T-cell Subset Profile in Kidney Recipients of Extended or Standard Donors

Balázs Nemes[a*], Réka P. Szabó[a], Dávid Péntek[a], Ildikó Nagy[a], Gergely Ivády[b], Bettina Kárai[b], Eszter Szánthó[b], Zsuzsa Hevessy[b], Sándor Sipka[c], Gergő J. Szőllősi[d], and Sándor Baráth[b]

[a]Department of Organ Transplantation, Institute of Surgery, Faculty of Medicine, University of Debrecen, Debrecen, Hungary; [b]Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary; [c]Institute of Internal Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary; and [d]Faculty of Public Health, University of Debrecen, Debrecen, Hungary

ABSTRACT

Introduction. The usage of extended-criteria donors (ECD) became a routinely accepted manner in the last decade. ECD is a potential risk factor for antibody-mediated rejection. Analysis of lymphocyte subsets might be a complementary diagnostic toolkit because there is limited knowledge about this term.

Method. Between May 12, 2016, and September 4, 2019, a total of 130 patients who had undergone kidney transplant were investigated. Patients were divided in ECD and standard criteria donor (SCD) groups. Blood samples were collected before the operation, then in the first week and after 30, 60, 180, and 365 days. Besides routine laboratory tests, multicolor flow cytometry was performed for lymphocyte subsets.

Results. ECD grafts were transplanted to older recipients. The number of CD4+ cells increased in the SCDs from the first week to until the end of first month, and then decreased. The number of CD4+ cells decreased from the beginning of the study until the end of first year to 66% of its original value in ECDs. At the first month, the number of CD19+ cells was higher in SCD compared with ECD cases; the number then decreased in both groups. T-regulatory cells had a drop at the first week that lasted until the first month. A bigger increase in SCD and a moderate increase in ECD group were then observed. The kinetics of CD19+ and CD19+ naive cells are similar in the ECD and SCD groups. In the SCD group, cell count decreased in both CD19+ (13%) and CD19+ naive (12%) between third and sixth month. The count of CD19+ cells decreased by 9%, but the count of CD19+ naive cells increased by 11% between the sixth month and first year.

Discussion. The prolonged postoperative uremic state caused by the poorer initial function, together with an aging immune system, explains the weaker immune response in ECD patients, which may be the cause of the decreased number of memory and regulatory T cells. Older patients with an ECD graft need a tailored, personalized, and less aggressive immunosuppressive treatment.

THE usage of extended-criteria donors (ECD) became a routinely accepted manner in the last decade. Many attempts have been made to precisely define ECD, and to evaluate the long-term clinical consequences of transplantations originating from an ECD. Besides the donor's age, and kidney function test (GFR), the long CIT, and low HLA matching were the main items used to determine ECD. In our settings, no differences in the final graft outcome of transplants from ECD and standard-criteria donors (SCD) were found [1]. However, there are few clear answers about whether ECD itself influences

*Address correspondence to Balázs Nemes, Institute of Surgery, University of Debrecen, Móricz Zsigmond krt. 22., H-4027 Debrecen, Hungary. E-mail: nemes.balazs@med.unideb.hu

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the immunologic outcome after kidney transplantation. The measurement of lymphocyte subsets reflects on the current state of the patient's immune system. Absolute number and proportion of subtypes of T, B, and NK cells changes dynamically during induction therapy and after transplantation. An interaction of these lymphocyte subsets manages the immunologic activation cascade. Helper T cells (CD4+) encourage the activation of cytotoxic T cells (CD8+) and the maturation of B cells into plasma cells. Cytotoxic T cells (CD8+) can eliminate malignant and viral infected host cells. However, they also can damage the cells of the donor organ in a direct or indirect manner [2]. It has been observed that in the case of chronic allograft nephropathy there is a significant decrease in the expression of CD8+ cells [3]. B cells act as antigen-presenting cells (APCs) for T cells and sources of antibody and cytokine release, and also function as the main store of memory cells. The rapid humoral answer is the production of plasmablasts (and IgM release), whereas the long-term answer is the formation of plasma cells (and IgG production). Memory cells can be central, effector, or resident, dependent on their state of residency (lymph node) and maturation stage [4]. In chronic allograft nephropathy there is a significant decrease in the expression of CD8+ cells [3], and calcineurin inhibitors diminish the amount of CD19+ cells and Il-10 production [5]. Long-term kidney allograft survival is positively correlated with the number of transitional B cells/CD19 (+) CD24 (hi) CD38 (hi)/. The 2 main groups of currently used immunosuppressive drugs (calcineurine inhibitors and mammalian target of rapamycine inhibitor) both inhibit the production of regulatory B cells [6]. Regarding certain CD cell-surface markers, there are significant differences between primary and retransplanted patients. The CD4+ naive absolute cell count increased in first transplants and did not decrease to its original value until the end of year 1. This is in contrast to retransplants, in which CD4+ naïve cell count rapidly dropped down below its original value, and remained low during the first year. CD8+ effector memory absolute cell count was higher in first transplants vs retransplanted patients at all time points by the end of month 1. The CD19+ naive absolute cell count increased in first transplants to 170% of its original value; however, it remained the same or decreased in second transplants. By the end of the first year, CD19+ naïve absolute cell count diminished to 70% of its original value in first transplants, and 38% in second transplants [7].

After our previous publication, we intended to identify other factors that might affect the kinetics of lymphocyte subsets in kidney transplantation. Recently we focused on the quality of donor organ as a target of the host immune system. We have presumed that organs from a compromised donor will not be as strong an immunologic target as those from an ideal donor.

Multicolor flow cytometry is a powerful tool in detection and analysis of multiple lymphocyte subsets from different samples. It might be an additive diagnostic tool in case of kidney transplantation. One of the advantages of this method is that a time-course profile can be drawn and compared with clinical symptoms with a simple blood test.

PATIENTS AND METHODS

Patients, Demographics, Clinical Groups

We enrolled patients who received kidney transplants between May 12, 2016, and September 4, 2019. Adult recipients of cadaveric and living related transplantations were included. The average recipient age was 51 ± 14 years, BMI 26 ± 4, 62% male, and 38% female. Primary (first) transplant candidates were the majority of participants (n = 112), whereas 18 recipients were retransplanted. Patients were first divided to ECD (45.4%) and SCD groups (54.6%). Donor data were evaluated according to our standard. ECD was defined according to kidney donor risk index. Recipient medical records were then evaluated regarding demographics, HLA matching, CIT, warm ischemia time, clinical follow-up data, and outcome at year 1. Homogeneity of groups was tested between ECD vs SCD. Induction therapy was given for 99% vs 81%. Anti-thymocyte globuline was given for 56.3% vs 47.5% of the patients; basiliximab was introduced for 42.4% vs 33.5%. The postoperative 1-, 3-, 9-, and 12-month glomerular filtration rate scores were 53 ± 4 vs 60 ± 5 mL/h, respectively, for the summarized population and 57 ± 4 vs 62 ± 6 for the first transplants for each group, respectively.

Groups then were compared regarding lymphocyte subset profile. Comparison was carried out first with the entire population, and then repeated with retransplanted patients excluded.

Data Collection and Measurements

After obtaining informed consent, we systematically collected blood samples of kidney transplanted patients at time of operation (0 sample), then at first week, first month, 3 months, 6 months, and 12 months. Besides routine laboratory tests, multicolor flow cytometry was performed. Percentage of lymphocyte subsets were determined by flow cytometry from ethylenediamine tetraacetic acid anticoagulated peripheral blood. Main lymphocyte subsets such as T, B, and NK cells were identified as CD3+, CD19+, and CD3-/CD56+ cells, respectively. To characterize these populations, CD20-FITC, CD5-PC5.5, CD8-APC-H7, CD4-PB (Becton Dickinson, San Jose, CA, USA), CD3-APC, CD56-PC7, CD19-PE (Beckman Coulter, Immunotech, Marseille, France), and CD45-PO (ExBio, Praha, Czech Republic) antibodies were used. Memory T and B cells were identified as CD4+/CD45RA+/CD62L+ naïve, CD4+/CD45RA-/CD62L+ central memory, CD4+/CD45RA-/CD62L- effector memory, CD8+/CD45RA+/CD62L+ naïve, CD8+/CD45RA-/CD62L+ central memory, CD8+/CD45RA-/CD62L- effector memory, CD8+/CD45RA+/CD62L- terminally differentiated memory T cells, CD19+/IgD+/CD27-naïve, CD19+/IgD+/CD27+ nonswitched, and CD19+/IgD-/CD27+ switched memory B cells. The following antibodies were used to determine memory cell subsets: CD45RA-FITC, CD27-PC5.5, CD19-PC7, IgD-APC, CD8-APC-H7, CD62L-PB (Beckman Coulter, Immunotech, Marseille, France), CD4-PE (Becton Dickinson, San Jose, CA, USA), and CD45-PO (ExBio, Praha, Czech Republic). We identified regulatory T cells by using CD4-FITC (BD Pharmingen, San Diego, CA, USA), CD25-PC5 (Beckman Coulter, Immunotech, Marseille, France), and CD127-PE (Beckman Coulter, Immunotech, Marseille, France) anti-human monoclonal antibodies. Our previous results showed that gated CD4+CD25brightCD127dim cells are positive for FOXP3. Samples were measured by BD FACSCanto II flow cytometer (BD Bioscience, San Jose, CA, USA), and data were analyzed using BD FACSDiva software, version 8.0.2 (BD Bioscience, San Jose, CA, USA). Lymphocytes were gated on the CD45 expression and side-scatter properties, and at least 100,000 events were analyzed from every sample.

Statistical Analysis

Statistical analysis was performed using SPSS-21.0 (SPSS; IBM SPSS Statistics 27.0.1.0, New York, United States). The results of the 2 main groups (primary vs retransplant patients) were compared pairwise at each time points after transplantation. Categorical parameters were analyzed by χ^2 test. The homogeneity of the continuous parameter was analyzed by Levene-test; a student t test was then performed accordingly. As for the kinetics of the subsets, they were explored with 3 statistical techniques. The comparison of the same parameter indicators between the 2 study groups (SCD vs ECD) was performed with Wilcoxon rank-sum test. A Kruskal-Wallis test was executed to explore the magnitude of change of the T subset within the subgroup (ie, whether CD4 changed significantly within ECD group by time). A post hoc Dunn's test was also performed. Significance level was $P < .05$.

RESULTS

Demographics of the study population are shown in Table 1. Our study aimed to determine the lymphocyte subset profile of ECD and SCD patients, differences between short (less than 17 hours) and long (more than 17 hours) CIT, as well as differences between full HLA DR vs lack of DR matching. Detailed results for ECD vs SCD are shown in Table 2. Cell numbers are expressed as absolute cell count (cell/uL).

Kinetics of T, B, and NK Cells

We found that the absolute number of CD4+ cells increased in the SCD group from first week to first month, and then decreased to its original value. In spite of this, the number of CD4+ cells gradually decreased from the beginning to year 1 to 66% of its original value in ECDs (Fig 1). These changes by time were significant within each study group (Kruskal-Wallis test). There was also a continuous significant difference between ECD/SCD groups in all time points from the first week to end of the first year (Table 2). The kinetics of CD8+ cells were similar; however, the difference disappeared by the end of the first year. The kinetics of CD19+ (B) cells are parallel in both groups (Fig 2). An increase was seen until the first week in ECDs, but until the first month in SCD recipients. Gradual decrease was found in both groups until the first year. These changes by time were significant within each study group (Kruskal-Wallis test). However, the difference between ECD/SCD group was significant only for the first month after transplant (Wilcoxon test) (Table 2). T-regulatory (T-reg) cells (CD4+/25+bright/CD127dim) had completely different kinetics. They dropped in the first week, and then a bigger increase in SCD and a moderate one in the ECD group were observed (Fig 3). These changes by time were significant within each

Table 1. Donor and Recipient Characteristics of the Compared Groups

			SCD	ECD	*P* Value
Donor	Sex	Male/Female, %	51/49	55/45	NS
	Age, y, mean ± SD		47 ± 13	56 ± 12	.01
	BMI (body weight kg/height cm^2)		25 ± 1,2	26 ±1,1(Fig 1)	
	Cause of BD, %	CVA	64	69	NS
		Not CVA	36	31	
	Serum creatinine, umol/L, mean ± SD		70 ± 26	88 ± 30	.01
Recipient	Original renal diagnosis, %	Hypertension	34	36	NS
		Diabetes	11	16	
		SLE	1	1	
		FSGS	4	3	
		Polycystic kidney disease	12	12	
		VUR	3	2	
		GI	9	7	
		Chronic pyelonephritis	2	2	
		Other undefined renal disease	15	26	
	Sex	Male/Female, %	60/41	62/38	
	Age, y, mean ± SD		45 ± 15	57 ± 10	.03
	BMI, mean ± SD		25 ± 4	26 ± 4	NS
	Dialysis modality and history, %	Hemodialysis only	57	47.2	NS
		CAPD only	20,3	31.9	
		HD and CAPD	16	12.5	
		None (predialytic)	6.9	6.3	
	GFR, mL/h, mean ± SD	At transplant	7 ± 3	8 ± 4	NA
		1 wk after KT	26 ± 18	18 ± 13	NS
		1 mo after KT	60 ± 20	47 ± 17	.031
		3 mo after KT	60 ± 19	47 ± 18	.042
		6 mo after KT	59 ± 20	47 ± 19	.05
		1 y after KT	60 ± 21	52 ± 17	.05

Abbreviations: BD, brain death; BMI, body mass index; CAPD, chronic ambulantory peritoneal dialysis; CVA, cerebrovascular accident (bleedng); ECD, extended-criteria donor; FSGS, focal segmental glomerulosclerosis; GFR, glomerular filtration rate; GS, glomerulosclerosis (unknown origin); HD, haemodialysis; KT, kidney transplantation; NA, not applicable; NS, non-significant; SCD, standard criteria donor; SD, standard deviation; SLE, systemis lupus erythematodes; VUR, vesico-ureteral reflux.

Table 2. Lymphocyte Profile (cells/uL) of ECD (n = 27) and SCD (n = 24) Transplants

			"At Transplant"	*P* value	1 Wk After KT	*P* Value	1 Mo After KT	*P* Value	3 Mo After KT	*P* Value	6 Mo After KT	*P* Value	1 Y After KT	*P* Value	Kruskal-Wallis Test (*P* Value)
CD3	CD4, cells/uL	SCD	622 ± 53	.1525	566 ± 104*	<.0001	999 ± 93*	<.0001	780 ± 66*	.0003	715 ± 55*	.0007	636 ± 49*	.0150	.0399
		ECD	678 ± 72		630 ± 102*		468 ± 74*		470 ± 581*		456 ± 52*		488 ± 52*		.0001
	CD8, cells/uL	SCD	366 ± 38*	.0176	272 ± 53*	<.0001	526 ± 50*	<.0001	469 ± 45*	.0003	516 ± 40*	.0026	560 ± 54	.2576	.0001
		ECD	421 ± 116*		300 ± 594*		264 ± 31*		275 ± 25*		350 ± 31*		460 ± 39		.0001
	CD19, cells/uL	SCD	116 ± 17	.4715	290 ± 38	.1614	335 ± 32*	.0069	217 ± 22	0.5115	157 ± 15	0.2187	116 ± 11	.3405	.0001
		ECD	141 ± 19		260 ± 34		243 ± 32*		188 ± 20		131 ± 14		111 ± 18		.0001
CD4	CD4+25+bright, cells/uL	SCD	37 ± 5*	.0309	4.5 ± 1.4	.9632	17 ± 3	.6250	39 ± 5*	.0084	37 ± 4*	.0068	32 ± 4*	.0303	.0001
		ECD	42 ± 5*		8.9 ± 3.4		20 ± 4		24 ± 4*		23 ± 3*		23 ± 3*		0.0001
	CD4+ naive, cells/uL	SCD	202 ± 18*	.0135	236 ± 52*	<.0001	465 ± 57*	<.0001	336 ± 33*	<.0001	301 ± 31*	.0003	248 ± 27*	.0030	.0226
		ECD	234 ± 31*		290 ± 59*		174 ± 35*		161 ± 26*		170 ± 28*		156 ± 25*		.0003
	CD4+ central memory, cells/uL	SCD	270 ± 32	.4173	217 ± 39*	.0002	338 ± 33*	.0001	275 ± 26*	.0011	254 ± 21*	.0032	225 ± 18	.0871	.0504
		ECD	281 ± 32		221 ± 38*		185 ± 31*		178 ± 26*		168 ± 17*		184 ± 18		.0001
	CD4+ effector memory, cells/uL	SCD	131 ± 12	.3941	102 ± 20*	.0001	182 ± 19*	.0001	156 ± 17*	.0062	146 ± 14*	.0230	149 ± 16	.4025	.0392
		ECD	146 ± 31		108 ± 19*		89 ± 12*		95 ± 10*		95 ± 8*		124 ± 12		.0001
CD8	CD8+ naive, cells/uL	SCD	102 ± 12*	.0004	107 ± 25*	<.0001	213 ± 23*	<.0001	181 ± 19*	<.0001	179 ± 16*	<.0001	155 ± 15*	.0059	.0055
		ECD	95 ± 13*		116 ± 22*		77 ± 11*		76 ± 10*		87 ± 10*		102 ± 18*		.0001
	CD8+ central memory, cells/uL	SCD	46 ± 6*	.0293	33 ± 6*	.0002	61 ± 6*	.0001	55 ± 5*	.0008	59 ± 6*	.0214	44 ± 4	.9832	.0044
		ECD	59 ± 12*		36 ± 7*		40 ± 8*		35 ± 5*		41 ± 5*		52 ± 10		.0001
	CD8+ effector memory, cells/uL	SCD	82 ± 19	.1374	54 ± 12*	.0021	125 ± 20*	.0001	120 ± 27*	.0038	123 ± 16*	.0161	144 ± 25	.5464	.0006
		ECD	137 ± 57		85 ± 22*		62 ± 9*		62 ± 8*		801 ± 10*		110 ± 15		.0001
	CD8+ t.d.e.m., cells/uL	SCD	136 ± 21	.2662	79 ± 18*	.0040	126 ± 21*	.0240	113 ± 15	.4796	155 ± 19	.4482	235 ± 31	.4510	.0001
		ECD	130 ± 55		63 ± 23*		86 ± 16*		100 ± 14		151 ± 20		200 ± 35		.0001
CD19	CD19+ naive, cells/uL	SCD	76 ± 13	.7360	159 ± 26	.2333	189 ± 26*	.0149	122 ± 14	.2342	87 ± 9	.1498	68 ± 8	.0810	.0001
		ECD	95 ± 14		142 ± 23		139 ± 24*		98 ± 13		72 ± 9		46 ± 7		.0027

Data presented as mean ± SD.

Abbreviations: CD8+t.d.e.m cells/μL, CD8+ terminally differentiated effective memory cells/μL; ECD, extended-criteria donor; KT, kidney transplantation; SCD, standard-criteria donor; SD, standard deviation.

* Indicates a timepoint at which the difference (with Wilcoxon test) is significant in all terms between ECD/SCD groups.

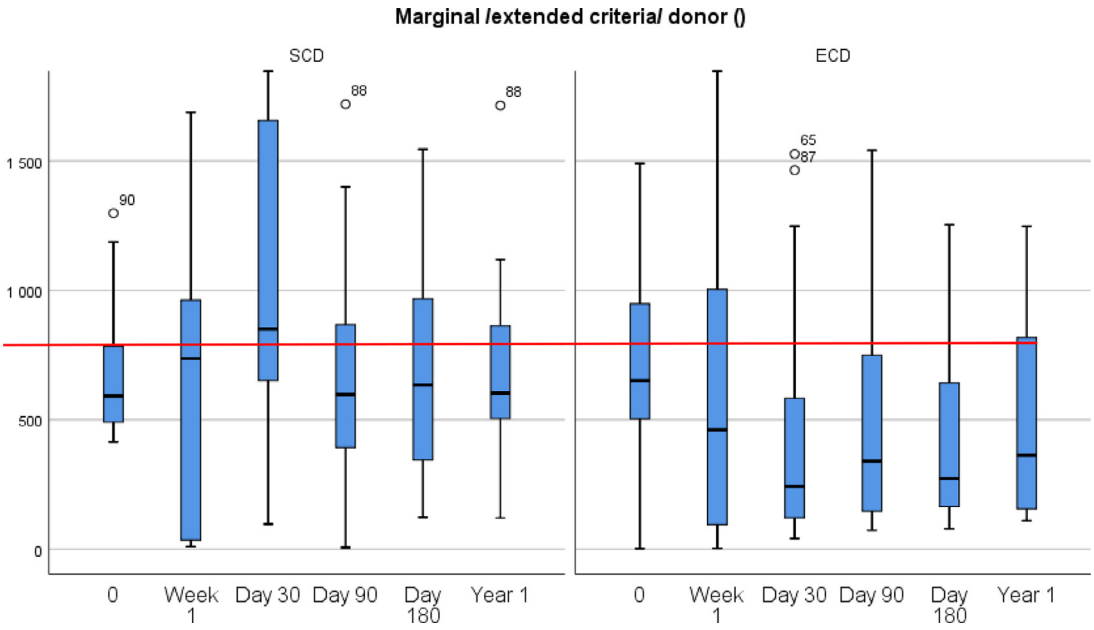

Fig 1. Kinetics of CD4+ cell count (cell/uL) in the standard-criteria donor vs extended-criteria donor group.

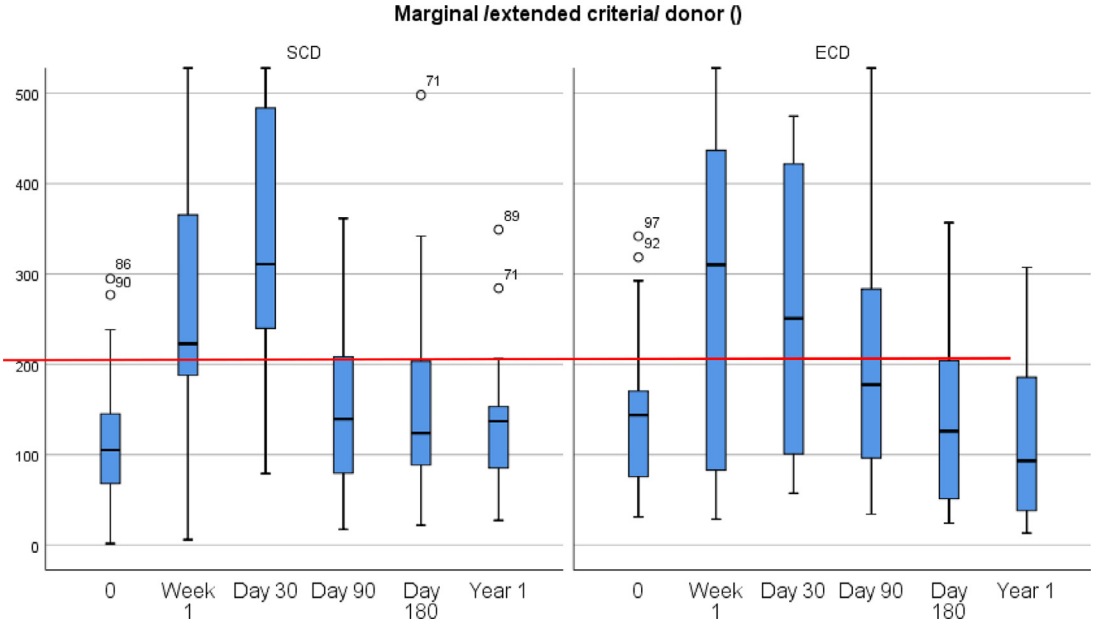

Fig 2. Kinetics of CD19+ cell count (cell/uL) in the standard-criteria donor vs extended-criteria donor group.

study group (Kruskal-Wallis test). The difference between ECD and SCD groups became significant only after the third month (Table 2). The kinetics of natural killer cells (CD3-/CD56+) are shown in Fig 4. We found that the absolute number of NK cells dropped under the pretransplant value in the first week after KT in both groups. The NK cell count then linearly increases back its original value until the first year. There were no significant differences between the 2 study groups. When these comparisons were repeated excluding retransplanted patients, we found the same results

Kinetics of Naive and Memory Cells

CD4± naives were found to increase in both groups until the first week, and kept increasing in the SCD group until the first month, but fall back in the ECD group. These differences were

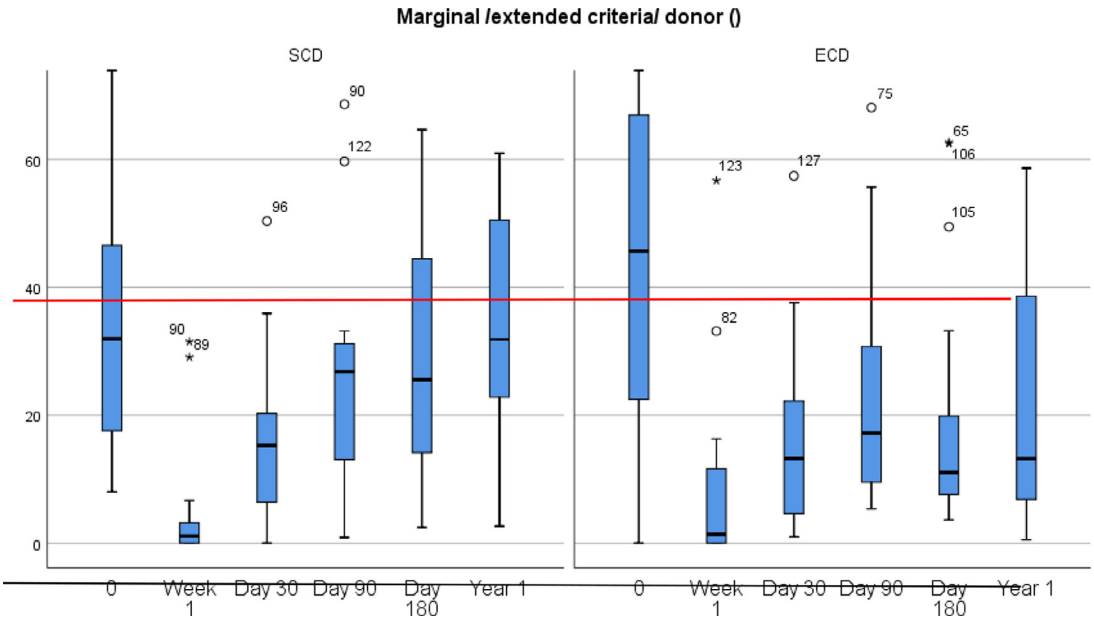

Fig 3. Kinetics of CD4+25+bright (T-regulatory) cell count (cell/uL) in standard-criteria donor vs extended-criteria donor group.

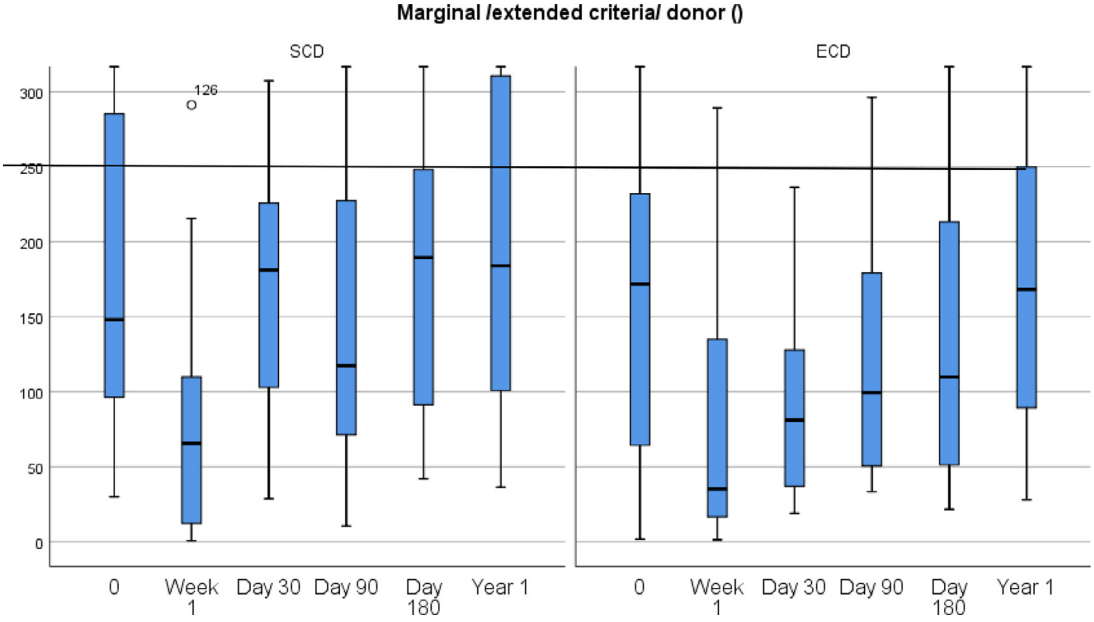

Fig 4. Kinetics of CD56+/3- (NK) cell count (cell/uL) in the standard-criteria donor vs extended-criteria donor group.

significant between the 2 study groups. CD4± central memory cells were found to decrease in both groups until the first week. This decrease continued significantly in ECD patients, but less so in SCD patients. The difference was also continuously significant between the ECD and SCD groups (Fig 5). The kinetics of CD4 ± effector memories were similar to those of CD4+ memories. The CD8 ± naive cells had similar kinetics as for CD4+ naive, and the difference was continuously significant between the ECD and SCD groups (Fig 6, Table 2). The CD8± central and effector memory cells had a similar curve to that of CD8+ naives in both groups, (Table 2). The CD19± naive cells were found to increase in a parallel fashion in both groups until the first month, then decreased below their original values by the end of the first year. These changes were significant within each study group by time (Kruskal-Wallis test). However, the difference between ECD and SCD groups was significant only at the first month after transplant (Fig 7, Table 2).

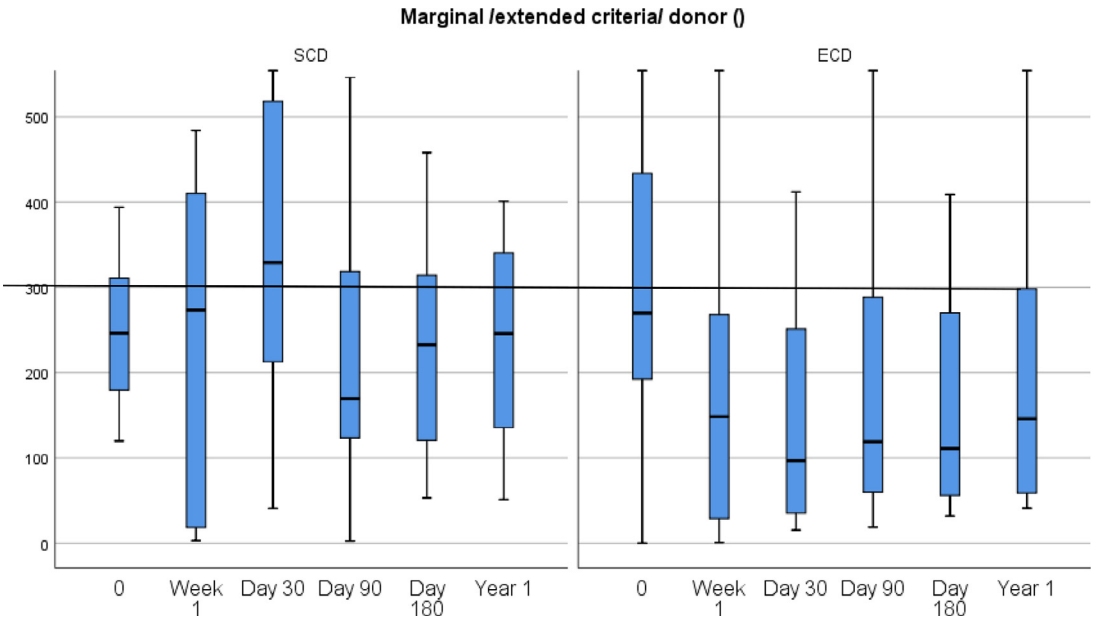

Fig 5. Kinetics of CD4+ *central memory* cell count (cell/uL) in the standard-criteria donor vs extended-criteria donor group.

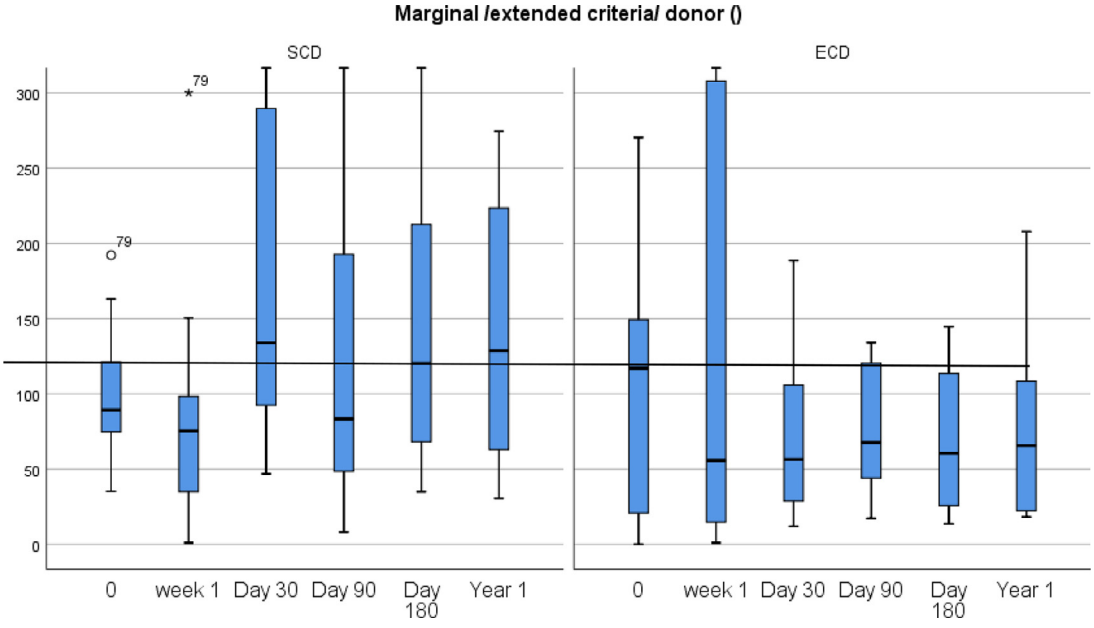

Fig 6. Kinetics of CD8+ *naive* cell count (cell/uL) in the standard-criteria donor vs extended-criteria donor group.

DISCUSSION

ECD donors are defined as unideal donors. Many attempts have been made to define ECD in kidney transplantation. The best approaches so far are the kidney donor risk index and kidney donor profile index scoring systems, which describe the extended criteria. Our center also uses this definition, as discussed earlier [1]. Groups were defined according to these criteria. When we compared the results with those of our previous publication, which compared first and retransplant cases [7], we observed 2 differences. Our previous results showed that in case of first transplants (without previous immunosuppression), both CD4+ and CD8+ cell counts increased and stayed high, but all these cell counts fell back very rapidly in retransplanted (previously immunosuppressed) patients. The recent findings showed a different kinetics, because the mentioned cell counts decreased only stepwise and slowly in ECD cases. We repeated the comparison between ECD and SCD after screening only in first

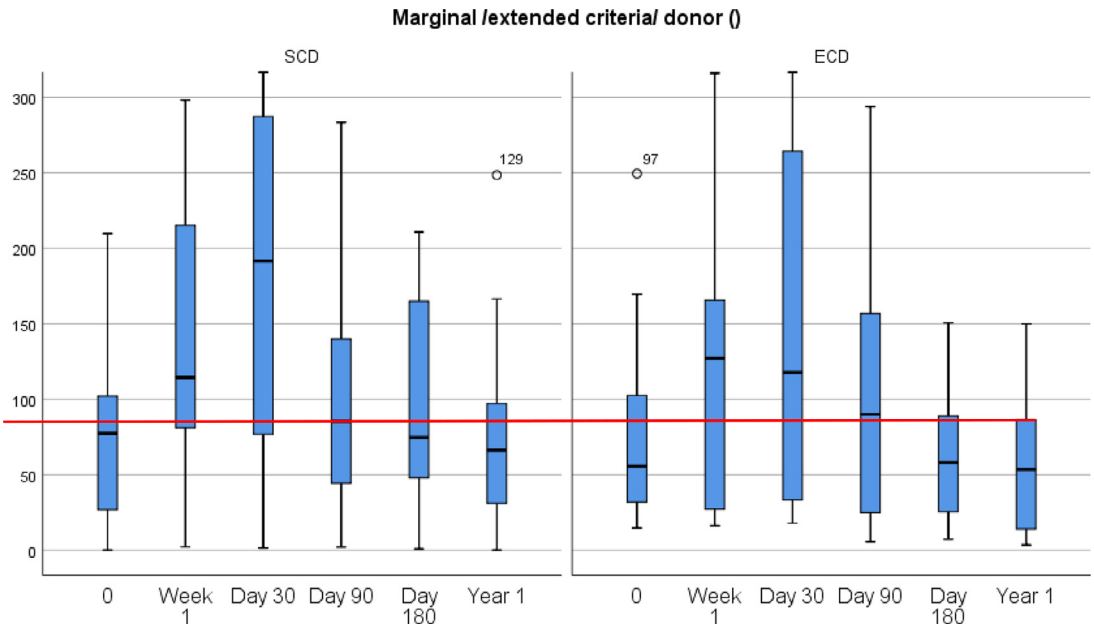

Fig 7. Kinetics of CD19+ *naive* cell count (cell/uL) in the standard-criteria donor vs extended-criteria donor group.

transplants. The kinetics and differences stayed as described above. A similar difference was identified in the kinetics of CD19+ cells. According to our previous results, the CD19+ naive absolute cell count increased in first transplants to 170% of its original value; by the end of the first month, however, it decreased by time in second transplants. In spite of this, the CD19+ cell count increased in both SCD and ECD recipients to a parallel degree (250% and 180% of their original values). Comparing further, we found that CD19+ naïve cell count diminished to 70% in first transplants, and to 38% in second transplants, by the end of the first year. In spite of this, CD19+ cells rose only to their start level, in both SCD and ECD recipients. We also repeated the comparison between ECD vs SCD by screening participants to include only first transplant. The results were the same. We found that the CD19+ cells and CD19+ naive cell dynamics and rates are similar in the ECD and SCD groups. Renal insufficiency might itself alter the characteristics of the lymphocyte population. Chronic kidney disease is associated with a lower naïve T-cell population and aberrant activation potential. Imbalance between suppressive regulatory T cells and T-helper 17 cells was also shown [8]. ECD donor kidneys have some similarity with kidneys in patients with renal impairment; hence some of these changes might also be present in ECD kidneys after transplantation [9]. Trzonkowski et al found that CD4+T cells are one of the critical players in acute rejection of allogeneic grafts in clinical transplantation. Their finding is that the aging immune system is characterized by senescent CD4+T cells, which may also define the features of immunity necessary to keep elderly patients free from acute rejection [10]. Moreover, data suggest that a balance between B cells producing IL-10 and a deficiency in plasma cells may encourage an environment favorable to tolerance maintenance. This hypothesis has been reinforced by the study of Shabir et al, who showed that an increase of transitional B cells (CD19+CD24 high; CD38 high) before and after transplantation could be protective against acute rejection in kidney transplant patients [11]. In the study of Kreijveld et al, 2 markers were associated with successful reduction of immunosuppression: low ratio of memory CD4 T cells/Treg prior to the start of tacrolimus reduction and depressed expression of naive T cells prior to the reduction compared with pretransplant values [12]. Others demonstrated that peripheral T-reg content was higher in the stable group compared with chronic rejection group [13-15]. Matignon et al published that T17-mediated cellular responses were more expressive in ECD recipients than in standard criteria donor kidneys. They have also revealed that there was a high expression of both RORgt and IL-17, strongly suggesting that IL-17 expression was associated with unsuccessful reversal independently of Foxp3 in ECD kidneys [16]. Our findings are consistent with other experimental data showing that aging is associated with a reduced cellular immunity and CD4+ T-cell response and a reduced ability to reject the skin allograft [17,18]. Diet et al did not find significant difference either in BPAR (biopsy-proven acute rejection) risk, or in long-term graft survival between ECD and standard-criteria donors [19]. Memory T cells have an important role in defense against pathogens, especially in immunocompromised patients; they are also important in transplant rejection. They are different from naïve cells because they are antigen-independent, and are able to be activated more easily than naïve T cells. Memory CD4+ cells have a faster responsiveness to antigens, and are highly reactive to donor antigens. A depletion of memory T cells after renal

transplantation might induce a state of delayed tolerance [20]. Memory T cells are less susceptible to depletion and are reconstituted rapidly by the naïve T cells. In fact, both the effector and central memory T cells recover to their pretransplant levels by the third month after kidney transplantation in patients who receive thymoglobulin induction. We can learn from the study of Taner et al that 6 months after kidney transplantation, the frequency of effector memory T cells have been shown to be similar in patients regardless of thymoglobulin induction, and similar to pretransplantation numbers [21].We might conclude that the quality of donor organ (ECD) and prolonged history of previous immunosuppression have similar depressive effects on the vast majority of lymphocyte subsets, except for strength of the effect on CD4+ and CD4+ memory cells, and regarding the overall kinetics of the CD19+ cells.

Our conclusion is that all CD4+cells (especially the CD4+ naïve and effector memory cells) and also CD8+ cells (especially CD8+ naïve and effector cells) become and remain underrepresented in patients transplanted with an ECD kidney, until 1 year, irrespective of the original immunosuppression.

The number of CD4+25+bright cells (T-reg) remained at the original value in SCD patients but decreased by 50% of the original value in ECD recipients. The CD4(+)CD25(+) regulatory T cells play a central role in the prevention of autoimmunity and in the control of immune responses by downregulating the function of effector CD4(+) or CD8(+) T cells [22]. The lower number of T-regs in ECD recipients might have less downregulation effect on cellular immunity. Within the ECD group, the number of CD19+ cells was much lower at the end of the first year compared to their original (0 time point) value. Schmitz et al discussed in detail the role of B cells in promoting or maintaining tolerance in kidney transplant recipients [23]. We know that tolerance induction in memory CD4 T cells requires 2 rounds of antigen-specific activation [24]. In the case of ECD donors, the depletion of memory CD4+ cells might have a benefit for the patient in avoiding late activation of the immune system; however, CD19+ depletion means a loss in terms of tolerance.

SUMMARY

In case of an ECD donor, the postoperative CD4+ (especially naïve and memory) cell counts as well as regulatory T cell counts fall down after transplantation and remain low. The CD19+ cell count increases rapidly until the 30th day, then falls. ECD grafts were usually given to older recipients, and the difference in initial kidney function was minor compared with SCDs (Table 1). It is conceivable that the prolonged postoperative uremic state caused by the poorer initial function, together with an aging immune system, explains the weaker immune response in ECD patients, which may be the cause of the decreased number of memory and regulatory T cells.

Our conclusion is that older patients with an ECD graft need tailored, personalized, and less aggressive immunosuppression. Further observations are also needed to determine whether underrepresentation of memory and regulatory cells in ECD patients may contribute to the risk for cellular and antibody-mediated rejection in the long run. Our results show that the recovery of the cellular components of the immune system is substantially different in SCD and ECD patients after transplantation.

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