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Cell-Free DNA as a Noninvasive Acute Rejection Marker in Renal Transplantation

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BACKGROUND: Acute rejection (AR) is a key conditioning factor for long-term graft function and survival in renal transplantation patients. The standard care with creatinine measurements and biopsy upon allograft dysfunction implies that AR is usually detected at advanced stages. Rapid noninvasive biomarkers of rejection are needed to improve the management of these patients. We assessed whether total cell-free DNA (tCF-DNA) and donor-derived cell-free DNA (ddCF-DNA) were useful markers for this purpose, both in plasma and in urine.

METHODS: Plasma and urine samples from 100 renal transplant recipients were obtained during the first 3 months after transplantation. tCF-DNA and ddCF-DNA were analyzed by quantitative PCR for the *HBB* (hemoglobin, beta) and the *TSPY1* (testis specific protein, Y-linked 1) genes, respectively. We observed 19 episodes of AR, as well as other complications, such as acute tubular necrosis, nephrotoxicity, and infections.

RESULTS: Plasma tCF-DNA concentrations increased markedly during AR episodes, often before clinical diagnosis, and returned to reference values after antirejection treatment. A cutoff plasma tCF-DNA concentration of 12 000 genome equivalents/mL correctly classified AR and non-AR episodes in 86% of posttransplantation complications (diagnostic sensitivity, 89%; specificity, 85%). Although similar increases were observed during severe posttransplantation infections, use of the combination of plasma tCF-DNA and procalcitonin (PCT), a specific marker of sepsis, significantly improved the diagnostic specificity (to 98%; 95% CI, 92%–100%), with 97% of the episodes being correctly classified. Use of transrenal DNA and ddCF-DNA concentrations did not add relevant information.

CONCLUSIONS: Given that renal biopsy is the gold standard for detecting AR, analysis of both plasma tCF-DNA and PCT could permit a more selective use of this invasive procedure.

Kidney transplantation is the most desirable and cost-effective modality of renal-replacement therapy for patients with irreversible chronic kidney failure. Kidney transplantation patients have the highest risk of complications in the early postoperative phase (3–6 months), and it is accepted that the first 3 months are crucial for the future of the graft. Medical causes of early graft dysfunction include a broad spectrum of diagnoses, such as acute rejection (AR),[4] acute tubular necrosis (ATN), drug nephrotoxicity (NTX), and post-transplantation infections *(1)*. An early and adequate diagnosis is very important because the treatments for some of these pathologies are very different and a mistaken therapeutic management can cause an obvious risk to the recipient. Graft loss caused by AR may be substantially reduced with better control of the adverse immunologic events that occur during the early post-transplantation period. Currently, monitoring of these patients is based on both measuring biochemical parameters with a very low diagnostic specificity, such as serum creatinine, and pathologic study of renal biopsy tissue. Renal biopsy, an invasive method with the potential for secondary complications, often yields inconclusive results (due to inadequate sampling, nonspecific findings, or poor reproducibility). Therefore, finding a new noninvasive and reliable marker for monitoring these patients would be very helpful. The presence of cell-free DNA (CF-DNA) in the circulation has been known since the 1950s. Although the mechanism of DNA release into the circulation remains unclear, cell death by apoptosis *(2)* or necrosis *(3)*, as well as active secretion by different types of activated cells of

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[4] Nonstandard abbreviations: AR, acute rejection; ATN, acute tubular necrosis; NTX, nephrotoxicity; CF-DNA, cell-free DNA; Tr-DNA, transrenal DNA or cell-free urine DNA; HUCA, Hospital Universitario Central de Asturias; tCF-DNA, total cell-free DNA; ddCF-DNA, donor-derived cell-free DNA; GE, genome equivalents; SRTP, stable renal transplantation patient; PCT, procalcitonin.

the immune system *(4, 5)*, may play an important role. Several previous studies have demonstrated the usefulness of CF-DNA quantification in monitoring patients with cancer, acute myocardial infarction, stroke, trauma, autoimmune diseases, or obstetric disorders. We hypothesized that because the clearance of circulating DNA is very fast *(6, 7)*, the measurement of CF-DNA concentrations could give useful complementary information for an early and noninvasive differential diagnosis of AR episodes.

Materials and Methods

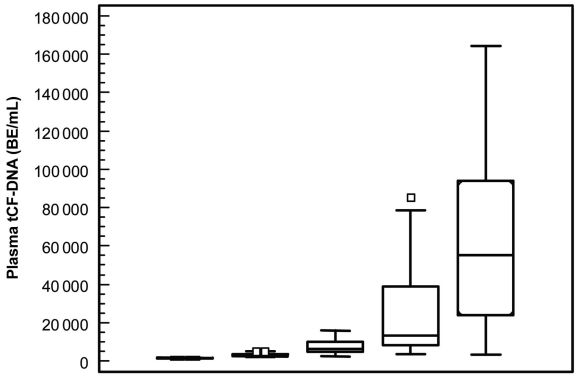
To establish reference values for plasma CF-DNA and cell-free urine DNA [or transrenal DNA (Tr-DNA)], we collected blood and urine samples from 125 healthy donors with stable renal function, who we recruited through the Blood Donation Center of the Hospital Universitario Central de Asturias (HUCA). Approximately half of these study participants were male (n = 61), and the age range was 21–64 years (mean, 42 years). The local ethics committee approved the study, and written informed consent was obtained from all patients and healthy donors.

Our study also included 100 renal transplantation patients (36 women, 64 men) who underwent their operations between January 2004 and December 2007 (age range, 16–76 years; mean, 47 years). During the first 3 months after transplantation, total CF-DNA (tCF-DNA) was measured by amplification of the *HBB*[5] (hemoglobin, beta) gene (n = 100). In addition, donor-derived CF-DNA (ddCF-DNA) released by the graft was measured by amplification of the *TSPY1* (testis specific protein, Y-linked 1) gene, but only in cases of women who received their graft from a male donor (n = 17). A mean of 24 serial plasma samples were analyzed per patient. Urine samples were also collected from 30 of these 100 patients for the quantification of Tr-DNA (mean, 7 samples/patient). Medical records were retrospectively reviewed. Thirty-one patients had a satisfactory clinical evolution, and 69 developed some posttransplantation complications (18 patients had >1 complication). We diagnosed 34 episodes of medical complications as ATN, 13 as anticalcineurinic NTX, and 21 as infections (8 cases of sepsis, 4 urinary tract infections, 2 respiratory tract infections, 5 other local infections, 1 cytomegalovirus infection, and 1 *Candida* infection). Also observed were 19 AR episodes, 13 (68%) of which were confirmed by biopsy. Five of the 6 remaining AR patients presented contraindications to performing a biopsy, and the sixth patient received a "nonconclusive" biopsy result. In these 6 cases, therefore, the AR diagnosis was based on clinical signs and symptoms, plasma creatinine concentrations, and a satisfactory response to empirical treatment with antirejection therapy. The staff of the Pathology Department, HUCA, evaluated all of the biopsies according to the Banff 97 classification for AR diagnosis in renal transplantation and without knowledge of the concentrations of biochemical markers obtained in the present study.

Blood samples were drawn into tubes containing tripotassium EDTA and processed within 2 h of venipuncture. All urine samples were transferred into tubes containing tripotassium EDTA as a nucleosome stabilizer. Both blood and urine samples were centrifuged in 2 steps (1800*g* for 10 min and then 16 000*g* for 10 min). The supernatants were stored at −80 °C until processing. CF-DNA was isolated with the QIAamp DNA Blood Mini Kit (Qiagen) according to the Qiagen blood and body fluids protocol (see handbook at http://www1.qiagen.com/HB/QIAampDNAMiniDNABloodMini). Sterile filter tips (spray-resistant) were used to avoid contamination. Each column was loaded with 400 μL plasma or 800 μL urine, and the extracted DNA was eluted with distilled water in a final volume of 50 μL. tCF-DNA and Tr-DNA were quantified by real-time PCR amplification of the *HBB* gene in an ABI Prism 7000 Sequence Detection System (Applied Biosystems). Target sequences were amplified in a 10-μL reaction volume containing 3 μL DNA solution, 5 μL ABsolute™ QPCR SYBR® Green ROX Mix (2×) (ABgene/Thermo Scientific), and 300 nmol/L of each oligonucleotide primer (354-F, 5′-GTG CAC CTG ACT CCT GAG GAG A-3′; 455-R, 5′-CCT TGA TAC CAA CCT GCC CAG-3′. The thermal profile was a first denaturation step at 95 °C for 15 min, followed by 45 cycles at 95 °C for 15 s and 62 °C for 1 min. Human genomic DNA was extracted, and extracts were quantified by measuring the absorbance at 260 nm. Serially diluted samples of this reference solution were then used to prepare a 6-point calibration curve (10 000, 5000, 1000, 100, 10, and 5 μg/L). Both calibrators and samples were analyzed in triplicate. A blank reaction and several negative controls were included in every run. Melting-curve analysis showed a single product-specific melting temperature with a mean (SD) of 82.7 °C (0.9 °C) for the *HBB* gene and 80.8 °C (0.6 °C) for the *DYS14* sequence. Results are expressed in genome equivalents (GE) per milliliter (1 GE = 6.6 pg DNA).

The same procedure was used to quantify ddCF-DNA concentrations by amplification of *DYS14* on the Y chromosome with primers 713-F (5′-CAT CCA GAG CGT CCC TGG-3′) and 880-R (5′-TTC CCC TTT GTT CCC CAA-3′). Details of the validation of

[5] Human genes: *HBB*, hemoglobin, beta; *TSPY1*, testis specific protein, Y-linked 1.



Diagnosis	vs	P
SRTP	ATN	0.000*
	NTX	0.003*
	I	0.004*
	AR	0.000*
ATN	NTX	0.026
	I	0.008*
	AR	0.000*
NTX	I	0.058
	AR	0.001*
I	AR	0.025

* $P < 0.01$

	SRTP	ATN	NTX	I	AR
n	31	34	13	21	19
p25, GE/mL	1035	2265	4668	8068	23 557
Median, GE/mL	1280	2944	6130	13 123	55 303
p75, GE/mL	1514	3338	9793	38 522	93 809

Fig. 1. Concentrations of plasma tCF-DNA on the day of clinical diagnosis of posttransplantation medical complications.

Box-and-whisker plots show the median, 25th and 75th percentiles, and the range. I, infection; p25, 25th percentile; p75, 75th percentile. Open squares represent outlier observations (points that are beyond the quartiles by one and a half the interquartile range).

the quantitative PCR are available in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol55/issue11.

We used time-resolved amplified cryptate emission (TRACE) technology in a Kryptor® autoanalyzer (BRAHMS) to measure plasma procalcitonin (PCT) concentrations in patients who developed an infection or AR on the day of the clinical diagnosis of these complications. Typical PCT concentrations in healthy individuals are usually <0.05 ng/mL *(8)*.

The results were analyzed with MedCalc® for Windows (version 9.2; MedCalc Software) and SPSS® (version 13.0; SPSS). Because the results were not normally distributed, nonparametric tests were used. A *P* value <0.05 was considered statistically significant. We constructed ROC curves to study the diagnostic performance of the biochemical markers.

Results

REFERENCE INTERVAL FOR PLASMA tCF-DNA AND Tr-DNA

The calculated reference interval for plasma tCF-DNA was 120 GE/mL (90% CI, 110–190 GE/mL) to 1800 GE/mL (90% CI, 1700–2200 GE/mL), with a median of 620 GE/mL (95% CI, 550–700 GE/mL). Typical reference Tr-DNA concentrations ranged up to 140 GE/mL (90% CI, 120–180 GE/mL), with a median of 55 GE/mL (95% CI, 43–68 GE/mL). No significant differences were found with respect to either the age or the sex of the donors (Mann–Whitney *U*-test).

UTILITY OF tCF-DNA AS A MARKER OF AR IN RENAL TRANSPLANTATION

Plasma concentrations of tCF-DNA in stable renal transplantation patients (SRTPs) (n = 31) were within the reference interval during the first 3 months after transplantation. Values for the intraindividual and interindividual CVs at different plasma tCF-DNA concentrations were calculated according to the procedure of Fraser and Harris *(9)* from measurements of samples obtained daily from these patients. We also calculated the reference change value to identify important changes in the state of patients during monitoring of their pathology *(10)*. The calculated intraindividual CV was 27%, the interindividual CV was 38%, and the reference change value was 81% and 114% at a *P* level of 0.05 and 0.01, respectively.

The remaining 69 patients developed some type of complication during the study period. An increase in the plasma tCF-DNA concentration was observed in most of the patients at the time of diagnosis of a complication. Fig. 1 shows plasma tCF-DNA concentrations observed on the day of the clinical diagnosis. Median values were higher in the NTX group than in the

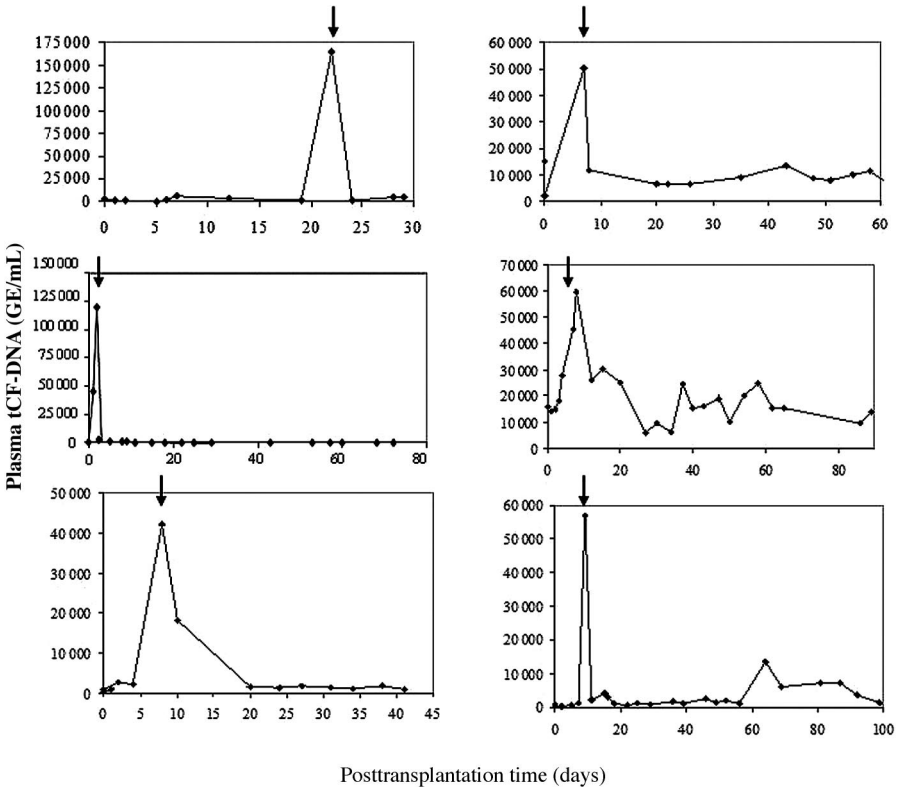


Fig. 2. Concentrations of plasma tCF-DNA in 6 patients who developed AR after renal transplantation.
Arrows indicate the time of clinical diagnosis.

ATN group and were higher in the infections group than in the ATN group or the NTX group. The highest plasma tCF-DNA concentrations were observed in AR patients. The differences between these groups were statistically significant ($P = 0.003$, Kruskal–Wallis test). Plasma tCF-DNA concentrations were significantly lower in SRTPs than in the groups of patients with complications (Mann–Whitney *U*-test with Bonferroni correction; Fig. 1, upper-right panel). On the other hand, plasma tCF-DNA concentrations were significantly higher in the AR group than in the ATN group ($P < 0.0001$) or the patients experiencing NTX episodes ($P = 0.001$). Differences in the plasma tCF-DNA concentration between the AR and infections groups were not statistically significant (Mann–Whitney *U*-test). In all of the groups with posttransplantation complications, the plasma tCF-DNA concentration was significantly higher on the day of diagnosis than on the day of previous evaluation (Wilcoxon test), with a mean increase of 90% in the ATN group ($P = 0.0001$), 131% in the NTX group ($P = 0.0049$), 544% in the infections group ($P = 0.0108$), and 6171% in the group with AR episodes ($P < 0.0001$). Thus, the AR cases, besides having the highest concentrations of all the medical complications, exhibited the highest increase from the day of previous evaluation to the day of diagnosis. In addition, plasma tCF-DNA appeared to be an early marker, because concentrations greater than the reference values were detected in 68% of the analyzed episodes of complications before these episodes were clinically diagnosed.

In 95% of the AR episodes (n = 18), the plasma tCF-DNA concentration increased significantly during the event ($P < 0.0001$, Wilcoxon test) and decreased after treatment had been established. Fig. 2 shows the changes in plasma tCF-DNA concentration with the time after transplantation for 6 patients with graft rejection.

These increases are not exclusive to AR, however, because large increases in plasma tCF-DNA concentration were also observed during infectious episodes (Fig. 3). Patients with systemic infections (median, 40 400 GE/mL) had significantly higher concentrations than those with local infections (median, 9200 GE/mL; $P = 0.032$, Mann–Whitney *U*-test). Patients in the AR group had significantly higher plasma tCF-DNA con-

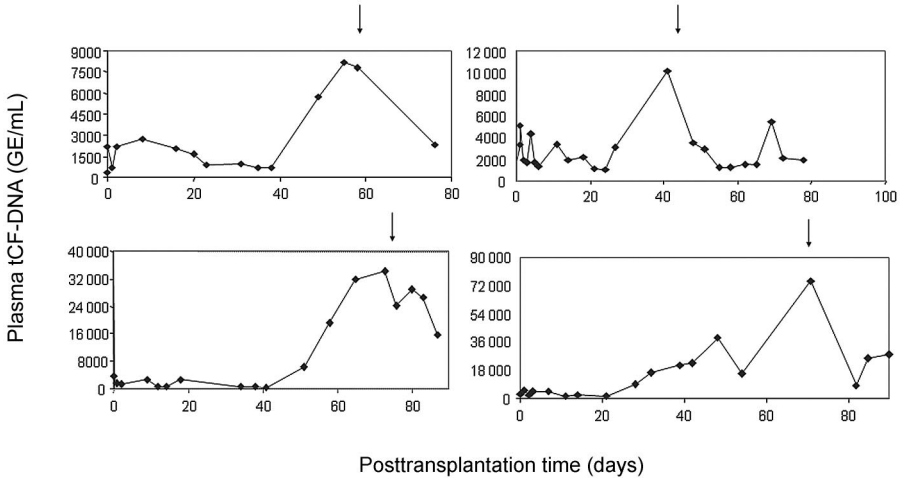


Fig. 3. Concentrations of plasma tCF-DNA in 4 renal transplant recipients who developed a local infection (top) or systemic infections (bottom).

Arrows indicate the time of clinical diagnosis.

centrations (median, 55 300 GE/mL) than those with local infections (median, 9200 GE/mL; $P = 0.001$, Mann–Whitney *U*-test); however, plasma tCF-DNA concentrations in the AR group and the sepsis group (median, 40 400 GE/mL) were not significantly different (Mann–Whitney *U*-test).

ROC curve analysis was used to study the diagnostic accuracy of the plasma tCF-DNA concentration to identify patients with AR. The area under the ROC curve was 0.925 (95% CI, 0.861–0.965), and the plasma tCF-DNA concentration with the highest diagnostic efficiency was 12 000 GE/mL, with a diagnostic sensitivity of 89% and a diagnostic specificity of 85%. This cutoff correctly classified 86% of the renal posttransplantation complications (AR vs non-AR patients), with 2 false-negative diagnoses. Positive and negative likelihood ratios, relative risks, and odds ratios are shown in Table 1.

Because plasma tCF-DNA was not able to differentiate between AR and sepsis episodes, we combined plasma tCF-DNA with a specific marker of systemic infection, PCT, the concentration of which is not expected to increase in response to an AR event *(11–16)*. PCT concentrations greater than the cutoff value for

Table 1. Discriminating power of measuring plasma tCF-DNA alone (cutoff, 12 000 GE/mL) and that of measuring plasma tCF-DNA (cutoff, 12 000 GE/mL) plus PCT (cutoff, 0.5 ng/mL) for diagnosing AR of the graft in renal transplant recipients.

	tCF-DNA (95% CI)	Combination of tCF-DNA + PCT (95% CI)
Episodes diagnosed correctly	86% (78%–91%)	97% (91%–99%)
Sensitivity	89% (65%–98%)	89% (65%–98%)
Specificity	85% (76%–91%)	98% (92%–100%)
PPV[a]	53% (35%–70%)	89% (65%–98%)
NPV	98% (91%–100%)	98% (92%–100%)
+LR	5.9 (3.6–9.7)	44 (11–180)
−LR	0.17 (0.03–0.46)	0.11 (0.03–0.40)
Relative risk	23 (5.6–93)	44 (11–180)
Odds ratio	48 (9.9–230)	410 (54–3100)

[a] PPV, positive predictive value; NPV, negative predictive value; +LR, positive likelihood ratio; −LR, negative likelihood ratio.

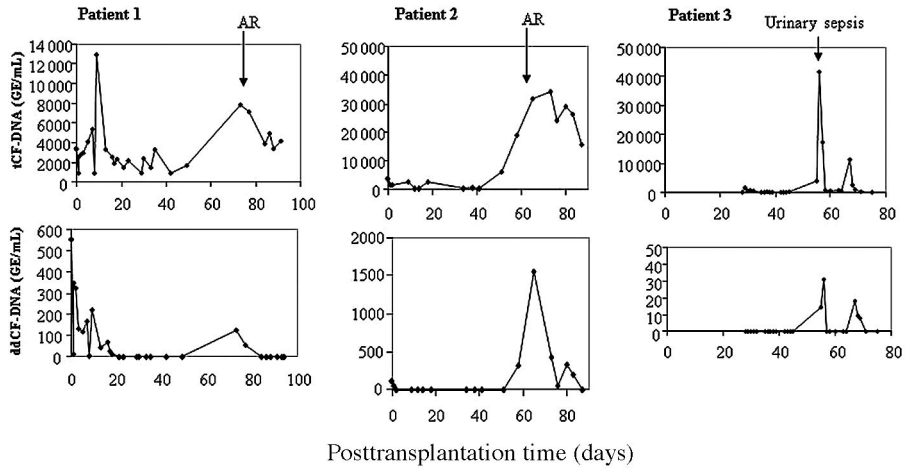


Fig. 4. Kinetics of tCF-DNA (top) and ddCF-DNA (bottom) observed in plasma samples from 2 women who developed AR (patients 1 and 2) and in urine samples from a woman who experienced urinary sepsis (patient 3).
Arrows indicate the time of clinical diagnosis.

the diagnosis of infectious processes (0.5 ng/mL) were not observed in AR patients; however, PCT concentrations were greater than this value in most of the infected patients with plasma tCF-DNA concentrations >12 000 GE/mL (only 2 patients had high plasma tCF-DNA concentrations at PCT concentrations <0.5 ng/mL; both of these cases involved minor local infections). Therefore, combining both biochemical markers improved the diagnostic efficiency for AR episodes (only 2 false negatives and 2 false positives were observed), and the diagnostic specificity increased from 85% to 98%. With this approach, 97% of the clinical complications were correctly classified as AR or non-AR (Table 1).

UTILITY OF Tr-DNA

DNA was also measured in urine samples in 30 of the 100 patients. An increase in Tr-DNA concentration was observed during the development of 2 AR episodes, and a rapid decrease was also observed after the start of antirejection treatment. High Tr-DNA concentrations were also evident in 2 urinary sepsis processes, however. In the remaining patients (without AR or urinary sepsis), no relationships were found between Tr-DNA and CF-DNA concentrations, or between these concentrations and clinical evolution.

UTILITY OF ddCF-DNA

We measured ddCF-DNA concentrations in 17 women who had received a graft from a male donor. ddCF-DNA was detected immediately after transplantation in 12 patients without AR and infection, and plasma concentrations were undetectable within the first week after transplantation. Two patients who developed AR showed a marked increase in the concentration of this graft-specific DNA, which became undetectable after an appropriate immunosuppressive treatment. Marked increases in ddCF-DNA were also observed in 3 patients who experienced graft infection. The kinetics of ddCF-DNA were similar to those of tCF-DNA in the AR and infections groups of patients, although lower ddCF-DNA concentrations were observed in both plasma and urine samples (Fig. 4).

Discussion

Kidney transplantation patients with AR showed increased plasma tCF-DNA concentrations before the clinical diagnosis. Mean plasma tCF-DNA concentrations observed in patients during AR episodes were 40 times higher than in SRTPs. Cellular destruction secondary to the immune attack on the graft could explain the release of DNA into blood circulation during an AR episode; however, this mechanism may not be the only source of this DNA because the plasma concentrations of ddCF-DNA and tCF-DNA are quite different. Thus, the cells of the recipient must also play an important role in this process. A second mechanism could involve the release of CF-DNA from effector receptor cells of the immune system that are activated during rejection. In fact, the release of CF-DNA has been observed in experimental systems *(17)* and is reduced by treatment with glucocorticoids. Glucocorticoids are known to inhibit the apoptosis of immune system cells *(18, 19)*, an effect that could be related to our own observations that the concentrations of cell-free plasma and urine

DNA are quickly reduced after the introduction of treatment with antirejection drugs.

We observed 19 AR episodes in the present study, 6 of which we documented without an available biopsy result, either because of major contraindications to performing a biopsy (n = 5) or because of a "nonconclusive" biopsy result (n = 1). These findings were considered representative of the actual clinical situation during follow-up of renal transplantation patients. Postbiopsy complications such as shock, bleeding, hematuria, anuria, and even loss of the graft (although this complication has been minimized in recent years) still constitute a high risk in some patients *(20)*. For this reason, patients with clinical features of rejection or contraindications to biopsy begin empirical treatment. The 3 major contraindications to biopsy are uncontrolled severe arterial hypertension, renal vascular anomalies, and acute pyelonephritis. Other relative contraindications include obesity, ascites, cystic kidney, and the presence of renal abscesses. On the other hand, approximately 10% of biopsies are of inadequate quality or demonstrate nonspecific, inconclusive changes; sometimes these alterations are present in the graft before transplantation *(21)*.

A plasma tCF-DNA cutoff concentration of 12 000 GE/mL correctly classified 86% of the renal complications after transplantation as AR or non-AR. Only 2 AR episodes showed plasma tCF-DNA concentrations less than this cutoff. The first was a lymphopenic HIV patient with a vascular AR proved by biopsy, a result that could support an immunologic origin of the tCF-DNA, at least in part. The second case was of a patient with clinically diagnosed and treated AR, but without a confirmatory biopsy result, suggesting the possibility of a questionable false-negative result.

An increased release of tCF-DNA into the circulation was observed in other causes of graft damage besides AR, such as ATN (median, 2900 GE/mL) and NTX (median, 6100 GE/mL). Significant increases in both plasma tCF-DNA and Tr-DNA concentrations were observed in patients with posttransplantation infections. The interquartile range of the plasma tCF-DNA concentration in AR episodes was 24 000–94 000 GE/mL, which overlaps with that observed in infectious processes (800–38 000 GE/mL). This finding suggests that the plasma tCF-DNA concentration is not able to effectively distinguish AR from posttransplantation infections. Therefore, we combined plasma tCF-DNA with PCT, a widely accepted specific marker of sepsis, to produce a useful tool for the differential diagnosis of systemic infection in the posttransplantation period, because PCT is not usually increased in AR episodes *(11, 16)*. Moreover, the high doses of corticosteroids usually administered to kidney transplant recipients do not influence the PCT concentration, whereas these drugs inhibit the synthesis of other new markers of sepsis, such as interleukin 6, proadrenomedullin, atrial natriuretic propeptide, or the peptide C-terminal pro–arginine vasopressin *(22)*. There is a transient increase, however, in the PCT concentration during the first days after transplantation that is caused by the surgery. In some patients, PCT concentrations increase by up to 50% during the first 3 days after surgery, compared with preoperative values. A maximum plasma concentration is reached at 24–48 h, and the PCT concentration returns to within the reference interval at the end of the first week after transplantation *(16, 23)*. In our study, AR episodes did not produce PCT concentrations >0.5 ng/mL. Combining the use of these 2 biochemical markers substantially improved the diagnostic specificity and the percentage of episodes correctly classified as AR or non-AR (approximately 97%). In addition, use of both plasma tCF-DNA and PCT improved the positive likelihood ratio more than 7 times, the relative risk by almost 2-fold, and the odds ratio by more than 8 times.

Although not shown in the results, 2 patients with AR showed markedly increased PCT concentrations (>16 ng/mL), results that are compatible with systemic infections; however, these increases were coincident not with the clinical diagnosis of AR but with the introduction of antirejection treatment with antibodies [anti-CD3 monoclonal antibody (OKT3 or muromonab) in one case and antithymocyte globulin in the other]. The same effect was also described by Sabat et al. *(24)* in renal transplantation patients and by Zazula et al. in heart and liver transplantation patients *(25)*. The regulatory process involved in these increases in the PCT concentration is still unknown. Consequently, monitoring the PCT concentration to demonstrate the presence of a bacterial infection or an AR would not be useful during the first days after the introduction of treatment with OKT3 or antithymocyte globulin. The PCT concentration should be carefully evaluated during subsequent days.

Both Tr-DNA and ddCF-DNA concentrations increased during AR. In urinary sepsis, the Tr-DNA concentration also increased substantially. Two patients who developed a severe gastrointestinal infection and a respiratory infection showed no increase in the Tr-DNA concentration. This observation could be explained by the fact that Tr-DNA is kidney specific. Unfortunately, no specific tests are available for its measurement in urine, and the unique analysis of Tr-DNA is not able to distinguish between rejection and urinary sepsis. This specificity of renal damage was also observed with ddCF-DNA. The concentration of this graft-specific DNA increased in severe processes that affect the integrity of the transplanted kidney, both in

AR and in graft infection; therefore ddCF-DNA shows no better efficiency than tCF-DNA for diagnosing AR.

Since the discovery of ddCF-DNA in the plasma of transplant recipients *(26)*, the usefulness of circulating nucleic acids in the transplantation field has remained unclear. Most of the literature has focused on describing the presence of ddCF-DNA (mainly in urine) and has ignored the potential value of tCF-DNA. Several qualitative studies have merely described the detection of this graft-specific DNA during AR and have not reported its detection in patients with stable renal function or after rescue treatment for the rejection *(27–29)*. Most of the quantitative studies were performed with very few patients. Zhang et al. described a high total Tr-DNA concentration in a patient with AR followed by a decrease in this marker after antirejection therapy *(30)*. Li et al. studied a renal transplantation patient with a satisfactory clinical outcome who showed high concentrations of total and donor-derived Tr-DNA just after transplantation that diminished during the first posttransplantation week *(27)*. Gadi et al. quantified ddCF-DNA in 65 serum samples on posttransplantation day 8 and found higher concentrations in the sera of patients with AR episodes (n = 31) than in the sera of patients without AR (n = 34) (median, 10.4 GE/mL and 0.9 GE/mL, respectively) *(31)*. Finally, Zhong et al. described increased concentrations of donor-derived Tr-DNA in 3 renal transplant recipients during the AR processes and a lack of detectable Tr-DNA at the end of the treatment period; however, as in the present work, these authors described a similar increase in a patient with a graft pyelonephritis *(32)*.

In conclusion, immunologic monitoring at the molecular level via serial quantification of plasma tCF-DNA and plasma PCT has the potential to detect complications of renal transplantation, such as AR or sepsis, in the early postoperative period. Although these biochemical markers will not replace renal biopsy as a definitive method for diagnosing medical complications after renal transplantation, they could help establish a diagnosis in patients with contraindications to biopsy. They could also help diagnose subclinical rejection episodes or rule out severe complications, such as AR or sepsis, thereby reducing the number of biopsies. Routine analysis of the plasma tCF-DNA concentration as a marker in the clinical laboratory would be facilitated by fully automating the entire process.

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