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ORIGINAL ARTICLE

Efficacy and safety of interleukin-1 blockers in kidney transplant recipients with familial Mediterranean fever: a propensity score–matched cohort study

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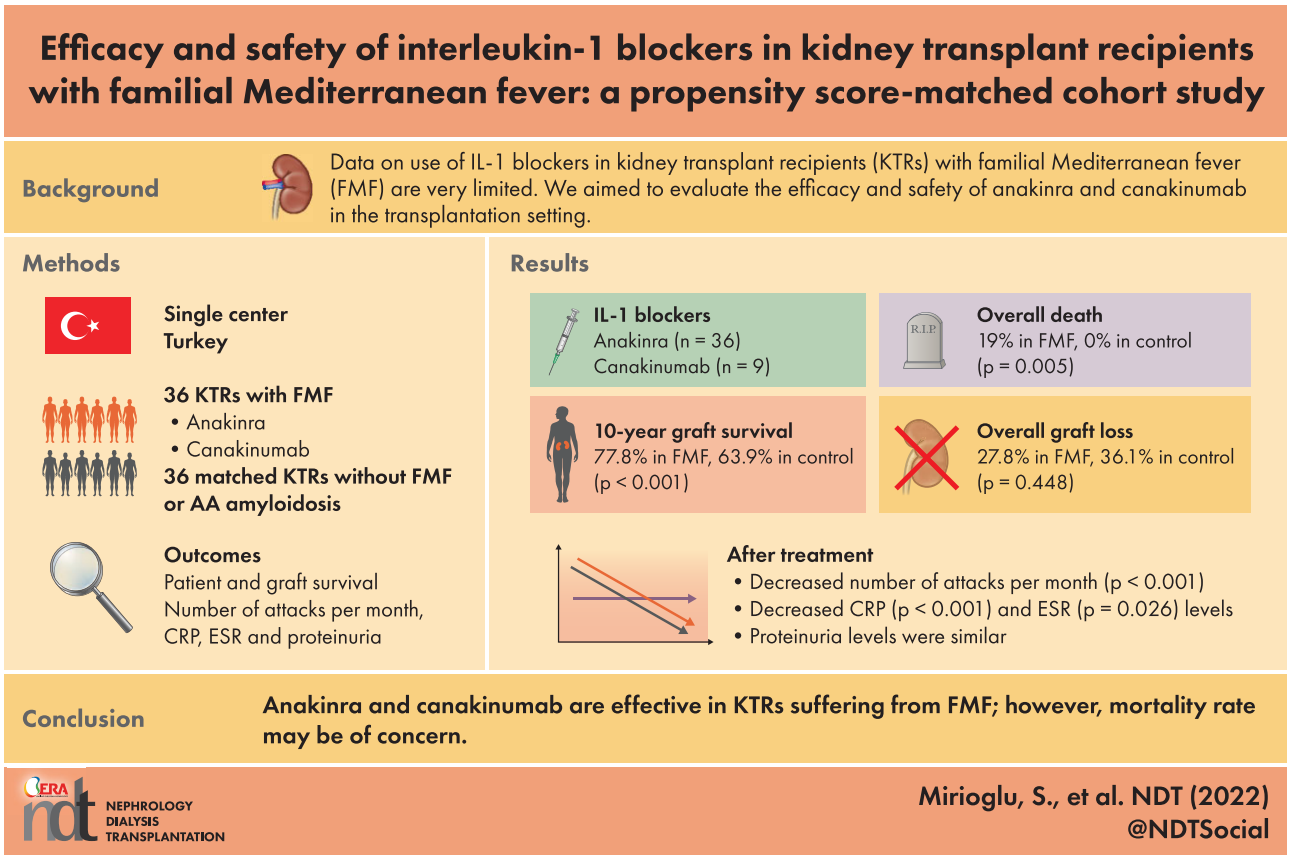
GRAPHICAL ABSTRACT



KEY LEARNING POINTS

What is already known about this subject?

- Familial Mediterranean fever (FMF), the most common inherited autoinflammatory disease with a high prevalence in populations originating from eastern Mediterranean, is characterized by recurrent episodes of fever and serositis accompanied by increased levels of several acute-phase reactants including serum amyloid A (AA) protein, and AA amyloidosis is the most severe complication which can result in kidney dysfunction and increased mortality.
- Several observational studies and two randomized clinical trials demonstrated the efficacy and safety of anakinra [a recombinant human interleukin (IL)-1 receptor antagonist] and canakinumab (a human IL-1β monoclonal antibody) in patients with colchicine-resistant/intolerant FMF in recent years.
- Only a handful of papers with very limited number of patients have reported use of IL-1 blockers in kidney transplant recipients (KTRs) with FMF so far.

What this study adds?

- This study evaluated the efficacy of anakinra and canakinumab in KTRs whose primary kidney diseases were AA amyloidosis caused by FMF and the safety of these agents using a propensity score-matched control group of KTRs without FMF or AA amyloidosis.
- Anakinra and canakinumab were found to be very effective in KTRs suffering from FMF with longer graft survival and lower rejection rates despite the increased number of deaths.
- The difference in patient survival might have partially originated not only from infections but also progression of the deposition of amyloid fibrils in various organs and tissues, especially cardiovascular and gastrointestinal systems.

What impact this may have on practice or policy?

- To the best of our knowledge, this is by far the largest study on use of anakinra and canakinumab for FMF in KTRs.
- These findings may provide some guidance for transplant nephrologists and rheumatologists in the care of KTRs with FMF.

ABSTRACT

Background. Data on use of interleukin (IL)-1 blockers in kidney transplant recipients (KTRs) with familial Mediterranean fever (FMF) are very limited. We aimed to evaluate the efficacy and safety of anakinra and canakinumab in the transplantation setting.

Methods. In this retrospective cohort study, we included KTRs who suffered from AA amyloidosis caused by FMF and treated with anakinra or canakinumab (study group, $n = 36$). Using propensity score matching, we selected 36 patients without FMF or amyloidosis from our database of 696 KTRs as the control group. Primary outcomes were patient and graft survival. Biopsy-confirmed graft rejection, changes in estimated glomerular filtration rate (eGFR), high-sensitivity CRP (hsCRP), erythrocyte sedimentation rate (ESR), proteinuria and number of monthly attacks were secondary outcomes.

Results. All KTRs with FMF began IL-1 blocker therapy with anakinra and nine (25%) were switched to canakinumab. Overall death was more frequent in the study group (19.4% vs 0%) ($P = .005$); however, overall graft loss was comparable between study (27.8%) and control groups (36.1%) ($P = .448$). Five- and 10-year graft survival rates were significantly higher in the study group (94.4% and 83.3%, respectively) than in the control group (77.8% and 63.9%, respectively) ($P = .014$ and $P < .001$, respectively). Rejections were numerically lower in study group (8.3% vs 25%), but it did not reach to statistical significance ($P = .058$). When compared with the pre-treatment period, with IL-1 blockers, the number of attacks per month ($P < .001$), and eGFR ($P = .004$), hsCRP ($P < .001$) and ESR ($P = .026$) levels were lower throughout the follow-up, whereas proteinuria levels were not.

Conclusions. Anakinra and canakinumab are effective in KTRs suffering from FMF; however, the mortality rate may be of concern.

Keywords: amyloidosis, anakinra, canakinumab, familial Mediterranean fever, kidney transplantation

INTRODUCTION

Familial Mediterranean fever (FMF) is the most common inherited autoinflammatory disease, and is characterized by recurrent episodes of fever and serositis with a high prevalence in populations originating from the eastern Mediterranean [1, 2]. FMF typically has an autosomal recessive pattern of inheritance and the causative gene, *MEFV*, encodes the pyrin protein [3, 4]. Variants of *MEFV* associated with the disease have been demonstrated to increase activation of inflammasome leading to interleukin (IL)-1β production [5]. Beyond its clinical manifestations including self-limited attacks of fever, peritonitis, pleuritis, synovial inflammation, erysipelas-like erythema and myalgia accompanied by increased levels of several acute-phase reactants like C-reactive protein (CRP) or serum amyloid A (SAA) protein, amyloid A (AA) amyloidosis is the most severe complication of FMF, which can result in multiple organ dysfunctions and increased mortality [6, 7].

Goals of treatment in FMF are to prevent inflammatory attacks and to control subclinical inflammation in between those attacks, thereby to avoid complications including AA amyloidosis [8]. Colchicine is the mainstay of the treatment with its well-established efficacy, whereas up to 10% of patients show inadequate response to colchicine with recurrent attacks and some patients are intolerant to colchicine mainly due to

its gastrointestinal adverse effects [2, 8]. Along with increasing burden of AA amyloidosis, these drawbacks triggered the research for new biologic therapies. As a result, several observational studies and two randomized clinical trials have demonstrated the efficacy and safety of anakinra (a recombinant human IL-1 receptor antagonist) and canakinumab (a human IL-1β monoclonal antibody) in patients with colchicine-resistant/intolerant FMF (crFMF) in recent years [2, 9, 10].

The number of kidney transplant recipients (KTRs) who suffer from end-stage kidney disease (ESKD) due to FMF-associated AA amyloidosis has increased considerably, and continuing attacks and graft loss due to recurrence of amyloidosis are a major concern in this population [7]. However, only a handful of papers with very limited number of patients have reported use of IL-1 blockers in KTRs so far [11–14].

Therefore, in this cohort study, we aimed to evaluate the efficacy of anakinra and canakinumab in KTRs whose primary kidney diseases were AA amyloidosis caused by FMF and the safety of these agents using a propensity score–matched control group of KTRs without FMF or AA amyloidosis.

MATERIALS AND METHODS

Study design

We examined data of adult KTRs (at least 18 years of age) who underwent kidney transplantation (KTx) due to ESKD caused by FMF-related AA amyloidosis and followed at our institution between 1992 and 2019. A total of 36 KTRs who were treated with anakinra and/or canakinumab were included in the study group. Patients were diagnosed to suffer from FMF according to Tel-Hashomer criteria [15]. Genetic analyses of the *MEFV* gene were recorded. In addition, KTRs who manifested with only AA amyloid deposition and harbored two pathogenic variants in the *MEFV* gene was considered to have "phenotype two" FMF [1]. Colchicine at a dosage of 1.5 mg/day was considered as initial therapy in patients with normal kidney functions. Resistance or inadequate response to colchicine was defined as recurrent clinical attacks (at least one attack per month over 3 months) and/or laboratory evidence of persistent inflammation in attack-free periods [16]. Starting in 2008, patients who showed inadequate response or significant side effects due to colchicine or had recurrent AA amyloidosis in the graft when using colchicine were first switched to anakinra, then canakinumab if necessary. Initiation dose was 100 mg/day for anakinra which was escalated to 200 mg/day if needed. Canakinumab was started as 150 mg/month. While using IL-1 blockers, patients continued to use colchicine with a target dose of 1–1.5 mg/day as long as they tolerated the drug.

Using the propensity score matching, 36 patients without FMF or AA amyloidosis were selected as a control group from a database of 696 KTRs followed at our institution. Primary kidney diseases in this group were as follows: five congenital anomalies of the kidney and urinary tract, three focal segmental glomerulosclerosis, three hypertensive nephrosclerosis, two chronic glomerulonephritis, two polycystic kidney disease, and one of each with chronic tubulointerstitial nephritis, chronic pyelonephritis, diabetes mellitus, fibronectin glomerulopathy and immunoglobulin A (IgA) nephropathy. Underlying kidney diseases in 16 KTRs were unknown; however, lack of any evidence (i.e. medical history, clinical examination and laboratory findings) in these patients helped to exclude FMF and amyloidosis. Flow chart of the study is shown in Fig. 1.

All transplant candidates with a history of amyloidosis were initially evaluated by echocardiography. A cardiac MRI was performed if the echocardiographic findings were suggestive or unclear for cardiac amyloidosis. Patients were eliminated from the transplant program if results were in line with cardiac amyloidosis and heart failure [17]. When patients with cardiac amyloidosis were asymptomatic or echocardiographic findings of heart failure were not detected, performing or avoiding KTx was decided after a consultation with an experienced cardiologist on a case-by-case basis.

All study procedures were conducted according to good medical and laboratory practices and the recommendations of the Declaration of Helsinki on biomedical research involving human subjects or its later amendments. This study was approved by the local ethical committee in our institution (2019/1345). All patients enrolled in the study provided written informed consent to extract their medical data into the center's research database. Reporting was carried out in line with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [18].

Follow-up principles and immunosuppressive management

Patients were followed at the transplantation outpatient clinic weekly in the beginning, and intervals were increased to 1 month after the first 6 months and 3 months at the end of the first year. Laboratory data including serum creatinine, albumin, high-sensitivity CRP (hsCRP), erythrocyte sedimentation rate (ESR), serum levels of tacrolimus (Tac) and cyclosporine (CsA), complete blood count and quantitative proteinuria measurements were retrieved from patients' charts. All laboratory values were measured using standard enzymatic procedures. Urinary protein-to-creatinine ratio in the first morning urine specimen was used to estimate quantitative proteinuria and reported as g/g. Estimated glomerular filtration rates (eGFRs) were calculated with Chronic Kidney Disease Epidemiology Collaboration formula [19]. Laboratory parameters measured during an acute infection episode were not included in the analyses. Graft biopsy was performed to patients in case of a graft dysfunction and/or proteinuria $\geq$1 g/g.

Induction therapy (ATG Fresenius, 2 mg/kg/day, for 3–7 days) was used in all transplantations from deceased donors. All patients received intraoperative methylprednisolone bolus injection at a dosage of 500 mg, and afterwards were treated by triple maintenance immunosuppressive regimen including a calcineurin inhibitor (CNi) (Tac or CsA), an antiproliferative drug [mycophenolate mofetil (MMF), mycophenolate sodium (MPS) or azathioprine (AZA)] and low-dose prednisolone. CNis were initiated 2 days and antiproliferatives 1 day before

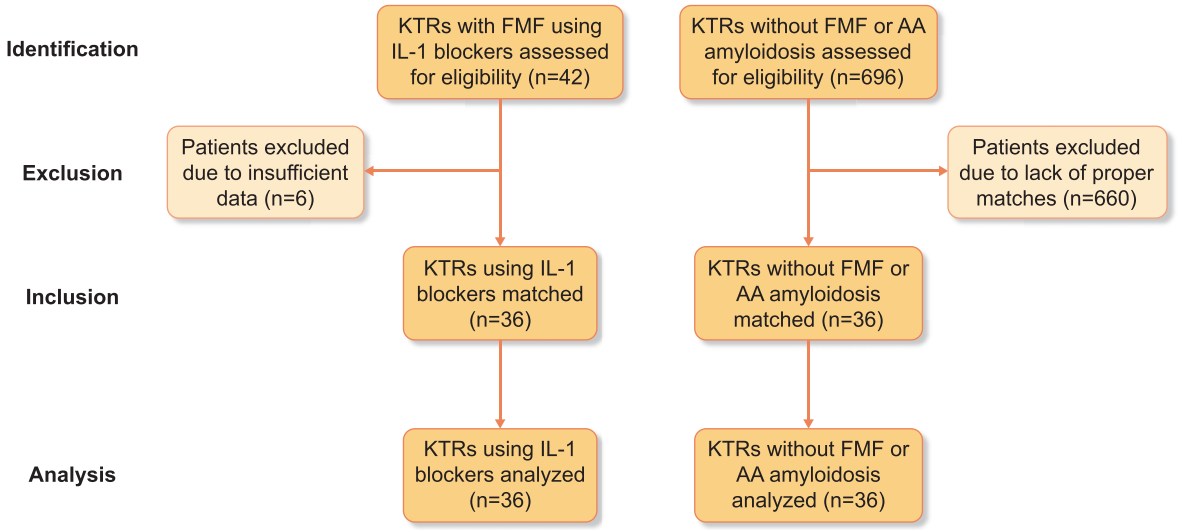


Figure 1: Flow chart of the study.

in living donor transplantations. Target trough blood levels of Tac and CsA were 8–12 ng/mL and 200–300 ng/mL for first 3 months, and 4–8 ng/mL and 50–150 ng/mL for subsequent months, respectively. MMF and AZA were administered at dosages of 2 g/day (1440 mg/day for MPS) and 1.5 mg/kg/day, respectively. On postoperative Day 1, patients received methylprednisolone 120 mg daily, with a rapid taper and reaching to maintenance dose of 10 mg prednisolone daily by the end of the first month and 5 mg daily within 3 months. If necessary, alterations were made in treatment strategies per immunologic risk and posttransplant complications. Since CsA is known to increase the level or effect of colchicine [20], Tac was chosen over CsA in patients using colchicine or colchicine dose was reduced in patients using CsA.

Histopathological evaluation

Adequate renal biopsy specimens, which were defined as having 10 or more glomeruli with at least two arteries, were evaluated. Three to four micrometer sections were used for all histochemical and immunohistochemical staining. 0.4–0.6 cm unfixed tissue was frozen with liquid nitrogen for immunofluorescence staining (IgG, IgM, IgA, C1q, C3, fibrinogen, and kappa and lambda light chains). Remaining tissues were fixed in Hollande's fixative, embedded in paraffin, and processed routinely for light microscopic evaluation (hematoxylin and eosin, periodic acid–Schiff, methenamine silver–periodic acid, Masson trichrome, Congo red). Banff criteria were used when evaluating the specimens by light microscopy [21–24]. C4d staining was performed by immunohistochemistry on paraffin embedded tissue blocks. Linear and circumferential staining in peritubular capillaries were regarded as positive according to the Banff scoring system (C4d >0) [21–24]. Histopathological diagnosis of AA amyloidosis in the kidney was determined by demonstration of an apple green birefringence under polarized light by Congo red staining and positive immunostaining with specific monoclonal antibodies to SAA.

Study outcomes

Primary outcomes were patient and graft survival rates. Graft loss was defined as the graft dysfunction necessitating dialysis or re-transplantation, or death with a functioning graft. Death-censored graft survival (DCGS) rates were evaluated separately. Secondary outcomes were biopsy-confirmed graft rejection, changes in eGFR, hsCRP, ESR and proteinuria levels, and monthly attack frequencies between the time of IL-1 blocker initiation and last follow-up. Infections, malignancies, leukopenia episodes and injection site reactions were recorded as adverse events. Impacts of various features of recipients and donors [including age at KTx, sex, donor type (living/deceased), donor age, donor sex, number of human leukocyte antigen (HLA) mismatches] on graft survival were also investigated.

Statistical analyses

Statistical analyses were performed by using SPSS for Windows (SPSS version 25.0, IBM Corp., Armonk, NY, USA). Propensity scores were generated using a multivariable logistic regression model based on recipient sex, donor type (living or deceased), donor sex and number of HLA mismatches, and the continuous variables of recipient age, donor age and follow-up duration (months) [25]. In order to obtain the most similar KTR control without FMF and amyloidosis for each case with FMF-associated amyloidosis, matching process was executed by using the nearest neighboring method in 1:1 ratio [25].

Results were expressed as mean ± standard deviation (SD) when normally distributed or as median [interquartile range (IQR)] otherwise. Categorical variables were shown as frequencies (%). Comparisons of continuous variables between the two groups were evaluated by *t*-test or the Mann–Whitney *U* test, where appropriate. Paired *t*-test or Wilcoxon signed-rank test was used to compare various features before and after treatment with IL-1 blockers. Differences in the proportions of different patient groups were compared using the chi-squared

Table 1: Demographic, clinical and laboratory characteristics of all patients.

Characteristics	Study group ($n = 36$)	Control group ($n = 36$)	P
Age at transplantation (years), mean ± SD	35.2 ± 12.3	34.7 ± 11.7	.861
Sex (male), *n* (%)	22 (61.1)	19 (52.8)	.475
Donor age (years), mean ± SD	45.1 ± 12.6	43.6 ± 10.7	.601
Donor sex (male), *n* (%)	16 (44.4)	14 (38.9)	.633
Donor type, *n* (%)			
Living	32 (88.9)	34 (94.4)	.394
Deceased	4 (11.1)	2 (5.6)	
Number of HLA mismatches, median (IQR)	3 (3–3.75)	3 (3–5)	.764
Duration of follow-up (months), median (IQR)	115.5 (80.8–138.5)	55.5 (33.3–61.8)	**<.001**
Transplantation year, *n* (%)			
1992–99	1 (2.8)	0 (0)	.561
2000–09	21 (58.3)	20 (55.6)	
2010–17	14 (38.9)	16 (44.4)	

HLA: human leukocyte antigen.

test or the Fisher's exact test. Paired *t*-test or Wilcoxon signed-rank test was used to compare various features of KTRs with FMF before IL-1 blocker initiation and at the last follow-up. Univariate survival comparisons were made by using the log-rank test. Patient and graft survival rates were analyzed using Kaplan–Meier curves and graft survival time for each patient was computed from KTx time to the last follow-up or the primary outcome. Variables previously found to affect graft survival in the univariate analyses ($P < .10$) were included in multivariate Cox proportional hazards model. Results of this model were described as hazard ratios (HRs) and 95% confidence intervals (CIs). Graphics were generated using MedCalc for Windows (MedCalc version 19.0, MedCalc Software, Ostend, Belgium). All tests were two sided and a *P*-value of ≤.05 was considered as statistically significant.

RESULTS

Baseline characteristics of all patients

In total, 72 KTRs (56.9% male) with a mean age of 34.9 ± 12 (range 18–61) years who were followed for a median duration of 64.5 (43.3–115.8) months after KTx were included in the study. Pretransplant panel reactive antibodies (PRA) were <10% in all patients. Demographic, clinical and laboratory characteristics of the patients were generally comparable between study and control groups (Table 1). Even though years of transplantations were similar ($P = .561$), the study group had a longer duration of follow-up as compared with the control group ($P < .001$).

Features of FMF

Thirty-five KTRs (97.2%) were diagnosed with FMF according to Tel-Hashomer criteria (phenotype 1) and one (2.8%) was considered to have phenotype two FMF, who was homozygous for M694V. Most of the patients (33, 92.1%) harbored an M694V variant of *MEFV*, 26 of whom (72.2%) were homozygous. Eleven patients (30.6%) underwent graft biopsy due to the described indications. Eight KTRs had recurrence of AA amyloidosis, two had T-cell mediated rejection, and one had borderline rejection. Biopsy details are provided in Supplementary data, Table S1. While using colchicine, 18 (50%) experienced gastrointestinal intolerance and 3 (8.3%) had elevations of aminotransferase levels. Inadequate response to colchicine (28, 77.8%) and recurrent AA amyloidosis in grafts (7, 19.4%) were the main causes of IL-1 blocker use. All KTRs used anakinra and nine (25%) were switched to canakinumab due to inadequate response ($n = 8$) or moderate to severe injection site reactions ($n = 1$). IL-1 blockers were initiated 29.9 ± 12.1 years after the onset of FMF. Two patients (5.6%) started to use anakinra 1 month and 24 months before KTx, whereas remaining 34 (94.4%) began IL-1 blocker therapy after a median of 75.3 (48.6–114.4) months after KTx. None of the patients was known to have been complicated by cardiac amyloidosis before KTx. Most of the KTRs (86.8%) were on a triple immunosuppressive regimen consisting of a CNi, an antiproliferative agent and low-dose prednisolone at the time of IL-1 blocker initiation. Various features of patients with regard to FMF are summarized in Table 2.

Study outcomes

Overall, seven patients (9.7%) died. Death was significantly higher in study group (7, 19.4%) as compared with the control group (0) ($P = .005$). Infections ($n = 4$) were the leading cause of death. One patient who was diagnosed with cardiac amyloidosis after KTx died due to sepsis following a graft loss caused by recurrent amyloidosis (Supplementary data, Table S2). Overall graft loss rates were comparable between the study (10, 27.8%) and control groups (13, 36.1%) ($P = .448$). However, death-censored graft loss rate was significantly lower in the study group (4, 11.1%) compared with the control group (13, 36.1%) ($P = .013$). Kaplan–Meier analyses revealed that 5-year graft survival rate was significantly higher in the study group (94.4%) than in the control group (77.8%) ($P = .014$) (Fig. 2a), and DCGS rates at 5 years were 100% and 77.8%, respectively ($P = .001$) (Supplementary data, Fig. S1). The graft survival rate was still significantly higher at 10 years in the study group (83.3% vs 63.9%) ($P < .001$) (Fig. 2**b**). DCGS at 10 years were 94.4% in the study and 63.9% in control groups ($P < .001$) (Supplementary data, Fig. S2).

In total, 12 KTRs (16.7%) experienced biopsy-proven graft rejection 57.7 ± 45.3 months after KTx. Even though it did not reach statistical significance, the rejection rate was numerically lower in study group (3, 8.3%) compared with the control group (9, 25%) ($P = .058$). In the study group, rejection episodes were seen 32.4 ± 27.8 months after IL-1 blocker initiation. No KTRs in study group had antibody-mediated rejection (Table 3).

KTRs in the study group were followed for 30.1 (9.6–57.7) months after IL-1 blocker initiation. Eight of them (22.2%) were tested for PRA and donor-specific antibodies (DSA) during the follow-up, and one patient had a 46% class I PRA, but no patients had DSA. One patient had a recurrence of AA amyloidosis in the graft while using anakinra, and hence was switched and responded well to canakinumab. Another KTR

Table 2: Various characteristics of patients regarding FMF.

Characteristics	Study group ($n = 36$)
FMF phenotype, *n* (%)	
Phenotype 1	35 (97.2)
Phenotype 2	1 (2.8)
Clinical manifestations, *n* (%)	
Fever	32 (88.9)
Serositis	33 (91.7)
Peritonitis	32 (88.9)
Pleuritis	12 (33.3)
Pericarditis	4 (11.1)
Arthritis	28 (77.8)
Distribution of *MEFV* variants, *n* (%)	
M694V homozygosity	26 (72.2)
M694V heterozygosity	5 (13.9)
M694V/V726A compound heterozygosity	2 (5.6)
Genetic analysis not performed	3 (7.9)
Indications for IL-1 blockers, *n* (%)	
Inadequate response to colchicine	28 (77.8)
Recurrence of AA amyloidosis in the graft	7 (19.4)
Gastrointestinal intolerance to colchicine	1 (2.8)
IL-1 blockers, *n* (%)	
Anakinra	36 (100)
Canakinumab	9 (25)
Indications for the switch to canakinumab, *n* (%)	
Inadequate response to anakinra	8 (22.2)
Moderate to severe injection site reactions with anakinra	1 (2.8)
Posttransplant colchicine dose (mg/day), median (IQR)	1 (1–1.5)
Age at IL-1 blocker initiation (years), mean ± SD	41.6 ± 12.8
Disease duration before IL-1 blocker initiation (years), mean ± SD	29.9 ± 12.1
Posttransplant duration before IL-1 blocker initiation (months), median (IQR)	73.8 (34.1–112.2)
Immunosuppressive regimen at IL-1 blocker initiation, *n* (%)	
CNi + MPA + prednisolone	18 (50)
CNi + AZA + prednisolone	13 (36.1)
mTORi + MPA + prednisolone	2 (5.6)
CNi + mTORi + prednisolone	1 (2.8)
Pretransplant	2 (5.6)

MPA: mycophenolic acid; mTORi: mTOR inhibitor.

who was started on anakinra due to recurrent AA amyloidosis in the graft was further diagnosed with cardiac amyloidosis. Number of attacks per month ($P < .001$) and levels of hsCRP ($P < .001$) and ESR ($P = .033$) decreased throughout the follow-up. The mean eGFR declined from 59.2 ± 26.2 to 50.2 ± 25.3 mL/min/1.73 m^2 ($P = .004$). Proteinuria and serum albumin levels remained quite similar (Table 4).

IL-1 blockade requires titration of anti-IL-1 treatment dose according to patient's IL-1 activity, but all patients received standard dose of 100 mg/day anakinra at the beginning. Nine patients in the study group (25%) were switched to canakinumab 150 mg/month, eight of whom (88.9%) responded quite well to the treatment possibly due to dose-dependent relatively higher IL-1 blocking activity. One KTR experienced graft loss caused by a rejection. The unresponsive patient who was homozygous for M694V died with a functioning graft due to pneumonia.

When the study group was analyzed with regard to the *MEFV* variants, KTRs with M694V homozygosity (26, 72.2%) were younger at the time of KTx (32.5 ± 10.5 vs 42.2 ± 14.5 years, respectively, $P = .032$) and more balanced in terms of distribution of sex (50% vs 90% males, $P = .027$) compared with the other patients (10, 27.8%). Most of the characteristics and study outcomes were generally similar between KTRs who were homozygous for M694V and those who were not (Supplementary data, Table S3).

Infection rates were comparable between study (17, 47.2%) and control groups (14, 38.9%) ($P = .475$). Pneumonia and other respiratory tract infections were more common in the study group (10 vs 3 patients), whereas urinary tract infections were mostly seen in the control group (3 vs 8 patients). One patient in each group developed malignancies which were non-Hodgkin lymphoma and cervical carcinoma *in situ*. Two patients in study group (5.6%) experienced leukopenia (Table 3). Four patients with FMF (11.1%) suffered from injection-site reactions with anakinra.

Predictors of graft loss

In regression models, we evaluated the effects of several variables (recipient and donor age and sex, donor type and number of HLA mismatches) present at the time of KTx

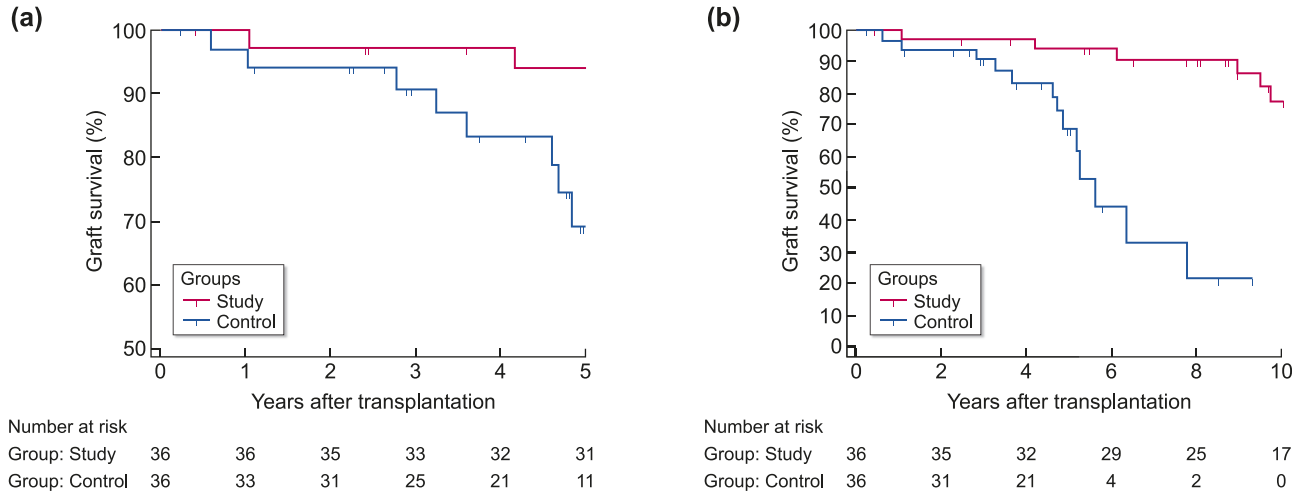


Figure 2: Graft survival rates at 5 (**a**) and 10 years (**b**) were significantly higher in study group when compared with the control group ($P = .014$ and $P < .001$, respectively).

and using IL-1 blockers on graft loss. In multivariate Cox regression analysis, usage of IL-1 blockers was significantly associated with reduced graft loss rates (HR 0.039, 95% CI 0.010–0.180, $P < .001$). Number of HLA mismatches (HR 1.817, 95% CI 1.174–2.813, $P = .007$) also predicted the graft loss (Supplementary data, Table S4).

For the study group, we calculated the effects of various confounders that were present at the time of IL-1 blocker initiation on graft loss for the time between IL-1 blocker initiation and last follow-up. Multivariate Cox regression analysis showed that only proteinuria at the time of IL-1 blocker initiation (HR 3.857, 95% CI 1.291–11.527, $P = .016$) significantly affected the outcome (Supplementary data, Table S5).

DISCUSSION

In the beginning of development of new pharmaceutical agents, patients with special conditions are generally excluded from clinical trials; therefore, observational data after administration of these agents becomes very important. The efficacy and safety of anakinra and canakinumab in patients with crFMF were demonstrated in two randomized controlled trials [9, 10, 26], whereas data are still scarce when it comes to the use of these agents in KTRs. In this observational study, we showed that death was more common in these patients; however, graft survival rates were significantly higher and rejection rates were lower. The number of infections was not higher in KTRs with FMF. Anakinra and canakinumab were also associated with decreased numbers of attacks and hsCRP levels.

KTx offers a significant survival benefit compared with dialysis in patients with AA amyloidosis despite the increase in mortality rates compared with patients without amyloidosis [7, 27]. Contemporary papers from various countries reported survival rates as 73%–84% at 5 years and 45–66% at 10 years, whereas DCGS was higher: 91%–94% for 5 years and 66%–78% for 10 years in the same series of AA amyloidosis [27–29]. In this study, we reported 72.2% as the overall survival rate; 5- and 10-year DCGS rates were 100% and 94.4%, respectively, which was significantly higher than previous studies. Additional immunosuppression caused by IL-1 blockers may have contributed to not only the higher DCGS, but also to higher mortality rates observed in our patients by increasing the immunosuppressive state. Indeed, infections were the leading cause of death in the FMF group followed by unknown causes, which might be associated with the progression of the amyloid deposition in cardiovascular and gastrointestinal systems despite the preserved kidney function. Even though only one patient was diagnosed with cardiac amyloidosis, subclinical amyloid deposition could not be ruled out in heart and other tissues. Supporting the latter view, we previously reported a higher risk of mortality in KTRs with AA amyloidosis possibly linked with the progression of extrarenal deposition of amyloid fibrils [7].

In the present study, graft rejection was lower in the FMF group compared with the control group as was the case in our previous report (8.3% vs 25%) [7]. Upregulation of IL-1 during an acute rejection was shown in murine models [30, 31], and IL-1 secreted by peripheral blood mononuclear cells and monocytes was associated with chronic rejection and transplant glomerulopathy [32, 33]. Therefore, use of IL-1 blockers on top of conventional maintenance immunosuppressive regimen may explain the lower rejection rates in the present series. However, only six patients (7.4%) used IL-1 blockers in our previous study [7], which suggests that there might be an additional factor in play. Various *in vitro* models of experimental autoimmune encephalomyelitis (EAE) revealed that activated $CD4^+$ T cells were targeted by amyloid beta ($A\beta$) [34, 35]. Amyloid fibrils also stimulated pathways which resulted in reduced IL-6, tumor necrosis factor-alpha and interferon-gamma (IFN-γ) levels [36, 37], and genetic deletion of amyloid precursor proteins exacerbated the EAE [34, 35]. A recent investigation demonstrated that adaptive T cells were hyporesponsive to $A\beta$ showing a weak activation and proliferation, reduced expression of IFN-γ and downregulation of antigen presentation function [38]. Extrapolating

Table 3: Study outcomes and various features of patients.

Outcomes	Study group ($n = 36$)	Control group ($n = 36$)	*P*
Death, *n* (%)	7 (19.4)	0 (0)	**.005**
Infections	4 (11.1)	0 (0)	
Unknown	3 (8.3)	0 (0)	
Graft loss, *n* (%)	10 (27.8)	13 (36.1)	.448
Death with a functioning graft	6[a] (16.7)	0 (0)	
Graft rejection	1 (2.8)	6 (16.7)	
Chronic allograft injury	0 (0)	4 (11.1)	
Recurrent AA amyloidosis	3 (8.3)	0 (0)	
Posttransplant glomerulonephritis	0 (0)	3 (8.3)	
Death-censored graft loss, *n* (%)	4 (11.1)	13 (36.1)	**.013**
Graft rejection, *n* (%)	3 (8.3)	9 (25)	.058
Borderline	1 (2.8)	2 (5.6)	
T-cell mediated rejection	2 (5.6)	5 (13.9)	
Antibody-mediated rejection	0 (0)	1 (2.8)	
Mixed	0 (0)	1 (2.8)	
eGFR at the last follow-up (mL/min/1.73 m^2), median (IQR)	50.4 (36.7–65.2)	51.6 (10.3–84.5)	.999
Infections, *n* (%)	17 (47.2)	14 (38.9)	.475
Urinary tract infections	3 (8.3)	8 (22.2)	
Pneumonia and other respiratory tract infections	10 (27.8)	3 (8.3)	
CMV infection	0 (0)	3[b] (8.3)	
Soft tissue infections	2 (5.6)	0 (0)	
Osteomyelitis	1 (2.8)	0 (0)	
Sepsis without a determined source	1 (2.8)	0 (0)	
Malignancies, *n* (%)	1 (2.8)	1 (2.8)	1.000
Leukopenia	2 (5.6)	0 (0)	-
Immunosuppressive regimen at the last follow-up, *n* (%)			
CNi + MPA + prednisolone	19 (52.8)	19 (52.8)	.058
CNi + AZA + prednisolone	8 (22.2)	2 (5.6)	
CNi + mTORi + prednisolone	2 (5.6)	0 (0)	
mTORi + MPA/AZA + prednisolone	1 (2.8)	1 (2.8)	
Double therapies	2 (5.6)	1 (2.8)	
CNi + prednisolone	1 (2.8)	0 (0)	
MPA + prednisolone	1 (2.8)	0 (0)	
AZA + prednisolone	0 (0)	1 (2.8)	
Withheld immunosupression due to graft failure	4 (11.1)	13 (36.1)	

[a]One patient died shortly after suffering from graft loss; [b]one patient had accompanying infective endocarditis.
CMV: cytomegalovirus; MPA: mycophenolic acid derivative; mTORi: mTOR inhibitor.

Table 4: Clinical and laboratory characteristics at the time of IL-1 blocker initiation and last follow-up in study group ($n = 36$).

Characteristics	Initiation of IL-1 blocker	Last follow-up	*P*
Number of attacks per month, median (IQR)	0.8 (0.2–2.3)	0 (0–0)	**<.001**
Serum creatinine (mg/dL), median (IQR)	1.3 (1.1–2)	1.5 (1.2–2)	**.003**
eGFR (mL/min/1.73 m^2), mean ± SD	59.2 ± 26.2	50.2 ± 25.3	**.004**
Serum albumin (g/dL), mean ± SD	4.2 ± 0.4	4.2 ± 0.6	.687
Proteinuria (g/g), median (IQR)	0.2 (0.1–0.8)	0.2 (0.1–0.8)	.283
hsCRP (mg/L), median (IQR)	20.2 (10.5–27.3)	3.1 (1.3–9.8)	**<.001**
ESR (mm/h), median (IQR)	21 (11–48)	10 (3–33)	**.033**

from these studies, we can speculate that amyloid deposition might blunt immune responses in KTRs which may have also contributed to the increased infection-related death rates in these patients.

Anakinra and canakinumab both performed well regarding manifestations of the disease in KTRs with crFMF. Attack frequencies and levels of hsCRP and ESR significantly declined after treatment, namely the number of attacks was decreased from a median of 0.8 to 0 per month. Proteinuria levels were very low at the time of IL-1 blocker initiation; therefore, no changes were observed, though various studies suggested a significant decline in proteinuria with IL-1 blockers [39, 40]. Despite the low levels, proteinuria still was the predictor of graft survival in the study group, which was previously shown [41]. eGFR levels demonstrated a decrease throughout the follow-up which can be expected in all KTRs [42], and FMF and control groups had similar eGFR levels at the end of the follow-up in our study. Both drugs were well tolerated and

adverse events including infection rates were generally similar with the control group, which was in line with the previous studies [9].

This study suffered from several limitations. First, it was designed as a retrospective study which led to the evaluation of only preexisting data. Second, the number of patients is quite limited; however, to the best of our knowledge, this is the largest study on use of anakinra and canakinumab for FMF in KTRs. To date, the number of patients has been <10 in the largest cohorts in the literature [12, 14]. Third, the study did not have a control group of KTRs with FMF using only colchicine, since all of these patients experienced ESKD due to AA amyloidosis and most of them carry deleterious M694V variants which together necessitate the use of anti-IL-1 therapies in this susceptible group. Fourth, even though we used propensity score matching when forming the control group, all characteristics were not collapsible: KTRs in the control group had a shorter duration of posttransplant follow-up, which may have partially caused an overestimation of patient and graft survival in the control group. Fifth, we could not select a control group suffering from AA amyloidosis due to other causes, since the major factor affecting the outcome was due to development and progression of amyloidosis, and the comparative analysis of amyloidosis patients with FMF and other inflammatory conditions could not be done. Sixth, we did not perform protocol biopsies in our institution; therefore, recurrence of AA amyloidosis and episodes of rejection might have been underdiagnosed. Seventh, 5- and 10-year graft survival rates in the control group could be considered as low, further perplexing the analysis with a possible underestimation. Finally, anti-IL-1 therapy was commenced in the study group after a median of 74 months after KTx creating an immortal time bias.

In conclusion, anakinra and canakinumab were effective in KTRs suffering from crFMF with longer graft survival and lower rejection rates. Although they were associated with high number of deaths, the difference in patient survival might have partially originated from not only infections but also progression of the deposition of amyloid fibrils in various organs and tissues, especially cardiovascular and gastrointestinal systems.

SUPPLEMENTARY DATA

Supplementary data are available at *ndt* online.

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None declared.

AUTHORS' CONTRIBUTIONS

S.M., A.G. and M.S.S. designed the study. S.M. had full access to all of the data and performed the analyses. Y.O. and I.K. carried out pathological examinations. All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be submitted.

DATA AVAILABILITY STATEMENT

Deidentified data are available upon reasonable request from the corresponding author.

CONFLICT OF INTEREST STATEMENT

Outside the submitted work, A.T. reported receiving honoraria from Novartis, and A.G. reported receiving honoraria and support for studies and meeting attendance from Novartis. The remaining authors have no relevant conflicts of interest to declare. The results presented in this paper have not been published previously in whole or part, except in abstract format.

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