Preliminary study evaluating the risk factors of kidney acute rejection

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Abstract

Background: *Acute rejection episodes (ARE) are a major determinant of renal allograft survival and still a major challenge for contemporary transplantation. Finding the factors contributing to ARE could help to determine the high-risk patients and greatly improve the transplantation outcomes by applying proper immunosuppressive drug regimens. The aim of this preliminary study was to evaluate various epidemiological, clinical and immunological parameters as potential risk factors increasing the probability of ARE occurrences.*

Material and methods: *The study included 44 patients undergoing kidney transplantation. During the 3-month period following the transplantation, ARE was diagnosed in 11 patients. Baseline clinical and epidemiological characteristics was examined. Baseline serum concentrations of IL-2, IL-4, IL-5, IL-10, IFN-γ, TNF-α were measured by means of flow cytometry. The logistic analysis was also performed to identify the potential risk factors.*

Results: *No significant differences in epidemiological and clinical baseline characteristics of NONARE (non-rejection patients) and ARE patients were observed. Cytometric analysis of serum Th1/Th2 cytokines showed significant differences in baseline concentrations of IFN-γ and IL-10 in NONARE vs. ARE patients ($p < 0.05$). Conducted logistic regression showed that age of the recipient and baseline serum concentrations of INF-γ, IL-10, IL-4 can be considered as risk factors of ARE. The calculated odds ratios (Exp(B)) were, as follows: age Exp(B) = 0.9, IFN-γ Exp(B) = 2.49, IL-10 Exp(B) = 2.41, and IL-4 Exp(B) = 0.11. Model classification was correct in 84.1% predictions.*

Conclusions: *Conducted analysis indicates that young age of the recipient and baseline serum concentrations of IFN-γ, IL-10 and IL-4 can be considered as risk factors for ARE.*

Key words: *kidney transplantation, acute rejection, risk factors.*

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Introduction

Acute rejection episodes (ARE) are a major determinant of renal allograft survival and still a major challenge for contemporary transplantation [1]. During the recent years the outcomes after kidney transplantation have greatly improved, mostly due to better immunosuppression therapies and monitoring techniques [2]. The majority of ARE occur within the first 3 months after transplantation. Finding the factors contributing to ARE could help to determine the high-risk patients and greatly improve the transplantation outcomes by applying proper immunosuppressive drug regimens.

The aim of this preliminary study was to evaluate various epidemiological, clinical and immunological baseline parameters as potential risk factors increasing the probability of ARE occurrences. Identifying those risk factors could help to improve the long-term allograft survival.

Material and methods

Approval for this study was obtained from the local Ethic Committee at the Poznan University of Medical Sciences in Poznan (Poland). The study included 44 patients undergoing kidney transplantation at the Department of Transplantation and General Surgery, the District Hospital in Poznan (Poland). During the 3-month period following the transplantation, ARE was diagnosed in 11 patients based on biopsy results applying the Banff classification. Inves-

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tigated patients were treated with standard doses of the following immunosuppressants: 1) cyclosporine (CsA) + mycophenolate mofetil (MMF) + glucocorticoids (Gs); 2) cyclosporine + azathioprine (AZA) + glucocorticoids; 3) tacrolimus (Tac) + rapamycin (RAPA) + glucocorticoids, and 4) tacrolimus + mycophenolate mofetil + glucocorticoids. We examined the baseline epidemiological and clinical characteristics including age, gender, race, time on dialysis, serum creatinine and urea concentrations, cold and warm ischemia times, degree of human leukocyte antigens (HLA) matching, % panel-reactive antibodies value (% PRA) and type of immunosuppressive therapy. Also the serum samples were collected 1 day before the transplantation. Each sample was tested for interleukin 2 (IL-2), IL-4, IL-5, IL-10, interferon γ (IFN-γ) and tumor necrosis factor α (TNF-α) concentrations using the human Th1/Th2 cytokine cytometric beads array (CBA) Kit (BD Biosciences Pharmingen, USA) according to the manufacturer's protocol. Data were analyzed by Mann-Whitney U and χ^2 test. Kaplan-Meier survival analysis for 90-day posttransplantation period was performed to evaluate the possible effect of applied immunosuppressive therapies upon the frequency of ARE occurrences. The logistic analysis was also performed to evaluate the potential of analyzed baseline parameters as risk factors increasing the probability of ARE in transplanted patients. Due to a relatively small group of patients ($n = 44$) the forced entry method was used to maximize the reliability of the obtained mathematical model. The following parameters (independent variables) were included in the model: age, gender, time on dialysis, baseline serum creatinine and urea concentrations, cold and warm ischemia times, degree of HLA matching, % PRA value, type of immunosuppressive therapy, baseline serum concentrations of IL-2, IL-4, IL-5, IL-10, IFN-γ and TNF-α. Data was analyzed with statistical package for the social sciences version 15 (SPSS, USA). Values were considered statistically significant when $p < 0.05$.

Results

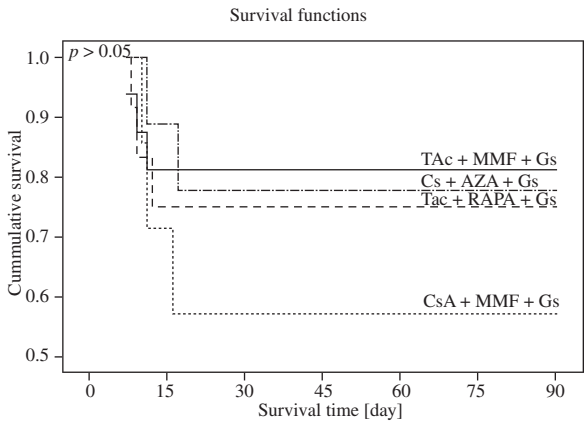
No significant differences in epidemiological and clinical baseline characteristics of NONARE (non-rejection patients) and ARE patients were observed ($p > 0.05$) (Table 1). Performed Kaplan-Meier survival analysis did not show any significant differences in effect of applied immunosuppressive therapies upon the frequency of ARE occurrences in investigated groups of patients in 90-day posttransplantation period ($p > 0.05$) (Fig. 1). Cytometric analysis of serum Th1/Th2 cytokines showed significant differences in baseline concentrations of IFN-γ (2.64 ±0.4 pg/ml vs. 4.53 ±1.1 pg/ml) and IL-10 (3.69 ±0.8 pg/ml vs. 6.87 ±0.4 pg/ml) in NONARE vs. ARE patients, respectively ($p < 0.05$) (Fig. 2). No significant differences between the two groups were observed in serum levels of IL-2, IL-4, IL-5 and TNF-α ($p > 0.05$). For the further reference, however, also the mean pretransplant concentrations of IL-4 in NONARE vs. ARE patients are given: 1.76 ±0.6 pg/ml vs. 1.83 ±1.3 pg/ml, respectively ($p > 0.05$).

To evaluate the potential role of analyzed parameters in acute rejection process, the logistic analysis was con-

Table 1. Baseline characteristics of patients prior to the kidney transplantation (mean ±SD). Data not included in the table: % PRA – close to zero, race – Caucasian

Parameter	NONARE	ARE	*p*
Age [years]	43.76 ±2.1	45.09 ±2.9	0.29
Sex			0.86
Male	20 (74.1%)	7 (25.9%)	
Female	13 (76.5%)	4 (23.5%)	
Dialysis time [years]	1.83 ±0.3	1.86 ±0.4	0.31
Serum creatinine [mg/dl]	6.94 ±0.4	6.89 ±0.5	0.95
Serum urea [mg/dl]	67.04 ±4.7	67.71 ±6.7	0.46
Immunosuppression			0.67
CsA + MMF + Gs	4	3	
CsA + AZA + Gs	7	2	
Tac + RAPA + Gs	9	3	
Tac + MMF + Gs	13	3	
HLA matching	3.12 ±0.2	3.09 ±0.3	0.95
Cold ischemic time [h]	20.57 ±6.0	23.38 ±3.6	0.08
Warm ischemic time [h]	0.41 ±0.1	0.48 ±0.1	0.07

ARE – rejection patients, AZA – azathioprine, CsA – cyclosporine, Gs – glucocorticoids, MMF – mycophenolate mofetil, NONARE – non-rejection patients, RAPA – rapamycin, Tac – tacrolimus



AZA – azathioprine, CsA – cyclosporine, Gs – glucocorticoids, MMF – mycophenolate mofetil, RAPA – rapamycin, Tac – tacrolimus

Fig. 1. Kaplan-Meier survival analysis for the 90-day post-transplantation period. The test of equality of survival distribution did not show significant differences in effect of applied immunosuppressive therapies upon the frequency of ARE occurrences in investigated groups ($n = 44$)

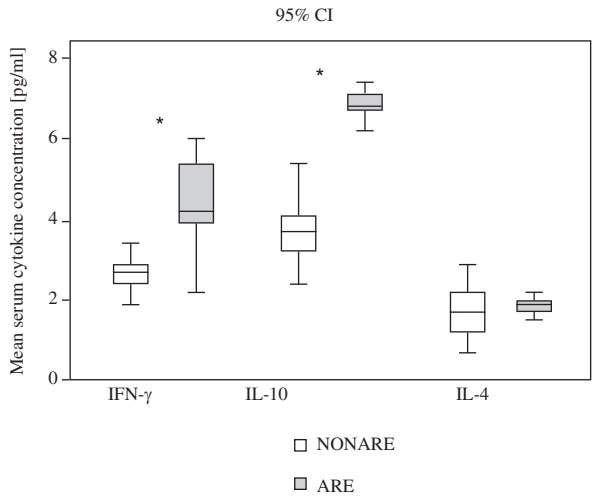


Fig. 2. Mean pretransplant serum cytokine levels in non-rejection (NONARE; $n = 33$) and rejection (ARE; $n = 11$) groups of patients. Statistically significant differences ($p > 0.05$) among investigated groups are marked with the asterisks (*)

Table 2. Independent variables (predictors) included in the model

	B	S.E.	Wald	df	Significance (*p*)	Exp(B)
Baseline IFN-γ	0.91	0.62	2.17	1	0.14	2.49
Baseline IL-10	0.88	0.41	4.49	1	0.03	2.4
Baseline IL-4	–2.17	1.12	3.73	1	0.05	0.11
Age	0.11	0.05	5.15	1	0.02	0.9

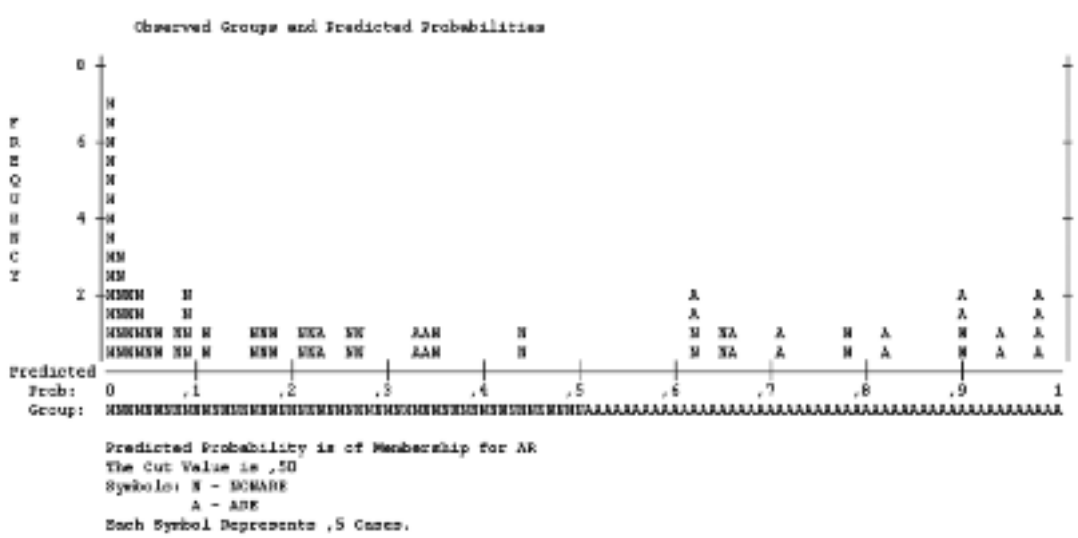


Fig. 3. Classification plot (correct and incorrect predictions under logistic regression)

ducted. Obtained mathematical model included statistically significant independent variables such as: age ($p = 0.02$), baseline serum IL-10 concentration ($p = 0.03$) and baseline serum IL-4 concentration ($p = 0.05$) (Table 2). Baseline serum IFN-γ was also included in the model, despite the fact the Wald statistic was higher than 0.05 ($p = 0.14$). The reason was the significant difference in mean baseline concentrations of serum IFN-γ in NONARE vs. ARE patients. The calculated odds ratios (Exp(B)) predicted by the model, which indicate the changes in odds of ARE resulting from the unit changes in the independent variables, were, as follows: age Exp(B) = 0.9, IFN-γ Exp(B) = 2.49, IL-10 Exp(B) = 2.41, and IL-4 Exp(B) = 0.11. Model classification was correct in 84.1% predictions (87.9% for NONARE and 72.7% for ARE) (Fig. 3). The overall fit of the model was statistically significant (-2 Log-likelihood $\chi^2 < 0.05$).

Discussion

Based on baseline characteristics of transplanted patients the mathematical model was built describing the effects of analyzed independent variables upon the dichotomous variable "acute rejection". The following parameters were included in the analysis: age, gender, time on dialysis, baseline serum creatinine and urea concentrations, cold and warm ischemia times, degree of HLA matching, % PRA value, type of immunosuppressive therapy, baseline serum concentrations of IL-2, IL-4, IL-5, IL-10, IFN-γ and TNF-α. The 3-month posttransplantation period of ARE occurrences was considered in the study. The conducted analysis using logistic regression showed that variables: young age of the recipient and baseline serum concentrations of IFN-γ, IL-10, IL-4 could be considered as risk factors for ARE. The odds ratios (Exp(B)) predicted by the model, which indicate the changes in odds of ARE occurrence resulting from the unit changes in the independent variables, were, as follows: age Exp(B) = 0.9, IFN-γ Exp(B) = 2.49, IL-10 Exp(B) = 2.41 and IL-4 Exp(B) = 0.11. Younger age of a recipient as a risk factor of ARE has been already documented (Exp(B) < 1) and has been linked to a stronger alloimmune response [3]. Based on our data it also seems that higher pretransplant concentrations of IFN-γ and IL-10 increase the probability of ARE (Exp(B) > 1) while the higher pretransplant concentrations of IL-4 lower the risk of ARE. The involvement of IFN-γ in the acute rejection process is consistent with other reports [4-6]. Interestingly, the higher IFN-γ concentrations were not accompanied by the elevation of other proinflammatory cytokines in the serum of ARE patients, suggesting an ongoing, nondetected, nonspecific Th1 response involving activated monocytes/macrophages and/or natural killer cells. It seems that the elevated pretransplant serum IL-10 concentrations accompanied by the defect of regulatory/suppressor T cells observed in ARE patients [7, 8] probably also result from the activation of monocytes/macrophages. In contrast, IL-4 in high concentrations during the pretransplantation period and shortly afterwards may play a protective role upon the allograft [9].

We are aware of limitations of this model due to the relatively small size of the investigated group. Therefore, it is necessary to continue the research on a bigger population. We believe that the results of this study could help to lower the frequency of ARE in kidney transplanted patients and improve the long-term allograft survival.

Conclusions

Conducted analysis indicates that young age of the recipient and baseline serum concentrations of IFN-γ, IL-10 can be considered as risk factors for ARE. In contrast, IL-4 in high concentrations during the pretransplantation period and shortly afterwards may play a protective role upon the allograft. Finding the factors contributing to ARE could help to determine the high-risk patients and greatly improve the transplantation outcomes by applying proper immunosuppressive drug regimens.

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