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Impact of cotrimoxazole prophylaxis on serum creatinine and potassium levels in renal transplant recipients

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Abbreviations

CTX: Cotrimoxazole

TMP: Trimethoprim

SMX: Sulfamethoxazole

PCP: Pneumocystis pneumonia

GFR: Glomerular filtration rate

eGFR: Estimated glomerular filtration rate

CKD: Chronic kidney disease

HIV: Human immunodeficiency virus

AIDS: Acquired Immunodeficiency Syndrome

CUSL: Cliniques Universitaires Saint-Luc

ACEI: Angiotensin Converting Enzyme inhibitor

ARB: Angiotensin II Receptor Blocker

NSAID: Non-Steroidal Anti-Inflammatory Drug

SD: Standard deviation

ESKD: End-stage kidney disease

CMV: Cytomegalovirus

HBs: Hepatitis B Surface antigen

HCV: Hepatitis C Virus

KDIGO: Kidney Disease : Improving Global Outcomes

CRP: C-reactive protein

BP: Blood pressure

Abstract - English

Administration of cotrimoxazole (CTX) 800/160 mg 3 times a week is the standard prophylaxis of pneumocystis pneumonia in renal transplant recipients as per KDIGO guidelines. The impact of CTX on serum creatinine and potassium is well quantified when used in doses of 800/160 mg daily and more. Whether the prophylactic regimen significantly influences serum creatinine and potassium is unknown, and our hypothesis is that it does. The objective of this study is thus to assess and quantify the impact of CTX prophylaxis on creatinine and potassium levels. The medical records of two hundred and eighteen consecutive recipients of a kidney transplant at CUSL between June 2012 until December 2015 were analyzed retrospectively. This cohort received a standard CTX prophylaxis during 6 months. With time of CTX discontinuation as the time-point of reference, monthly lab results - before and after stop - were compared. Continuous variables were compared using a t-test with unequal variances. Multivariate analysis was conducted with a panel data linear fixed effects model for periods and robust cluster variance estimation. Between period 6 (last intake of CTX) and 7 (1 month after last intake of CTX) serum creatinine decreased significantly (-0.111 mg/dl.) This 8% decrease in serum creatinine contrasts with the absence of significant change of serum creatinine in the months before 6 or after 7. By multivariate statistical analysis we found that impact of CTX on serum creatinine was dependent on baseline serum creatinine (p-value <0.001), which is consistent with the fact that tubular secretion of creatinine (blocked by CTX at higher doses) is known to increase as serum creatinine increases. In addition, serum urea did not drop at the time of CTX discontinuation, a finding further supporting the causal link between CTX discontinuation and serum creatinine decrease. Serum potassium decreased significantly by -0.093 mmol/L (CI95% -0.173 to -0.014 mmol/L) after CTX discontinuation. For clinicians, the significant reversible impact of low dose CTX prophylaxis on serum creatinine should be considered when interpreting the evolution of serum creatinine over time. The absence of such a drop at the time of CTX discontinuation or a seemingly mild increase should trigger the suspicion of a problem. As to the potassium results, clinicians facing hyperkalemia in KTRs should seriously consider stopping low dose CTX prophylaxis, as it reversibly contributes to hyperkalemia. For registries, and large databases, the main implication is that comparisons of creatinine based eGFR between studies or registries are not completely reliable if some units continue low dose CTX prophylaxis indefinitely whereas others stop after 3 or 6 months (like at CUSL).

Abstrait – Français

Administration de cotrimoxazole (CTX) 800/160 mg 3 fois par semaine est la prophylaxie standard de pneumonie à pneumocystis chez des transplantés rénaux selon les recommandations KDIGO. L'impact du CTX sur la créatinine sanguine et la kaliémie est bien quantifiée pour des doses journalières de 800/160 mg ou plus. Si le CTX influence ces taux sanguins à des doses prophylactiques, ce que nous pensons être vrai, est actuellement inconnu. L'objectif de cette étude est donc d'élucider et quantifier l'impact du CTX à des doses prophylactiques sur la créatinine sanguine et la kaliémie. Les rapports médicaux consécutifs de deux-cent dix-huit receveurs de greffe rénale aux CUSL entre Juin 2012 et Decembre 2015 ont été analysés rétrospectivement. Cette cohorte recevait une prophylaxie standard de CTX durant 6 mois. Avec temps d'arrêt de CTX comme référence, résultats laboratoires mensuels – pré et post arrêt – ont été comparés. Des variables continues ont été comparées en utilisant des t-tests avec des variances inégales. Des analyses multivariées ont aussi été effectuées. Entre période 6 (dernière prise CTX) et 7 (1 mois après dernière prise CTX) la créatinine sanguine a diminué significativement (-0.111 mg/dl). Cette diminution de 8% de la créatinine contraste avec l'absence de changement significatif dans les mois avant p6 ou après p7. On a trouvé, par analyse statistique multivariée, que l'impact du CTX sur la créatinine sanguine était dépendante de la créatinine sanguine de base (p-value <0.001), ce qui est consistent avec le fait que la sécrétion tubulaire de créatinine (bloquée par CTX à des doses plus hautes) augmente lorsque la créatinine sanguine augmente. L'urée sanguine n'a pas changé à l'arrêt du CTX, ce qui renforce le lien causal entre l'arrêt du CTX et la diminution de la créatinine sanguine. La kaliémie diminuait significativement de -0.093 mmol/L (CI95% -0.173 to -0.014 mmol/L) après l'arrêt du CTX. Pour les cliniciens, l'impact réversible significatif de la prophylaxie low-dose à CTX sur la créatinine sanguine devrait être considéré lors de l'interprétation de l'évolution de la créatinine sanguine à travers le temps. L'absence de cette diminution lors de l'arrêt de CTX ou une légère augmentation doit faire suspecter un problème rénal réel. Concernant le potassium, le clinicien en face d'une hyperkaliémie chez un greffé rénal doit sérieusement considérer d'arrêter la prophylaxie CTX, car elle contribue réversiblement à l'hyperkaliémie. Pour registres et bases de données grandes, l'implication principale est que la comparaison d'eGFR à base de créatinine entre études ou registres ne sont pas complètement fiables si certaines unités continuent une prophylaxie CTX low-dose indéfinitivement et d'autres l'arrêtent après 3 ou 6 mois (comme CUSL).

Introduction

Cotrimoxazole (CTX) is an old (1968) antibiotic drug composed of one part of trimethoprim (TMP) and 5 parts of sulfamethoxazole (SMX). It is one of the most commonly prescribed drugs on the planet and is on the WHO list of essential medicines. It is commonly used in nephrology to treat urinary tract infections. It is also used for prophylaxis and treatment of pneumocystis pneumonia (PCP), a serious opportunistic pulmonary infection – caused by the ubiquitous fungus *Pneumocystis jirovecii* [6] In a study of 544 patients with proven PCP infections in 17 hospitals in France, 41% had AIDS and the other 59% suffered from other immunosuppressive disorders. [6] Renal transplant recipients fall into the category of immunosuppressed patients, and as such receive a prophylactic regime of CTX after kidney transplantation. Patients at Cliniques Universitaires Saint Luc (CUSL) receive CTX 800/160 mg 3 times-a-week during 6 months following kidney transplantation, as first-choice prophylaxis in line with the KDIGO recommendation of 3 to 6 months prophylaxis following transplantation. [14]

PCP and recent outbreaks

Pneumocystis jirovecii (formally known as Pneumocystis carinii) is an opportunistic fungal pathogen that can cause life threatening pneumonia in immunocompromised patients, such as kidney transplant recipients, with a fatality rate around 50% even when adequate treatment is administered. [14, 15] The typical clinical picture of pneumocystis pneumonia is a patient with hypoxemia, cough and dyspnea which are disproportionate to physical and radiologic findings. [7] Symptoms typically manifest 6 to 8 weeks following the start of immunosuppressive therapy in kidney transplant recipients. [14] PCP diagnosis is made upon organism detection in lung tissue or in lower respiratory tract secretions. [14] When treating PCP, CTX is given in high doses, usually 15 to 20 mg/kg of TMP and 75 to 100 mg/kg of SMX daily, typically given in three to four equally divided doses for 3 weeks, and parenterally if PaO2 is under 70 mmHg, and the administration of corticosteroids should be considered. [1, 15]

But what about PCP prophylaxis? A recent systematic review of PCP outbreaks found thirty outbreaks between 1980 and March 2015 in India, Australia, France, UK, Japan, Switzerland,

Germany, Netherlands, Puerto Rico, Norway, USA. [3, 4, 5] Solid organ transplant recipients were concerned in twenty-five (83%) outbreaks, primarily renal transplant recipients (77% of total outbreaks). [5] It is important to note that no affected patient was receiving optimal PCP prophylaxis, an indirect demonstration that PCP prophylaxis is effective. [7] If no PCP prophylaxis is given, circa 1 in 20 kidney transplant recipients will acquire PCP. [15] As stated, CTX is the antibiotic of choice in PCP prophylaxis. [14] CTX prophylaxis is well-tolerated and is used near-universally to prevent pneumocystis pneumonia. It has the additional advantage to prevent other infections caused by common urinary, respiratory, gastrointestinal pathogens and uncommon opportunistic microorganisms. [7]

CTX pharmacology

CTX is available globally in tablet, oral suspension, or in intravenous form. Tablets exist in doses of either single strength, 400mg SMX and 80mg TMP, or double strength, 800mg SMX and 160mg TMP. The administration of the standard oral 5:1 ratio ensures adequate *in vivo* peak serum levels. [1]

KDIGO guidelines recommend that kidney transplant recipients receive PCP prophylaxis with daily TMP-SMX for 3-6 months after transplantation. [14] Similarly the EBPG Guidelines on Renal Transplantation recommend that kidney transplant recipients receive CTX 400/80 mg daily or 800/160 mg every other day for at least 4 months. [15] CTX at a dose of one double-strength tablet three times weekly is an acceptable alternative dosing schedule that may result in fewer adverse effects. [1]

TMP and SMX inhibit 2 successive steps in the pathway for folic acid production [1]. This does in theory pose a problem with human folic acid biosynthesis but because CTX inhibits the bacterial enzyme versions at a 50 000 – 60 000 times higher power than the mammalian enzyme and because humans use dietary folate, CTX use is not problematic in humans for folic acid levels.[1]

TMP and SMX *per os* are well absorbed and distributed, and serum levels are similar to those seen after intravenous administration. [1] Max serum levels of TMP and SMX are seen around 10 hours after administration, and their half-life range from 11 to 17 h depending on kidney function, and in CKD patients can be doubled or even tripled. [1, 11, 12] Doses must be modulated for patients with lowered renal function, as drug blood levels are considerably higher and half-life increases up to 20 to 30 hours or more [1].

Concerning their metabolization, approximately 70% of SMX is acetylated and glucuronide-conjugated in the liver. [1, 11] SMX is mostly protein bound (70%), although a significant part of the drug circulates as free drug. [1] The vast majority of SMX (85%) is excreted in the urine, mostly as its acetylated metabolite. [1] As for TMP, it is excreted in the urine without any metabolization. [1, 11]

CTX side effects

CTX is, like all active compounds in medicine, not without side effects. Some of these are potentially life-threatening, which warrants further studies to elucidate its cost-benefit profile. Uncommon and severe side effects include aseptic meningitis as described in case reports, toxic epidermal necrolysis, teratogenicity, hematological toxicity and acute renal toxicity (acute interstitial nephritis, obstructive tubulopathy). [8] Numerous drug interactions have been identified and result from its P450 inhibiting effect. Notable interactions include ACEIs, ARBs, warfarin, methotrexate, NSAIDs, oral hypoglycemic drugs, fluvastatin and phenytoin [8] Hypoglycemia is common as a drug-drug interaction with sulphonylureas or meglitinides. [8] The two common side effects which are of particular interest to our study are hyperkalemia and GFR-independent serum-creatinine elevation.

Creatinine-elevation

The renal elimination of creatinine, the universally used marker for GFR, is modified during CTX treatment. The nephrotoxic effect of SMX was first described in 1973 by Kalowski et al, in a study of 16 patients who had received CTX therapy dosed at 1 to 4 single-strength tablets

daily. [13] A serum creatinine increase from 3.0 ± 0.56 to 5.6 ± 0.92 mg/dl and creatinine clearance decrease from 40 to 16 ml/min was seen in all patients, and was irreversible in 3 patients, 2 of which had acute tubular necrosis upon kidney biopsy. [13] SMX was determined as the causal agent because of the well-recognized nephrotoxic effects of sulphonamides.

In 1975, the first important study on the effect of TMP on serum creatinine was published. [12] When 21 patients were treated with CTX 1600/160 mg daily for 12 days, Berglund et al. noted a reversible (5 days after treatment discontinuation) increase of serum creatinine by an average of 0.22 mg/dl or 20%, with increases ranging from 0 to 0.4 mg/dl, which upon further study could be produced with TMP alone but not with SMX alone. [17] Interestingly, the higher the baseline serum creatinine, the more important the increase in serum creatinine tended to be, which was interpreted as a competitive inhibition of the tubular secretion of creatinine. This conclusion was further strengthened when oral administration of creatinine before CTX administration potentiated its creatinine elevating effects. [17] To elucidate whether this increase in serum creatinine was due to actual kidney toxicity or not, the team used an exogenous reference GFR marker (iothalamate) to measure both the creatinine clearance and GFR in 4 healthy subjects before, during and after CTX therapy and they discovered that the creatinine-related changes were not associated with any significant change in GFR. [17] Numerous studies have used an exogenous GFR marker but they have all studied therapeutic daily doses of CTX.

A review article published by Delanaye et al. shows that no study to date has revealed any "nephrotoxicity" by TMP in monotherapy. [12] Current data shows that TMP does in fact, 2 to 6 hours after administration, induce a GFR-independent reversible increase in serum creatinine through tubular creatinine secretion inhibition, which, in healthy subjects, accounts for 13 to 23% of total kidney creatinine elimination. [12] [10] Just like Berglund and al.

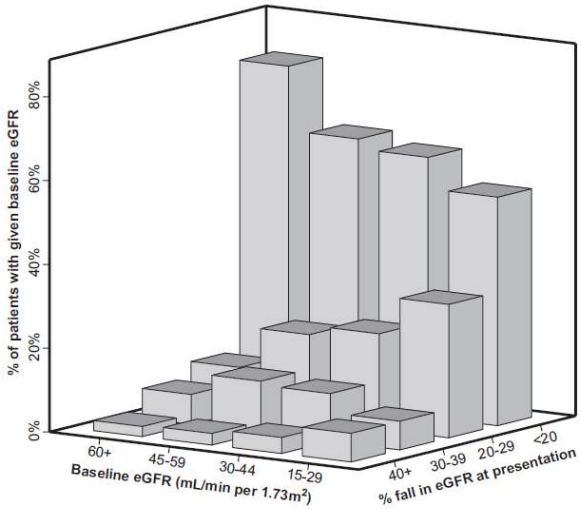

Figure borrowed from Mitsides et al. [18] showing the percentage fall in eGFR from baseline to presentation, divided by base-line eGFR

proposed, current knowledge shows that the effect of TMP is more pronounced in CKD patients, leading to over 35% increase in serum creatinine. [12]

The finding in Delanaye et al.'s review article that alerted us as to the relevance of our study was the fact that the lowest dose of TMP studied at the time of publication (2011) was 160 mg per day, which means that the effect of TMP on creatinine at any dose lower than 160 mg daily is unknown. [12] Since Delanaye's study was published, a few studies on the effects of CTX at prophylactic levels have emerged. Recent studies of the effect of TMP on creatinine in renal transplant recipients [18, 20] tried to answer similar questions as to our current one but because of the structure of their studies were unable to give a precise answer. Mitsides et al found a rise in serum creatinine in 22% of renal transplant recipients under low-dose (<80mg TMP daily) CTX prophylaxis. [18] They found a creatinine increase of 13% after 6 months of CTX prophylaxis in patients without adverse effects when compared to serum creatinine at start of CTX prophylaxis. It is important to note that no post-CTX-discontinuation control period was measured which is crucial for the drawing of a solid conclusion on degree of elevation due to CTX.

Our study is pertinent in that we are aiming to pinpoint the degree of effect of CTX on blood creatinine levels at a prophylactic dose of CTX of 800/160 mg 3-times-a-week. Our hypothesis is that CTX has a creatinine-elevating effect at prophylactic dosing. While guidelines for PCP prophylaxis for HIV patients [2] are specific and universally accepted, there is no consensus regarding PCP prophylaxis for other immune-compromised patients. Because CTX is a drug with adverse effects, some of which can be life-threatening, there is a need for its study to further understand its risk-benefit profile.

Hyperkalemia

Another interesting question was the potassium-elevating effect of CTX. TMP has been shown to be a reversible inhibitor of sodium channels in the amiloride-sensitive collecting duct of the nephron. [10] At a physiological level, the intracellular transport of sodium is linked to the extracellular transport of potassium. Because the sodium channels and potassium channels in the

collecting duct have an interlinked functioning, the administration of CTX results in a potassium-sparing diuretic effect and therefore entails a risk of hyperkalemia.

Velasquez et al found that the administration of high-dose TMP (20mg/kg per day) to PCP-infected AIDS patients brought forth an elevation of blood potassium levels by a mean +0.6mEq/L despite normal adrenocortical function and GFR, and that hyperkalemia, defined as a serum potassium levels over 5 mEq/L, was diagnosed in 15 of the 30 patients. [10] Severe and potentially life-threatening hyperkalemia (potassium > 6.0 mmol/L) occurred in three patients (10%).

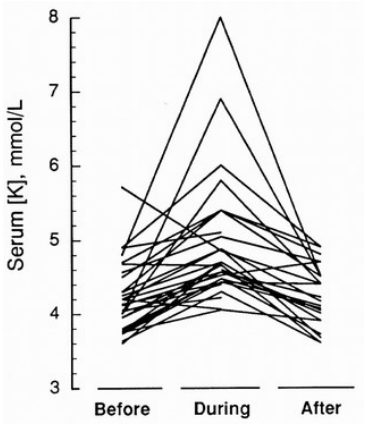

Figure borrowed from Velasquez et al. [10] showing the change in kalemia with high-dose TMP (20 mg/kg daily)

Hyperkalemia can be lethal as a result of cardiac arrythmia, etc. Hyperkalemia is common, and tends to develop after several days of therapy, particularly in patients with renal insufficiency, high-dose CTX, older age, diabetes, AIDS, and administration of ACEi, ARB, spironolactone or NSAIDs. [8] A population-based case–control study, with a 14-year study period, involving 439677 patients taking ACEIs or ARBs found the risk of hospital admission for hyperkalemia to be 7 times higher among those taking CTX compared with those taking other antibiotics used for urinary tract infections. [9]

In a retrospective study, electrolyte disorders, defined as Na < 135 mEq/L and/or K > 5.0 mEq/L, were found in fourteen of the fifty-three oncology patients (26.4%) after 5 days of CTX treatment, at an average dose of 145.7 +/- 24.9 mg/day. [11] The patients were divided into 3 groups on the basis of TMP dose: Low-dose group (under 80 mg daily), stadard-dose group (80-120 mg daily), high-dose group (over 120 mg daily). Higher doses were associated with a greater risk of electrolyte disorders. Forty-five out of fifty-three received CTX prophylactically. Mori et al. also found that the increase in electrolyte

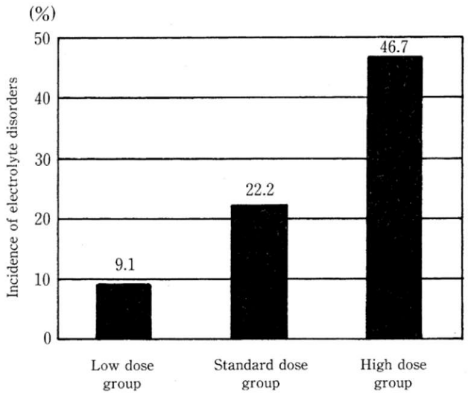

Figure borrowed from Mori et al. [11] illustrating the incidence of electrolyte disorders (Hyponatremia and/or hyperkalemia) in three groups. Numbers are the percentage of patients with electrolyte disorders.

disorders caused by CTX is dose-dependent and that a greater TMP dose increased the risk of electrolyte disorder with an odds ratio of 2.35. Electrolyte disorders were seen in 9.1% and 22.2% of patients given the low dose (TMP < 80 mg) or standard dose (TMP 80-120 mg) of CTX, respectively. [11] Renal dysfunction was strongly correlated with the risk of electrolyte disorders (85.7% prevalence with renal dysfunction compared with 17.5% prevalence among patients with normal renal function) with an odds ratio of 80.29. [11] Multiple linear regression analysis revealed a significant correlation between changes in Na concentration and the dose of TMP, although the level of K was not correlated with the dose of TMP but with renal function. [11]

Higashioka et al. [19] studied PCP prophylaxis by means of CTX at under 80mg daily TMP in a Japanese immune-deficient population and found that 17.2% developed hyperkalemia, defined as blood potassium levels over 5mEq/L. They also found that blood potassium reached the same values in patients under prophylactic doses and patients under therapeutic doses of CTX. Because this is a single-center study conducted in a Japanese study without a control population, and the study population was not kidney transplant recipients, we believed that further study of the potassium-modulating effects of CTX at a prophylactic dose was reasonable.

Methods

Patients

In this retrospective study, we included patients having benefited from a kidney transplantation at Cliniques Universitaires Saint Luc (CUSL) in Brussels, Belgium from June 2012 until December 2015. The long-term post-transplant follow-up of these patients was done in the outpatient Nephrology clinic unit of CUSL. All kidney transplant recipients at CUSL receive a 6-month post-transplant CTX-prophylaxis as per KDIGO guidelines, unless there is any contraindication. [14] They come to the outpatient nephrology clinic at CUSL twice a week during 2-3 months then at least once a month during the first year post-transplantation, and are subject to a blood test every visit.

The patients meeting the following characteristics were excluded from the study: a. minors (under 18 years old) at the time of transplantation, b. Pregnant women, c. Combined organ transplant recipients, d. Patients receiving therapeutic doses of CTX (800/160 mg or more daily) e. CTX discontinuation before 3 months of prophylaxis f. Re-start of dialysis or death before one month of post-CTX-discontinuation period.

Since this is a retrospective study, information was collected through the patients' electronic medical records via 'Medical Explorer' at CUSL. Two hundred and eighteen patients were included in the study. Surgical protocols, laboratory results, and renal transplantation outpatient clinic and hospitalization reports were studied.

The following renal transplant recipient characteristics were noted Date of birth, Age at transplantation, Gender, Number of renal transplants, cause of ESKD, Pre-operative treatment (Hemodialysis, Peritoneal dialysis, or No dialysis), Duration of dialysis if present, Weight, Height, Allergies, ABO blood group, Viral status (HBs, HIV, HCV, CMV, Syphilis), HLA Compatibility, Plasmapheresis use and duration.

The following donor characteristics were noted: Living or deceased, Relationship to recipient, Graft donor location origin (internal/external), Type of preservation fluid, ABO blood group, CMV status.

The following transplantation intra-operative data was noted: Duration of operation, Duration of ischemia.

The following biological data, with data collected from time of transplantation until time of data extraction, was studied: Serum creatinine, Serum urea, Serum uric acid, Serum sodium, Serum potassium, Serum bicarbonate, Serum chloride, blood hemoglobin, Serum CRP.
We also collected sequential Systolic BP, Diastolic BP, BMI, medication names and dosages, through the Nephrology clinic outpatient consultation reports.

The biological data was collected at the time of stop of CTX prophylaxis, which usually was at 6 months post-transplantation. Then data at 1 month after stop of prophylaxis was collected, at 2 months post stop, at 3 months post stop, and at 6 months post stop. Data was also collected 1 month before stop of prophylaxis, 2 months before stop, 3 months before stop, 4 months before stop and 5 months before stop.
Name and dosage of medications at time of outpatient clinic was collected from the medical records.

Statistical methods

Continuous variables were compared using a t-test with unequal variances**.** Multivariate analysis was conducted with a panel data linear fixed effects model for periods and robust cluster variance estimation**.** All analyses were conducted in Stata v15. P-values <0.05 were considered as statistically significant.

Results

Table 1 : Patient population description

Variable	Value
Number of patients	218
Gender, Male	145 (66.5%)
Mean age at transplantation (years)	51 (18y min, 82y max)
Cause of ESKD	
- **Polycystic kidney disease**	34 (15.6%)
- **Alport disease**	10 (4.6%)
- **Congenital nephro-uropathy**	6 (2.8%)
- **IgA nephropathy**	18 (8.3%)
- **Diabetic nephropathy**	18 (8.3%)
- **Autoimmune/inflammatory disease**	4 (1.8%)
- **Nephrosclerosis**	9 (4.1%)
- **Other glomerulonephritis**	32 (14.7%)
- **Vasculitis**	4 (1.8%)
- **Chronic interstitial nephritis**	16 (7.3%)
- **Drug nephrotoxicity**	7 (3.2%)
- **Idiopathic**	36 (16.5%)
- **Other**	24 (11.0%)
Serum creatinine level (mg/dl)	
- **3 months before stop**	1.51
- **At stop**	1.50
- **3 months after stop**	1.37
Serum potassium level (mmol/L)	
- **3 months before stop**	4.14
- **At stop**	4.09
- **3 months after stop**	3.99
Pre-transplant dialysis	
- **Hemodialysis**	159 (72.60%)
- **Peritoneal dialysis**	22 (10.05%)
- **No dialysis**	38 (17.35%)
Number of current kidney transplantation	
- **1**	197 (90.37%)
- **2**	20 (9.17%)
- **3 or more**	1 (0.46%)
Relationship of recipient to donor	
- **Living donor, related**	34 (15.74%)
- **Living donor, unrelated**	21 (9.72%)
- **Deceased**	161 (74.54%)

Creatinine part

The mean value of serum creatinine in this study across all periods was 1.472 ± 0.557 mg/dl (mean ± SD). When dividing the patients into 2 periods of either receiving CTX or not, without taking into account other variables, the mean serum creatinine was 1.529 ± 0.594 mg/dl (mean ± SD) in the period of CTX prophylaxis whereas after CTX stop the mean serum creatinine was 1.383 ± 0.481 mg/dl (mean ± SD).

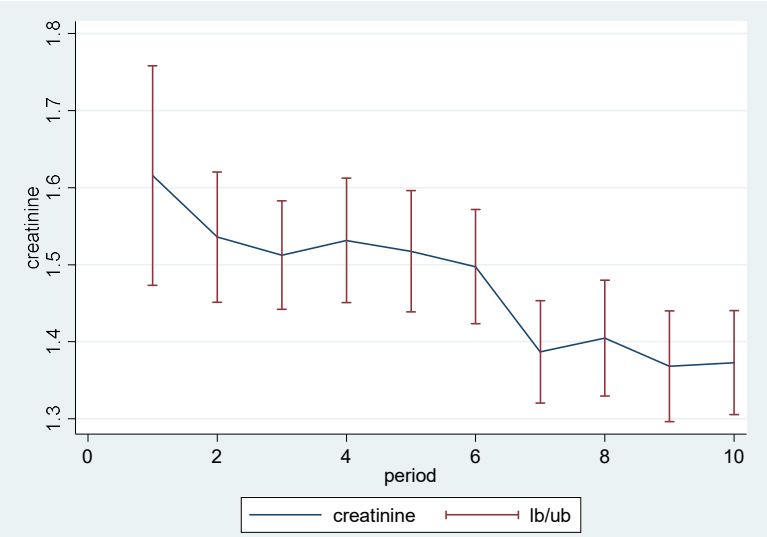

Figure 1 : Mean serum creatinine (mg/dl) over time period; Period 6 corresponds to time of CTX discontinuation; Periods 1-9 are separated monthly; Period 9 and 10 are separated by 3 months; lb = lower border of CI95%; ub = upper border of CI95%

The value of serum creatinine per period of observation is illustrated in figure 1, and the serum creatinine difference between sequential periods of observation is illustrated in figure 2.

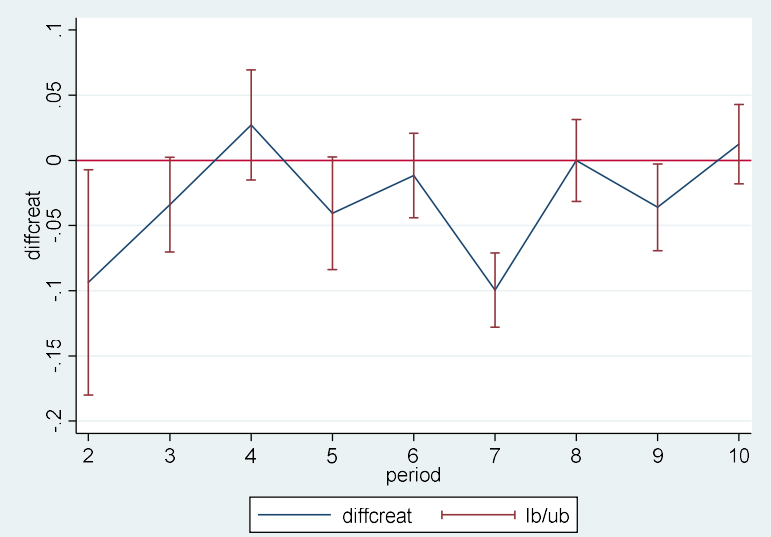

Figure 2 : Mean serum creatinine sequential difference (mg/dl) compared to previous period over time period; Period 6 corresponds to time of CTX discontinuation; Periods 1-9 are separated monthly; Period 9 and 10 are separated by 3 months; lb = lower border of 95% CI; ub = upper border of 95% CI

T-test of serum creatinine change between period 6 (last intake of CTX) and 7 (1 month after last intake of CTX) was statistically significant with a measured change in serum creatinine of -0.111 mg/dl (CI95% -0.210 to -0.012 mg/dl, p-value 0.029). This corresponds to a +8% decrease in serum creatinine

Regression equations showed a mean general effect of CTX on serum creatinine, without taking into account the period, of +0.157 mg/dl (CI95 0.128-0.186 mg/dl) or +11.4% increase from the mean baseline serum creatinine level of 1.376 mg/dl (CI95 1.359-1.394 mg/dl, p-value <0.001).

Upon statistical analysis of creatinine by CTX intake and baseline serum creatinine we also found that the impact of CTX on serum creatinine was statistically significantly larger if the baseline serum creatinine was higher. Upon multivariate statistical analysis of serum creatinine as a function of period, CTX intake and baseline serum creatinine, we found that the serum creatinine change by period was -0.012 mg/dl (CI95 -0.018 to -0.007 mg/dl). We also confirmed that the impact of CTX on serum creatinine was indeed dependent on base serum creatinine.

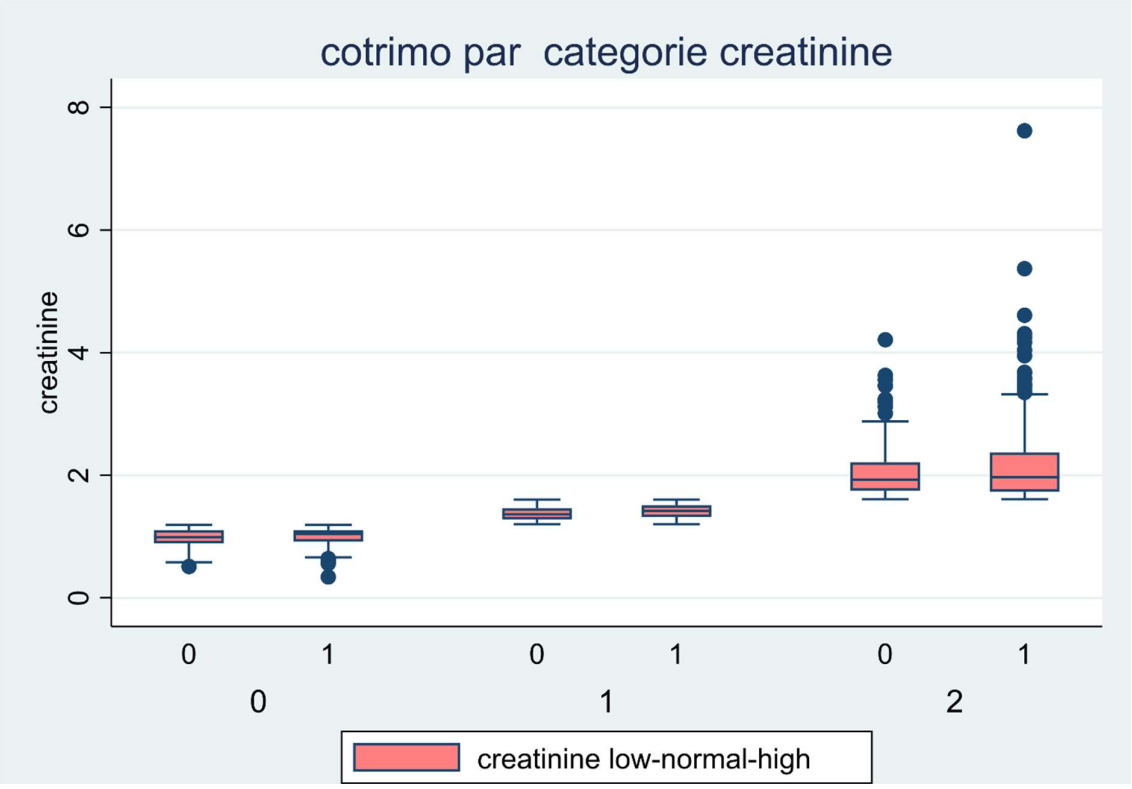

Figure 3 : Box-plot of mean serum creatinine before and after CTX discontinuation [1 or 0] with sub-categorization by function of baseline serum creatinine [0 or 1 or 2] into a a low creatinine group (<1.11 mg/dl), an intermediate group or a high creatinine group (>1.67 mg/dl); Contains median, interquartile range, min, max, outliers.

We found that the change in serum creatinine in patients with a baseline serum creatinine under 0.80 mg/dl was -0.082 mg/dl (n=5) between time of CTX discontinuation and 1 month after discontinuation. On the other hand, patients with a baseline serum creatinine over 1.3 mg/dl had a mean change in serum creatinine of -0.115 mg/dl (n=89) in that same time frame.

When repeating the multivariate analysis of the determinants of creatinine level as a function of CTX intake, period, baseline creatinine and additionally dividing the patient population into 3 groups based on serum creatinine - a low creatinine group (<1.11 mg/dl), a high creatinine group (>1.67 mg/dl), and an intermediate group – we found that CTX elevated serum creatinine by +0.142 mg/dl (CI95% 0.099 to 0.184 mg/dl, p-value <0.001) in the high creatinine group, and that the effect of CTX on the low and intermediate groups were statistically insignificant (p-value 0.088).

Table 2 :Mean serum potassium and creatinine by period of observation

	-5mo	***-4mo***	***-3mo***	***-2mo***	***-1mo***	***STOP***	***+1mo***	***+2mo***	***+3mo***	***+6mo***
Mean creatinine	1.616	1.536	1.512	1.532	1.517	1.498	1.387	1.405	1.368	1.373
Mean potassium	4.409	4.221	4.144	4.151	4.138	4.093	3.999	4.036	3.985	3.946

Potassium part

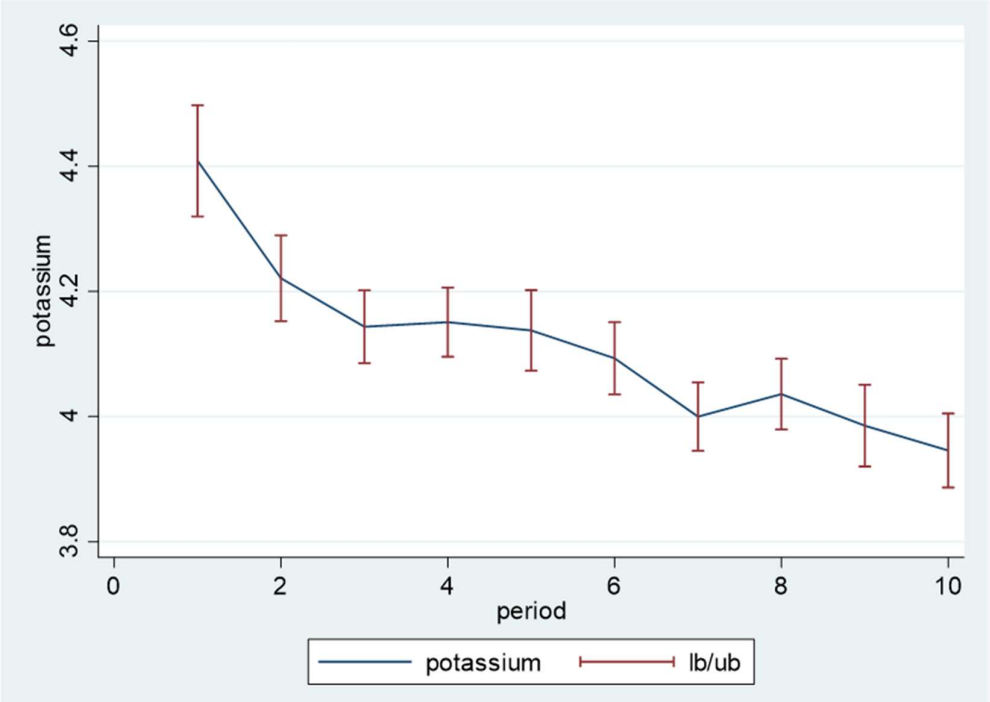

Figure 4 : Mean serum potassium (mmol/L) over time period; Period 6 corresponds to time of CTX discontinuation; Periods 1-9 are separated monthly; Period 9 and 10 are separated by 3 months; lb = lower border of CI95%; ub = upper border of CI95%

The mean value of serum potassium in this study across all periods was 4.104 ± 0.444 mmol/L (mean ± SD). When dividing the results into 2 periods of either receiving CTX or not, without taking into account other variables, the mean serum potassium was 4.177 ± 0.454 mmol/L (mean ± SD) in the CTX period whereas in the no-CTX period the mean serum potassium was 3.992 ± 0.405 mmol/L (mean ± SD). The mean change in serum potassium starting from the CTX to the no-CTX period was therefore -0.185 mmol/L (CI95% -0.224 to -0.145). The mean difference between serum potassium levels in period 6 (last intake of CTX) and 7 (1 month after last intake of CTX) was statistically significant with a decrease of -0.093 mmol/L (CI95% -0.173 to -0.014 mmol/L, p-value 0.021).

Upon regression analysis of serum potassium with CTX use we found that serum potassium increased by +0.179 mmol/L (CI95% 0.148 to 0.210 mmol/L) under CTX.
Upon regression analysis of serum potassium with CTX use and serum potassium we found the effect of CTX on serum potassium was indeed dependent on the serum potassium (p <0.001).

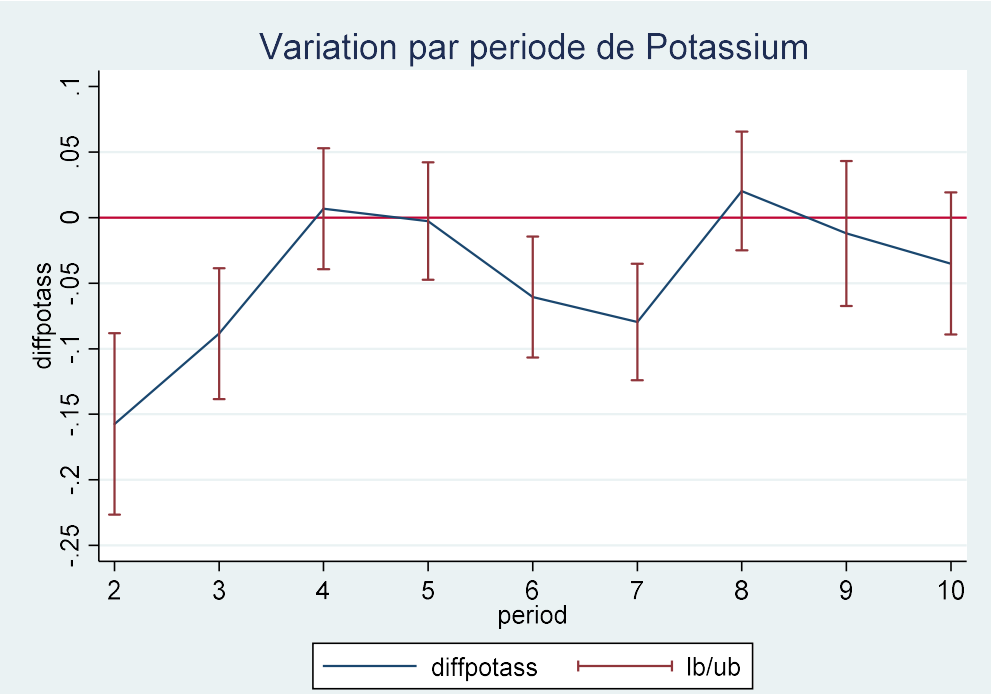

Figure 5 : Mean serum potassium sequential difference (mmol/L) compared to previous period over time period; Period 6 corresponds to time of CTX discontinuation; Periods 1-9 are separated monthly; Period 9 and 10 are separated by 3 months; lb = lower border of CI95%; ub = upper border of CI95%

The other biological variables (sodium, uric acid, CRP, etc.) showed no or minimal linear correlation with serum creatinine with the exception of urea, as expected. However, urea did not drop between period 6 and 7 which was the case with serum creatinine.

Upon regression analysis of risk of hyperkalemia (>5 mmol/L) and administration of the drugs mentioned in figure X, we found that NaHCO3 administration was a risk factor (OR 5.89, p-value 0.002) for hyperkalemia, as was insulin administration (OR 4.25, p-value 0.046). The odds ratios for all other drugs were not statistically significant. However, ACEI use (OR 2.78, p-value 0.102), beta-blocker use (OR 2.79, p-value 0.077), and kayexalate use (OR 5.49, p-value 0.058) were close to being statistically significant and are probably correlated with risk for hyperkalemia if a larger patient population was studied. Thiazide (observations = 7) and everolimus (observations = 4) use could not be analyzed by regression analysis because of small sample sizes. As described in Table 4, when comparing intake of medication during period 3 (month 3 prior to CTX discontinuation) with medication during period 9 (month 3 after CTX discontinuation) the number of patients being prescribed the same drug was roughly equivalent.

Table 3 : Relationship between drug and risk of hyperkalemia (>5mmol/L)

	Odds ratio	***Std. Err.***	***Z-test***	***P-value***	***CI 95%***
Advagraf	0.36	0.694	-0.53	0.596	0.008 - 15.490
Medrol	1.45	2.690	0.20	0.841	0.038 - 55.065
ACEI	2.78	1.737	1.63	0.102	0.815 - 9.464
Sartan	2.62	2.266	1.11	0.266	0.480 - 14.277
Loop diuretic	0.42	0.382	-0.95	0.342	0.073 - 2.480
Thiazide	-	-	-	-	-
Beta-blocker	2.79	1.615	1.77	0.077	0.896 - 8.674
NaHCO3	**5.89**	3.345	3.12	**0.002**	1.932 - 17.929
Vichy	2.66	1.583	1.65	0.100	0.830 - 8.536
Insulin	**4.25**	3.090	1.99	**0.046**	1.025 - 17.666
Kayexalate	5.49	4.926	1.90	0.058	0.947 - 31.859
Everolimus	-	-	-	-	-

Table 4 : Description of number of medication prescriptions per period

	Number of patients being prescribed X drug											
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
Period 3	140	138	20	16	24	1	55	22	26	12	3	2
Period 9	164	163	36	15	24	3	70	24	31	16	4	1

Abbreviations 1 : A. Advagraf ; B. Medrol ; C. ACEI ; D. Sartan ; E. Loop diuretic ; F Thiazide ; G. Beta-blocker ; H. NaHCO3 ; I. Vichy ; J. Insulin ; K. Kayexalate ; L. Everolimus

Male gender was shown to be statistically significantly correlated with higher serum potassium when compared to female gender, by +0.128 mmol/L (IC95% 0.028 to 0.228 mmol/L, p-value 0.012), with female patients having a mean serum potassium of 4.023 mmol/L (IC95% 3.941 to 4.105 mmol/L).

When dividing the patients into two groups, based on age at time of transplantation, of either A. under 65 years of age or B. patients aged 65 and older we found no statistically significant added risk of hyperkalemia in either group. Patients in group B had an increase in serum potassium of +0.331 mmol/L (IC95% -0.086 to 0.152 mmol/L, p-value 0.585) compared to group A. However, we found a statistically significant increase of mean serum potassium by +0.004 mmol/L (IC95 0.0004 to 0.007 mmol/L) with every added year of age at transplantation.

Only 81 out of the 218 patients presented a complete series of data across all periods. We therefore verified the results on those complete cases (810 observations). This shows virtually identical results.

Discussion

Our hypothesis proved correct: CTX at a dose of 800/160 3 times weekly has an elevating and reversible effect on serum creatinine. +8%, or +0.111 mg/dl (CI95% -0.210 to -0.012 mg/dl, p-value 0.029), with a clear drop in serum creatinine after CTX discontinuation. We also found that the impact of CTX on serum creatinine was statistically significantly larger if the baseline serum creatinine was higher, which is consistent with the evidence that TMP is a reversible inhibitor of tubular creatinine secretion. The proportion of tubular creatinine secretion is 13-23% in normal subjects whereas that proportion increases to over 35% in CKD patients. [12]

The specific creatinine tubular effect is further supported by the fact that urea serum level did not change significantly between T 6 and 7.

We recorded a downward trend in serum creatinine from time of transplantation and onwards. The monthly decline in serum creatinine was steepest between time of CTX discontinuation and 1 month later. Between 3 months before CTX discontinuation and 5 months before CTX discontinuation (i.e. a month after TP), the decline in serum creatinine was also relatively high. This decline is largely observed between month 1 and 2 after TP, and likely reflects progressive recovery of kidney function after TP (as a result of recovery from cold/warm ischemia etc…) to the question we are trying to answer This is irrelevant and at the very least unnecessarily confounding the questions of this study.

As for serum potassium we confirmed the reversible potassium-elevating effect of CTX. Serum potassium followed a similar but milder and less accentuated progression compared to the evolution of serum creatinine within the same time period.

We found that the serum potassium elevating effect of CTX was dependent on the kalemia. Previous studies have demonstrated that renal insufficiency is a risk factor for hyperkalemia. Several pathophysiological factors favor the development of hyperkalemia in CKD patients: (1) reduced urine output impairs renal secretion; (2) metabolic acidosis, a common complication of ESKD, affects potassium distribution between the ICF and ECF; (3) comorbidities, such as

diabetes though plasma hyperosmolality; (4) drugs such as ACE inhibitors or ARBs. [21] We found a correlation between serum creatinine and serum potassium, which tends to be consistent with studies showing that renal insufficiency is a risk factor for hyperkalemia.

We found that use of NaHCO3 and/or insulin were statistically significant risk factors for hyperkalemia. As aforementioned, we can only conclude a correlation and not causation between the risk for hyperkalemia and use of drug. Just like kayexalate, insulin is a treatment and not a cause for a hyperkalemia-related disorder. Diabetes, through its plasma hyperosmolarity effects and risk for acidosis, increases the risk for hyperkalemia, and insulin is a late-stage treatment necessity in type 2 diabetes patients.

We couldn't find a statistically significant added risk of hyperkalemia with use of ACEI, ARB, etc. ACEIs in particular are drugs that have been demonstrated to be risk factors for hyperkalemia with concomitant prophylactic doses of CTX. [19]
An interesting finding was that male gender was shown to be statistically significantly correlated with higher serum potassium when compared to female patients, by +0.128 mmol/L (IC95% 0.028 to 0.228 mmol/L, p-value 0.012), with female patients having a mean serum potassium of 4.023 mmol/L (IC95% 3.941 to 4.105 mmol/L). Especially interesting was that Higashioka et al found male gender to be an additional risk factors for hyperkalemia in the subpopulation of patients with renal insufficiency. [19] So this is the second time that male gender has been found to be correlated with risk for hyperkalemia in patients receiving under 80 mg TMP daily, and further study into the cause for this association would be interesting.
On a general note, regarding the interpretation of our results, it is important to understand that correlation does not equal causation. Kayexalate administration was correlated across all studied periods with high kalemia, but of course the kayexalate treatment is not the cause, but the treatment for the high kalemia. With this example in mind, it is evident that from a purely statistical standpoint we cannot draw conclusions regarding causation but only correlation.

One issue encountered during this study was the fact that the information available to us in the medical records was not standardized. Since the outpatient clinic records at CUSL are handwritten, and by various different doctors, there arises a risk for error in interpretation which

may sometimes have been the case in this study. Two doctors can write the same written note on the consultation sheet, and the intended message might be different. In this study, this issue was present in the written note regarding CTX discontinuation. Some wrote "-> STOP CTX", others wrote "STOP CTX", others "-> CTX FIN BOITE" and so on. These statements are up for interpretation by the reader and may have led to errors in this study, that in turn negatively influence the precision of our analysis of the degree of creatinine elevation due to CTX. My reasoning is that this has led to an underrating of the creatinine elevation due to CTX since some of the time-points of CTX discontinuation will be located outside of the period 6-7 comparison range which is important to this study. That being said, this imprecision would thus have tended to blur our results toward the null hypothesis, whereas we found a significant impact of low dose CTX prophylaxis discontinuation

Another issue was the unverifiability of patient compliance to prescribed medication. Here again, this source of imprecision would have tended to blur our results toward the null hypothesis, whereas we found a significant impact of low dose CTX prophylaxis discontinuation

Overall, the implications of our results are both for clinicians and registries.
For clinicians, the significant impact of low dose CTX prophylaxis on serum creatinine should be taken into account when interpreting the evolution of serum creatinine over time. The absence of such a drop at the time of CTX discontinuation or a mild increase (not triggering otherwise any concern) should trigger the suspicion of a problem (including rejection, BK virus nephropathy, etc…). As to the K results, clinicians facing hyperkalemia in KTR should seriously consider stopping low dose CTX prophylaxis, as it contributes to hyperkalemia.
For registries, and large databases, the main implication is that comparisons of creatinine based eGFR between studies or registries are not completely reliable if some units continue low dose CTX prophylaxis indefinitely whereas others stop after 3 or 6 months (like at CUSL).

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