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A success story we should all celebrate: 45 years of kidney transplantation in Slovenia

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Transplantation is a recent phenomenon. Many of the big developments in this discipline have taken place within the past 50 years. Much of the story of transplantation is a story of barriers and how modern scientific medicine overcame those barriers. Major developments being perfected that helped keep renal patients alive and kidney transplantation a story of success are the development and refinements of dialysis and surgical techniques, proper organ preservation solutions, and most importantly anti-rejection immunosuppressive drug medications. This time line gives a brief outline of how transplantation progressed in Slovenia and became a story of success.

A major historic landmark in kidney transplantation in Slovenia was the establishment of the national histocompatibility laboratory in 1969. Since the beginning, this biochemical laboratory was part of the Blood Transfusion Centre of Slovenia. Its founder was prof. Mateja Bohinjec (1, 2). She was influenced by the work of Jean Dausset, a French scientist who in 1965 described the first group of antigens, which is now known as HLA-system (human leukocyte antigens). Her work was also inspired by dr. Jon J. van Rood from Leiden, who proved that matching in the HLA-type of donor and recipient had a positive effect on the transplantation outcome and who founded Eurotransplant in 1967 (3). Another important historic achievement was the introduction of dialysis treatment at the University medical centre Ljubljana. Already in 1959, the urologists at the Department of Urology started treating patients with acute renal failure. Based on their pioneering work, the first chronic hemodialysis unit was opened in 1970. This hemodialysis center was part of the Department of nephrology (4). The existence of the hemodialysis unit was an important prerequisite to start with the regular transplantation program. The fundamental transplant persons were the urologists, prof. Slavko Rakovec and prof. Ludvik Ravnik, and a nephrologist, prof. Saša Luzar, who started to assess potential living kidney donors to evaluate their suitability for donation (5). Finally, on April 16th 1970 first living-related kidney transplantation was performed at the University Medical Centre Ljubljana. The surgical team consisted of prof. Slavko Rakovec and prof. Ludvik Ravnik as well as cardiovascular surgeon prof. Miro Košak (2, 5). Since the first successful case, regular transplant activity from living-related donors continued (6) and 126 transplant procedures were performed between 1970 and 2014. The highest number of such transplants per year was 23, performed in 1986 (7). Because living-related kidney transplants were most commonly performed in the patients from other republics of former Yugoslavia, the number of these procedures decreased after Slovenia declared independency. Living donation dropped further after January 2000 when Slovenia joined Eurotransplant and the number of deceased donor kidney transplants significantly increased (7).

In 1985, the first transplant law was passed in Slovenia and paved the way for solid organ transplantations from deceased donors (8). Deceased donor kidney transplants have been regularly performed since 1986. In March 1998 a National Transplant Network was established and there has been a significant organizational change in deceased donation and solid organ transplantation in Slovenia (8). Besides the University Medical Centre Ljubljana,

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nine donor hospitals have been included in the national program for deceased donor organ transplantation. The establishment of a National Transplant Network composed of a centrally located kidney transplant centre, donor centers, a tissue-typing center, a national transplant waiting list, and a central coordinating institution, Slovenija-transplant, resulted in a significant increase in the number of deceased-donor organ transplants. After this successful organizational change Slovenia was accepted into Eurotransplant, followed by the implementation of activities as of January 1st 2000. The greatest credit for this important organizational change belongs to dr. Jasna Vončina. Joining Eurotransplant meant positive changes and is regarded as a success story. Both donor numbers and transplant possibilities increased and equal chances are assured for our patients on the common Eurotransplant waiting list. Nowadays in Slovenia, which has an area of 20,000 km2 and a population of 2 million, there is one Kidney transplant center located at the Department of Nephrology of the University Medical Center Ljubljana and one Tissue typing center at the Blood Transfusion Center of Slovenia, also located in Ljubljana.

Since the first successful kidney transplant in 1970, our urologists have performed more than 1000 kidney transplant procedures. In the period between 1970 and 2014, 126 patients received living donor and 968 patients received deceased donor kidney transplants. The great majority of living donor kidney transplants (124) were performed before joining Eurotransplant. On the other hand, until the end of December 1999, 239 patients received kidney grafts from deceased donors, while 729 patients were transplanted from deceased donors after January 2000 when Slovenia joined Eurotransplant. It should be noted that the total number of kidney transplants in the last 15 years was 2 times higher than the number of kidney transplants in the 30 years before 2000. This also means a 2.8 times higher number of transplants per year in the last period. Furthermore, membership in a successful organization is a permanent incentive for keeping pace with the other members of such an organization. With more than 60 deceased donor kidney transplants performed yearly during the last years, we have reached and also exceeded the average number of these transplants per million population of the Eurotransplant. Up to December 31, 2014, the 1-year and 5-year patient survival rates were 98% and 94%, respectively. The concomitant graft survival rates were 94% and 88%, respectively. This short-term and medium-term results of kidney transplantation in our country have been very good, much better than in the past and entirely comparable to those presented by the most successful countries worldwide.

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Pre-emptive kidney transplantation – benefits and concerns

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Pre-emptive kidney transplantation is widely considered as the best renal replacement therapy for patients with advanced chronic kidney disease. Benefits are better patient and graft survival, less delayed graft function, better quality of life, avoidance of vascular access surgery, easier return or continuation of work, less costly treatment. There are also concerns: limited lifespan of kidney graft, which consumption begins before it is truly needed, rapid loss of native kidney function after transplantation, premature operative risk, high cost of transplantation in the first year, organ shortage. In conclusion, taking all the pros and cons into account, pre-emptive kidney transplantation is the optimal renal replacement therapy for many patients with advanced chronic kidney disease. The optimal timing for transplantation allowing the maximal use of native kidney function and accounting for the limited lifespan of kidney graft seems to be as late as possible in the course of chronic kidney disease (just before dialysis) or shortly after dialysis initiation. No survival benefit was demonstrated if pre-emptive transplantation was performed at a higher level of glomerular filtration rate.

Keywords: chronic kidney disease; dialysis; kidney transplantation; pre-emptive kidney transplantation; renal replacement therapy

Pre-emptive kidney transplantation (before starting maintenance dialysis) is widely accepted as the best renal replacement therapy for many patients with advanced chronic kidney disease (CKD) (1,2). It is suggested that patients with CKD and estimated glomerular filtration rate (eGFR) < 30 ml/min/1.73 m2 should be referred to transplant center for work-up for enrollment for transplantation, either from living or deceased donor (3). In the previous European guidelines pre-emptive transplantation was suggested when GFR was below 15 ml/min (4). In the recent US study it was shown that trends in pre-emptive transplantation have been moved towards higher level of eGFR, however, without survival benefit in patients transplanted earlier (5). It has also been shown that there was no significant difference in patient survival between the patients transplanted pre-emptively or during the first year from dialysis initiation (6). Similar findings were found in pediatric study, showing no survival benefit in children transplanted pre-emptively or even more than 2 years from dialysis initiation (7). Study from French transplant network failed to find association between pre-emptive transplantation from a deceased donor or time on dialysis with patient and/or graft survival (8). However, in the very recent large US study, similar patient but decreased 5-year graft survival (death-censored) was found in patients dialyzed less than 1 year as compared to pre-emptively transplanted patients (9).

The aim of this review is to evaluate benefits and concerns and suggest the optimal timing for pre-emptive kidney transplantation.

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Pre-emptive kidney transplantation: benefits

Benefits are numerous. Better patient (10) and graft survival (9,11,12) as compared to transplantation after dialysis was demonstrated. Pre-emptive transplantation offers better quality of life, avoidance of vascular access or peritoneal dialysis catheter surgery, easier continuation of employment, lower cost of

renal replacement therapy because of dialysis avoidance, attenuation of uremic complications. Organ shortage is one of the main problems preventing its wider use. Hence, it is not surprising that living donors are often the main source of organs. However, in the recent years, especially in US, deceased donors are becoming increasingly important source of organs for pre-emptive transplantation (5).

Pre-emptive kidney transplantation: concerns

In parallel with numerous advantages, there are also concerns. Lifespan of the kidney graft is limited. In the large study from US, analyzing 252 910 patients with kidney graft, it was shown that half-life of kidney graft from the ideal deceased donor in 2005 was 8.8 years, from living donor 11.9 years and from extended criteria deceased donor 6.4 years (13). Long-term graft survival in Europe is better than is US (estimated 10-year graft survival from a deceased donor was 56.5%), however, still limited (14). In the case of pre-emptive transplantation, consumption of the limited lifespan of kidney graft begins before it's truly needed.

Kidney transplantation is a major surgical intervention. In the pre-emptive transplantation patient is exposed to early risk of surgical and other complications. Rate of residual native kidney function loss may be increased after transplantation, as a consequence of surgery, calcineurin toxicity and other mechanisms (»renal counterbalance«). It is often difficult to predict in an individual patient with eGFR 15-20 ml/min/1.73 m2 how long the patient will live without the need for renal replacement therapy. Cost of kidney transplantation during the first years is high, even in the absence of complications, so postponing the transplantation towards lower eGFR level and maximizing the use of residual native kidney function may decrease overall renal replacement therapy cost during lifetime of an individual patient.

Last, but not least, ethical problems may arise. Is it justified to transplant organs from deceased donors to the patients not yet on dialysis instead to offer them primarily to the patients already on dialysis, who are hardly waiting for transplantation? In Eurotransplant, contrary to US, pre-emptive kidney transplantations from deceased donors are relatively rare. Dialysis patients have the advantage in the scoring system when competing for the organ. However, in the countries like Slovenia, with a waiting time for kidney transplantation being relatively short and transplant activity stable or increasing, the possibility for pre-emptive first or second kidney transplantation from a deceased donor may be a real option.

Rapid loss of native kidney function after preemptive transplantation and »renal counterbalance«

Two important observational studies have shown that the advantage of higher eGFR (at transplantation) is lost soon after pre-emptive transplantation. In the first study 671 pre-emptively transplanted patients were studied. Patients with eGFR >15 ml/min/1.73m2 at the time of transplantation were compared with the patients with eGFR <10 ml/min/1.73m2. Before transplantation the difference in eGFR between the groups was 16.7 ml/min/1.73m2. One year after transplantation the difference in eGFR has decreased to only 4.5 ml/min/1.73 m2 (15). In another study, 4046 patients with pre-emptive transplantation from a living kidney donor were analyzed. One of the main findings of the study was, that there was no association with eGFR before and 6 months after transplantation. In the conclusion of the study the authors suggested to perform pre-emptive kidney transplantation as late as possible in the course of chronic kidney disease, providing that the patient is not suffering from uremic symptoms and dialysis can be safely avoided (16). Both studies suggested that residual native kidney function was rapidly lost after transplantation.

One of the possible explanations for rapid loss of native kidney function after pre-emptive transplantation may be found in a brilliant experimental study published more than 40 years ago. The study was performed on male Lewis rats (17). In the first group of 11 rats, kidney transplantation was performed with preserved both native kidneys. No immune mechanism was involved, as it was the case of isograft. This was the earliest kidney transplantation as early can be: kidney transplantation in the rat with normal kidney function. The other group of 12 rats had kidney graft transplanted, however, with binephrectomy performed simultaneously with transplantation. After approximately 5 weeks the animals were sacrificed. In all 11 rats with transplanted kidney and preserved

native kidneys graft atrophy was found, with decrease in weight by 64%. Histology revealed intensive interstitial inflammation with fibrosis, while glomeruli and vessels were relatively spared. On the contrary, in 12 rats with simultaneous transplantation and binephrectomy, compensatory hypertrophy of the graft was found, with increase in weight by 68%. In the third group of 8 rats, kidney was transplanted with simultaneous right nephrectomy. Kidney graft atrophy was observed in this this group as well as in the first group, however, to a lesser degree, with decrease in graft weight of 41%. In parallel with graft atrophy, remaining left kidney weight increased by 32%. The mechanism helping to explain this observation was described almost a century ago (1923) by Hinman F. as »renal counterbalance« or »disuse atrophy« (18). In the earlier paper from 1919 he wrote »I venture the opinion… that a healthy kidney, once thoroughly accustomed to doing all the work, will, if left alone, continue to do it in spite of any attempt to relieve its burden. A crippled kidney, though potentially capable of some work, would be completely ignored when brought into competition with its big active and efficient fellow». It seems also that a kind of cross-talk between the kidneys exists. Theory of »renal counterbalance« was used for the explanation of progressive unilateral atrophy in chronic pyelonephritis, despite the absence of active infection. Today this theory may help us to understand rapid loss of native kidney function after pre-emptive kidney transplantation, demonstrated by observational studies.

Clinical evidence pointing in the same direction, however, from the opposite side, was demonstrated in the case report of two pre-emptively transplanted patients, published more than 10 years ago (19). Combined kidney and pancreas transplantation in one and kidney transplantation alone in the other patient was performed. Both patients had diabetic nephropathy with nephrotic proteinuria (6 and 9 grams per day) and rather high creatinine clearance at the time of transplantation (58 in 62 ml/min, respectively). Soon after transplantation nephrotic proteinuria decreased to less than 1 gram per day (0.3 and 0.7 gr, respectively). Renal scintigraphy performed one month after transplantation demonstrated almost nonfunctional native kidneys (it was expected, related to GFR level at the time of transplantation, that native kidneys will function for approximately 5 years). So high creatinine clearance one month after transplantation of 97 and 117 ml/min, respectively, was completely originated from the transplanted kidney. In this situation transplanted kidney was »big and active fellow kidney« and native kidneys were »crippled kidneys«. Major surgery, calcineurin toxicity and other mechanisms may adversely influence native kidney function after transplantation.

Pre-emptive kidney transplantation – when?

Level of GFR for enrollment for pre-emptive kidney transplantation is completely arbitrary. As we have seen, there are arguments from observational as well as experimental studies, that pre-emptive transplantation should be performed as late as possible in the course of chronic kidney disease, just before the necessity for dialysis initiation. With such approach native kidney function would be maximally exploited as well as limited lifespan of the kidney graft. In practice, we should target the approximate period for timely arteriovenous fistula creation or insertion of peritoneal dialysis catheter. Of course, exact moment of kidney transplantation including all medical and logistic complexity is difficult to target with such precision, especially in the case of deceased donor. Optimal timing is much easier to achieve with a living donor. Previously mentioned studies in the adult and pediatric patients has shown that there are no significant differences in survival between the patients transplanted one or two years after starting dialysis as compared to pre-emptively transplanted patients (6,7). These findings may help increasing some room from maneuver in achieving optimal timing for kidney transplantation, that is especially important for transplantation from a deceased donor.

However, we must be aware that even in the absence of survival benefit between pre-emptively transplanted or patients transplanted soon after dialysis initiation, other benefits of pre-emptive transplantation remain, like dialysis avoidance including dialysis access avoidance and better quality of life. As a consequence, during planning optimal timing for transplantation all effort should be invested to realize the possibility of pre-emptive transplantation in suitable patients.

Recent position statement by the Descartes working group and ERBP (European Renal Best Practice) suggest that optimal timing for pre-emptive transplantation (from a living donor) should be »shortly or a few months before the need to initiate dialysis« (20). Further, they comment that in line with IDEAL study (21), this usually happens when GFR is between 7 and 10 ml/min. They also emphasize that pre-emptive transplantation should be performed only in recipients who have renal disease that is definitively irreversible and clearly progressive, and that timing should not be based on a fixed, predetermined level of GFR but rather should take into account both clinical and biochemical evidences.

Early dialysis – a parallel to early transplantation?

Idea of early dialysis, a kind of parallel to pre-emptive kidney transplantation, was popular in the late seventies and following decades, with some observational studies reporting on survival benefit, resulting in including early dialysis approach into the guidelines (US National Kidney Foundation Dialysis Outcomes Quality Initiative guidelines). The idea was challenged after NECOSAD study, trying to address for lead-time bias, in addition with some other observational studies, has showed that early dialysis may not improve survival (22). The concept of early dialysis was finally abandoned after randomized clinical study IDEAL was published in 2010 comparing early and late dialysis start, finding that early dialysis did not improve patient survival (21).

Problems with outcome measurement in pre-emptive kidney transplantation

Lead-time bias. When performing pre-emptive transplantation, we transplant a kidney into the patient not needing either dialysis or transplantation. The indication for transplantation is (or was) usually based on arbitrarily defined level of GRF, without specific clinical problem requiring the introduction of renal replacement therapy. Patient and graft survival are counted from the day of transplantation and compared with a survival of patients transplanted after dialysis initiation. In the latter group the survival is usually counted from the day of transplantation as well and not from the day of the first dialysis (that is usually started at GFR less than 10 ml/min). Studies reporting survival benefit of pre-emptive kidney transplantation usually were not corrected for such a bias (»lead time bias«), which may be difficult or impossible (23). True comparison of pre-emptively and late (after dialysis) transplanted patients would be the comparison from the start of renal replacement therapy (either dialysis or transplantation), which was initiated at the similar level of GFR (and not survival comparison from the day of transplantation).

Delayed graft function. Some papers report on decreased incidence of delayed graft function in pre-emptively transplanted patients. Delayed graft function is defined as necessity for dialysis in the first week after transplantation. However, the pre-emptively transplanted patient did not need dialysis before transplantation anyway. It is also possible that true delayed graft function may be masked by residual function of the native kidneys.

Early work-up for enrollment for kidney transplantation

Work-up for enrollment for kidney transplantation is often time-consuming. The are probably no arguments against completing work-up before dialysis initiation. Such approach can significantly decrease waiting time for transplantation and enable transplantation just before or soon after starting dialysis. Work-up for kidney transplantation may also serve as a kind of psychotherapy in patients being stressed as approaching dialysis. Instead of being helpless and terrified observer of increasing serum creatinine level, the preoccupation of patient with advanced chronic kidney disease is shifted towards diagnostic and sometimes corrective procedures during pretransplant work-up and to positive focus on expecting the new kidney.

Pre-emptive second transplantation

Pre-emptive kidney transplantation in the patients with kidney graft failure have numerous benefits. Such patients are already receiving immunosuppressive therapy and are usually distressed with the need for dialysis (re)initiation. Early retransplantation may enable avoidance of dialysis and vascular access surgery (if needed). Transplantectomy, a major surgical procedure sometimes needed for graft intolerance syndrome after graft failure, may be avoided by pre-

emptive retransplantation and continuation of immunosuppressive therapy. The latter may also be helpful in avoiding further sensitization. Of course, early re-transplantation is a possibility for patients without significant comorbidities. Recent study comparing 3509 early recipients of the second kidney graft with 14075 non-early recipients of the second graft has shown that early re-transplantation is associated with better survival if the first graft has functioned for al least 1 year (24).

Conclusions

Taking into account all benefits and concerns, pre-emptive kidney transplantation is the optimal renal replacement therapy for many patients with advanced chronic kidney disease. Optimal timing for transplantation, enabling maximal use of the residual native kidney function and limited lifespan of kidney graft is as late as possible in the course of chronic kidney disease, just before dialysis initiation or in the first year of dialysis treatment. Transplanting earlier in the course of chronic kidney disease (at the higher level of GFR) was not associated with better survival.

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Pre-emptive kidney transplantation from a deceased donor in Slovenia

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Background. Kidney transplantation before starting dialysis (pre-emptive) is widely considered as the best renal replacement therapy for patients with advanced chronic kidney disease (CKD). The aim of our report is to analyze the frequency and outcome of pre-emptive kidney transplantation from a deceased donor in Slovenia in the period between 2006 and 2015 (October 27).
Methods. Sixteen patients with advanced stage 5 CKD, 8 males, aged 46.5 ± 11.3 years, underwent pre-emptive kidney transplantation from a deceased donor (one patient had combined liver-kidney, and one combined pancreas-kidney transplantation). Fifteen transplantations were performed in the period between 2011 and 2015 (October 27), representing 5.4% of all (n=279) kidney transplantations performed in that period. Data on graft function and outcome were analyzed, obtained from our transplant center's database (the only one in the country).
Results. The mean waiting time was 7.0 ± 4.6 (range 0.16-16.2) months, pretransplant serum creatinine 589 ± 149 (range 365 - 914) μmol/L and eGFR calculated from the MDRD formula 8 ± 3 (range 4 - 15) ml/min/1.73 m2. Two patients had delayed graft function, and in two surgical revision was required. During follow-up (mean time 631±515 days, range 36 - 1607 days), four recipients experienced seven episodes of acute cellular rejection. BK virus nephropathy was diagnosed in one recipient. Two grafts were lost during follow-up. One kidney had to be removed 36 days after transplantation because of graft thrombosis and bleeding as a consequence of percutaneous intervention for sub-occlusive renal artery stenosis. Another patient lost the graft 4.2 years after transplantation after the treatment of two acute cellular rejections. Both patients are currently treated by maintenance hemodialysis, while the remaining 14 patients have functioning grafts.
Conclusions. Pre-emptive kidney transplantation from a deceased donor is a realistic option for patients with advanced CKD in Slovenia. All patients had stage 5 CKD when transplanted. Average waiting time was 7 months. Timely enrollment on the waiting list during the course of CKD is crucial for enabling this preferred modality of renal replacement therapy.

Keywords: kidney transokantation;pre-emptive kidney transplantation; deceased donor

Pre-emptive kidney transplantation, or transplantation before the initiation of dialysis, has been associated with optimal outcomes in terms of patient and graft survival (1–5) when compared with maintenance dialysis, making it the preferred therapy for end-stage renal disease patients (6). It has been proven that, for suitable candidates, it can improve quality of life (7,8), has higher return-to-work rates (9), and lowers long-term medical costs when compared to transplantation after the initiation of dialysis (10). Long waiting time on dialysis resulted in worse outcomes in comparison with both living and deceased donor transplantation, thus pre-emptive kidney transplantation might also be beneficial by avoiding the morbidities of dialysis, such as catheter-associated complications and dialysis-associated cardiovascular events (11,12).

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If a living donor is available, optimal timing for pre-emptive kidney transplantation is not difficult to achieve. However, pre-emptive transplantation is a challenge in the case of deceased donors when patients not yet on dialysis are competing with patients already undergoing dialysis who had been accumulating waiting time for a transplant.

Consequently, it is not surprising that only 2.5% of patients with end-stage renal disease undergo transplantation as initial renal replacement therapy (13).

The aim of our report is to analyze the frequency and outcome of pre-emptive kidney transplantation from a deceased donor in Slovenia in the last 10 years.

Methods

Data from our Centre for kidney transplantation database (Department of Nephrology, University Medical Centre Ljubljana) were used for this retrospective clinical study. Time period between 2006 and 2015 (October 27) was studied and pre-emptively listed and transplanted patients were analyzed, including data on graft function and outcome for the latter group. As this is the only transplant center in the country, data represent the complete national cohort.

During this time 16 patients with advanced stage 5 chronic kidney disease (CKD), 8 males, aged 46.5 ± 11.3 (range 18-63) years, underwent pre-emptive kidney transplantation from a deceased donor (one patient had combined liver-kidney, and one combined pancreas-kidney transplantation), representing 3.2% of all (n=505) kidney transplantations. Fifteen of these patients were transplanted from 2011 to 2015 (October 27), which presents 5.4% of all (n=279) kidney transplantations during this time period.

Results

The causes of end-stage renal failure were IgA nephropathy (n=6), membranoproliferative glomerulonephritis (n =2), polycystic kidney disease (n=2), diabetic nephropathy (n=1), other nephropathy (n=3) and undefined nephropathy (n=2). The number of pre-emptive kidney transplantations performed yearly is presented in Table 1. Waiting time, pre- and post-transplantation serum creatinine and estimated glomerular filtration rate (eGFR) calculated from the MDRD formula are shown in Table 2. The outcome in patients placed on the waiting list for pre-emptive kidney transplantation from 2013 to 2015 is presented in Table 3.

Table 1. Number of pre-emptive kidney transplantations performed per year.

Year	2006	2011	2012	2013	2014	2015
N	1	2	1	2	7	3

Two patients experienced delayed graft function, and in two recipients' surgical revision was required. During follow-up (mean time 631±515 days, range 36 to 1607 days), four recipients experienced seven episodes of acute cellular rejection. There were no cases of humoral rejection. BK virus nephropathy was diagnosed in one recipient.

Two grafts were lost during follow-up. One kidney had to be removed 36 days after transplantation because of graft thrombosis. In this patient, thrombosis of the renal vein occurred immediately after transplantation, but it was successfully treated surgically. In the postoperative period the function of the transplanted kidney remained impaired, and further examinations with Doppler ultrasound revealed a sub-occlusive renal artery stenosis. Percutaneous intervention was performed, followed by dissection, graft thrombosis and massive renal artery bleeding, which required the immediate removal of the transplanted kidney. Soon after graft removal, arteriovenous fistula was created and maintenance dialysis program was initiated. Another patient lost his graft 4.2 years after transplantation after the treatment of two acute cellular rejections. Both patients are currently treated by maintenance hemodialysis, while the remaining 14 patients have functioning grafts.

Discussion

In this study we explored stage 5 CKD patients pre-emptively transplanted from the deceased donor in the last 10 years in Slovenia. Our results showed that pre-emptive kidney transplantation from a deceased donor is possible, with increased number of patients

Table 2. Serum creatinine, estimated glomerular filtration rate and waiting time of patients pre-emptively transplanted. Data are presented as means ± standard deviation (range).

Prior to transplantation (n =16)	
Cr (µmol/L)	589 ± 149 (365 - 914)
eGFR (MDRD) (ml/min/1.73m^2)	8 ± 3 (4 - 15)
Waiting time (months)	7.0 ± 4.6 (0.16-16.2)
After transplantation	
After hospital discharge (n=15)	
Cr (µmol/L)	125 ± 37 (78 - 208)
eGFR (MDRD) (ml/min/1.73m^2)	51 ± 19 (29 - 90)
After 3 months (n=15)	
Cr (µmol/L)	104 ± 17 (76 - 128)
eGFR (MDRD) (ml/min/1.73m^2)	60 ± 14 (37 - 90)
After 6 months (n=12)	
Cr (µmol/L)	104 ± 37 (61 - 192)
eGFR (MDRD) (ml/min/1.73m^2)	61 ± 14 (45- 90)
After 12 months (n=10)	
Cr (µmol/L)	110 ± 26 (80 - 170)
eGFR (MDRD) (ml/min/1.73m^2)	57 ± 11 (47 - 79)

Table 3. Outcome in patients placed on the waiting list for pre-emptive kidney transplantation.

	All patients on the waiting list	**Candidates for pre-emptive transplantation**	**Pre-emptive transplantation**	**Onset of dialysis**	**Awaiting transplantation**
Year 2013	63	11.1% (n=7)	57.1% (n=4)	14.3% (n=1)	28.6% (n=2)
Year 2014	85	20% (n=17)	29.4% (n=5)	35.3% (n=6)	35.3% (n=6)
Year 2015 (until Oct. 27)	44	15.9% (n=7)	28.6 % (n=2)	14.3% (n=1)	57.1% (n=4)

transplanted in the last years. The average waiting time for transplantation was 7 months.

Further expansion of this preferred renal replacement therapy modality requires additional effort in pretransplant work-up of patients with advanced CKD and possible motivation of living donors, the option with low activity in Slovenia in the last 25 years, partly (or mainly) due to relatively short waiting time for transplantation from a deceased donor.

The timing for pre-emptive transplantation was stage 5 CKD in all patients. Just-before-dialysis period should be targeted according some observational studies and recent position statement from European Renal Best Practice Advisory Board (14), with further comment that eGFR is may be expected to be between 7-10 ml/min, as derived from IDEAL study comparing early and late initiation of dialysis (15). Position statement is, however, related to living-donor pre-emptive transplantation, where planning and realizing transplantation is much easier to achieve. Nevertheless, targeting lower level of GFR should be keep in mind when listing the patient, to allow maximal use of residual native kidney function

as well as maximal use of limited lifespan of kidney graft.

Exposing the patient to the premature operative risk is one of the concerns related to pre-emptive kidney transplantation. In our patients two of sixteen lost the graft prematurely, one of them in the early posttransplant period because of vascular complications and bleeding. This may be another argument not to perform transplantation too early in the course of CKD.

In the last years up to 20% of newly enlisted patients for the deceased donor transplantation were candidates for pre-emptive transplantation, with up to 35% of them requiring dialysis treatment before they could be pre-emptively transplanted. Timely listing in the course of advanced CKD, of course, necessary, however, it can not guarantee that transplantation will be realized before dialysis initiation. Exploring the possibilities for living donor may increase the chances for these patients to be pre-emptively transplanted.

In conclusion, pre-emptive kidney transplantation from a deceased donor is a realistic option for patients with advanced CKD in Slovenia. All patients had stage 5 CKD when transplanted, with average waiting time of 7 months. Timely enrollment on the waiting list during the course of CKD is crucial for enabling this preferred modality of renal replacement therapy. Potential living donors should be explored and encouraged to increase chances for listed patients to be transplanted before dialysis initiation.

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Clinical biomarkers for kidney allograft rejection

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Kidney allograft rejection is still the major risk factor for kidney transplant failure and is becoming a leading cause of new cases of end-stage renal disease. Rejection phenotypes are classified as T-cell-mediated or antibody-mediated. There has been growing awareness of the transplant community over the last decade on the profound negative impact of antibody-mediated rejection on kidney transplant outcomes. The standard test for the diagnosis of rejection is the renal biopsy. However, non-invasive test would be preferable. Furthermore, noninvasive screening that foretells rejection before loss of kidney function is clinically detectable might reduce rejection-associated allograft damage. Several investigators have attempted to identify molecular markers of immune function in blood, urine, and the graft itself, to distinguish between rejection phenotypes, as well as to detect these adverse events before they can reduce kidney function. At present time these molecular signatures of transplant rejection cannot be widely used as they rely on high-quality testing and are associated with high cost. Therefore, clinical biomarkers for predicting the risk of allograft injury or for indicating preclinical damage are needed. The objective of this review is to provide contemporary data on the diagnostic role of laboratory test-based functional monitoring assays that can be used in everyday clinical practice and use non-invasive samples, such as peripheral blood or spot urine samples that might help distinguish rejection versus non-rejection episodes and between different rejection phenotypes as well as to predict functional recovery after anti-rejection therapy.

Keywords: antibody-mediated rejection; kidney transplantation; non-invasive biomarkers

Despite advances in the prevention of rejection, long-term outcomes after kidney transplantation have only modestly improved during the last decades. Survival rates remained quite stable, with approximately 50% of kidneys from deceased donors still functioning 10 years after transplantation (1). Kidney allograft rejection is still the major risk factor for transplant failure and is becoming a leading cause of new cases of end-stage renal disease (2,3). Rejection phenotypes are classified as T-cell-mediated (TCMR) or antibody-mediated (ABMR). There has been growing awareness of the transplant community over the last decade on the profound negative impact of ABMR on kidney transplant outcomes (4).

Rejection and its phenotype are diagnosed by means of needle biopsy. This invasive procedure has become safer, and biopsy interpretation more standardized (5). Nevertheless, biopsy is most commonly indicated in sudden deterioration of kidney function when rejection-associated graft injury already occurs. Protocol biopsies that may help to predict the subsequent graft dysfunction and failure pose challenges, including feasibility and cost (6). A noninvasive screening that foretells rejection phenotype before loss of kidney function would be preferable.

Several investigators have attempted to identify molecular markers of immune function in blood, urine, and the graft itself, to distinguish between different rejection phenotypes, as well as to detect these adverse events before they can reduce kidney function (7-9). At present time these molecular signatures of transplant rejection cannot be widely used as they rely on high-quality testing, skilled laboratory personnel and are associated with high

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cost. Therefore, clinical biomarkers for predicting the risk of allograft injury or for indicating preclinical damage are needed. Applications that require an invasive biopsy limit the clinical applicability of identified biomarkers, and functional monitoring assays that can be used in everyday clinical practice and use non-invasive samples, such as peripheral blood or patient urine, are more favorable (for patients and economically).

Clinical laboratory test-based biomarkers in the peripheral blood

Advances in immunosuppressive therapy and improved patient monitoring have decreased the incidence of rejection episodes after kidney transplantation. However, the lack of non-invasive biomarkers in the peripheral blood makes early diagnosis and optimized treatment regimens difficult, leading to approximately 10 to 30% of all transplant patients being diagnosed and treated for acute rejection within the first year after transplant, on top of a high number of undetected subclinical episodes. Chronic ABMR that represents a major risk factor for long-term allograft loss is even more difficult to diagnose early (4,10).

Pre-transplant HLA and non-HLA antibodies

Currently, a match between the human leukocyte antigen (HLA) in the sera of the donor and the recipient and presence of preformed anti-HLA panel reactive antibodies (PRA) represent the best pre-transplant biomarkers (11). Yet even in the case of a total match, the risk of clinical or subclinical rejection and or chronic allograft damage cannot be excluded.

More recently, integrative proteogenomic analyses have identified tissue-specific novel non-HLAs that led to serological responses in kidney transplant recipients. Antibodies against MHC class I polypeptide related sequence A (MICA) in the recipients that recognized antigens specific to the renal pelvis and the renal cortex were identified (12). The association of such novel non-HLA antigens with clinically relevant phenotypes could identify specific immunogenic epitopes in allograft rejection and chronic allograft dysfunction. In addition, other antibody-based biomarkers have been identified by investigating non-HLA antigen responses after transplantation, which have a greater role in allograft outcome than previously thought and thus represent novel diagnostic and predictive biomarkers. Of note are the agonistic antibodies against the angiotensin II type 1 receptor described in renal allograft recipients with severe vascular types of ABMR (13). Pre-transplant sensitization against angiotensin type II type 1 receptor was associated with more acute rejections episodes. Furthermore, such presensitization constituted an independent risk for long-term allograft failure, independently from the other standard clinical determinants, such as donor age, PRA or delayed graft function (14).

Serum creatinine

Post-transplant biomarkers include functional parameters that are routinely measured in the peripheral blood at the protein level, such as serum creatinine. An increase in serum creatinine level is often the first clinical indicator of kidney allograft rejection and it is still the best surrogate marker for it. However, it lacks sensitivity and specificity. The limitations associated with monitoring rejection by measurements of serum creatinine have been recognized previously by the observation that 30% of allograft biopsies performed in patients with stable kidney function or in patients who were considered to have been successfully treated for rejection reveal histological features of TCMR (15). These subclinical TCMR appear biologically relevant, since treatment better preserves the structure and function of kidney allografts (16). More recently, subclinical ABMR has been reported in patients with anti-HLA antibodies (17). In subclinical ABMR, the serum creatinine level was stable, but protocol kidney graft biopsy specimens showed glomerulitis, peritubular capillary infiltration by leukocytes, and diffuse or focal staining of peritubular capillaries with an anti-C4d antibody. The early recognition of these histological changes may provide the opportunity to modify the immunosuppressive regimen and potentially improve long-term graft survival.

De-novo donor-specific anti-HLA antibodies

One of the most important advances in kidney transplantation medicine has been the recognition that de-novo anti-HLA antibodies are destructive and

various studies over the past decade have indicated that the alloimmune response, mediated by anti-HLA antibodies, plays a key role in the development of ABMR and failure of kidney allografts (18,19). Although anti-HLA antibodies are considered to be harmful, there is a wide spectrum of graft injury related to these antibodies, ranging from no recognizable damage to florid rejection. Since the pioneering discovery that anti-HLA antibodies are lymphocytotoxic, activation of the complement cascade has been considered to be a key component of ABMR, and C4d deposition in renal capillaries has been considered the footprint of antibody-mediated allograft damage (20,21). Just recently, it has been demonstrated that the presence of complement-binding anti-HLA donor-specific antibodies with C1q binding capacity after transplantation is strongly associated with antibody-mediated allograft injury and loss and that incorporation of this risk factor improves risk stratification for allograft failure (22). However, surveillance antibody testing using single-antigen beads technology (LuminexÒ) is expensive and the cost of frequent testing may not be justified in all transplant recipients.

Clinical laboratory test-based biomarkers in the urine

Transplantation initiates the processes responsible for rejection episodes and biomarkers of different subtypes of rejection injury that indicate damage are passed into the urine and might help distinguish rejection versus non-rejection episodes and between different rejection phenotypes as well as to predict functional recovery after anti-rejection therapy.

Urine protein and terminal complement complexes excretion

Increased urine protein excretion has been associated with progressive kidney disease, allograft failure and mortality in kidney transplant recipients (23). Two main mechanisms can lead to proteinuria: an increased passage of albumin and/or protein with higher molecular weight because of a disruption of glomerular filtration barrier or an inadequate reabsorption of small proteins from tubular cells (24). Inflammation in kidney allograft rejection can occur in the glomeruli, tubulo-interstitial and arterial compartment (25) and may result in an increase in urine protein excretion before deterioration of graft function occurs. As noted above, the presence of antibodies directed against antigens expressed in donor kidney, results in an antibody-mediated immune injury of the transplanted organ. De-novo DSA attack the endothelial cells of the allograft, resulting in complement activation, formation of terminal complement C5b-9 membrane attack complexes and glomerular injury (19-22). Therefore, ABMR may result in a grater increase in urine protein and complement excretion when compared with TCMR or non-rejection findings. A study from Fotheringham et al. (26) demonstrated that protein excretion estimation from spot urine samples is associated with DSA detection and is likely to be an important factor that determines the risk for ABMR, decline in kidney function and earlier allograft loss. When compared to antibody testing, regular proteinuria testing is inexpensive, can easily be performed at every clinic visit, and is associated with glomerular injury that accounts for 37% of graft loss (27). Centers that consider intensive antibody surveillance unaffordable might consider using data on proteinuria to identify the patients in whom the yield of antibody screening will be higher.

The clinical utility of urine protein and terminal complement complexes excretion as early biomarkers for kidney allograft rejection were tested in a national -based observational historic cohort and prospective validation study of kidney transplant recipients in whom indication allograft biopsies were performed. We hypothesized that increased urine protein and complement excretion are predictive of allograft rejection and its phenotype and can be identified before loss of kidney function is clinically detectable.

In a historic cohort study we have assessed the diagnostic accuracy of measuring the estimated protein excretion rate (ePER) from spot urine samples in kidney allograft recipients undergoing first indication biopsy beyond 3 months after transplant. Kidney biopsy was considered in any case of serum creatinine increase >20% form the baseline without other evident causes (i.e., obstruction, graft artery stenosis). From 616 patients that received a deceased donor kidney transplant between 2000 and 2012, 151 recipients (25%) had an indication biopsy

beyond 3 months after transplant. Urine protein/creatinine ratios (UPCR) were measured using spot urine samples at baseline (time of lowest serum creatinine before biopsy), 3 months before biopsy, at the time of biopsy and 3 months thereafter. ePER was calculated by multiplying UPCR and estimated creatinine excretion rate (8.8 mmol/day/1.73m2). We then investigated the association of ePER at different time points with different histological phenotypes. Biopsy specimens were evaluated according to the revised Banff classification of renal transplant histopathology (28,29). Among the 151 patients with an indication biopsy beyond 3 months after transplant, 109 patients were classified as having rejection (72%), while 42 patients (28%) had no evidence of rejection. TCMR was diagnosed in 77 patients and phenotypes were reported as borderline (9 patients), tubulo-interstitial (53 patients) or vascular (15 patients). The ABMR was diagnosed in 32 patients and was classified as acute (13 patients) or chronic (19 patients). Non-rejection diagnoses were classified as no major abnormalities (15 patients), recurrent glomerular disease (11 patients), calcineurin inhibitor nephrotoxicity (9 patients), BK virus-associated nephropathy (4 patients) or pyelonephritis (3 patients). Median ePER values 3 months before biopsy and at the time of biopsy were significantly greater in 109 patients with rejection than in 42 patients with no rejection (Figure 1A; P<0.001). When compared with the baseline, the ePER levels increased 3 months preceding allograft biopsy and further increased at the time of biopsy. Except for the baseline values, the levels of ePER from patients with ABMR were significantly higher than the levels in patients with TCMR or no rejection (Figure 1B; ANOVA P<0.001). In contrast with an increase in ePER, group comparisons showed that eGFR 3 months before the biopsy remained stable when compared with the baseline and did not differ between patients with biopsy specimens showing ABMR and those showing TCMR or no rejection (baseline eGFR 56±18 vs. 59±17 vs. 55±18 ml/min/1.73m2, and 3 months before biopsy 54±18 vs. 57±16 vs. 53±18 ml/min/1.73m2, respectively). At the time of biopsy, the eGFR levels were lower in patients with ABMR than in patients with TCMR or no rejection (25±14 vs. 32±11 vs. 37±12 ml/min/1.73m2, P<0.001). Receiver operator characteristic (ROC) analysis revealed that in a comparison of the group of patients who had biopsy specimens showing rejection with the group of patients who had no signs of rejection, the area under

Figure 1. Box-and-whisker plots show the estimated protein excretion rate (ePER) values of longitudinally collected urine samples at baseline, 3 months before biopsy, at the time of biopsy, and 3 months after biopsy from patients with biopsy readings showing rejection versus no rejection (A) and a comparison of the ePER values from patients classified as having no rejection, an episode of T-cell mediated rejection (TCMR) or an episode of antibody-mediated rejection (ABMR) (B).

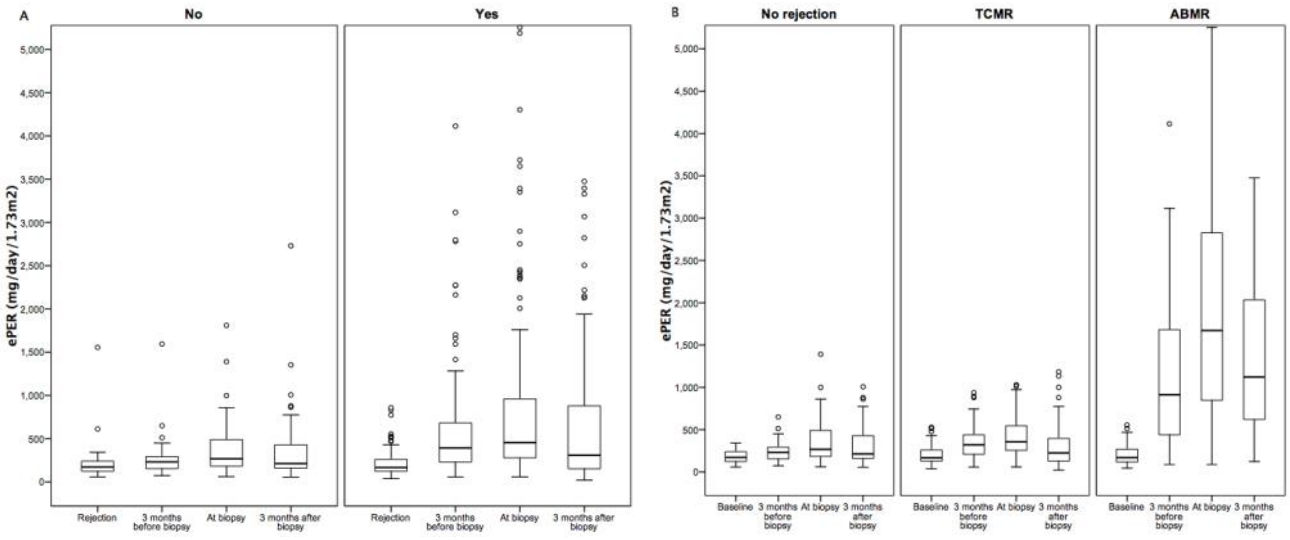

Table 1. 24-hour urine protein and terminal C5b-9 complement complexes excretion in 169 prevalent proteinuric kidney transplant recipients with indication biopsy showing antibody-mediated rejection (ABMR), T-cell mediated rejection (TCMR) or no rejection, and control patients.

Variable	ABMR (*n* = 29)	TCMR (*n* = 26)	No rejection (*n* = 14)	Control patients (n = 99)	*P* value
24-hour proteinuria					
median (mg/day)	1480	500	450	400	< 0.001
interquartile range (mg/day)	740 – 2080	308 – 740	308 – 925	220 – 680	
C5b-9 complexes					
N of patients (%)	19 (66%)	6 (23%)	3 (21%)	4 (4%)	< 0.001
median (ng/ml)	68	0	0	0	< 0.001
interquartile range (ng/ml)	6 – 208	0 – 37	0 – 9	0 – 0	

the curve (AUC) was 0.73 (95% CI, 0.65 to 0.81). In a comparison of the group of patients with biopsy specimens showing ABMR with the group of patients who had TCMR or no rejection, the AUC was 0.84 (95% CI, 0.75 to 0.93).

The relationship between urinary excretion of proteins, complement C5b-9 membrane attack complexes, and graft histology was explored in a prospective study in a national cohort of kidney transplant recipients with increased proteinuria beyond 3 months after transplant. In January 2013, 190 patients with persistent proteinuria (UPCR >20 mg/mmol) were identified from 572 prevalent kidney transplant recipients (33%). We measured 24-hr proteinuria and urinary excretion of C5b-9 membrane attack complexes (by ELISA) in 168 patients who consented for the study and had stable allograft function. In 69 patients (41%) with significant proteinuria (>1g/day) or subsequent allograft dysfunction (increase in serum creatinine >20% form baseline), kidney biopsy was performed and presence of DSA was determined. Patients in whom biopsy was performed had significantly greater 24-hr proteinuria as compared with control patients (1126±818 vs. 508±377 mg/day; P<0.001). Among the 69 proteinuric patients with an indication biopsy, 29 patients were classified as having ABMR, 26 were diagnosed with TCMR, and 14 patients had no evidence of rejection. The levels of 24-hour urine protein and C5b-9 membrane attack complexes excretion were significantly greater in patients with a diagnosis of ABMR than in patients with TCMR, no rejection or control patients (Table 1). Patients with ABMR and positive DSA (21/29) had greater median C5-b9 levels than those with ABMR and no detected DSA (130 vs. 11 ng/ml; P=0.028). ROC analysis revealed that 24-hour proteinuria and urinary excretion of C5b-9 complexes had good discriminatory ability to predict ABMR with AUC of 0.85 (95% CI, 0.78 to 0.93) for proteinuria and 0.80 (95% CI, 0.69 to 0.91) for urinary C5b-9 excretion.

Conclusion and future perspectives

The ultimate goal of biomarker studies in kidney transplantation is to find laboratory test-based non-invasive biomarkers of transplant pathologies using patient urine or blood that indicate changes at the molecular level, before the development of allograft dysfunction, and that predict disease phenotype, allograft outcome or response to therapy. Once transferred to the clinic, recent advances in biomarkers studies will eventually lead to

personalized transplantation medicine, including improved donor-recipient matching, individual immunosuppressive regimens, and individual risk assessment for kidney allograft rejection or chronic dysfunction. Finally, these changes will be reflected by increased allograft survival and decreased patient morbidity.

Our data indicate that in kidney transplant recipients undergoing indication biopsy urinary excretion of proteins and complement C5b-9 membrane attack complexes appears to be associated with allograft rejection and rejection phenotype. This simple diagnostic tools measured in spot urine specimens obtained longitudinally from patients with biopsy-confirmed TCMR or no rejection was relatively flat and distinct from the progressive increase observed in patients in whom ABMR was diagnosed later. This finding is important given that spot urine protein and C5b-9 membrane attack complexes excretion can be easily measured and followed after kidney transplantation. The detection of increased urinary protein and complement excretion before the deterioration of graft function raises the prospect of improving clinical risk stratification. Further studies are needed to prospectively validate these findings.

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Dyslipidemia after kidney transplantation

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Cardiovascular disease (CVD) is the leading cause of mortality in kidney transplant recipients. Pathogenesis of CVD involves atherosclerotic process that is accelerated already in the pretransplant time. An important risk factors for the development of atherosclerosis is dyslipidemia. In kidney transplant patients with good graft function it is mainly a consequence of several immunosuppressive drugs, i.e. calcineurin inhibitors (CNI), steroids and everolimus. As these drugs in different combinations represent the mainstay of modern immunosuppressive regimens, management of hyperlipidemia will remain an important part of posttransplant cardiovascular diseases prevention in patients with good graft function. When, however, the graft function starts to decline, uremia specific factors add to dyslipidemia profile again and accelerate the process of atherosclerosis.

The beneficial effect of statins on cardiovascular outcomes in kidney disease is less firm than in general population, where reductions in serum LDL-cholesterol have been proved to significantly reduce both morbidity and mortality of patients in secondary prevention. In the randomized controlled study ALERT (1) fluvastatin lowered LDL-cholesterol by 32%, but risk reduction for the primary endpoint (cardiac death, non-fatal myocardial infarction (MI) or coronary intervention procedure) was not significant, although there were fewer cardiac deaths or non-fatal MI in the fluvastatin group compared to the placebo group. In the open-label ALERT extension study total mortality and graft loss did not differ significantly between the group taking fluvastatin and the placebo group (2). Fluvastatin, however, produced a safe and effective reduction in LDL-cholesterol associated with reduced risk in major adverse cardiac events (cardiac death, MI or coronary intervention procedure) and a 29% reduction in cardiac death or definite non-fatal MI. Although it is possible that cholesterol-independent pleiotropic effects of statins contribute to the reduced cardiovascular death, it seems more probable that it is predominantly the lower LDL-cholesterol that mediates this effect. In a recently published IMPROVE-IT trial (3) treatment with ezetimibe as an add-on drug to statin resulted in incremental lowering of LDL cholesterol and improved cardiovascular outcomes in general population. Additional cardiovascular benefit that was gained through lowering of LDL to levels (1.4 mmol/L) below previous targets (1.8 mmol/L) can be ascribed to either reduced LDL-cholesterol which is in line with a lipid hypothesis or to possible pleiotropic effects of ezetimibe for which, however, there is no scientific evidence (4). Taking advantage of synergistic effects of ezetimibe as an add-on to a weak statin (fluvastatin) or to a reduced dose of a potent statin therefore seems very reasonable. Although in kidney transplant patients' causal relationship between lower LDL-cholesterol and cardiovascular mortality has not been proven it is reasonable to extrapolate data from the general population and aggressively treat posttransplant dyslipidemia.

There are several potential reasons for which lipid lowering drugs are less effective in kidney patients than in general population. An important player in the cardiovascular risk profile of CKD patients apart from LDL cholesterol may be the HDL cholesterol. Molecular composition of HDL correlates strongly with its function. Its atheroprotecive properties are severely impaired in chronic kidney disease. Studies have found that HDL may function as an acceptor, transporter and inactivator of oxidized LDL lipids. HDL has also other nonlipid related mechanisms. In

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CKD patients, however, HDL does not show its protective role as in general population. In addition to that restoration of renal function does not correct impairment of uremic HDL properties (5). Structural and functional properties of HDL in stable renal transplants remain dysfunctional after renal transplantation. Cholesterol acceptor capacity and antioxidative activity are suppressed in kidney transplant recipients regardless of graft function and are comparable with levels in ESRD patients. Although HDL proteins in patients with good allograft function recover partly toward healthy phenotype, several HDL proteins that are high in ESRD remain in higher concentration also after kidney successful transplantation. It is also proven that HDL in children with CKD promotes endothelial dysfunction. HDL strongly inhibits nitric oxide production, promotes superoxide production and reduces cholesterol efflux from macrophages. The effects on endothelial cells correlates with CKD grade, with most pronounced changes in dialysis patients. Partial recovery of HDL is observed in patients after renal transplantation (6).

Additional reason for lower efficacy of lipid lowering drugs in CKD patients may be the finding that atherosclerosis is no longer a disease attributed mainly to the high lipid content of the body, but mainly to endothelial damage and free radicals including reactive oxygen species (7). In chronic kidney disease uremia specific toxins add to endothelial dysfunction which begins early in CKD (8). Targeting the the LDL cholesterol in CKD patients may therefore be an insufficient strategy. An optimal approach still remains to be discovered.

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Polyomavirus nephropathy in renal transplant recipients: an update on diagnostic approach

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Polyomavirus infection is widespread in the human population and generally remains asymptomatic through the life of healthy individuals. However, in immunocompromised individuals, such as renal transplant recipients, it can be associated with various patterns of tissue injury, of which polyomavirus nephropathy (PVN) is the most common. PVN is a major complication after renal transplantation, leading to loss of renal grafts in approximately 43% of patients. Since polyomavirus (PV) viruria and viremia can be seen without renal injury and viral nephropathy, a diagnosis of PVN must be confirmed by renal biopsy. However, characteristic viral inclusion and tubular injury might be focally observed in the biopsy specimens, and varying degrees of tubulointerstitial inflammation reminiscent of T-cell mediated acute rejection make accurate diagnosis difficult. The intriguing concepts of immune reconstitution injury and extensive inflammation in resolving PVN after reducing immunosuppression need further investigation. Reduction of baseline immunosuppression remains the common therapeutic strategy of PVN but is associated with increased risk of rejection. Since unrecognized PVN diagnosed late after transplantation causes chronic tissue injury and graft failure, the goal of screening protocols and classifications schemes of PVN is to characterize early disease grades that respond to therapeutic intervention and may heal without progressing to chronic graft injury. Novel diagnostic assays, such as the urinary polyomavirus-haufen test, provide non-invasive strategies for accurate diagnosis and assessing the severity of PVN in voided urine samples.

Keywords: kidney transplantation; polyomavirus infection; polyomavirus nephropathy

Polyomavirus infection and disease in humans

Polyomaviruses are non-enveloped, double-stranded ubiquitous DNA viruses living in birds and mammals as natural hosts. They were named for their ability to produce tumors (Greek poly- many, multiple; -oma, tumors), particularly in rodents and experimental models (1). In humans, seroprevalence ranges from 20-90%, depending on the viral strain and patient age. After infection, PV persist latent in the renourinary tract and may undergo periods of self-limiting transient asymptomatic activation with viruria and viremia without causing disease (1, 2). Polyomavirus infection therefore represents serological or virological evidence of virus exposure without distinguishing among replicating, latent and transforming patterns. Manifest viral disease, defined as histological evidence of polyomavirus-mediated organ pathology, is mainly limited to immunocompromised patients, such as transplant recipients (2, 3).

Three PV strains, the BK virus, JC virus and simian virus (SV-40), were considered to be pathogenic in humans. Infections with SV40 were detected following administration of contaminated polio vaccines in the late 1950s, without known clinical manifestation (1). BK and JC viruses, named after the initials of infected patients, were isolated in 1971 from a patient with ureteral stenosis after kidney transplantation and from a patient with progressive multifocal leukoencephalopathy. Both strains are characterized by productive viral infection with tissue injury showing specific tropism for the renourinary

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tract or central nervous system (1, 3, 4). There is increasing evidence that BK virus may be involved in tumorigenesis of bladder carcinoma in renal transplant recipients and salivary gland inflammation and sclerosis in HIV patients (5, 6). Recently, several new strains, such as trichodysplasia spinulosa-associated polyomavirus and Merkel cell carcinoma polyomavirus, have been detected, probably related to proliferative lesions and neoplasms without productive viral replication (7).

Polyomavirus nephropathy

PVN is a major complication after renal transplantation, affecting 1-10% of patients (8). During the cyclosporine era, only sporadic cases were reported and PVN remained forgotten for nearly 25 years. Despite modern immunosuppressive drugs introduced in the 1990s enabling improved allograft survival, they were responsible for the occurrence of previously uncommon side effects, including PVN and hemorrhagic cystitis (3). Before screening protocols for PV reactivation were routinely used, PVN was diagnosed late after transplantation, in an advanced grade, with chronic tissue changes leading to allograft loss in 50-90% of cases (9, 10). Potential misdiagnosis of concurrent rejection resulting in increased immunosuppression might contribute to accelerated allograft failure.

The specific mechanisms of viral activation remain unknown. PVN is typically caused by the BK strain and only rarely by simultaneous activation of BK and JC viruses (3). It seems that the transplant microenvironment promotes viral reactivation, because there has only been sporadic detection of PV in native kidney of patients with other organ transplants or in immunodeficient patients (11, 12). PVN also commonly occurs in patients with post transplantation complications, including delayed graft function and acute rejection. Other risk factors are male gender, older recipient age, diabetes, prolonged ureteral stent placement, smoldering subclinical graft inflammation and/or abnormalities of dendritic cell and NK cell/T-cell activation. Relative over-immunosuppression by modern immunosuppressive drugs, though, is considered the main risk factor (3, 8, 13, 14).

In order to confirm intrarenal PV replication, renal biopsy remains the gold standard for a definitive diagnosis of PVN (8). A minimum of two cores including the medulla are recommended to make a correct diagnosis (8, 15, 16). Biopsy findings can be focal and presented only in the medulla, so PVN can be missed due to sampling error. Morphologically, PVN is characterized by intrarenal viral replication, mainly in tubular epithelial cell nuclei (intranuclear inclusions), causing tubular injury and host cell lysis (Figure 1A). Viral replication in tubular epithelial cells can induce various nuclear changes: an amorphous ground-glass inclusion body (type 1), a central irregular inclusion body surrounded by a halo (type

Figure 1. Polyomavirus nephropathy. Virally induced tubular epithelial cell injury and lysis (left). Intranuclear viral inclusion bodies: an amorphous ground-glass inclusion body and vesicular nuclear changes with coarsely clumped viral inclusions (right) (Hematoxylin and eosin, 600x).

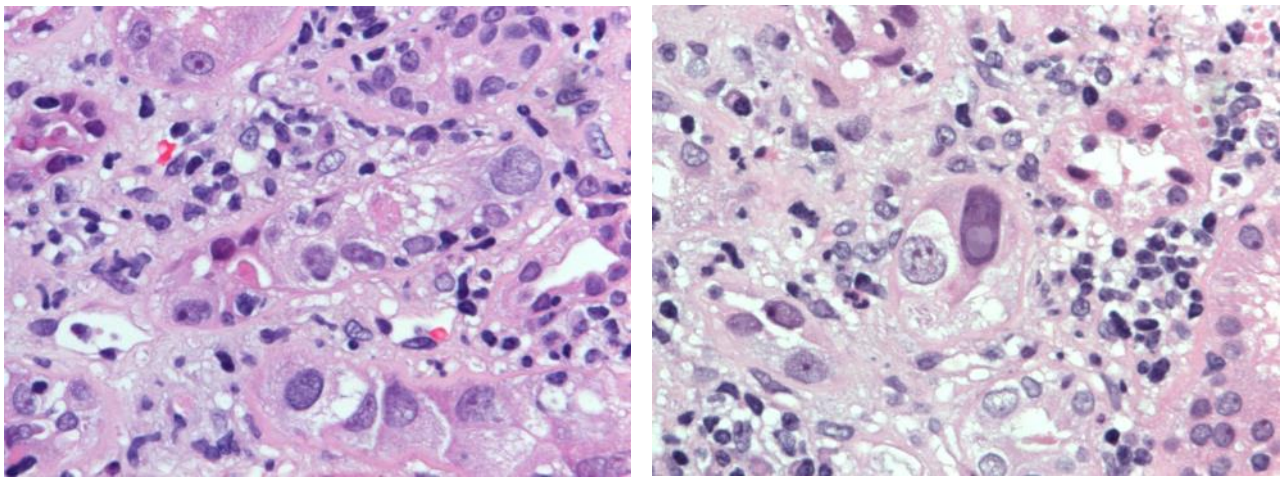
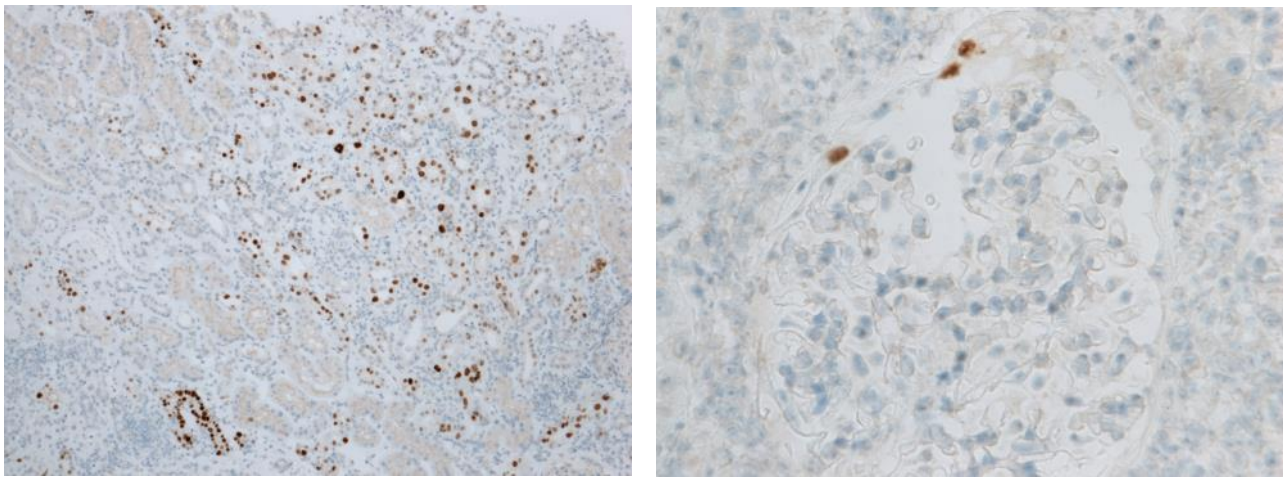


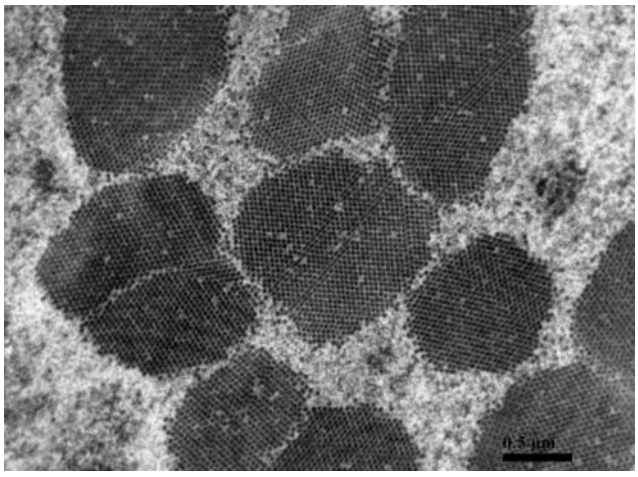
Figure 2. Detection of SV-40 antigen in tubular epithelial cells (left) (200x) and epithelial cells of Bowman's capsule (right) by immunohistochemistry (SV-40, 400x).



2), finely granular nuclear alterations (type 3) and vesicular changes with coarsely clumped viral inclusions (type 4) (Figure 1B). In rare cases, the ascending PV infection can affect epithelial cells of Bowman's capsule. Diagnostic confirmation can easily be achieved by immunohistochemistry (Figure 2A, B) or immunofluorescence, with antibodies directed against the polyomavirus T antigen, VP capsid proteins or detection of intracellular virions of 40-50 nanometer in diameter by electron microscopy (Figure 3) (15, 16).

In early stages of PVN with focal and minimal tubular changes without tubular injury and characteristic intranuclear inclusions, the diagnosis can only be established by immunohistochemistry with antibody directed against SV40-T antigen (16). Later in the course of the disease, many cases of PVN may show numerous infected cells and an inflammatory lymphocytic infiltrate mimicking T-cell mediated acute rejection. Recognition of PVN is critical, since the proper therapy is reduction, rather than enhanced immunosuppression. Glomeruli and vessels must be carefully examined in order to exclude glomerulitis and vasculitis, which would strongly suggest concomitant rejection. Advanced disease often shows marked interstitial fibrosis/tubular atrophy, while interstitial inflammation and viral replication may be variable.

Figure 3. Intracellular virions of 40-50 nanometer in diameter detected by electron microscopy.



PVN must be differentiated from other rare viral infections, including CMV, herpes simplex virus and adenovirus. However, the most important differential diagnosis, particularly in PVN after reduction of immunosuppression, remains T-cell mediated acute rejection (10, 16, 17).

Little is known about the natural course of PVN. Some authors have reported that biopsies obtained after reduction of immunosuppression during decrease of the plasma viral load may show severe interstitial infiltrate and tubulitis reminiscent of T-cell mediated acute rejection but the outcome of renal grafts was good despite prolonged reduction of immunosuppression (9, 10). In subsequent biopsies, the virus was cleared from renal tissue and inflammation resolved without the presence of marked interstitial fibrosis. They suggested that such tubulointersitial nephritis might be immune

reconstitution-associated graft inflammation, enabling the resolution of PVN.

Clinical presentation, prognosis, and therapy

Several studies have shown that differences in PVN morphology may predict the clinical presentation and outcome of the disease (18-21). In order to provide optimal diagnostic and prognostic information of PVN, the Banff working group on PVN proposed three clinically significant disease grades based on the severity of polyomavirus replication and the degree of interstitial fibrosis (3, 22). Polyomavirus replication was defined as the histologic viral load, estimated by % of virally infected epithelial cells detected by immunohistochemistry (22). It ranged from scattered SV-40 positive cells in PVN grade 1 to numerous in grade 2 and grade 3. In addition to SV-40 positive cells, grade 3 is characterized by interstitial fibrosis, which is responsible for irreversible tissue injury leading to graft failure (3, 22). It has been shown that disease grade depends on the time of the diagnosis: PVN grade1 was diagnosed in the first 5 months after transplantation, usually presenting with normal renal function and associated with a favorable outcome in 85-90% of cases. In contrast, grade 2, and particularly grade 3, were detected 12 months after transplantation, associated with an increase of creatinine and graft failure in 25% and 50% of cases, respectively. Since PVN has limited treatment options, the early detection of PVN has a major impact on the prognosis of the disease and therefore on allograft survival.

Early diagnosis of PVN is difficult, because early stage PVN does not show any signs of systemic infection or proteinuria or hematuria and renal function remains normal, particularly when only the medulla is involved (16). The first step of viral reactivation shown in almost all patients is characterized by detection of characteristic polyomavirus inclusion-bearing cells in the urine – decoy cells. Initial viruria may be followed by detection of PV in plasma and onset of PVN after a 6-12 week window period (8).

The goal of screening is to facilitate early diagnosis of patients when viruric or viraemic, and it was considered that pre-emptive reduction of immunosuppression prior to the development of overt nephropathy might be beneficial (8, 10). Current guidelines recommend a urinary cytology test initially and then plasma test by PCR if they consistently find urinary decoy cells (8). While PVN is most common in the first year after transplantation, screening at least every 3 months during the first two years and after anti-rejection treatment seem appropriate to cover the majority of PVN cases (8). The advantages of cytology urine tests include high negative predictive value to rule out a diagnosis of PVN, lower costs and a window period between viral reactivation and PVN. However, several studies have shown that only a few patients with urinary shedding of virus progressed to PVN (23). In patients without biopsy-proven PVN, pre-emptive long lasting reduction of immunosuppression could be potentially harmful due to increased risk of acute rejection. Notably, BK viruria and viremia may represent transient asymptomatic activation or can originate from extrarenal sites, usually along the lower urinary tract. On the other hand, preemptive reduction of immunosuppression does not always prevent the development of PVN in viremic patients (24).

Not surprisingly, PCR-based BK viremia correlate only moderately well with the severity of the intrarenal disease, ranging between 25-75% (10, 25). The exact range of viral load that would indicate PVN cannot be defined because some patients may present with very low BK virus copy numbers at the time of PVN diagnosis.

In order to enable non-invasive diagnosis of definitive PVN without the risk of renal biopsy, a novel urine based assay, called the urinary polyomavirus-haufen test, has recently been introduced (3). Polyomavirus-haufen are tight cast-like three dimensional viral aggregates, detected by negative staining electron microscopy of the voided urine sample. Because polyomavirus-haufen admixed with uromodulin are formed in tubular lumens, they specifically predict intrarenal disease (26). Moreover, the titer of polyomavirus-haufen tightly correlates with the degree of intrarenal polyomavirus replication, providing additional information on the severity of PVN (27). The urinary polyomavirus-haufen test seems therefore to be a sensitive and specific biomarker for intrarenal viral disease, with positive and negative predictive value of higher than 90%.

A BKvirus VP1 mRNA, another PVN urine biomarker, was recently described with high sensitivity and specificity for PVN (28). Detection of additional urine

biomarkers not only offers additional strategies for noninvasive PVN diagnosis but also predicts graft outcome.

Management of PVN is still very limited. Reduction of the baseline immunosuppression as the common therapeutic strategy is associated with clinical acute rejection rates of 8-14% (3, 29). Some patients with BK viremia subsequently develop definitive PVN despite pre-emptive reduction of immunosuppression (24). Many of the therapeutic agents, including leflunomide, quoinolone and cidofovir, have been involved in PVN treatment with undetermined antipolyomavirus effect (16). It was recently shown that IVIG administration may be effective in treatment of BK viremia and PVN in patients who have failed to respond to immunosuppression reduction and leflunomide therapy (30).

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Surgical complications can compromise kidney allograft outcome. Can they be avoided?

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Renal transplantation is from a surgical point of view, a stage process, which includes obtaining a kidney graft for a suitable candidate, the preparation of obtained kidneys for transplantation, kidney inserting itself into the body of the recipient and possible surgical complications recognized later in postoperative period. The surgical techniques for the transplant are well established and the procedure is associated with high success rates, as such, complications are less frequent than in transplantation of other organs. However, the detection and accurate diagnosis of surgical complications is important in maintaining the function of the transplanted kidney. Late diagnosis may compromise graft function threats the life of transplant recipient and even risks the graft loss.

Depending on the stage of the transplant process these complications may have an origin in back-table work to prepare the allograft, the dissection of the renal bed and vascular anastomosis or in the restoration of the continuity of the urinary tract. Despite the experience of the surgeon, there are often on the recipient risk factors such as the elderly recipients, atheromatosis vessels, obesity, associated heart disease or they have to be treated with antiplatelet agents which may predispose to perioperative bleeding complications. For most of these complications surgical intervention is required, and sometimes also radiological intervention, or in a combination of both methods. As with all surgical procedures complications such as bleeding and infections of surgical wounds can occur. In a further, however, they are divided into the vascular complications, complication relating to urological urinary diversion and to lymph drainage (1,2).

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Infections and complications with wound healing

The incidence of these complications is about 5%. The main factor is poor or delayed wound healing due to illness on the part of the recipient, bleeding wounds and wound infections. Recipients receiving intensive immunosuppressive therapy, therefore they have the possibility of complications significantly higher. Wound infections are often associated with bleeding in or below the layers of the wound, despite the inserted drainage, sometime the tube can be plugged. Stalled hematoma around the kidney may later be the source of infection, so it is advisable to remove large hematoma, usually surgically, rarely percutaneous drainage can be successful because of organized blood cloths formed around the graft (3).

Vascular complications

They are rare in the postoperative period. Renal artery thrombosis occurs in 0.2 to 3.5% and usually results in graft loss. This is due primarily to inadequate surgical technique, bending of artery intima injury or anastomosis stenosis due to atherosclerotic plaque. Renal vein thrombosis in the early period is slightly more common, the incidence is 0.3 -3%. The causes are usually surgical as folding renal vein, especially if it is too long, too narrow anastomosis, damage to the vein wall or a number of tiny veins on graft. It appears as a sudden cessation of diuresis and pain in the area of the graft. If we soon recognize this situation, by quick surgical intervention we can manage to remove fresh thrombus from vein or re-wash renal vein with a solution to keep the graft. Graft artery stenosis is the most common vascular complication and represents

almost 75% of vascular complications. The reason may be inadequate surgical technique of anastomosis between graft artery and recipient artery, knicking artery, and damage to the artery intima due to atherosclerotic plaques. If it is recognized at an early stage, the best solution is reanastomosis or later radiological interventions using angioplasty technique (4.5).

Urological complications

Urine leakage

The incidence is 1 to 4.3% and may occur in the renal pelvis, along the ureter of renal graft, on bladder wall or at the site of ureteral anastomosis to bladder. Leakage of urine from the renal pelvis is rarely encountered. Damage to renal pelvis can be done during preparing the donor kidney prior to transplantation. Also, leakage of urine from the bladder is less often using catheter inserted for a longer period and Lich-Gregoire technique of anastomosis. More often resulting leakage mainly in the lower part of the ureter when it is connected to the bladder, because this part of the ureter is poorly blood supplied. Namely graft ureter have blood supply only thorough the branches of renal artery in hilar part of kidney artery or from the artery which arises from the lower pole of the kidney. Other causes of urine leak include immunosuppressive therapy poorly placed splint or clogged urinary catheter. Symptoms appear 3-4 days later as leaking urine through drainage, reflecting mainly as technical failure. Urine leakage which appears 10-14 days later usually means ureteral necrosis anywhere on his length from kidney to the bladder, most often on the lower part. Urine may drain even into abdominal cavity, which extends the abdominal cavity, but more often into retroperitoneal place around the graft, what makes the pressure on the blood vessels and ureter, diuresis is reduced, and around the kidney there appears urinoma. Diagnosis can be established by contrast CT urography, cystography or scintigraphy. Minor leakage from the bladder can be healed spontaneously leaving urinary catheter for a longer period, otherwise, according to most authors the optimal solution is reoperation and surgical removal of the necrotic part of the ureter and reanastomosis. At the same time this raises the question of how can we exactly localize urine leakage. Contrast CT urography having good renal function, can show the place of urine flow. If there exists even ureteral stenosis, the best solution is nephrostomy insertion and contrast imaging through nephrostomy. Also we can apply filling the bladder with blue liquid to see the place of urine escaping. Rarely we can endoscopically insert a ureteral catheter into the new formed orifice for retrograde contrast imaging to detect the place of urine leakage from ureter.

Ureteral stenosis and obstruction

These are the most common urological complication that occur in 3 to 7%. Causes can be the pressure on the ureter due to hematoma, urinoma or other surrounding structures. Most frequent the cause is in the ureter due to ischemia, necrosis, proliferation of connective tissue in the wall, but also due to knicking of the ureter due to the excessive length. The causes which have origin in the ureter are presented in a later period after transplantation and can be beside ureteral necrosis also urinary stones, necrotic renal papilla, blood clots. Obstruction of the ureter may be presented early after transplantation, but usually during 1-3 months, possibly even a few years later, with a gradual rise in serum creatinine and hydronephrosis of the graft. If it occurs very early in the postoperative period, it is usually technical in nature as may be edema, tight stiches, pressure of overlying funiculous, hematoma, urinoma, or seroma. Inserting nephrostomy catheter and contrast imaging displays the location and the length of stenosis, or necrosis on ureter. Anterograde insertion of stent or balloon dilation are only a temporary measure used sometime. Standard way is the surgical approach with resection of the narrowed and necrotic ureter and neoimplantation, usually constructing Boar flap from the bladder and psoas hitch. In some cases, the process of ureter necrosis continues and more than two thirds of ureter becomes necrotic. In such case we can use patient´s own ureter and make anastomosis on graft ureter side by side or end to end. Use of an isolated part of the intestine or appendix (Monti or Mitrofanoff procedure) to bridge the defect when ureter is to short is very rare method (1,2,8).

Vesicoureteral reflux

Vesicoureteral reflux (VUR) can occur in any post-transplant period and has a very wide range of incidence, influenced by the technique of ureter implantation and when the antireflux technique of ureter implantation is not properly done. Most patients having VUR are asymptomatic, particularly in case if urinary tract infection is not present. It is not yet completely clarified the connection between VUR, urinary tract infection and impact on graft survival. Today, many believe that VUR in short term and with transient infestation of urinary tract does not affect the function of the graft. Following the frequent recurrences and long-term treatment of infections, resistant to antibiotic treatment occurs, in such case identified VUR could require surgical intervention. Surgical intervention is also required if the recipient has recurrent episodes of pyelonephritic attacks with apparent high-grade VUR (grades IV III), which threatens allograft function. Possible surgical techniques is ureter re-implantation using antireflux Lich-Gregoire or Politano Leadbetter technique. Good results are also achieved by tunneling ureter, if it is long enough, under bladder mucosa which is a kind of Cohn´s technique modification used in children having reflux. Endoscopic interventions with injecting some bulking substance under new formed ureteral orifice to prevent reflux because of poor results were not implemented in clinical practice.

Lymphocele

Lymphocele means the accumulation of lymphatic fluid, mainly from recipient´s disrupted lymphatic vessels situated around iliac vein and artery in an enclosed extraperitoneal space. It occurs in 0.6% to 18%, which is largely dependent on surgical techniques, careful ligation of lymphatic vessels and tissue preservation technique. To a large extent, the formation of lymphocele also depends on immunosuppressive therapy, whereas mTOR inhibitors (everolimus and sirolimus) and steroids act antilimphangiogenic, preventing lymphatic channels healing. Other risk factors include acute rejection, diuretics, low molecular weight heparins or recipient obesity. Lymphocele occurs in a period of few weeks or months after transplantation, it can be spontaneously absorbed or remain small, asymptomatic. Larger lymphocele can put pressure on the recipient iliac vein or graft vein, ureter, bladder, and can cause hydronephrosis, edema in legs, urinary urgency or even urinary retention. Diagnosis is established by ultrasound examination, though lymphocele may sometimes be confused in the early period with urinoma. A single puncture and evacuation of liquid, and determination of creatinine and electrolytes help us to establish correct diagnosis. The derivation of lymphocele by simple percutaneous drainage is not recommended because lymph flows out for extended period and recipient can lose high-protein fluid. The most successful method is the laparoscopic or open method of lymph derivation through the open peritoneal window from extraperitoneal place into the abdominal cavity, where it is absorbed. Using omentum inserted into the peritoneal opening prevents closing down the small window in peritoneum (9,10,11).

Rupture of the allograft

This is extremely rare and dangerous complication that requires urgent surgical intervention, because it threatens not only the transplant graft but also the recipient. It develops only a few days in postoperative period, or later during the first weeks. The causes can be acute rejection, acute tubular necrosis, renal vein thrombosis, renal biopsy, overuse of low molecular weight heparin. Clinically appears as a sudden severe pain over the graft, swelling, anuria, hypotension, a drop in hemoglobin. Diagnosis is established by ultrasound or CT investigation. An immediate surgical intervention is needed, focused on graft nephrectomy versus salvaging. In cases where graft rupture site is surgically manageable, the bleeding can be controlled sparing the renal graft in situ and not compromise the patient survival. If the recipient is hemodynamically unstable with low blood pressure and red blood count, nephrectomy should be done as the best solution in order to preserve the recipient survival.

Conclusion

The survival and successful functioning of the transplanted kidney in the period of first weeks and months after transplantation largely depends on proper and well established surgical technique of transplantation, and rapid identification and

resolution of postoperative complications. Most complications are well known and are well manageable using established surgical techniques.

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Health related quality of life after kidney transplantation

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Health related quality of life (HRQoL) is defined as a sense of well-being and ability to function productively. In posttransplantation patients it represents not only an important outcome measure but also an effective assessment of treatment effectiveness and a predictor of adverse outcomes (1). Nephrologists are aware that patients with end-stage renal disease (ESRD) have lower HRQoL than general population. Three decades ago a mile-stone study was published comparing HRQoL in 11 dialysis and transplant centers in US in 859 patients. It was found that 79.1 per cent of the transplant recipients were able to function at nearly normal levels, as compared between 47.5 and 59.1 per cent of the patients treated with dialysis (home-hemodialysis, in-center hemodialysis, CAPD). Nearly 75 per cent of the transplant recipients were able to work, as compared with between 24.7 and 59.3 per cent of the patients undergoing dialysis. In respect to life satisfaction, well -being, and psychological affect transplant recipients had a higher HRQoL than patients on dialysis (2).

There are a lot of questionnaires for generating the profile of HRQoL. The most widely used SF-36 (Short Form 36-Item Health Survey) consists of eight dimensions: 1)Physical Functioning, 2)Role Limitations due to Physical Functioning, 3)Bodily Pain, 4)General Health Perception, 5)Vitality, 6)Social Functioning,7)Role Limitations due to Emotional Functioning, and8)Mental Health. Row scores are transformed into a score between zero and hundred for each dimension. Higher score indicates better health (3).

Analysis of 53 articles including 36582 patients found that the scores of SF-36 health dimensions were not significantly different between hemodialysis and peritoneal dialysis patients, but the scores of renal transplant patients were higher than those of dialysis patients, except for the dimensions of Mental Health and Bodily Pain (4).

A systematic review of 110 studies with a total 1 922 300 participants revealed in most studies significantly lower mortality associated with transplantation, reduced risk of cardiovascular events and substantially better quality of life in regard to chronic dialysis patients (5).

Brazilian national study with representative sample of 3036 patients on hemodialysis (HD), peritoneal dialysis (PD), and after renal transplantation RTx using the SF-36 questionnaire showed that patients after RTx have the best quality of life of the three treatment modalities. RTx patients achieved the best mean score in the physical component of quality of life. There were no significant differences among treatment groups regarding the mental component. Older patients had better mental quality of life but worse physical quality. Patients in a higher socioeconomic class and patients that were not hospitalized are also reported better quality of life. Also the dialysis units and transplant centers influenced the patients' quality of life (6).

Patients with kidney transplant or receiving intensive HD report a higher quality of life than patients on conventional HD. There is insufficient evidence to determine whether there are significant differences in the quality of life between these treatments. Individual concerns about the relative risk and benefits of renal transplantation may drive some patients to choose to stay on dialysis (7). The data from Slovenian Renal Replacement Therapy Registry showed that considerable group of dialysis patients refusing RTx. Patients on PD refuse RTx less often than HD patients (8).

In prospective prevalent cohort study in 879 RTx

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patients HRQoL was assessed by SF Questionnaire, and depressive symptoms using the Center Epidemiologic Studies –Depression Scale. Every 10-point increase in SF-36 Physical Composite Score and Physical Functioning and General Health Perception scores was associated with 18%, 11% and 7%lower risk of mortality, respectively (9).

Little is known about the changes of HRQoL on long term outcomes in RTx. Repeat SF-36 and Transplant Effects questionnaire in 102 RTx survivors after 6 years showed improved emotional HRQoL (Mental Component Score, Mental Health, Energy). Physical HRQoL deteriorated (Physical Component Score, Pain). Living related RTx recipients had greater decline in physical functioning compared to cadaver recipients. Worry about the transplant and feeling of responsibility increased significantly over time (10).

In a cross-sectional multicenter study in France, including 1061 RTx patients (72.5% responded) SF-36 and ReTransQoL questionnaires were used to assess HRQoL. The variables which decrease scores were: female, unemployment, lower education, living alone, high BMI, diabetes, recent critical illness and hospitalization, non-compliance, a long duration of dialysis, treatment side effects, dismissal and recent surgery on the graft (11).

Assessment of HRQoL (SF-36, End-Stage Renal Disease Symptom Checklist) at 3 months after RTx and after 10 years was performed in 151 patients. Higher physical HRQoL at baseline was associated with younger age, less severe perceived side effects of immunosuppressive treatment and higher efficacy in stopping unpleasant emotions. Higher mental HRQoL was associated with older age, less severe perceived side effects of immunosuppressive treatment, and higher efficacy in stopping unpleasant emotions and lower efficacy in getting support from family and friends. Older age, higher kidney function and both higher physical and mental HRQoL at baseline significantly improved the odds of graft and patient survival over a period of 10 years (1).

Gastrointestinal (GI) symptoms in RTx patients seem to be very frequent and can impair QoL. Ekberg et al. (12) reported of study conducted in 4232 patients (70% responded). Compared to the general population, 92 % of patients complained on GI troubles and 53% had impaired HRQoL. On the other hand, 145 nephrologists (79 responded) from the same centers answered that their patients had GI troubles in 20% of cases and lower HRQoL in 36%. The similar results were reported in Italian RTx patients (n= 1130) using an Italian translation of the Gastrointestinal Symptom Rating Scale (GSRS) and Gastrointestinal Quality of Life Index (GIQLI) questionnaires. In the physician interview, 39.2% of patients had one or more GI symptoms vs. 88.3% of patients in the self administered questionnaire (13).

Immunosuppressive drugs may have influence on HRQoL. Mycophenolic acid (MPA) formulations (MMF, Mycophenolate mofetil; EC-MPS, enteric-coated mycophenolate sodium) are part of maintenance immunosuppressive regimen and may express adverse GI effects, particularly diarrhea and abdominal pain. A randomized, multicenter, 12 weeks study was conducting. 115 RTx patients were randomized to continue MMF (n=56), or change to EC -MPS (n=59). Incidence of GI complications was significantly lower in the EC-MPS group and better HRQoL was recorded (using a GSRS and GIQLI instruments). Switching from MMF to EC-MPS also enable an increase in the maximum tolerated dose of MPA (14).

In the sub-study of the Symphony Study SF-36 Health survey was completed at baseline, 3, 6, and 12 months in 156 RTx patients with the aim to evaluate HRQoL with different low-toxicity regimens post-transplantation. There were no differences between groups in SF-36 at baseline or at month 12. Low tacrolimus (Tac) showed higher scores at month 3 than standard dose or low dose of cyclosporine (CsA). Patients with serum creatinine less or equal 1.5 mg/ dL had better HRQoL at 6 and 12 months. Proportion of these patients was higher in low-Tac at 6 months. Physical component summary of patients increased during follow-up, but mental did not. Patients with acute rejection showed lower mental component summary at 6 months. No HRQoL differences were identified among groups, but the low Tac group showed the fastest improvement (15).

A randomized, multi-country, open-label clinical trial was performed in 430 RTx patients randomly assigned to sirolimus (SRL) + corticosteroid (ST) (n=215) or SRL+CsA+ST (n=215) therapy after initial 3-months period of combined SRL+CsA+ST

treatment. HRQoL was measured using the Kidney Transplant Questionnaire (KTQ) and SF-36 Health survey at month 3, 12, 24, 36 post-transplantation. It was shown that SRL-based therapy with early CsA-elimination results in fewer appearance-related problems, less fatigue, greater vitality, and improved general health status and social functioning compared with continuous SRL+CsA+ST treatment (16).

HRQoL was assessed in 128 stable RTx and in 102 chronic kidney disease patients (CKDP) using SF-36 health survey. RTx patients with estimated (MDRD) creatinine clearance > 60 mL/min versus <60 mL/ min showed higher scores among 7 of 8 SF-36 categories: physical function, role physical, bodily pain, general health, vitality, role emotional, and mental health. These were not observed in the control group of CKDP. The explanation of difference is perhaps in a fear of failing graft and approaching to dialysis again with the memories of unpleasant experiences (17).

Patients after kidney graft failure starting dialysis again have not only reduced HRQoL but also higher mortality rate in respect to transplant naïve patients wait-listed for kidney transplantation (18,19).
Living donor kidney transplant recipients are reported on average of better HRQoL and societal participation than deceased donor kidney transplant recipients in the first year after transplantation and thereafter also. Probably, the most benefit is for an early living-donor renal transplantation (20, 21,22).

Patients with type l diabetes mellitus and ESRD have after simultaneous pancreas kidney transplantation better HRQoL when compared with remaining on waiting list. Significant positive effect was shown on diabetes-related HRQoL (Diabetes Quality of Life, DQOL; SF-36; Quality of Well-Being questionnaires) , which was sustained longitudinally but it was difficult to show an overall improvement in general HRQoL; however, up to one third was reported even a decrease (23,24).

Comparing HRQoL in older RTx recipients aged 65 years or more (n=150) and younger than 65 years (n=1544) a lower physical QoL was found in older patients but mental QoL was maintained and was higher than in younger recipients. Longer time since transplantation in elderly was associated with having significantly impaired physical QoL, but no predictors were associated with significantly impaired mental QoL. In younger recipients, rejection, diabetes mellitus, delayed graft function, coronary artery disease, and longer time on dialysis were associated with impaired physical QoL. Rejection, smoking, diabetes mellitus, and longer time on dialysis were predictors of impaired mental QoL (25).

Symptom Checklist-90 subscales of depression and anxiety, the Nottingham Extended ADL scale, and the Duke Health Profile questionnaire in total of 100 RTx and 63 HD patients demonstrated that depression and anxiety are more prevalent among HD patients compared with RTx subjects (26).
Cross-sectional cohort of 100 wait-listed pre-RTx and 100 post-RTx patients completed validated fatigue, sleep, mood and QoL questionnaires. Pre- RTx patients had higher levels of fatigue frequency, fatigue severity, and fatigue disruptiveness than post-RTx patients and also more difficulty with sleep quality, latency, duration, efficiency, and disturbance, and were more likely to have poor sleep quality (27).

Anemia in post RTx patients has negative impacts on HRQoL. Scoring mental and physical QoL by SF-36 is useful to identify groups of patients whose QoL could be improved by rHuEPO (28).
Despite controversy, reviewed results showed significant improvement of sexual functioning after receiving RTx. Identified determinants associated with improvement are decreased prolactin serum level, age younger than 45 years, and onset of dialysis less than 6 moths (29).
Regular sport activity significantly improves different dimensions of HRQoL among RTx patients and its benefits go beyond the impact on physical health and involve psychological and social component of quality of life (30).

Annual income may also influence HRQoL in some areas where post-transplant immunosuppressive drugs are under government–sponsored medical coverage only partly, and was positively correlated

with General Health (31).

It seems reasonable to have an enough long-lasting national plan for improving the quality of life also in dialysis and transplant patients. Comparison of two national quality of life survey for patients with ESRD between 2005-2007 and 2011 showed slightly decreased indicators (32).

In a daily life it is important how good are we living, not only how long. Patients with ESRD have lower HRQoL than general population. Majority of studies showed that RTx can offer the highest level of HRQoL among the patients with ESRD. We are aware that development of the other RRTs have also achieved a degree which can offer a quite satisfied life and a decision, which modality of RRT is the most appropriate for the patient is mutual with medical staff and the patient after giving a full information of the treatments. How to measure HRQoL is also important. Most frequently used SF-36 questionnaire is validated and enough reliable. In our department a retrospective study using a simple 5-degree scale was accomplished with report of better HRQoL after RTx in respect to the period on HD (33). In the current cross sectional study in all RTx recipients in Slovenia (more than 600 patients) using Short Form-36 (SF-36), and End Stage Renal Disease Symptom Checklist – Transplant Module (ESRD-SCL) questionnaires, with already finished two pilot studies, we have the intention to evaluate HRQoL, with deeper insight into selected subgroups of patients, and to reveal the factors of possible impact. It may be worthy continuously or periodically follow up the HRQoL with the aim of improving potential influencing factors on HRQoL which would perhaps have also the impact on patient and graft survival. Considering European Renal Best Practice Guideline on kidney donor and recipient evaluation and perioperative care may also have influence on HRQoL later (34). Further studies are needed to answer this conjecture.

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Long term outcome of renal transplantation in Slovenian children

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Background. Renal transplantation is the preferred treatment modality of end-stage renal disease in children. We analyzed the outcome of renal transplantation with emphasis on long-term impact on socio-economic status in pediatric renal graft recipients.
Methods. In our cross-sectional study medical records of all Slovenian pediatric transplant patients were reviewed retrospectively. Altogether, 46 patients were included. At the time of survey five of them had died, four were lost from follow-up and one patient refused cooperation. Twenty-fife of the remaining 36 patients had reached adulthood. Information regarding their present status was obtained by telephone interview.
Results. Forty-seven transplantations were performed in 46 different patients, younger than 20 years. 30% of patients received a living related donor graft and 70% a deceased donor graft. The average recipient age was 13.6 years. The most frequent complication was biopsy-proven acute graft rejection (42%) 5-, 10-, 15- and 20-year graft survival was 78%, 68%, 45% and 25%, respectively. Only 28% of all patients were employed. Eight percent of patients graduated from a university, 76% were meeting friends regularly and 32% were married or were involved in a steady relationship. Twelve percent of patients have children. Quality of life was rated as excellent or good in 48% and 36%, respectively. Twenty-five percent of patients are worried about their financial status and employment.
Conclusions. Long-term graft survival in Slovenian children is comparable to reviewed literature and is improving. Acute graft rejection is the most common complication. In adulthood, concerns are mostly related to the patient's economic situation, including employment.

Keywords: kidney transplantation; children, complications; outcome; socio-economic status

End stage renal disease (ESRD) in children is a rare condition. Among pediatric patients aged <20 years at the onset of renal replacement therapy (RRT) from 16 countries contributing to the ERA-EDTA Registry, the incidence of ESRD has remained stable at 8-10 per million age-related population (pmarp) since 2001 (1). Kidney transplantation is the preferred treatment modality of ESRD in children because it improves growth, increases life expectancy compared to dialysis and provides a better quality of life (2-4). Despite all advantages related to transplantation, there are multiple factors that can affect renal transplant outcome and patient survival. In addition, a long-term impact on socio-economic status has been reported in renal graft recipients (5). The aim of our study was to analyze complications and outcome of renal transplantation with emphasis on long-term impact on socio-economic status in pediatric renal graft recipients.

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Methods

Study population

In our cross-sectional study medical records of all Slovenian pediatric renal graft recipients (<20 years of age) who underwent kidney transplantation

between November 1984 and September 2014 were reviewed retrospectively. All patients have been treated at the University Medical Centre in Ljubljana. Altogether 46 pediatric patients were included during that time and majority of them received a deceased donor graft. We assessed complication rate of renal transplantation during childhood in 43 patients, in whom the data were available because three of the patients left Slovenia immediately after transplantation. At the time of survey five of our patients had died, four were lost from follow-up and one patient refused cooperation. Twenty-five of the remaining 36 patients had reached adulthood and information regarding their present socio-economic status was obtained by telephone interview.

Outcome and socio-economic status assessment

We analyzed graft survival after 5, 10, 15 and 20-years and evaluated all complications related to transplantation. The diagnosis of acute rejection was based on abrupt increase of serum creatinine concentration and renal biopsy results.

We also performed a socio-economic analysis in those patients, who received a renal transplant during childhood and reached adult age. The assembled information included employment and educational degree, spare-time activities and socializing, marital status and having children. The patients were also asked to assess their quality of life and to express their most troublesome worries.

Statistics

Descriptive statistics were applied, whereby age was expressed as mean value ± standard deviation. First graft survival was assessed by Kaplan-Meier analysis.

Results

Forty-seven pediatric transplantations were performed in Slovenia between November 1984 and September 2014 in 46 different children younger than 20 years (19 girls and 27 boys). The average recipient age was 13,6 ± 3,8 years (range 4,6-19,8 years). Fourteen patients (30%) received a living related donor graft and 32 patients (70%) received a deceased donor graft.

Complications of renal transplantation

The most frequent complication was biopsy-proven acute graft rejection (humoral, cellular od combined), found in 18/43 patients (42%), followed by bacterial infection in 11/43 patients (26%), adverse effects of immunosuppressive medication in 4/43 patients (9%) and viral infections in 4/43 patients (9%). There were some surgical complications in 3/43 patients (7%) and lymphoceles in 2/43 (5%). All other complications occurred in only one patient each (2%), namely, de novo FSGS, biopsy proven calcineurin-inhibitor nephrotoxicity, recurrence of native disease, systemic aspergillosis, malignancy (PTLD), vascular thrombosis, serious renal artery stenosis and renal stones.

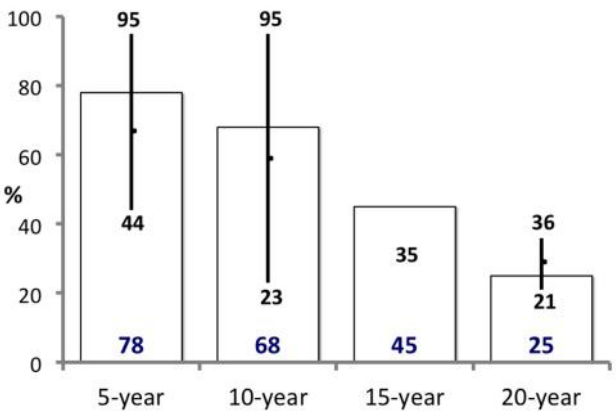
Outcome

Five-, 10-, 15- and 20-year graft survival was 78%, 68%, 45% and 25%, respectively. The longest period of normal graft function was 20,8 years, and this graft was still functioning normally at the time of our survey. Graft survival was comparable to data published by other authors (Figure 1). Graft survival was not influenced by the source of the graft (i.e., living related graft, deceased donor graft) (Figure 2). Only one patient received a second graft during childhood. Five patients (11%) died, two of them younger than 19 years.

The socio-economic survey

Two of 25 patients (8%) graduated from university, 11/25 patients (44%) finished vocational school,

Figure 1. 5-, 10-, 15- and 20-year graft survival in Slovenian pediatric renal graft recipients (white bars). Our results are compared to the data from existing literature (black high-low lines) (9).



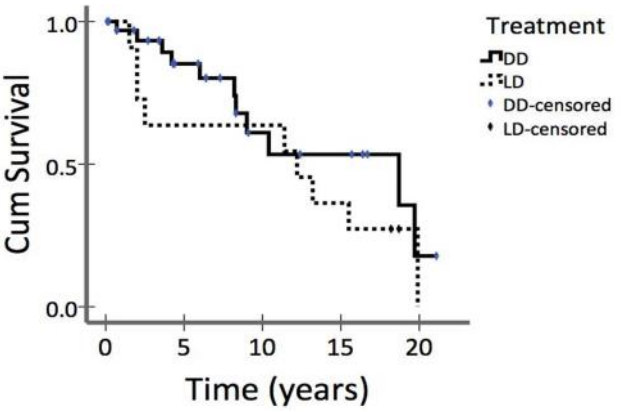


Figure 2. Kaplan-Meier analysis for first graft survival in Slovenian pediatric transplant patients. Data are stratified for deceased donor (DD) and living-related donor (LD) grafts. The censored cases represent grafts in which the observer event (i.e., failure) has not occurred in the time of analysis. Of note, only 2/14 LD grafts were functioning at survey, compared to 23/32 of DD grafts. This discrepancy is explained by the fact, that almost exclusively DD grafts have been transplanted during the past 15-years.

6/25 patients (24%) finished high school, 5/25 patients (20%) finished elementary school and 1/25 patient has not completed any educational program. Nine of 25 patients (36%) were employed (i.e., full time, part time, self employed). Nine of 25 patients (36%) of were retired, 3/25 patients (12%) remained unemployed and 4/25 patients (16%) were still students. Ten of 25 patients (40%) were regularly involved in sport activities. Seventy-six percent of all patients were meeting friends regularly. Eight of 25 patients (32%) were married or were involved in a steady relationship. Three of 25 patients (12%) had one or two children. The quality of life was rated as excellent, good, fair and poor by 48%, 36%, 8% and 4% of patients, respectively. Fifteen of 25 patients (60%) had no worries at all, whereas worries related to financial status, general health, school performance and graft function were expressed by 20%, 8%, 8% and 4% of patients, respectively.

Discussion

Kidney transplantation is the treatment of choice in children with ESRD and has been shown to improve life expectancy and to provide better quality of life compared to hemodialysis or peritoneal dialysis (2-4). Slovenia regularly provides extended data to the ESPN/ERA-EDTA registry, which collects data on RRT at an annual basis via the national and regional renal registries in Europe. Currently, 35 countries are participating in the registry, providing information on more than 10,000 patients who started RRT before the age of 20, between 1997 and 2013. According to 2013 data, the incidence for RRT in Slovenian children younger than 15 years was 6,7 pmarp, which is comparable to the overall registry incidence of 5,2 pmarp (6). Considering the relatively small number of children in Slovenia, approximately 1-3 children per year start RRT in our country. Early transplantation is accepted as the best treatment option in our institution. However, only one preemptive transplantation was performed until now.

According to the results of our study, the majority of Slovenian children received renal grafts from deceased donors, which is contrary to numerous other reports, where the majority of grafts were provided by living donors (7). One possible reason for this observation could be the relatively small number of children, resulting in a relatively short waiting time for deceased donor organs.

Although, all children received potent immunosuppressive medication according to generally accepted guidelines, the most frequent complication of renal transplantation observed in our cohort of patients was biopsy proven acute rejection (e.g., humoral, cellular or combined) (42%). Our results are in accordance with NAPRTCS 2006 data, showing that 46,7% of renal transplant recipients experience at least one rejection episode (8). All but one episodes of acute rejection in our patients were successfully treated and did not result in organ failure. Despite the relatively high number of acute rejection episodes, long-term graft survival in our cohort of transplant patients was somehow better than the reported average, with the exception of the 20-year graft survival (9).

According to our results, bacterial infections (26%) were more common than viral infections (e.g., Cytomegalovirus (CMV), Epstein-Barr virus) (9%). This is in contrast to published literature, reporting higher numbers of viral infections (20%-60% of CMV infections) (7,10). Unfortunately, our study was not designed to provide an explanation for this finding.

Long-term social integration and economical status of our pediatric patients who reached adulthood were evaluated as well. Seventy-six percent of patients have accomplished a certain educational level, which increases the chances for employment. Sixteen percent of our patients were still students and only 36% were employed at the time of analysis. The employment rate was lower compared to data from similar studies (54%-86%) (11-13), which can be partially explained by a relatively high percentage of students in our study population who will apply for a job only after finishing school. Besides, the high unemployment rate can also be explained by the ever-increasing general unemployment rate in Slovenia and other European countries, especially in the young population. Nevertheless, the results of Groothoff et al. (14) confirm our findings that patients with childhood onset ESRD are more often unemployed than the age matched population. Thirty-six percent of our patients have been receiving a government pension and were classified as nonemployees.

Thirty-two percent of our patients were involved in a steady relationship but only 12% of them have offspring. Similar results were reported by other authors (12). A substantial number of our patients (76%) have been meeting friends regularly. This is in agreement with the fact that 60% of our patients expressed no particular worries in our survey and more than three quarters of them rated the quality of their life as good or even excellent. We assume that their answers were honest and not merely the consequence of a relaxed and cozy domestic environment during the telephone interview. Although concerns were not frequently declared by our patients, they were mostly related to their financial situation and employment and not to their health status. This is not surprising, considering the high level of unemployment and consequent low income in this group of patients, superimposed on the current unfavorable general economic situation in the society.

Conclusions

Long-term graft survival in Slovenian children is comparable to reviewed literature and is improving despite the fact that the majority of transplanted organs came from deceased donor. Acute graft rejection is the most common complication, followed by bacterial and viral infections and adverse effects of immunosuppressive therapy. In adulthood, less than half of the patients were employed therefore concerns are mostly related to the patient's economic situation. Nevertheless, most patients with a renal graft seem to be optimistic regarding their future.

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Medication burden in children with renal grafts

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Background. Transplanted patients are exposed to complex drug regimens. A recent analysis of Slovenian adult renal graft recipients revealed a substantial daily medication burden. The aim of our study was to evaluate whether this applies to pediatric renal graft recipients as well.
Methods. All Slovenian pediatric patients with a functioning renal graft were included in our retrospective cross-sectional study. The number of drugs and the corresponding number of pills were obtained by reviewing the medical charts at the last visit to our Transplant Unit. The burden of immunosuppressive drugs was analyzed separately.
Results. Fourteen renal graft recipients, 9 boys and 5 girls, aged 12.2±3.9 years (range 4.6-19.6) participated in the study. The average time, elapsed between transplantation and data recording, was 2.7±2.1 years (range 0.2 -6.9). The average number of different drugs prescribed per day was 9.5 (median 10, range 6-14). The mean number of pills prescribed per day was 22.6 (median 23, range 15-31). Only 2/14 (14%) patients have been regularly taking two immunosuppressive drugs per day, whereas the majority of patients were receiving three different immunosuppressive drugs. Forty-one percent of the pill burden was represented by immunosuppressive medicine, with an average daily number of pills of 9.3 (median 9, range 6-15).
Conclusions. The daily medication burden in pediatric renal graft recipients is high. Fifty percent of patients are receiving more than 10 different drugs daily, resulting in over 23 pills per day. Immunosuppressive treatment is responsible for more than one third of the medicine load.

Keywords: medication burden; adherence, kidney transplantation; children

Kidney graft recipients face a life-long intake of immunosuppressive medication to prevent rejection of the transplanted kidney. Although strict adherence to the prescribed therapy is crucial for long-term graft survival, recipients do not always adhere perfectly to their regimen. Nonadherence to immunosuppressant medication is recognized to be associated with a high risk of acute rejection and graft loss after renal transplantation (1). It has been shown that the odds of graft loss are increased seven-fold in nonadherent patients compared with adherent patients (2).

The success rate of kidney transplantation has improved in the past decades, mainly due to the use of potent immunosuppressive regimes to prevent graft rejection (3). The most common long-term combination immunosuppressive regimen consists of steroids, tacrolimus, and mofetil mycophenolate (4). However, additional medication is usually required due to comorbid conditions (e.g., antihypertensive drugs, magnesium and phosphate supplementation, osteoporosis prophylaxis and bicarbonate, etc.) Specific treatment is included according to the primary diagnosis and clinical situation. Coadministration can result in potential drug-drug interactions.

Transplanted patients are exposed to a high drug and pill burden, which increases the risk of nonadherence and adverse treatment effects, which can both influence graft survival (5). A recent analysis of a Slovenian cohort of 634 adult renal graft recipients

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showed that the average daily number of prescribed drugs and pills was 10.5 and 20, respectively (6). The aim of our study was to evaluate whether this applies to pediatric renal graft recipients as well.

Methods

All Slovenian pediatric patients with a functioning renal graft who were followed in our outpatient clinic at the time of survey were included in our retrospective cross-sectional study, which was conducted in October 2014. The number of different drugs and the corresponding number of pills were documented by reviewing the medical records at the last visit to our transplant unit.

The term »pill« was chosen to represent all the different drug forms used (e.g., tablets, capsules, drops, solution for injection) regardless of the route of administration. In case of liquid formulations, the whole amount of the medication taken at one time (e.g., drops, milliliters) was considered the equivalent of one pill. The composite number of pills was acknowledged in case that the prescribed dose involved different formulations or strengths of the same drug (e.g., Prograf 1.5 mg is composed of one capsule containing 1 mg and one capsule containing 0.5 mg of the active substance).

The burden of immunosuppressive and non-immunosuppressive drugs was analyzed separately and the results compared. Furthermore, data on primary renal disease, estimated glomerular filtration rate (eGFR) according to Schwartz formula, time on chronic renal replacement therapy (RRT) prior to transplantation and the time period between transplantation and our survey were recorded. Data were analyzed by using descriptive statistics.

Results

Fourteen renal graft recipients were included in our study. There were 9 boys and 5 girls, aged 12.2 ± 3.9 years (median 12.5 years, range 4.6-19.6 years). The primary renal diseases leading to end stage renal disease were as follows: congenital anomalies of the kidney and urinary tract 50% (7 patients), nephronophtysis 14.3% (2 patients), primary hyperoxaluria 14.3% (2 patients), hypoxic injury 14.3% (2 patients) and cystinosis 7.1% (1 patient). The average time period, elapsed between transplantation and data recording, was 2.7 ± 2.1 years (median 4.7 years, range 0.2-6.9 years). The average time spent on RRT before transplantation was 2.9 years (median 2.4 years, range 0.1-6.7 years). The average serum creatinine at the time of analysis was 77.1 µmol/L (median 76.5 µmol/L, range 44-124 µmol/L), resulting in a mean eGFR of 112.2 ml/min/1.73m2 (median 112.0 ml/min/1.73m2, range 64-188 ml/min/1.73m2).

The average number of different drugs prescribed per day was 9.5 (median 10, range 6-14). Thirty percent of the drug burden was composed of immunosuppressive medication. Only 2/14 (14%) patients have been regularly taking two immunosuppressive drugs per day, whereas the majority of patients (12/14, 86%) were receiving three different immunosuppressive drugs. According to our own transplant protocol, immunosuppression consisted of steroids, tacrolimus, and mofetil

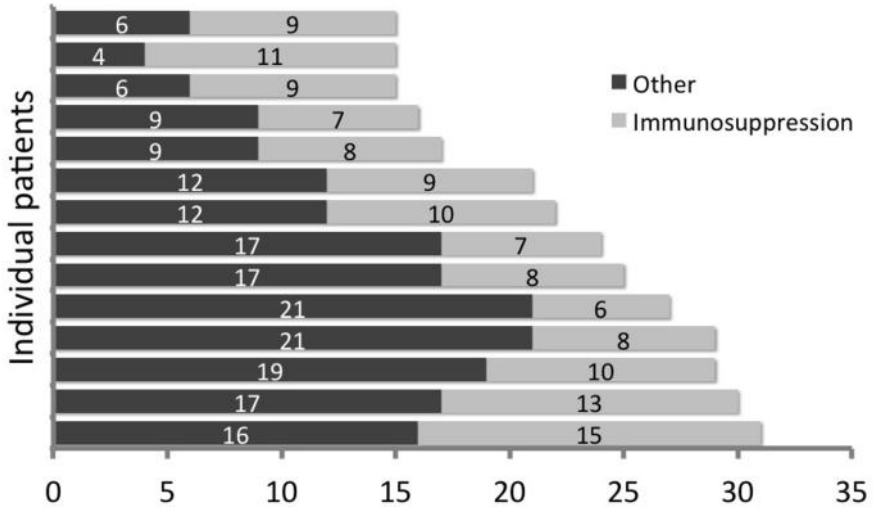

Figure 1: Total pill burden (whole bars) with numbers representing pill count per individual patient. The fractions participated by immunosuppressive medication (black bars) and other drugs (gray bars) are highlighted separately.

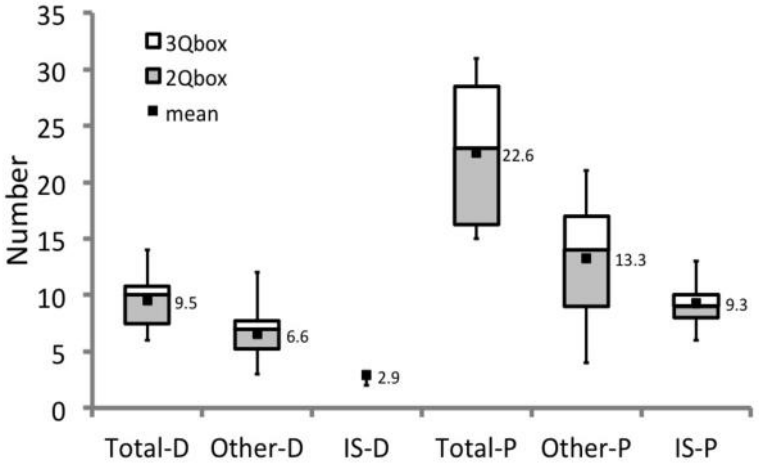

Figure 2: Summary quartile distribution of two groups of data displaying the number of daily drugs prescribed (first half of the graph) and the corresponding total daily pill burden (second half of the graph). Each group consists of three subgroups (i.e.; total number of drugs/ pills, immunosuppression drugs/pills and other drugs/pills) which are displayed in separate box and whisker bars.D=drug; IS=immunosuppression; P=pill; Q=quartile.

mycophenolate. All patients also received induction therapy with anti-interleukin-2 receptor blocking antibodies. The average number of daily non-immunosuppressive drugs was 6.6 (mean 7, range 3-12).

The mean number of pills prescribed per day was 22.6 (median 23, range 15-31). Forty-one percent of the pill burden was represented by immunosuppressive medicine, with an average daily number of pills of 9.3 (median 9, range 6-15) (Figure 1). The average daily number of non-immunosuppressive pills prescribed was 13.3 (median 14, range 4-21). A summary quartile distribution of the daily drug and pill load is presented in Figure 2.

Discussion

Medication remains an integral part of chronic disease management. Complex medication regimens have been shown to contribute to poor medical adherence in the general population (7). This can also be observed in patients with a renal graft, which face a life-long intake of immunosuppressive medication. Of note, transplanted patients often have several comorbid conditions, adding to polypharmacy. A recent analysis of a Slovenian cohort of adult renal graft recipients showed that the average number of prescribed drugs and pills was 10.5 and 20, respectively (6).

The results of our study showed that the daily drug and pill burden is high also in Slovenian pediatric renal graft recipients. Fifty percent of children have been receiving more than 10 different drugs daily, resulting in over 23 pills per day. Forty-one percent of the pill burden was represented by immunosuppressive medication, with an average daily number of pills of 9.3. The remaining part (59 %) was composed of drugs prescribed for comorbid conditions. We are aware that our findings are limited by the small sample size. However, since all Slovenian pediatric renal graft recipients are followed in our institution, our results bear a national character.

Obviously, the medication burden imposed by immunosuppressive drugs in renal graft recipients depends on the time lag between transplantation and data recording. Immunosuppression is most intense immediately after transplantation and declines subsequently according to immunosuppressive protocols. Hence, the more time elapses after transplantation, the smaller is the medication burden. Unfortunately, the number of children included in our cross-sectional study was too small to allow for a stratified analysis of the drug burden according to the individual time lag. The median time lag in our study was 2.4 years, with a range of 0.1-6.7 years.

We can also expect the medication burden to be higher in patients with more comorbid conditions. As already mentioned, almost 60 % of the drug burden in our study was not related to immunosuppression. An independent, graded association has been shown to exist between a reduced eGFR and the risk of chronic kidney disease (CKD) related complications (8). Therefore, it can be assumed that a decreased eGFR in renal graft recipients is associated with more comorbid conditions and results in a bigger drug burden. Recently, the median eGFR in adult Slovenian

renal graft recipients was reported to be 61 ml/ min/1.73 m2 (range: 4-(>90) ml/min/1.72 m2) according to MDRD formula. Approximately one tenth of these patients had significantly reduced graft function, corresponding to advanced stages (4-5) of CKD (6). In our study, the median eGFR in pediatric renal graft recipients was 112 ml/min/1.73 m2 (range: 64-188 ml/min/1.73 m2). Less than one third of our pediatric patients had CKD grade 2, whereas the remaining patients had normal graft function (CKD grade 1). Of note, this difference in graft function is merely the result of a shorter follow-up time, since all pediatric transplant patients are transferred to the adult transplant unit when they come of age and are recognized as adults.

However, despite of the difference in graft function, the drug and pill burden was similar in Slovenian pediatric and adult transplant patients. This observation could be attributed to the causes of end-stage renal disease (ESRD) in pediatric patients, which differ from those in adults. It could be speculated that some primary renal diseases in pediatric graft recipients involve specific treatment resulting in an increased drug burden. However, the correlation between primary renal disease and medication burden was not analyzed in our study. In addition, the prevalence of comorbid conditions generally increases with patient age, which would suggest an increased drug burden in elderly patients (9).

Since comorbid conditions differ between pediatric and adult renal graft recipients, one would expect this to be reflected by a different assortment of non-immunosuppressive drugs used in the relevant groups of patients. However, according to our results, only a few drugs have been acknowledged to be used exclusively in either pediatric or adult patients. The use of antiaggregation or anticoagulant drugs and insulin are often prescribed in adult patients and almost never in children, whereas the opposite holds through for growth hormone and drugs prescribed for the treatment of inborn metabolic diseases (e.g., Cystagon). The use of anticoagulant drugs in children is usually restricted to the immediate post transplant period and insulin is used transiently in case of drug-induced diabetes related to high doses of immunosuppressant medication. On the other hand, one can expect more patients with inborn errors of metabolism reaching adulthood due to the ever-improving graft and patient survival in these diseases. Two major complications can be associated with polypharmacy and high drug burden, namely, nonadherence and drug-drug interactions, influencing pharmacodynamics of individual drugs and enhance adverse effects. Obviously, the more drugs prescribed, the more side effects can be expected. On the other hand, adverse effects can provoke nonadherence and increase the risk of early graft loss. In addition, individuals with lower medication adherence are more likely to experience treatment related adverse events (10).

In pediatric renal graft recipients, nonadherence correlates with age and is most common in adolescents (3, 11-13). Adolescent immaturity may lead to conflict and nonadherence, as a misguided assertion of independence. The desire for autonomy is unique to adolescents, but difficult to achieve when grappling with chronic illness (14). We have previously reported that adolescents treated with chronic dialysis, especially girls, might refuse transplantation, because of fear of changes in body appearance inflicted by immunosuppressive medication (15).

Complex medication regimens were found to be associated with lower drug adherence in adult CKD patients (16). It has been shown that the prescribed number of doses per day is inversely related to drug adherence, and less frequent dosing regimens result in better adherence. Adherence was reported to be significantly higher for once daily versus 3-times daily regimens but not for once daily versus twice daily regimens (17). Hence, reducing dosing frequency can result in overall improvements in adherence, patient satisfaction, quality of life and costs (18). In our experience, however, dosage simplification of the treatment regimen with introducing once daily dosing can be challenging in adolescents with adherence difficulties. Namely, missing a once daily dose means 24 hours without immunosuppressant medication, whereas missing one dose of twice daily drug means only 12 hours uncovered. These concerns have also been raised by other authors (19).

Our results confirm that pediatric renal graft

recipients are exposed to a high medication burden. Many of the prescribed drugs exhibit a narrow therapeutic index, where even small differences in dose or blood concentration may lead to serious therapeutic failure or adverse events. Of note, age related changes in pharmacokinetics have been described, as the absorption, distribution, metabolism and excretion of drugs change with age (20). Infants generally have a higher metabolic capacity and may require higher doses or reduced dosing intervals for many drugs, and the toxicity profile can also be different (21). Hence, therapeutic drug monitoring when available and individual dosage adaptation is mandatory (22).

Pharmacogenomics offer an additional means of further individualizing drug therapy by incorporating genetic information to guide safer and more effective treatment decisions. Pharmacogenomics aims to integrate an individual's genetic variability with the pathophysiology of disease, pharmacokinetic drug disposition, and predicted disease outcomes to predict therapeutic efficacy and likelihood of adverse drug reactions (4, 23).

Conclusions

The daily medication burden in pediatric renal graft recipient is substantial and can trigger nonadherence or manifest with treatment related adverse effects, both affecting graft survival. Strategies to reduce drug and pill burden should be explored in the future in order to avoid these complications. If the number of medication cannot be reduced, we should at least aim towards closer monitoring and individualization of the treatment. The use of pharmacogenomics might further enhance drug safety and treatment efficacy.

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Medication and pill burden in kidney graft recipients: a national cohort study

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Background. Medication and pill burden in kidney graft recipients is high. The aim of this retrospective clinical study was to assess drug and pill burden in Slovenian cohort of kidney graft recipients.

Methods. All Slovenian adult patients with functioning kidney graft at the end of 2012 participated in the cross-sectional study. Number of drugs and pills reported in medical chart from the last outpatient visit in 2012 were counted. Serum creatinine concentration, estimated glomerular filtration rate (eGFR/1.73 m2, MDRD), duration od renal replacement therapy (RRT) and time after transplant were evaluated in the multivariate model.

Results. Six hundred and twenty-nine kidney graft recipients, 55.8% males, aged 53 ± 12 years (median 55, range 18-78) participated in the study. Mean eGFR was 59 ± 20 ml/min/1.73m2 (range 4 to >90 ml/ min/1.73m2). Mean serum creatinine concentration was 118 ± 74 micromol/l (range 42 to 781 micromol/l). The mean number of drugs prescribed per day was 10.3 ± 3.2 (range 2 to 22). The mean number of pills prescribed per day was 19.8 ± 7.0 (range 3 to 43). In multiple linear regression analysis recipient age, male gender, diabetes, eGFR and time after transplantation were identified as independent predictors of number of medications, while time on RRT was not. Independent predictors of number of pills per day were male gender, eGFR and time after transplantation.

Conclusions. Drug and pill burden in kidney graft recipients is high, with average of 20 pills prescribed per day. Strategies to reduce drug and pill burden should be explored in the future, to avoid drug interactions, improve patients' adherence and make life easier.

Keywords: adherence; kidney transplantation; medication

Introduction

High pill burden is frequently associated with chronic diseases. One of the highest pill burden reported for any chronic disease state was 19 pills per day for maintenance dialysis patients. In a cross-sectional study analyzing 233 dialysis patients, it was found that pill burden was associated with lower health-related quality of life (1).

Pill burden in the patients with transplanted kidney is high. This is a consequence of immunosuppressive therapy in addition to drug medications prescribed to treat or to prevent complications of immunosuppression or comorbidities. Data on medication and pill burden in kidney graft recipients are scarce. In the longitudinal study analyzing pill burden in 68 patients with kidney or combined kidney-pancreas transplantation the median pill burden one year after transplantation was 16 pills per day. The highest pill burden was reported one month after transplantation (25 pills per day) (2).

Kidney graft has a limited lifespan (3,4). Humoral rejection and non-adherence were recognized as a major causes of graft failure (5). High pill burden may contribute to non-adherence, especially in adolescent patients (6). Interventions aimed to improve adherence in transplant recipients include intensified inpatient and outpatient pharmaceutical care and counseling by a dedicated clinical pharmacist (7).

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Various monitoring strategies of medication adherence were reported, including pill counting, review of prescription records and incorporation of electronic monitoring devices, even ingestible sensor systems (8-12).

Increased pill burden in kidney graft recipients is rarely addressed as a major problem. Our group reported data on medication and pill burden in Slovenian kidney graft recipients (13). Having only one transplant center in the country, taking care of all kidney graft recipients from transplantation until graft failure, we have a unique opportunity to study the complete national cohort of kidney graft recipients.

The aim of our cross-sectional retrospective study was to evaluate medication and pill burden in Slovenian adult kidney graft recipients.

Patients and Methods

In this retrospective, cross sectional study, we included all Slovenian adult transplant recipients with a functioning kidney graft in December 2012. Laboratory values at the last outpatient visit in 2012 were recorded, including estimated glomerular filtration rate (eGFR), calculated with 4-variable Modified Diet in Renal Disease (MDRD) formula, and serum creatinine. Total duration of renal replacement therapy (RRT) and time with a functioning transplant were determined from the national Renal Replacement Therapy Registry.

Number of drugs/medications and pills were counted from the medical records of the last outpatient visit in 2012. The total number of medications was determined as the sum of different oral medications the subject was taking at home and parenteral medications, administered either at home (e.g., insulin, epoetin) or in the transplant unit (e.g., intravenous iron supplements). The total pill burden was defined as the total number of pills the subject took daily. For medications prescribed to be taken less frequently than daily or as needed, a fraction was assigned, based on known or estimated frequency of usage. Separately, we determined whether patients were prescribed aspirin, clopidogrel, epoetin, warfarine or other anticoagulant medication.

Statistica 7.0 (StatSoft, Tulsa, USA) was used for statistical analyses. Data are presented as mean ± standard deviation (SD) with ranges or as percentages, as appropriate. The number of medications and pills was correlated to different predictors using Pearson product-moment correlation coefficient and multiple linear regression analysis.

Table 1. Patients' characteristics

Parameter	Value
N	629
age [years]	53 ± 12 (18 – 78)
male gender	55.8%
diabetes mellitus	9.9%
serum creatinine [micromol/l]	118 ± 74 (42 – 781)
eGFR (MDRD) [ml/min/1.73 m2]	59 ± 20 (4 – 91)
average time after transplantation [years]	8.1 ± 6.2 (0 – 36.6)
duration of RRT [years]	14.4 ± 7.9 (0.5 - 38.7)
number of medications per day	10.3 ± 3.2 (2 - 22)
number of pills per day	19.8 ± 7.0 (3 - 43.1)

Table 2. Multiple linear regression analysis of factors associated with the number of medications (overall model fit $R^2 = 0.20$, $p < 0.001$) and number of pills per day (overall model fit $R^2 = 0.28$, $p < 0.001$).

Variable	Medications / day		Pills / day	
	beta	p value	beta	p value
age (years)	0.21	<0.001	0.06	0.08
male gender	0.14	<0.001	0.19	<0.001
diabetes mellitus	0.11	<0.001	0.02	0.51
eGFR (ml/min/1.73 m2)	-0.26	<0.001	-0.10	<0.01
RRT vintage (days)	0.08	0.16	-0.02	0.70
Transplant vintage (days)	-0.34	<0.001	-0.48	<0.001

Results

On Dec 31st 2012, there were 629 adult kidney transplant recipients with a functioning graft in Slovenia and all were included in the study. Their clinical characteristics as well as medication and pill burden are presented in Table 1.

Patients were taking 10.3 ± 3.2 medications on average per day with average pill burden of 19.8 ± 7.0 pills daily. In addition, 28.8% of patients were taking acetylsalicylic acid, 1.7% clopidogrel, 6.4% were on anticoagulant medications and 16.1% were receiving epoetins. The number of medications positively correlated with CKD stage ($r = 0.22$, $p <0.001$) and negatively with eGFR ($r = -0.24$, $p <0.001$) and time after transplantation (expressed in days: $r = -0.22$, $p <0.001$, or as a time group: $r = -0.07$, $p=0.07$). On the other hand, the number of pills per day inversely correlated with the time after transplantation (expressed in days, $r = -0.49$, $p <0.001$, or as a time group: $r = -0.22$, $p <0.001$), but not with CKD stage ($r = 0.04$, $p=0.29$) or eGFR ($r = -0.05$, $p=0.23$) (Figures 1 and 2).

In multiple linear regression analysis age, male gender, diabetes, eGFR and time after transplantation were identified as independent predictors of number of prescribed medications, while time on RRT was not (Table 2). Independent predictors of the number of pills taken daily were male gender, eGFR and time after transplantation.

Discussion

In this study we have explored medication and pill burden in Slovenian national cohort of kidney graft recipients. High medication and pill burden was found, with average of 20 pills per day. Male gender, eGFR and time after transplantation were independently associated with pill burden.

Despite high pill burden, non-adherence is not perceived as a major problem in Slovenian transplant recipients. The patients are thoroughly educated by nurses and physicians both during pre-dialysis education as well as pre-transplant work-up and after transplantation. Single transplant center enables uniform approach to transplantation, immunosuppressive protocols and patients' follow-up. After outpatient visit laboratory data are immediately communicated to the patient and necessary actions, if needed, are performed. However, high medication and pill burden are not contributing to better quality of life and may be a potential source of serious interactions.

Strengths and limitations of the study

Analyzing a complete national cohort of transplant recipients being treated in a single center is major strength of the study. All medications were prescribed under the same protocol and policy. Therapy was regularly supervised, checked and documented with great precision, so quality of data

Figure 1: Medication and pill burden versus time after transplantation

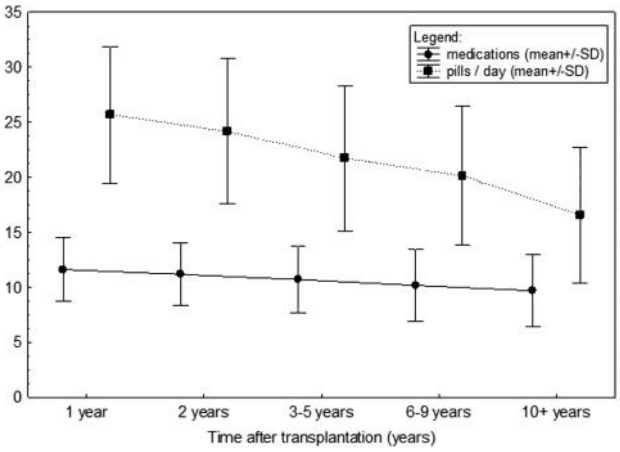
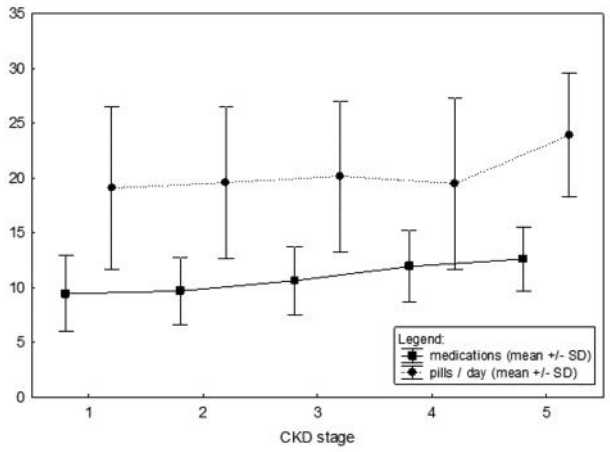


Figure 2: Medication and pill burden versus stage of chronic kidney disease (CKD)

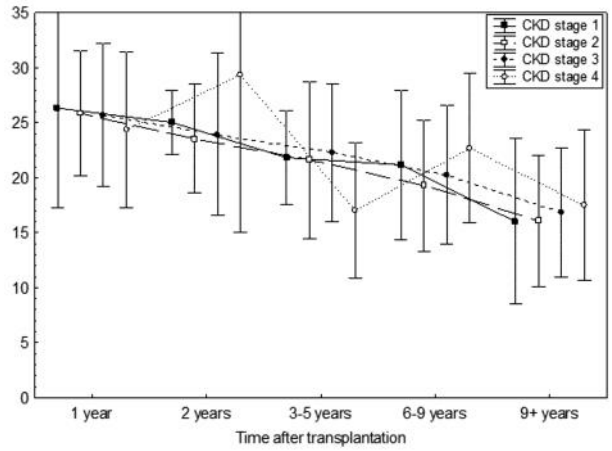


was high. However, this was cross-sectional study and not longitudinal. The type of medications was not analyzed in detail, so strategies to reduce pill burden may not be precisely guided by the results of the study.

Explanation of study findings

Association of male gender with medication burden after adjusting for age, diabetes and other covariates may be explained by higher comorbidity of males with end-stage renal disease. As well known from the registry data, males are significantly younger when initiating RRT as compared to women. In 2012 in Slovenia, mean age of incident RRT patients was 65.5 ± 14.6 (median 68 years) for men and 69.9 ± 12.8 (median 70.5 years) for women, respectively (14). Decreasing pill burden with time after transplantation may be explained by decreasing immunosuppression and prophylactic antimicrobial therapy prescribed early after transplantation. Increasing pill burden with the stage of CKD is probably associated with CKD complications needing additional therapy. Putting together, it seems that pill burden is more significantly associated with time after transplantation than with CKD stage (Figure 3).

Figure 3: Pill burden versus time after transplantation, stratified by stage of chronic kidney disease (CKD)



Implications for clinical practice and research

Our study results may increase focus on strategies to reduce pill burden. It was already shown in ADMIRAD study that lowering pill burden may improve adherence (10). Once-a-day formulations may be further expanded in the future. Monitoring of adherence may work for many, even majority of patients, however, not for all. Longitudinal studies on medication burden and risk of interaction may be desirable in future, with detailed analysis of specific drugs contributing to pill burden. Such a focus is especially important in the reality of the increasingly complex kidney graft recipients with more comorbidities as compared to the past.

In conclusion, medication and pill burden in kidney graft recipients is high, with the average of 10 medications and 20 pills prescribed per day. Male gender, CKD stage and time after transplantation

were independent predictors of the number of pills prescribed. Strategies to reduce medication and pill burden should be explored in the future to avoid drug interactions, improve patients' adherence and make their life easier.

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