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Delayed graft function in renal transplantation

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Citation
Boom, H. (2005, January 19). *Delayed graft function in renal transplantation*. Retrieved from https://hdl.handle.net/1887/579

Version:	Corrected Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
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Note: To cite this publication please use the final published version (if applicable).

CHAPTER 4

CALCIUM LEVELS AS A RISK FACTOR FOR DELAYED GRAFT FUNCTION

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Transplantation 2004; 77(6): 868-873

Background Delayed graft function (DGF) occurs in upto 50% of renal transplants. Hypercalcemia and hyperparathyroidism are associated with impaired renal function. Little is known on the effects of serum calcium levels on delayed graft function. This issue was addressed in the current study.
Methods Patients receiving a cadaveric renal transplant between 1986 -1996 were studied. Data on calcium metabolism and histological characteristics of nephrocalcinosis, acute tubular necrosis (ATN) and acute rejection in biopsies taken within the first week, were related to the occurrence of DGF.
Results The incidence of DGF in a cohort of 585 cadaveric transplants was 31%. DGF correlated independently with serum calcium levels (OR 1.14 (95% CI 1.04-1.26) per 0.1 mmol/l). The use of calcium channel blockers before transplantation protected against DGF (OR 0.5 (95% CI 0.29 – 0.87). In this selected group we found an association with histological signs of ATN and DGF. However, most of the biopsies also had features of acute rejection or nephrocalcinosis. Nephrocalcinosis was found in 12 of 71 biopsies and was not associated with serum calcium levels or the occurrence of DGF.
Conclusions In this study, serum calcium levels were independently associated with DGF. This could not be explained by the presence of microscopic nephrocalcinosis. Therefore DGF is attributed to high intracellular calcium levels. As calcium supplementation and vitamin D analogues are commonly used in dialysis practice, hypercalcemia influences long-term graft outcome by its effect on DGF. The pretransplant use of calcium channel blockers has a protective effect on the occurrence of DGF.

INTRODUCTION

The pathogenesis of acute tubular necrosis (ATN) in human kidneys remains enigmatic despite great scientific interest and investigative efforts. The poor correlation between the clinical occurrence of acute renal failure and the morphological manifestations of ATN in the renal biopsy has hampered progress in this field (1). The regular occurrence of delayed graft function (DGF) immediately following transplantation of postmortal kidneys has renewed this interest. However, the circumstances in which DGF occurs in renal allografts differ from the conditions in which acute renal failure develops in native kidneys. Conditions during explantation and implantation of the donor organ as well as calcineurin inhibitor toxicity and rejection episodes immediately following transplantation may be responsible for or contribute to the development of DGF (2). The interest in the occurrence of DGF was heightened by the finding that rejection episodes are more likely to occur in association with DGF (3,4). In combination with rejection episodes DGF also influences the long term prognosis of graft function (4,5).

The study of the morphological manifestations of DGF in renal allografts is facilitated by the fact that graft biopsies are more readily obtained than biopsies from native kidneys. It is conceivable that the discrepancy between DGF and ATN in allografts is not only explained by factors related to the transplantation procedure, including calcineurine toxicity, but also by elevated serum phosphate – and / or serum calcium levels at the time of transplantation.

The mechanism by which hypercalcemia causes acute renal failure remains largely hypothetical. In dogs and rats, acute renal failure has been reported as a result of experimentally induced hypercalcemia (6). In animal models 3 types of nephrocalcinosis are distinguished: chemical nephrocalcinosis, microscopic nephrocalcinosis and macroscopic nephrocalcinosis (7). The latter is characterized by gross calcium deposits found on radiographic investigations. Microscopic nephrocalcinosis is characterized by microscopic calcium deposits, mainly located in the lumen of the tubules. Its effect on renal function is thought to be caused by tubular obstruction and tubular back-leak. Chemical nephrocalcinosis, assumed to be present when macroscopic and microscopic nephrocalcinosis are excluded, affects glomerular filtration rate by vasoconstriction and natriuresis induced volume constriction (8). Calcium is a co-factor in the activation of proteolytic enzymes that are linked with tubular integrity. Several factors have been described to play a role in the development of tubular damage caused by oxygen deprivation such as abnormal calcium homeostasis, reactive oxygen species and activation of enzymes (9). Hypoxia has been found to reduce the cellular pool of adenosine triphosphate (ATP) leading initially to accumulation of adenosine di- and monophosphate and further catabolism to hypoxanthine and xanthine and the generation of reactive oxygen species (10). As a consequence of ATP depletion, cells are no longer able to extrude calcium. The influx of calcium occurs predominantly during reperfusion and reoxygenation. High cytosolic concentration of free calcium has been shown to activate calcium-dependent enzymes such as phospholipases, nucleases, and cysteine proteases (11). The wide spread use of vitamin D analogues in dialysis patients and the experimental evidence for a nephrotoxic effect of hypercalcemia prompted us to investigate the role of serum calcium in the occurrence of DGF.

METHODS

Patients

Patients who received a cadaveric renal transplant between January 1986 and December 1996 were included in this study. Twenty-six patients experiencing primary non-function (PNF) were excluded from the study. PNF was defined as grafts that never functioned and led to transplantectomy. A total of 585 transplants were analyzed. DGF was defined when serum creatinine levels increased, remained unchanged or decreased less than 10% per day in three consecutive days in the first week after transplantation. Primary function (PF) was present when they did not meet the definition of DGF and acute rejection was excluded. All patients that received anti-rejection treatment in the first week were categorized as primary function and acute rejection. Cold ischemia time (CIT) was calculated from the Eurotransplant report; warm ischemia time (WIT) was registered by the attending transplant physician. Only three patients received a graft from a non heart beating donor.
The standard immunosuppressive regimen consisted of prednisone and Cyclosporine-A (Sandimmune® soft gelatin capsules). CsA was administered intravenously in a dose of 3 mg/kg /day for the first 48 hours, starting at the onset of surgery. The initial oral dose of CsA was 8 - 10 mg/kg/day from day 2 onward, divided in three daily doses and subsequently tapered. Doses were adjusted according to CsA trough level monitoring. All patients received 20 mg of prednisone starting on day one. Rejection episodes were treated with 1 gram of methylprednisolone intravenously for 3 days or rabbit anti-thymocyte globulin for 10 days, as described previously (12).

Calcium, phosphate, parathormone (PTH)

Concentrations of calcium, phosphate, parathormone (PTH) and albumin were determined on serum samples, obtained on admission for transplantation. Calcium values were corrected for protein binding. Intact PTH was measured with a two-side radioimmunometric assay (IRMA) from Nichols Institute, San Juan Capistrano CA, USA.

Histological examination

During the study period 85 biopsies were taken within 7 days after transplantation. Fourteen biopsies were excluded because sufficient material was not available for analysis. A total of 71 biopsies could be studied. Transplantectomy specimens were not included in the analysis. Needle biopsies were taken to detect or exclude early graft rejection, not to confirm ATN. Three wedge biopsies were taken during surgical re-interventions.
Kidney sections stained by hematoxylin-eosin (HE), periodic acid-Schiff (PAS) and silver-methenamine were scored for manifestations of ATN, according to Olsen et al (13). Signs and severity of rejection in allograft biopsies were taken from the light microscopic description of these biopsies in the clinical records. Acute rejection episodes were retrospectively classified based on the criteria described in the Banff 1997 classification of renal allograft pathology (14).

In order to detect calcium deposits, paraffin sections were stained according to Von Kossa (15) and scored independently by JAB and LES. Nephrocalcinosis was defined when Von Kossa positive material was seen at the same location in at least two consecutive sections (fig.2).

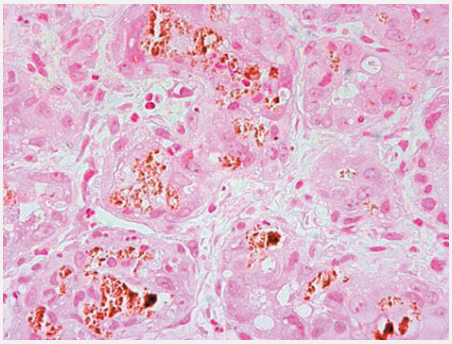


Figure 2

Representative slide showing nephrocalcinosis (Von Kossa staining).

Statistical analysis

Characteristics among groups were compared using Student's t-test for independent continuous variables and Pearson chi-square test for categorical variables. Binary logistic regression analysis was used to assess the risk for occurrence of DGF for each variable separately. Multivariate binary logistic regression was used to adjust for confounding. All analyses were performed using SPSS statistical software package (version 10.07;SPSS Inc. Chicago Ill.)

RESULTS

DGF occurred in 183 (31%) of the 585 patients studied (Table 1). Sex of either donor or recipient did not correlate with DGF, but a higher donor age correlated significantly ($p = 0.0005$) with the occurrence of DGF, whereas recipient age had no influence. Warm ischemia time (WIT) was longer for patients with DGF, but this difference was not significant, whereas cold ischemia time (CIT) was significantly longer in recipients with DGF. Recipients who used calcium channel entry blockers before transplantation experienced less frequent DGF ($p = 0.01$).

Calcium, phosphate and PTH

Recipients with DGF had significantly higher total serum calcium ($p = 0.001$) and albumen corrected calcium levels ($p = 0.01$) than recipients without DGF (Table 1). In a logistic regression model in which several risk factors for the occurrence of DGF were included the Odds Ratio for serum albumen corrected serum calcium was 1.14 for each 0.1 mmol/l increase of serum calcium. When serum calcium levels were stratified, calcium levels of 2.75 mmol/l or higher were associated with a higher occurrence of DGF compared to levels of less than 2.55

mmol/l (OR: 2.51; 95% CI: 1.59 – 3.98; p < 0.0005) (Table 2). However, the individual calcium levels of recipients with and without DGF shows a considerable overlap (fig.1). The relation between corrected calcium levels and the predicted probability of DGF did not show a critical value above which DGF was more likely to occur. Serum phosphate levels did not correlate with the occurrence of DGF and neither did the calcium phosphate product or the albumin corrected calcium-phosphate product. Serum PTH levels were higher in recipients with DGF, but this difference was not significant (p = 0.81).

Table 1: Transplantation characteristics and of calcium and phosphate metabolism of 585 kidney transplant recipients

	Total n = 585	PF n = 402	DGF n = 183	P-value
Transplantation characteristics				
Sex recipient male (%)	63	62	66	0.34
Sex donor male (%)	58	59	56	0.66
Recipient age (years)	46.2 (12.8)	45.9 (12.5)	47.1 (13.5)	0.68
Donor age (years)	38.4 (15.0)	36.7 (14.6)	42.3 (15.2)	<0.0005
Number of transplants	1.20 (0.48)	1.22 (0.49)	1.17 (0.46)	0.34
Pre-Tx CCB's (%)	17.5	20	11.4	0.01
CIT (hours)	28.7 (6.7)	28.0 (6.6)	30.1 (6.7)	< 0.00001
Pre-Tx dialysis mode (% HD)	292 (50)	193 (47)	99 (54)	0.70
Time on dialysis (months)	40.5 (69)	43 (80)	35 (31)	0.61
Preservation fluid (% EC)	41	40	43	0.62
WIT (minutes)	27.8 (9.3)	27.5 (9.1)	28.4 (9.6)	0.58
Laboratory parameters				
Serum calcium (mmol/l)	2.57 (0.24)	2.54 (0.24)	2.63 (0.25)	< 0.001
Corrected serum calcium (mmol/l)	2.47 (0.22)	2.46 (0.22)	2.51 (0.21)	0.01
Serum phosphate (mmol/l)	1.67 (0.51)	1.68 (0.50)	1.66 (0.52)	0.72
Calcium*Phosphate product	4.27 (1.28)	4.23 (1.24)	4.34 (1.35)	0.39
Corrected Calcium* Phosphate product	4.11 (1.26)	4.10 (1.23)	4.14 (1.33)	0.72
Serum PTH (pmol/l)*	18.5 (23.6)	18.3 (24.8)	19.1 (21.1)	0.81

*Since February 1993 (n = 179); () mean (SD) () Mean (SD); CCB's : calcium channel blockers; EC: Euro Collins

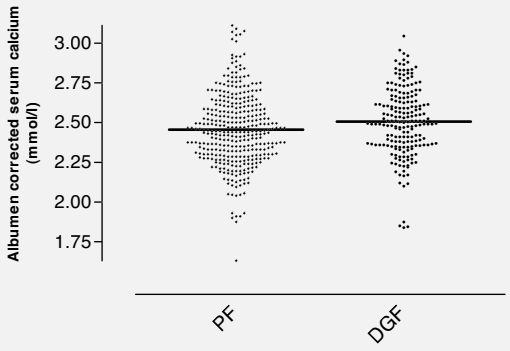
Table 2: Independent risk factors for DGF in the cohort 585 patients transplanted in the period 1986 - 1996

	OR	95% CI	p-value
Adjusted serum calcium			
< 2.54	1		
2.55 – 2.74	1.54	0.98 – 2.43	0.062
> 2.74	2.51	1.59 – 3.98	< 0.0005
CIT > 28 h	1.70	1.16 – 2.48	0.007
Donor age >=50 years	1.48	0.975 - 2.23	0.07
Use of CCB	0.49	0.28 – 0.85	0.01

*CCB: Calcium Channel Blocker

Figure 1

The distribution of albumen corrected serum calcium levels, showing a considerable overlap between patients with PF and DGF



Histological examination

To determine whether the results could have been influenced by the biopsy strategy used to take graft biopsies, characteristics of biopsied (n = 85) and non-biopsied (n = 500) patients were compared (Table 3). No differences were found, except for the serum phosphate levels (p = 0.05). Despite the significant higher phosphate levels in the biopsied patients, hyperphosphatemia was not associated with DGF.

In a total of 71 biopsies, 34 were taken from patients with DGF and 37 were taken from patients with PF. DGF correlated significantly with the presence of ATN in the renal biopsies (Table 4A). However most biopsies, except six had other morphological changes such as acute rejection or nephrocalcinosis. The association of these changes with DGF was therefore analyzed (Table 4B and 4C). DGF did not correlate with histological signs of acute rejection, except when it was accompanied with ATN (Table 4B). Nephrocalcinosis did not correlate with DGF, but it could have been associated with other histological abnormalities. It did not occur in the absence of other abnormalities like acute rejection or ATN (Table 4C).

Table 3: Characteristics of 585 patients with an allograft biopsy or no biopsy within 7 days after transplantation

	Biopsy n = 85	No biopsy n = 500	p-value
Sex recipient male (%)	65	63	0.82
Sex donor male (%)	57	59	0.91
Age recipient	44 (13)	47 (13)	0.14
Age donor	38 (15)	39 (15)	0.58
CIT (hours)	23.0 (6.6)	28.6 (6.8)	0.67
WIT (min.)	26.8 (8.5)	28.0 (9.4)	0.26
DGF	29 (36 %)	178 (36%)	0.94
Use of CCB's (%)	18	17	0.77
Serum calcium (mmol/l)	2.54 (0.22)	2.58 (0.25)	0.21
Corrected Calcium (mmol/l)	2.44 (0.22)	2.48 (0.22)	0.11
Serum phosphate (mmol/l)	1.77 (0.49)	1.65 (0.51)	0.05
Calcium / phospate product	4.47 (1.24)	4.23 (1.28)	0.12
Calcium / phospate product (albumen corr.)	4.32 (1.28)	4.07 (1.26)	0.10
PTH (pmol/l) *	25.9 (32.2)	17.3 (21.0)	0.16

() = mean (SD); *since February 1993 (N=179); CCB: Calcium channel blocker

Table 4 A: Histological characteristics of ATN and initial graft function, showing a relation between the presence of ATN and DGF, especially in combination with signs of acute rejection or nephrocalcinosis

	PF n = 37	DGF n = 34	p-value
No ATN	33 (89)	19 (56)	0.002
ATN total	4 (11)	15 (44)	0.002
ATN only	2 (5)	6 (18)	NS
ATN + rejection	2 (5)	4 (12)	0.04
ATN + NC*	0	5 (18)	0.001

* NC: nephrocalcinosis; (%)

Table 4 B: Histological characteristics of acute rejection and initial graft function, showing a lack of correlation in the absence of signs of ATN.

	PF N = 37	DGF n = 34	p-value
No rejection	11 (30)	14 (41)	NS
Total rejection	26 (70)	20 (59)	NS
Rejection only	21 (57)	12 (35)	NS
Rejection + ATN	2 (5)	4 (12)	0.04
Rejection + NC*	3 (7)	4 (12)	NS

* NC: nephrocalcinosis; (%)

Table 4 C: Histological characteristics of nephrocalcinosis and initial graft function, showing a lack of correlation in the absence of signs of ATN.

	PF n = 37	DGF n = 34	p-value
No NC	34 (92)	25 (73)	0.05
Total NC	3 (11)	9 (27)	0.05
NC only	0	0	
NC + ATN	0	5 (15)	0.001
NC + Rejection	3 (7)	4 (12)	NS

NC: nephrocalcinosis; (%)

Role of calcineurin inhibitors on DGF

In the current study with the use of continuous infusion of Cyclosporin all patients received a standardized systemic exposure to cyclosporin in the first postoperative days. To asses a possible confounding effect of this intravenous regimen, we extended our study with a cohort of patients transplanted between 1997 and 2002, who received triple immunosuppressive therapy with the micro-emulsion formula of cyclosporin (Neoral ®), Prednison and Mycophenolate Mofetil (CellCept ®). The oral doses of cyclosporine were adjusted according to 12 hours serum trough level monitoring, aiming at 250 – 350 μg/l. Fifty five out of 222 patients (25%) experienced DGF. Cold ischemia time (CIT) and donor age were again significantly associated with DGF. Also in this cohort, the increased pretransplant serum calcium levels were associated with the occurrence of DGF (PF vs. DGF: 2.49 mmol/l vs. 2.54 mmol/l ; p: 0.11). The results of the multivariate analysis are shown in table 5. The increased serum calcium levels were associated with an increased occurrence of DGF (OR: 3.12: calcium > 2.75 mmol/l vs. serum calcium < 2.55 mmol/l; 95% CI: 1.18 – 8.26; p: 0.02). Patients who used calcium channel blockers prior to and at the time of transplantation were protected against DGF (OR 0.57; 95% CI: 0.26 – 1.23; p = 0.15), although no statistical significance was reached, probably because of the limited number of patients that could be analyzed in this additional cohort of patients (Table 5).

Table 5: Independent risk factors for DGF in the cohort 222 patients transplanted in the period 1997 - 2002

	OR	95% CI	p-value
Serum calcium			
< 2.55 mmol/l	1		
2.55 – 2.74 mmol/l	1.60	0.73 – 3.51	0.24
>2.75 mmol/l	3.12	1.18 – 8.26	0.02
CIT > 28 h	2.00	0.93 – 4.30	0.07
Donor age > 50 yrs	3.49	1.71 – 7.11	0.001
Use of CCB	0.57	0.26 – 1.24	0.16

*CCB: Calcium Channel Blocker

DISCUSSION

In this retrospective study, the occurrence of DGF was 35%. We also found that among other known risk factors, like donor age and CIT, pre-transplant serum calcium level was independently associated with DGF. Although the differences between the two groups seem to be small (0.09 mmol/l in serum calcium levels and 0.05 mmol/l for the albumen corrected serum calcium levels), the risk of DGF increased 14 %, with each 0.1 mmol/l rise in serum calcium level (OR 1.14 [95%CI: 1.04 -1.26]). Dialysis mode, duration of dialysis before transplantation and the preservation fluid used on the other hand did not correlate with DGF. When a commonly used definition for DGF (e.g. the need of at least 1 dialysis treatment in the first week), was used similar results were obtained. However the need of dialysis treatment in the first week also includes patients that are dialyzed for other reasons than DGF (16). In our study 12 out of 358 patients (3.4%) were dialyzed in the first week after transplantation for other reasons than DGF.

Also the possibility of calcineurin toxicity as a risk factor for the occurrence of DGF was considered. Although histological proof of acute cyclosporin toxicity was only found in 2 biopsies in the PF group, we cannot exclude a role of cyclosporin in the development of DGF. However differences in cyclosporin assays over time and the high inter patient variability of systemic exposure to cyclosporin preclude any meaningful retrospective evaluation of systemic exposure. (17,18). To asses a possible confounding effect of this intravenous regimen, we analyzed an other group of patient that was transplanted in the period 1997-2002 and that were treated with Neoral® soft gelatin capsules twice daily, assuming that the occurrence of high serum levels is limited. Again albumen corrected serum calcium levels tend to be higher in the DGF group (OR 3.12; p: 0.02 calcium > 2.75 mmol/l vs. <2.55 mmol/l). We also found that the use of calcium channel blockers (CCB) prior to the transplantation protected against the occurrence of DGF. Unfortunately the level of significance was not reach, probably because of the limited number of patients that could be analyzed.

No relation was found between microscopic nephrocalcinosis and serum calcium levels (2.43 mmol/l in each group). Acute renal failure in native kidneys has been reported in patients with serum calcium levels above 3.5 mol/l. In native kidneys, these conditions occur in the milk alkali syndrome (19), severe hyperparathyroidism, PTH-related proteins (PTHrP) associated conditions(20), multiple myeloma (21) or vitamin D intoxication (22). Although the corrected serum calcium exceeded the serum level of 2.7 mmol/l in only 19% of the cases, we found an effect of serum calcium levels on initial graft function. Since we did not find a critical value above which DGF occurred more often, it is difficult to predict DGF on the basis of serum calcium levels alone.

Little is known about the effects of hypercalcemia on the initial graft function. Torregosa et al. (23) reported a significant effect of elevated PTH levels on the incidence of DGF, whereas serum Vitamin D levels and serum calcium levels did not differ. Therefore we analyzed the pre-transplant PTH-levels that were routinely measured; 179 pre-transplant PTH levels were measured but no significant differences were found between the two groups (PF vs. DGF: 18.3 pmol/l vs. 19.1 pmol/l ; p= 0.81).

Vitamin D analogues and calcium-containing phosphate binders are prescribed on a regular basis in patients that are on renal replacement therapy to prevent renal bone disease.

Serum calcium levels and the total body calcium load therefore will rise. This is associated with a higher incidence of calcifications of the coronary arteries and possibly with inferior patient survival (24,25). Since DGF is associated with inferior long-term graft outcome (26) this prophylactic regimen may not only influence patient survival but also graft survival. Unfortunately Vitamin D levels were not routinely measured in our study. A prospective study is required to determine the risk of developing DGF as a consequence of calcium and vitamin D supplements in transplant recipients.

Of the 26 biopsied patients with DGF only 13 biopsies showed signs of ATN whereas in the other 13 biopsies no signs of ATN were observed. Therefore attempts were made to find other morphological explanations for DGF in these patients. ATN was only associated with DGF when it coincided with acute rejection or nephrocalcinosis. Acute rejection and nephrocalcinosis alone or in combination with other histological features than ATN were not associated with DGF. This means that DGF and ATN are firmly correlated in this cohort. In contrast to the whole group of patients, we found no differences in serum calcium levels between the PF and DGF group that had renal biopsies in the first week ($p = 0.3$).

This study can not explain the effects of high pre-transplant serum calcium levels on initial graft function by the development of calcium depositions. Very few clinical studies have looked at the morphological substrate of hypercalcemia in the kidney. Prospective studies have not been performed and on the basis of the relative benign outcome of DGF it will be hard to justify protocolized prospective renal biopsies in recipients with and without DGF. In animal models 3 types of nephrocalcinosis can be distinguished: chemical nephrocalcinosis, microscopic nephrocalcinosis and macroscopic nephrocalcinosis (7). The latter is characterized by gross calcium deposits found on radiographic investigations. As macroscopic nephrocalcinosis is not present in this study, microscopic and chemical nephrocalcinosis might explain the effect of high serum calcium levels on initial graft function. Microscopic nephrocalcinosis is characterized by microscopic calcium deposits, mainly located in the lumen of the tubules. It is supposed to be a transitional phase between chemical and macroscopic nephrocalcinosis. Microscopic nephrocalcinosis is associated with increased calcium x phosphate product and with chronic renal failure. Especially high serum phosphate levels seem to trigger this process. Its effect on renal function is thought to be caused by tubular obstruction and tubular back-leak. Chemical nephrocalcinosis, assumed when macroscopic and microscopic nephrocalcinosis are excluded, affects glomerular filtration rate by vasoconstriction and natriuresis induced volume constriction (8) and is histological characterized by areas of focal necrosis in the distal tubules and medullary collecting duct. High calcium content of the medullary area was found and the functional substrate was characterized by impaired function of the distal tubules. The role of cytoplasmatic calcium as an intracellular messenger in many important cell functions might explain these functional changes associated with the high cytoplasmatic calcium content. Calcium dependent enzymes that were analyzed in vitro in this respect are the cystein proteases, like calpaine and the caspases (27). In this study, we found a correlation of DGF with serum calcium levels but not with calcium deposits. This suggests that chemical nephrocalcinosis rather than microscopic nephrocalcinosis is the underlying cause of the effect of serum calcium levels on initial graft function.

In this context our finding that the use of pre-transplant calcium channel blockers (CCB's) protects against DGF is interesting. CCB's are reported to ameliorate initial graft function

(28). The mechanisms by which CCB's have this protective effect may be related to an increase of renal perfusion because of vasodilatation of the glomerular arterioles (29). On the other hand it is conceivable that the CCB induced reduction of calcium influx into tubular cells during ischemic and reperfusion periods and thereby reduce the generation of oxygen-free radicals and activation of cystein proteases (11).
In this study we found that serum calcium levels were independently correlated with the occurrence of DGF. In contrast to other studies a significant correlation was found between DGF and ATN, but not with microscopic nephrocalcinosis. Therefore DGF should be attributed to high intracellular calcium levels (chemical nephrocalcinosis). As calcium supplementation and vitamin D analogues are commonly used in dialysis practice, hypercalcemia might cause a higher incidence of DGF and therefore influence long-term outcome of renal transplantation. The use of calcium channel blockers protects against the occurrence of DGF.

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