frequent in the CD group (36.0% vs. 30.9%; RR, 1.16; 95% CI, 1.03-1.30; P=0.01).
Conclusions: Based on current evidence, CD MMF administration cannot be recommended as routine practice for kidney transplant recipients. Therapeutic drug monitoring for MMF may be targeted toward high-risk recipients, who should be identified in future studies.

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

Am J Transplant. 2013 May;13(5):1253-61. doi: 10.1111/ajt.12191. Epub 2013 Mar 8.

Bouamar R, Shuker N, Hesselink DA, Weimar W, Ekberg H, Kaplan B, Bernasconi C, van Gelder T.

ABSTRACT:

Therapeutic drug monitoring (TDM) 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. Data from three large trials were pooled. We used univariate and multivariate analysis to investigate the relationship between biopsy-proven acute rejection (BPAR) and Tac predose concentration at five time points (day 3, 10 and 14, and month 1 and 6 after transplantation). A total of 136/1304 patients experienced BPAR, giving an overall incidence of 10.4%. 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. We did not find an association between the Tac predose concentrations measured at five time points after kidney transplantation and the incidence of acute rejection occurring thereafter. Based on this study it is not possible to define the optimal target concentrations for Tac.

70. A review on therapeutic drug monitoring of immunosuppressant drugs.
Iran J Basic Med Sci. 2011 Nov;14(6):485-98.
Mohammadpour N, Elyasi S, Vahdati N, Mohammadpour AH, Shamsara J.

Immunosuppressants require therapeutic drug monitoring because of their narrow therapeutic index and significant inter-individual variability in blood concentrations. This variability can be because of factors like drug-nutrient interactions, drug-disease interactions, renal-insufficiency, inflammation and infection, gender, age, polymorphism and liver mass. Drug monitoring is widely practiced especially for cyclosporine, tacrolimus, sirolimus and mycophenolic acid.

Cyclosporine: Therapeutic monitoring of immunosuppressive therapy with cyclosporine is a critical requirement because of intra- and inter-patient variability of drug absorption, narrow therapeutic window and drug induced nephrotoxicity.

Mycophenolic acid (MPA): Some reasons for therapeutic drug monitoring of MPA during post-transplant period include: relationship between MPA pharmacokinetic parameters and clinical outcomes, Inter-patient pharmacokinetic variability for MPA despite fixed MMF doses, alternations of MPA pharmacokinetics during the first months after transplantation, drug- drug interaction and influence of kidney function on MPA pharmacokinetic.

Sirolimus: A recent review of the pharmacokinetics of sirolimus suggested a therapeutic range of 5 to 10 µg l−1 in whole blood. However, the only consensus guidelines published on the therapeutic monitoring of sirolimus concluded that there was not enough information available about the clinical use of the drug to make recommendations.

Tacrolimus: Sudies have shown, in kidney and liver transplant patients, significant associations of low tacrolimus concentrations with rejection and of high concentrations with nephrotoxicity. Although the feasibility of a limited sampling scheme to predict AUC has been demonstrated, as yet, trough, or pre-dose, whole blood concentration monitoring is still the method of choice.

71. KHA-CARI guideline: KHA-CARI adaptation of the KDIGO Clinical Practice Guideline for the Care of Kidney Transplant Recipients

Nephrology (Carlton). 2012 Mar;17(3):204-14. doi: 10.1111/j.1440-1797.2011.01559.x. No abstract available.

Chadban SJ, Barraclough KA, Campbell SB, Clark CJ, Coates PT, Cohney SJ, Cross NB, Eris JM, Henderson L, Howell MR, Isbel NM, Kanellis J, Kotwal SS, Manley P, Masterson R, Mulley W, Murali K, O'Connell P, Pilmore H, Rogers N, Russ GR, Walker RG, Webster AC, Wiggins KJ, Wong G, Wyburn KR; Kidney Health Australia Caring for Australians with Renal Impairment (KHA-CARI).

72. The KDIGO clinical practice guidelines for the care of kidney transplant recipients.
Transplantation. 2010 Mar 27;89(6):644-5. doi: 10.1097/TP.0b013e3181d62f1b.
Chapman JR.

ABSTRACT:
The clinical guideline for care of renal transplant recipients was written by a committee of 15 people from nine countries, supported by an evidence review team The scope of the review was care of the patient after a renal transplant-not evaluation or selection of recipients and donors, focusing on the issue specific to the immunosuppressed transplant patient. A total of 12,327 articles comprising 3168 randomized controlled trials, 7543 cohort studies, and 1609 reviews were selected by a formal search. Each article was formally evaluated for the quality of the data from A to D. A consistent set of statements were based on the strength of the evidence. Level 1 evidence: "we recommend" means that if you were a patient, most people would want to do this; if a clinician, you should recommend this course of action to most patients; and if a policy maker, you should adopt this as a reasonable standard. Level 2 evidence: "we suggest" means the majority of patients would want do this; to the clinician, it means that different solutions may well be needed for different patients; whereas to the health policy maker, this is a strong warning to engage stakeholders in the creation of a particular local policy. Because 69% of the advice is "suggested" on the basis of level C or D evidence, one outcome of this work is to make it clear where the current evidence for clinical decisions runs out of data.

73. Optimal everolimus concentration is associated with risk reduction for acute rejection in de novo renal transplant recipients.

Transplantation. 2010 Jul 15;90(1):31-7. doi: 10.1097/TP.0b013e3181de1d67.
Chan L[1], Hartmann E, Cibrik D, Cooper M, Shaw LM.

ABSTRACT:

Background: Everolimus (Evl) plus tacrolimus (Tac) in de novo renal transplantation is effective and safe. Whether the concentration of Evl affects efficacy and safety in a Tac-based regimen has not been previously reported.
AIM: To evaluate whether the concentration of Evl affects biopsy-proven acute rejection (BPAR), renal function, adverse events (AEs); and to assess for pharmacokinetic (PK) interactions.
Methods: Data were from a prospective, multicenter, open-label, randomized, exploratory 6-month study of 92 renal transplant patients treated de novo with concentration-controlled Evl (target trough levels > or =3 ng/mL) plus low-dose Tac or Evl plus standard-dose Tac; both groups received basiliximab and corticosteroids. Data were pooled across study arms to examine BPAR rates in patients with Evl trough levels less than 3 (n=26), 3 to 8 (n=62), or more than 8 ng/mL (n=4). Groups were stratified by both Evl and Tac trough levels to evaluate glomerular filtration rate and AEs. Evl and Tac PK interactions were evaluated in a subset of 14 patients.
Results: Evl trough level of more than or equal to 3 ng/mL was associated with significantly lower rates of BPAR as compared with a trough level of less than 3 ng/mL. Glomerular filtration rate was similar at 6 months for both the low and standard Tac groups. No apparent PK interactions were observed between Evl and Tac. AEs were infrequent and did not seem to be associated with the Evl or Tac level.
Conclusions: Evl trough levels > or =3 ng/mL plus Tac are associated with low rates of BPAR without adversely affecting renal function. No evident PK interaction exists between Evl and Tac.

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Secretaría Científica Saatchi & Saatchi Health | A Publicis Healthcare Communications Group Company
C/ Ramírez de Arellano, 21 - 4ª planta - 28043 - Madrid
Tel +34 917 684 700 - Fax +34 917 684 713

Secretaría de la SET | C/ Calvo Sotelo 19, 3º, Of. 3, 39002 Santander
Tel. +34 902 116 513 - Fax +34 942 231 058

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