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Cytokines signatures in short and long-term stable renal transplanted patients

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ABSTRACT

Despite the evidences showing the relevance of regulatory immune-mediated mechanisms to guarantee the stable graft function in renal transplanted patients, studies focusing on the immune response observed over a long-term period after renal transplantation are still limited. Several efforts have been done to establish novel biomarkers with relevant predictive values that could be used as prognostic laboratorial tools to monitor the complex network triggered through time after kidney transplantation. In this study, we have evaluated the pro-inflammatory and regulatory patterns of plasma cytokines in a group of 120 renal transplanted patients with stable graft function ranging from 1 to 160 months. Our data demonstrated an overall predominance of regulatory cytokines short-term after renal transplantation (1–24 months) with peaks of IL-4, IL-5 and IL-10. Moreover, a slight peak of TNF-α was observed 25–60 months after renal transplantation. Following a gap of stable cytokine profile (61–120 months), peaks of pro-inflammatory cytokines IL-8, IL-6, IL1β, TNF-α and IL-12 were observed later on (>120 months) after renal transplantation. Additionally, the categorical analysis of "low" or "high" cytokine producers re-enforce the occurrence of an overall regulatory status early-after stable renal graft function with a predominant pro-inflammatory pattern later on long-term renal transplantation. Taken together, our data suggest that IL-5 is a good biomarker associated with short-term stable renal function, whereas IL-12 seems to be a relevant pro-inflammatory element in long-term renal transplanted patients.

1. Introduction

Renal transplantation is the better choice for treatment of end stage renal disease. Nevertheless, after renal transplantation patients need permanent immunosuppressive treatment to prevent graft rejection and loss. Despite great improvements in renal allograft survival over the last three decades, long-term graft loss, particularly through antibody-mediated rejection, remains the great challenge of renal transplantation [1–3].

Recent studies have suggested that cytokines plasma assess after renal transplantation could allow a better understanding of the renal allograft rejection pathogenesis, as well as to predict future rejection process [2–7]. Besides, cytokines gene polymorphisms have been correlated with increased production of these cytokines [8,9].

Cytokines are generally responsible for TH1 and TH2 responses in renal transplantation. TH1 lymphocytes produce IL-2, TNF-α and IFN-γ. By activation of macrophages, these lymphocytes participate in delayed hypersensitivity and cytotoxic activity. TH2 lymphocytes produce IL-4, IL-5, IL-6, IL-10, IL-13 and participate in the production of antibodies. Deregulated production of pro-inflammatory or regulatory cytokines plays an important role in the disease susceptibility and progression of renal transplantation [5–7]. Therefore, induction of specific immunologic tolerance with suppression of IL-6, IL-12, TNF-α and others proinflammatory cytokines remains as an important goal of organ transplantation [10–12].

The aim of this study was to evaluate the cytokines plasma levels in renal transplanted patients with stable graft function. It should be highlighted that this is the first study evaluating a wide cytokine profile in distinct periods post-transplant. This study can contribute to expanding the understanding about the immune response after renal transplantation and provide novel biomarkers with relevant predictive values that could be used as laboratorial tools for prognostic and monitoring purposes.

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2. Materials and methods

2.1. Patients

A total of 120 renal transplanted patients were selected at two Renal Transplant Units, in Belo Horizonte, Minas Gerais, Brazil from 2010 to 2011. The study population comprises 82 males and 38 females with age ranging from 19 to 73 years and time post-transplantation ranging from 1 to 160 months. All patients have received kidney from living organ donors. Pre-transplant panel reactive anti-HLA antibody was negative in 49 receptors, positive in 8 and not available for 63 patients. All patients were under corticosteroid therapy at their inclusion in this study. Triple immunosuppressive protocol with tacrolimus + mycophenolate mofetil + prednisone was given to 51.6% of patients and ciclosporin + mycophenolate mofetil + prednisone to 35.0% of patients. A small group of patients (13.4%) were treated with prednisone associated with other immunosuppressive drugs. Seven out of 120 patients presented previous CMV infection short-term after renal transplant. These patients were included in the present investigation since they showed negative CMV antigenemia at the blood collection. The inclusion criterion was stable graft function and the exclusion criteria were acute or chronic allograft rejection. Rejection was defined by an increase in creatinine plasma levels by 0.3 mg/dL from baseline that was not attributed to other causes with subsequent return to baseline after treatment with pulse steroids or anti-lymphocytic antibodies [13]. The study population was categorized into four subgroups according to the time after transplantation and included G1: patients within 1–24 months; G2: patients within 25–60 months; G3: patients within 61–120 months and G4: patients 121–160 months post-transplant. These subgroups typify distinct posttransplantation phases including early (G1), intermediate (G2 and G3) and long-term (G4) survival with stable graft function. Four out of 29 patients from the G4 subgroup presented previous and sporadic episodes of renal dysfunction after renal transplantation, all of them were considered to present stable graft function at the time of their inclusion in the present study. The major demographic and clinical features of these subgroups are presented in Table 1.

2.2. Ethics

This study was approved by the Ethics Committee at Federal University of Minas Gerais and informed consent was obtained from all participants. The research protocol did not interfere with any medical recommendations or prescriptions.

2.3. Plasma samples

Five mL whole blood samples were drawn in EDTA-K_3 1.8 mg/mL (Vacuette®) and centrifuged at 1300*g* for 20 min at 4 °C to obtain the plasma samples. Plasma aliquots were stored at −70 °C until use for flow cytometric cytokine measurements.

2.4. Cytometric beads array for cytokine measurements

Cytokine plasma levels were determined using commercially available kits, including Human Th1/Th2 Cytometric Beads Array – CBA (BD Biosciences Pharmingen, USA) to quantify IFN-γ, IL-4 and IL-5 along with the Human Inflammation kit to quantify IL-1β, IL-6, IL-8, IL-10, TNF-α and IL-12.

The CBA immunoassay uses 7.5 μm polystyrene microbeads, assembled in distinct fluorescent sets, unique on their type 4 fluorescence intensity (FL-4). Each microbead is coupled to monoclonal antibody (MAb) against a given cytokine. Following incubation with the test sample, the bead-captured cytokines were detected by direct immunoassay using a "detection cocktail" of distinct MAbs labeled with type 2 fluorescence, phycoerythrin-PE (FL-2).

The method was carried out as recommended by the manufacturer, modified as follows: briefly, 25 μL of undiluted plasma samples or standards (previously diluted) were added to 15 μL of bead-mix and incubated for 90 min at room temperature in the dark. The cytokine standard curves were run daily for each assay. After incubation, the samples and standards were washed with 500 μL of wash buffer and centrifuged at 600*g* for 7 min at room temperature. Subsequently, 20 μL of detection cocktail were added to each tube and the bead-mix re-incubated for 90 min at room temperature in the dark. Following incubation, the samples and standards were washed again with 500 μL of wash buffer and centrifuged at 600*g* for 7 min at room temperature to remove unbound detector reagent. After washing, 250 μL of wash buffer was added to each tube. Data acquisition and analysis was performed in dual-laser FACScalibur™ flow cytometer (BD Biosciences Pharmingen, San Jose, CA, USA), using the BD Bioscience CBA software. Although the fluorescently labeled particles in the BD CBA immunoassay are designed to be excited by the 488 nm and 532 nm lasers on other BD flow cytometers, they can also be excited by the red diode laser 633 nm on dual-laser BD FACSCalibur instruments. The detection of beads emission at FL-4 channel simplifies the instrument set-up procedure and reduces the need for fluorescence compensation. Thus, a total of 1800 beads/tube were acquired after proper set-up of a flow cytometer. Results were expressed as mean fluorescence intensity (MFI) for each cytokine.

2.5. Analysis of "overall cytokine patterns" and "cytokine signatures"

The plasma cytokine levels were initially analyzed as the mean fluorescent intensity provided by the CBA immunoassays and referred as "overall cytokine patterns". Each cytokine pattern was first expressed as moving average of plasma cytokines according to the time after renal transplantation. Following study population sub grouping categorization, the median overall cytokine patterns were compared amongst G1, G2, G3 and G4 subgroups. Additional analysis referred as "cytokine signatures" were also performed as previously proposed by Luiza-Silva et al. [14]. Briefly, the global median value for each cytokine was calculated taking the whole data universe from all renal transplanted patients. The global median cut off were used as the cut-off edge to tag each patient as they display "Low levels" (▭ for all cytokines), high levels of pro-inflammatory (■ for IL-8, IL-6, IL-1β, TNF-α, IL-12, IFN-γ) or high levels of regulatory (▬ for IL-4, IL5 and IL-10) cytokines. After assembling of gray-scale diagrams for each study subgroup, the frequency (%) of patients showing "High cytokine levels" was calculated. This strategy allowed for computation of the percentage

Table 1
Demographic and clinical features of the study population[a].

Groups	Time post-transplant (months)	*N*	Creatinine levels (mg/dL)	Gender (M/F)	Age (years)
G1	1–24	31	1.7 ± 0.9	22/09	36 (19–63)
G2	25–60	30	1.5 ± 0.6	19/11	45 (21–66)
G3	61–120	30	1.5 ± 0.5	21/09	49 (33–73)
G4	121–160	29	1.6 ± 0.5	20/09	47 (33–64)
Total	1–160	120	1.6 ± 0.7	82/38	44 (19–73)

[a] Time post-transplant are expressed as min–max, creatinine levels as mean ± SD and age as median (min–max).

of patients displaying high cytokine levels. Following, the "cytokine signature" for each subgroup was then assembled as the ascendant frequency of high cytokine levels of G1, G2, G3 and G4.

2.6. Statistical analysis

The cytokine patterns were first evaluated considering the moving average of plasma cytokine levels, expressed as mean fluorescence intensity (MFI), along to the time post-transplantation to identify, for each cytokine, the post-transplant period associated with peaks of maximum MFI. Upon study population subgrouping categorization, comparative analysis amongst subgroups were performed by Kruskal–Wallis followed by Dunn's post-test and differences considered significant at $P < 0.05$. Prior statistical analysis, the normality of data distribution was evaluated by the Kolmogorov–Smirnov test. All statistical comparisons were performed using the program GraphPad PRISM (version 5.0).

An additional strategy of data analysis were used to tag each patient as they present "Low" or "High" cytokine levels, taking the global median MFI value from all data universe of a given cytokine as the cut-off. The frequency of patients with High cytokine levels were then compiled to establish the cytokine ascendant profile referred as "cytokine signatures". Relevant cytokine frequency was considered when the percentage of patients with high cytokine levels was above the 50th percentile. Further, comparative analysis of the cytokine signatures among subgroups were performed by overlapping the ascendant cytokine curves of each subgroup of renal transplanted patients. Relevant differences in the ascendant cytokine signatures among subgroups were identified by comparative analysis, considering for each subgroup only the cytokines with frequency above the 50th percentile.

3. Results

3.1. Analysis of "overall cytokine patterns" and "cytokine signatures"

An overall predominance of regulatory cytokines is observed short-term after renal transplantation, while increased levels of pro-inflammatory cytokines are found later on long-term renal transplantation.

Aiming to characterize the overall cytokine pattern of renal transplanted patients, we have first evaluated the moving average of plasma cytokine levels according to the time after transplantation to identify, for each cytokine, the post-transplant period associated with increased MFI (Fig. 1). Our finding demonstrated that there was an increase tendency of IL-4, IL-5 and IL-10 in renal transplanted patients within 1–24 months post-transplantation. On the other hand, a tendency of increased levels of IL-8, IL-6, IL-1β and TNF-α was observed in patients with more than 120 months of renal transplantation. Similarly, a tendency of increased TNF-α was also observed in patients within 25–60 months post-transplantation. IFN-γ levels remained steady along the time post-transplantation (Fig. 1).

3.2. The categorical analysis of low or high cytokine producers highlighted the occurrence of an overall regulatory status early-after stable renal graft function with a predominant pro-inflammatory pattern later on long-term renal transplantation

Aiming to further characterize the cytokine profile, we have used a novel strategy to analyze the frequency of renal transplanted patients with distinct levels of plasma cytokines, categorized as "low" or "high" cytokine producer, as previously proposed by Luiza-Silva et al. [14]. The global median values was determined for each cytokine, including IL-8 = 1.8, IL-6 = 8.4, IL-1β = 2.4, TNF-α = 4.5, IL-12 = 5.3, IFN-γ = 4.0, IL-4 = 1.3, IL-5 = 1.4 and IL-10 = 6.0, all expressed in mean fluorescence intensity (MFI). These standards were further used as a cut-off to tag each patient as they present "low" (▭) or "high" levels of pro-inflammatory (■) or regulatory (■) plasma cytokines (Fig. 2A). Following, gray-scale diagrams were created to calculate for each study subgroup, the frequency (%) of patients displaying high cytokine levels (Fig. 2B). Our data highlighted that whereas G1 (1–24 months) displayed increased frequencies (upper than 50th percentile) of patients with regulatory status (IL-4 = 71%, IL-5 = 71% and IL-10 = 52%), the subgroup G2 (25–60 months) showed prominent frequency of patients with pro-inflammatory pattern (IL-1β = 57%, TNF-α = 60% and IFN-γ = 64%). Although G3 (61–120 months) and G4 (121–160 months) presented a mixed cytokine profile, the G3 showed a balanced profile of pro-inflammatory and regulatory cytokines (IL-8 = 53% and IL-10 = 57%), whereas G4 displayed a predominant pro-inflammatory profile (IL-8 = 62%, IL-6 = 62%, IL-1β = 66%, TNF-α = 59%, IL-12 = 76% and IL-5 = 64%) (Fig. 2B).

3.3. Cytokine signatures pointed out IL-5 as a putative biomarker associated with short-term stable renal function and IL-12 as a relevant pro-inflammatory event in long-term renal transplanted patients

To further evaluate the cytokine mediated immune response in renal transplanted patients, we have generated the inflammatory/regulatory ascendant cytokine signatures for each subgroup, using the 50th percentile as the significance baseline (Fig. 3A). Our data showed that the outstanding regulatory status of G1 (1–24 months) was characterized by IL-5 = IL-4 > IL-10 with no significant contributions of any other cytokine evaluated. On the other hand, G2 (25–60 months) displayed a pro-inflammatory cytokine profile signed by IFN-γ > TNF-α > IL-1β. The G3 (61–120 months) subgroup was characterized by a balanced cytokine signature composed by IL-10 > IL-8. Finally, predominant pro-inflammatory cytokine signature was observed in G4 (121–160 months) with higher than 50th percentile for IL-12 > IL-1β > IL-5 > IL-8 = IL-6 > TNF-α (Fig. 3A).

The analysis of the overlapping cytokine ascendant curves further illustrated the relevant role of IL-5 in the early phase of stable graft renal function after transplantation and the prominent involvement of IL-12 as a pro-inflammatory element later during long-term renal transplantation (Fig. 3B).

3.4. Semi-quantitative analysis revealed that plasma levels of IL-5 and IL-12 are useful laboratorial tools for prognostic and monitoring purposes in renal transplanted patients

The comparison of the median cytokine plasma levels observed at distinct time ranges after renal transplantation is shown in Fig. 4. Our findings demonstrated higher levels of IL-5 ($P = 0.008$) in G1 (1–24 months) as compared to G2 (25–60 months). Moreover, higher levels of IL-12 ($P = 0.015$) was observed in G4 (121–160 months) as compared to G3 (61–120 months). No significant differences were observed for the median plasma levels of all other cytokine evaluated.

4. Discussion

Tolerance or rejection to transplanted allografts usually refers to the adaptation of the recipient's immune system to recognize donor alloantigens and controls alloreactivity or the establishment of an intense reactivity to the transplanted graft. Both mechanisms are mediated by cellular immune response supported by cytokine-mediated events [15]. The pro-inflammatory/regulatory cytokine network is known to play a crucial role controlling this

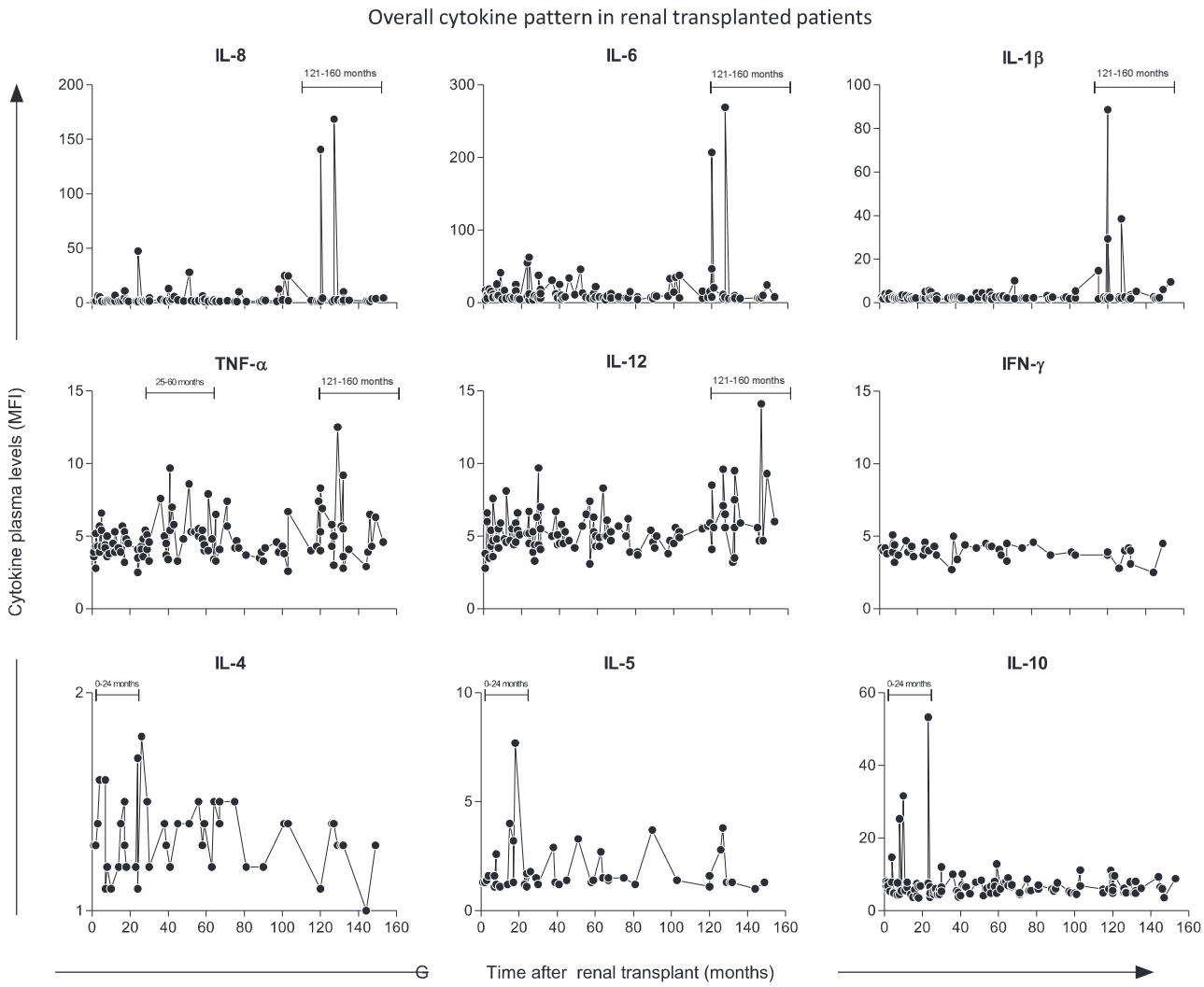

Fig. 1. Overall cytokine patterns in renal transplanted patients. Data are expressed as mean fluorescence intensity (MFI) and presented as moving average of IL-8, IL-6, IL-1β, TNF-α, IL-12, IFN-γ IL-4, IL-5 and IL-10 plasma levels, along to the time after renal transplantation (1–160 months). The range lines referring to 1–24, 25–60, 61–120 and 121–160 months highlight the post-transplant periods associated with peaks of maximum MFI values for each cytokine. These peaks were used to support the subgrouping categorization of the study population.

phenomenon. Following renal transplantation, changes in the systemic cytokines profile can be observed as early as 6–24 h after surgery and are known to drive the immune response towards rejection or tolerance [5,6,10,16,17]. Pro-inflammatory cytokines such as IL-2 and IL-15 mediates the rejection process, whereas modulatory cytokines, such as IL-10 and TGF-β participate in the tolerance mechanisms. These findings are critical to the fail or success of renal grafting, especially in the first year after transplantation [5,6,11].

The most of others publications were performed short-term after transplantation. It should be highlighted that a few studies evaluating cytokines plasma levels over a long period after renal transplantation were found on the literature. The understanding of the immune response associated with short and long-term renal transplantation would provide knowledge and new insights about the mechanisms underlying the tolerance or rejection of kidney grafts.

The availability of plasma panels from renal transplanted patients has enabled an unprecedented comparative analysis of plasma cytokines during early and late phases of stable renal transplantation. Aiming to identify putative immunological features to be used as complementary biomarkers to monitor stable renal transplantation, we have performed a cross-sectional analysis of changes in pro-inflammatory/regulatory plasma cytokines, according to the time of renal transplantation.

In the present investigation, we have observed an overall predominance of regulatory cytokines short-term after renal transplantation (1–24 months), with peaks of IL-4, IL-5 and IL-10 being the major findings (Fig. 1). In fact, IL-5 has been identified as a significant biomarker associated with short-term stable renal function (Fig. 4). Involvement of IL-5 in immune tolerance is not fully understood. It has been admitted that high levels of IL-5 in recent post-transplant period is related to immunosuppressive therapy for immunological tolerance induction, which is mainly used in this period [16]. At this time, no evident predominance of pro-inflammatory profile could be observed. Consistent with this data it has been shown that in the first 2 years after transplantation only mild degree of inflammation is usually observed and also mild fibrosis that do not qualify for the diagnosis of rejection [17,18].

A slight peak of TNF-α was observed 25–60 months after renal transplantation. After 2 years post-transplanted the immunosuppression is usually reduced, which justify the decrease in regulators cytokines and the shift towards a more pro-inflammatory profile. Assessment of TNF-α plasma levels in patients with

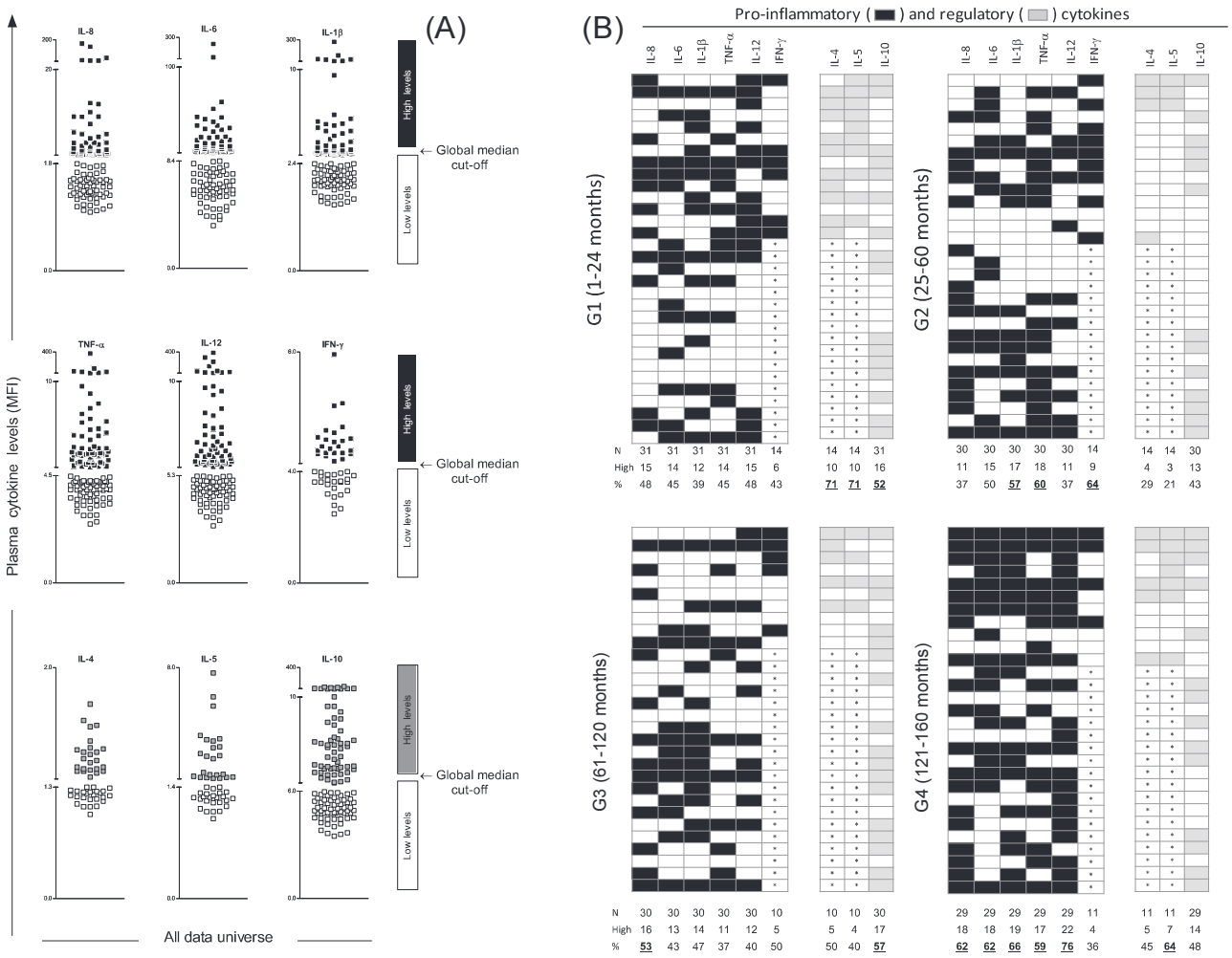

Fig. 2. Categorical analysis of low or high cytokine producers in subgroups of renal transplanted patients. The global median values was determined for each cytokine, including IL-8, IL-6, IL-1β, TNF-α, IL-12, IFN-γ, IL-4, IL-5 and IL-10, all expressed in mean fluorescence intensity (MFI). These standards were further used as a cut-off to tag each patient as they present "low" (▭) or "high" levels of pro-inflammatory (■) or regulatory (▭) plasma cytokines (A). Gray-scale diagrams were used to compile the patients displaying high cytokine levels of each subgroup of renal transplanted patients, including G1 (1–24 months), G2 (25–60 months), G3 (61–120 months) and G4 (121–160 months) (B). The sample size of each subgroup (N), the number (sum of high) and the frequency (%) of patients displaying high cytokine levels are provided in the figure. Prominent cytokine frequencies, above the 50th percentile, are highlighted in bold underline.

5 ± 3 years post-transplantation have revealed higher levels comparing to control group (18). In our study, no significant differences were observed in the plasma levels of TNF-α amongst the subgroups evaluated (Fig. 4). It is possible that subject-specific particularities in the degree of post-operative healing, inflammation and interstitial fibrosis in the studied subgroup could count for these findings, since although it is expected that 5 years after transplantation most patients will present some level of inflammation and fibrosis, both glomerulopathy and inflammation is significantly variable within these groups of patients [17–19].

Following a gap of stable cytokine profile (61–120 months), our data demonstrate that peaks of pro-inflammatory cytokines IL-8, IL-6, IL1β, TNF-α and IL-12 can be observed later on (121–160 months) after renal transplantation (Fig. 1). However, only IL-12 was a significant pro-inflammatory element in long-term renal transplanted patients (Fig. 4). Again, we believe that changes in the immunossupressive treatment protocol applied to late-stable renal transplanted patients, with lower modulatory properties are the events underlying the up-regulation of IL-12 observed. Immunosuppressive drugs are known to enhance the production of suppress the production of IL-12 [20]. One possibility is that the dosage of immunosuppression directly affects the balance of regulatory/pro-inflammatory cytokines, as previously reported for the frequency of circulating dendritic cells expression IL-10 and IL-12 [21]. Consistent with this pro-inflammatory plasma cytokine pattern observed, it has been reported that plasma IL-10 is down-regulated late post-transplant as compared with early post-transplant [21]. However, in the present investigation no change in the levels of IL-10 was observed. Recent studies have demonstrated that down regulation of pro-inflammatory cytokines, especially IL-12, is associated with short-term transplant tolerance. On the other hand, increase in pro-inflammatory cytokines may be associated with the decline of renal function, especially in the long term [15,22].

Complementary data analysis was applied to evaluate the plasma cytokine profile amongst groups, using the general concept of "Low" and "High" cytokine producers (Fig. 2A) as proposed by Luiza-Silva et al. [14]. Following data assembling on multi-cytokine diagrams, the frequency of High cytokine producers was calculated for each group (Fig. 2B). The comparative analysis of High cytokine producers amongst the subgroups of renal transplanted patients was performed using the 50th percentile as a limit to identify relevant differences [14]. The comparative analysis of cytokine signatures pointed out that early after renal transplantation there is an

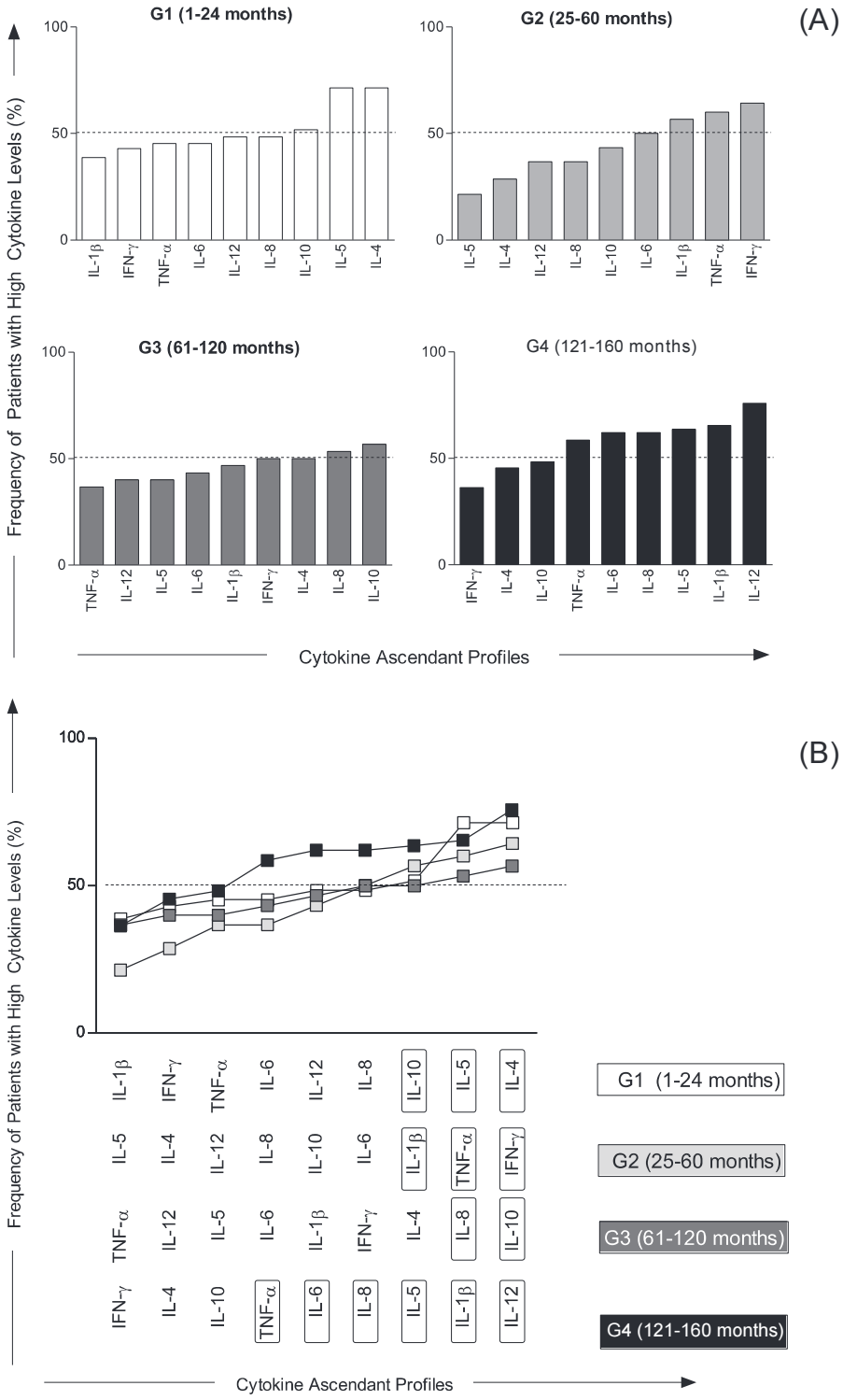

Fig. 3. Cytokine signatures of subgroups of renal transplanted patients. The cytokine pattern was expressed as the ascendant frequency of high cytokine producers for each subgroup of renal transplanted patients, including G1(▭ 1–24 months), G2(▭ 25–60 months), G3(▭ 61–120 months) and G4(▭ >120 months). Column charts (A) and overlay curves (B) were used to identify changes in the overall cytokine signature amongst subgroups. Prominent cytokine frequencies, above the 50th percentile dotted line, are highlighted by rectangles (B).

enhanced frequency of patients with high levels of IL-10, IL-4 and IL-5, consistent with the peaks observed in the analysis of moving average showed in the Fig. 1. In fact, this regulatory cytokine microenvironment maybe expected at 1–24 months post-transplantation, since at the beginning of post-transplant immunossupression the patients undergo to the "induction therapy", referred as "state of immunossupression" combining classic immunossupressive drugs (corticosteroids, CSA, TAC, AZA, MMF) with supportive therapy for CMV [23].

A balanced cytokine signature was observed in patients within 61–120 months after transplantation, characterized by enhanced frequency of patients with high plasma levels of pro-inflammatory

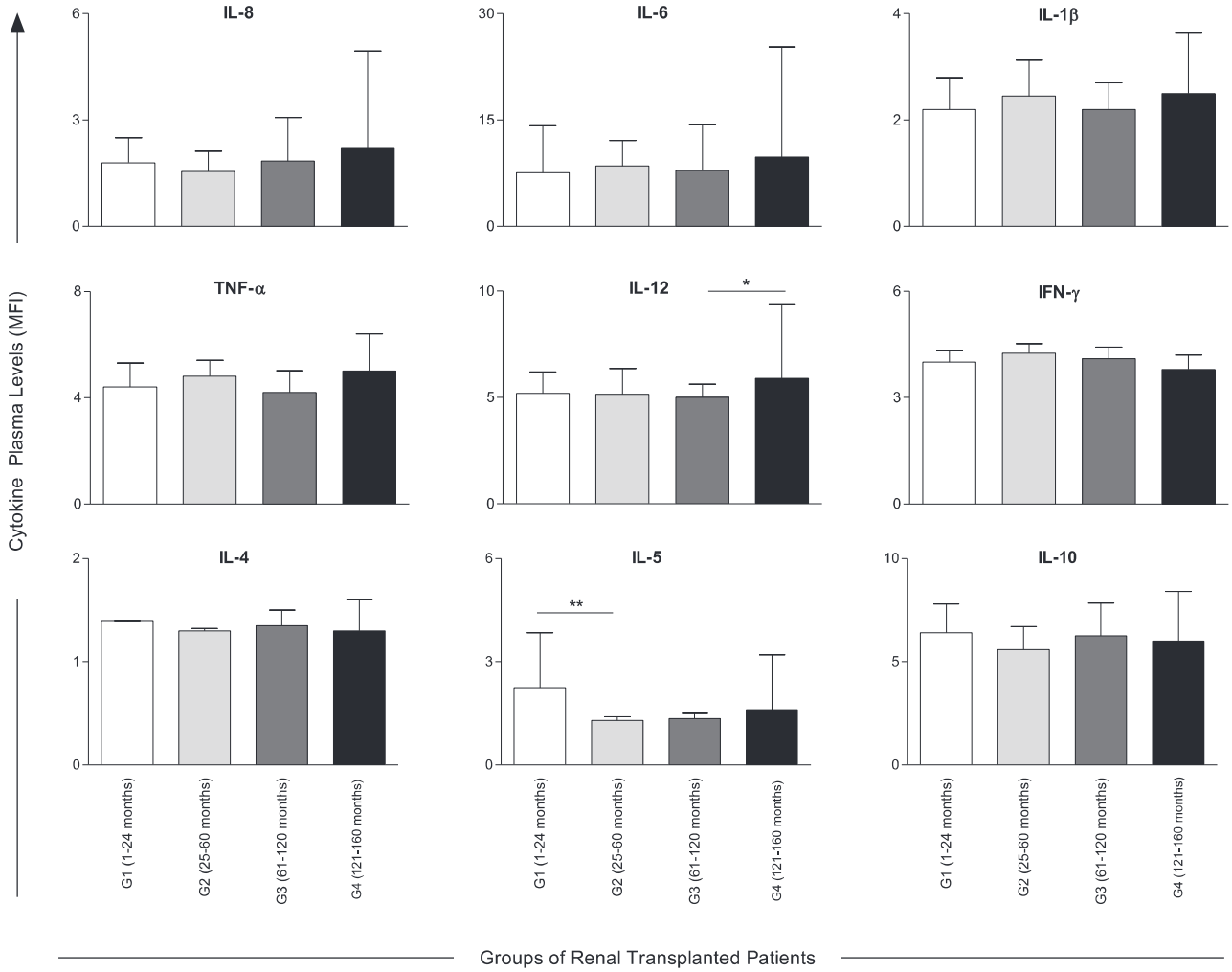

Fig. 4. Cytokines plasma levels in subgroups of renal transplanted patients. Data are expressed as mean fluorescence intensity (MFI) and presented as median + inter-quartile range of IL-8, IL-6, IL-1β, TNF-α, IL-12, IFN-γ IL-4, IL-5 and IL-10 plasma levels observed in G1(▭ 1–24 months), G2(▭ 25–60 months), G3(▭ 61–120 months) and G4(■121–160 months). Comparative analyses amongst subgroups were performed by Kruskal–Wallis followed by Dunn's post-test. Significant differences at $P < 0.05$ are highlighted by connecting lines.

IL-8 along with increased percentage of patients with outstanding levels of the regulatory cytokine IL-10. It is possible to infer that these patients who survive more than 5 years post-transplantation reached equilibrium between pro-inflammatory and regulatory cytokines, which will be broken with the aging of the kidney transplanted.

In fact, more than 50% of patients within the 121–160 months subgroup showed increased plasma levels of most pro-inflammatory cytokines evaluated (IL-8, IL-6, IL-1β, TNF-α and IL-12) (Fig. 3). This cytokine signature analysis corroborate the analysis of moving average (Fig. 1) that showed peaks of pro-inflammatory cytokines IL-8, IL-6, IL1β, TNF-α and IL-12 as the most prominent changes in the cytokine network elements observed late post-transplant. De Serres et al. [7] have also demonstrated that long-term glomerulopathy in renal transplanted patients (14 years after transplantation) is associated with higher plasma levels of pro-inflammatory cytokines, including IL-1β, IL-6 and TNF-α. It is noteworthy that later on after renal transplantation, it is expected that patients become carelessness with the immunosuppressive medication, since they are living for a long time in good conditions with the transplanted kidney, favoring the overcome of a pro-inflammatory response.

The major advantage of applying the cytokine signature model for data analysis was the opportunity to detect, with higher sensibility, putative minor changes in the cytokine profile not detectable by conventional statistical approaches.

Additional longitudinal studies are still necessary to characterize the plasma cytokine profile in patients showing distinct postrenal grafting outcome to clarify the applicability of these biomarkers as a prognostic tool within complex immunological alterations after renal transplantation. Moreover, as only plasma samples were available from the donors studied here, it was not possible to address the cellular sources of the elevated levels of cytokines. However, it is important to notice that the pattern of changes in the cytokine levels observed according to the time following renal grafting would be consistent with a theoretical expected response involving distinct immunosupression therapy applied at different after transplantation.

It is important to mention that the cross-sectional design of the present investigation may offer restrictions to define causal relationship between the immunological and clinical findings. However, it should be emphasized that cross-sectional studies are essential to provide insights and guide further longitudinal studies.

5. Conclusions

Taken together, our data suggest that IL-5 is a good biomarker associated with short-term stable renal function, whereas IL-12 seems to be a relevant pro-inflammatory element in long-term renal transplanted patients.

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