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NONINVASIVE DIAGNOSIS OF RENAL-ALLOGRAFT REJECTION BY MEASUREMENT OF MESSENGER RNA FOR PERFORIN AND GRANZYME B IN URINE

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ABSTRACT

Background Acute rejection is a serious and frequent complication of renal transplantation, and its diagnosis is contingent on the invasive procedure of allograft biopsy. A noninvasive diagnostic test for rejection could improve the outcome of transplantation.

Methods We obtained 24 urine specimens from 22 renal-allograft recipients with a biopsy-confirmed episode of acute rejection and 127 samples from 63 recipients without evidence of acute rejection. RNA was isolated from the urinary cells. Messenger RNA (mRNA) encoding the cytotoxic proteins perforin and granzyme B and a constitutively expressed cyclophilin B gene were measured with the use of a competitive, quantitative polymerase-chain-reaction assay, and the level of expression was correlated with allograft status.

Results The log-transformed mean (\pm SE) levels of perforin mRNA and granzyme B mRNA, which encode cytotoxic proteins, but not the levels of constitutively expressed cyclophilin B mRNA, were higher in the urinary cells from the 22 patients with a biopsy-confirmed episode of acute rejection than in the 63 recipients without an episode of acute rejection (perforin, 1.4 ± 0.3 vs. -0.6 ± 0.2 fg per microgram of total RNA; $P < 0.001$; and granzyme B, 1.2 ± 0.3 vs. -0.9 ± 0.2 fg per microgram of total RNA; $P < 0.001$). Analysis involving the receiver-operating-characteristic curve demonstrated that acute rejection can be predicted with a sensitivity of 83 percent and a specificity of 83 percent with the use of a cutoff value of 0.9 fg of perforin mRNA per microgram of total RNA, and with a sensitivity of 79 percent and a specificity of 77 percent with the use of a cutoff value of 0.4 fg of granzyme B mRNA per microgram of total RNA. Sequential urine samples were obtained from 37 patients during the first nine days after transplantation, and measurements of the levels of mRNA that encoded cytotoxic proteins identified those in whom acute rejection developed.

Conclusions Measurement of mRNA encoding cytotoxic proteins in urinary cells offers a noninvasive means of diagnosing acute rejection of renal allografts. (N Engl J Med 2001;344:947-54.)

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RENAL transplantation is the treatment of choice for most patients with end-stage renal disease. However, because of the wide disparity between the supply of organs and the demand,¹ many patients wait three to four years for a suitable organ. Allograft failure is one of the four most common causes of end-stage renal disease in the United States² and is an important factor in the organ-shortage problem. Indeed, about 20 percent of the patients in the United States who are on the waiting list are those with a failed graft,¹ and about 15 percent of the procedures performed are repeated transplantations.³

Acute rejection, defined as a sudden deterioration in renal-allograft function as a result of the recipient's immune response to the donor organ, is a major risk factor for allograft failure.³⁻⁸ About 35 percent of allograft recipients have an episode of acute rejection in the first year after transplantation.⁴ Acute rejection is associated with a 20 percent reduction in the one-year survival rate of cadaveric grafts, and the projected half-life of the allografts is four years shorter in patients who have had an episode of acute rejection than in patients who have not had an episode of acute rejection.⁸

Needle biopsy of allografts is the standard test for the diagnosis of acute rejection. Recent refinements have reduced but not eliminated biopsy-associated complications, such as hematuria, anuria, perirenal hematoma, bleeding and shock, arteriovenous fistulas, and graft loss.⁹⁻¹¹ Sampling errors pose an additional problem, and multiple samples are therefore needed

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to increase diagnostic accuracy.¹²⁻¹⁵ The development of an accurate, noninvasive diagnostic test that also provides insights into the mechanisms of rejection would be of considerable value.

We have developed a competitive, quantitative polymerase-chain-reaction (PCR) assay that permits the noninvasive diagnosis of allograft rejection at the molecular level. We assessed the diagnostic accuracy of measuring the levels of perforin and granzyme B messenger RNA (mRNA) in urinary cells from renal-allograft recipients. We measured the mRNA of perforin, a pore-forming protein,¹⁶ and the mRNA of granzyme B, a serine peptidase,¹⁷ because these proteins are integral components of the lytic machinery of cytotoxic cells,¹⁸⁻²³ and cytotoxic cells are often present in allografts that are undergoing acute rejection.²⁴

METHODS

Collection of Urine Samples and Renal-Biopsy Specimens

We collected 151 urine specimens (110 in the first month after transplantation, 24 one to six months after transplantation, and 17 more than six months after transplantation) from 85 renal-allograft recipients. Thirty-eight of these patients underwent needle biopsy (yielding 44 specimens) to identify the basis for graft dysfunction; urine was collected before each biopsy. The biopsy specimens were fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin, periodic acid-Schiff, or Masson's trichrome. The specimens were reviewed and classified with use of the Banff 97 classification¹² by a single pathologist who did not know the results of the molecular studies. On the basis of histologic findings, 24 renal-biopsy specimens from 22 recipients (mean [\pm SD] age, 44 \pm 15 years; 10 women and 12 men) were classified as showing acute rejection, 5 specimens from 5 recipients (mean age, 52 \pm 17 years; 3 women and 2 men) were classified as showing chronic allograft nephropathy, and 15 specimens from 11 recipients (mean age, 50 \pm 13 years; 2 women and 9 men) were classified as showing other findings according to the Banff 97 classification. Seven of the 15 samples showed toxic tubulopathy, 4 had nonspecific changes, 3 showed acute tubular necrosis, and 1 had signs of renal-vein thrombosis.

The remaining 107 urine specimens were from 47 patients who were classified as having stable allograft function after transplantation (mean age, 47 \pm 12 years; 12 women and 35 men). In these patients, the serum creatinine levels either had decreased or had not changed by more than 0.2 mg per deciliter (18 μ mol per liter) during the seven days before and the seven days after urine collection. Sequential urine samples were obtained from 37 patients (mean age, 46 \pm 12 years; 14 women and 23 men) during the first nine days after transplantation.

Eleven of the 85 renal-allograft recipients (mean age, 52 \pm 13 years; 1 woman and 10 men) had impaired graft function in the first week after transplantation and required dialysis therapy at that time. This group was classified as having delayed graft function.

Immunosuppression consisted of a cyclosporine-based or tacrolimus-based regimen, with antilymphocyte antibodies (muromona-CD3 [OKT3] or antithymocyte globulin) given for episodes of glucocorticoid-resistant acute rejection.²⁵ The study was approved by the institutional review board at the Weill Medical College of Cornell University, and each patient gave written (or oral, if only urine samples were involved) informed consent.

Isolation of RNA

Urine was centrifuged at 10,000 \times *g* for 30 minutes at 4°C. RNA was extracted from the pellet with use of a commercial kit (RNeasy minikit, Qiagen, Chatsworth, Calif.). More than 95 percent of urine specimens yielded RNA suitable for PCR. For each

sample, 1 μ g of RNA was reverse-transcribed to complementary DNA (cDNA).²⁶

Construction of Gene-Specific DNA Competitors and Quantitative PCR

The design and construction of gene-specific DNA competitors are shown in Figure 1. The cDNA of granzyme B, perforin, or cyclophilin B was amplified with different concentrations of DNA competitors. The PCR products were resolved by electrophoresis, stained with ethidium bromide, and scanned by laser densitometry.²⁶ We quantified the concentrations of naturally occurring gene transcripts by measuring the ratio of the cDNA band to the band of the specific competitor. Transcript levels were expressed in femtograms of specific mRNA per microgram of total RNA.

Statistical Analysis

We used SAS software (SAS, version 7.0, SAS Institute, Cary, N.C.) for data analysis. Before we compared the steady-state levels of mRNA in the various groups, we examined the normality of the distributions of transcript levels. The levels of perforin mRNA, granzyme B mRNA, and cyclophilin B mRNA deviated significantly from the normal distribution ($P < 0.001$), and the extent of the deviation was substantially reduced through the use of a log transformation. We used the natural logarithm (\ln) of mRNA levels as the dependent variable in a one-way mixed-level analysis of variance²⁷ to identify any differences among the four groups. We then used Dunnett's test for multiple comparisons to control for the risk of a type I error while comparing the mRNA levels in the acute-rejection group with those in the group with other findings, the group with chronic allograft nephropathy, and the group with a stable course after transplantation. We used a conventional receiver-operating-characteristic (ROC) curve to analyze mRNA levels in order to determine the cutoff points that yielded the highest combined sensitivity and specificity with respect to distinguishing patients with an episode of acute rejection from those without such an episode. We calculated the area under the curve and used generalized estimating equations²⁸ to reestimate the sensitivity and specificity at the selected cutoff point after adjustment for the lack of independence resulting from the inclusion of multiple urine specimens from some patients.

RESULTS

Histologic Classification of Renal-Allograft-Biopsy Specimens

The Banff 97 classification¹² was used to categorize the biopsy specimens as showing acute rejection in 24 specimens from 22 patients, chronic allograft nephropathy in 5 specimens from 5 patients, and other findings in 15 specimens from 11 patients. Of the 24 biopsy specimens that showed acute rejection, 2 were graded as borderline, 6 as grade IA (focal moderate tubulitis), 8 as grade IB (severe tubulitis), 5 as grade IIA (mild-to-moderate intimal arteritis), 2 as grade IIB (severe intimal arteritis), and 1 as grade III (transmural arteritis). Among the 22 patients with biopsy evidence of acute rejection, the clinical diagnosis, as assessed by the response to antirejection therapy with glucocorticoids or antilymphocyte antibodies in 20 patients and by histologic analysis of nephrectomy specimens in 2 patients, was consistent with the biopsy diagnosis. Two of the biopsy specimens showing acute rejection had features of chronic allograft nephropathy: one had severe interstitial fibrosis, tubular atrophy, and tubular loss (grade III chronic allograft nephrop-

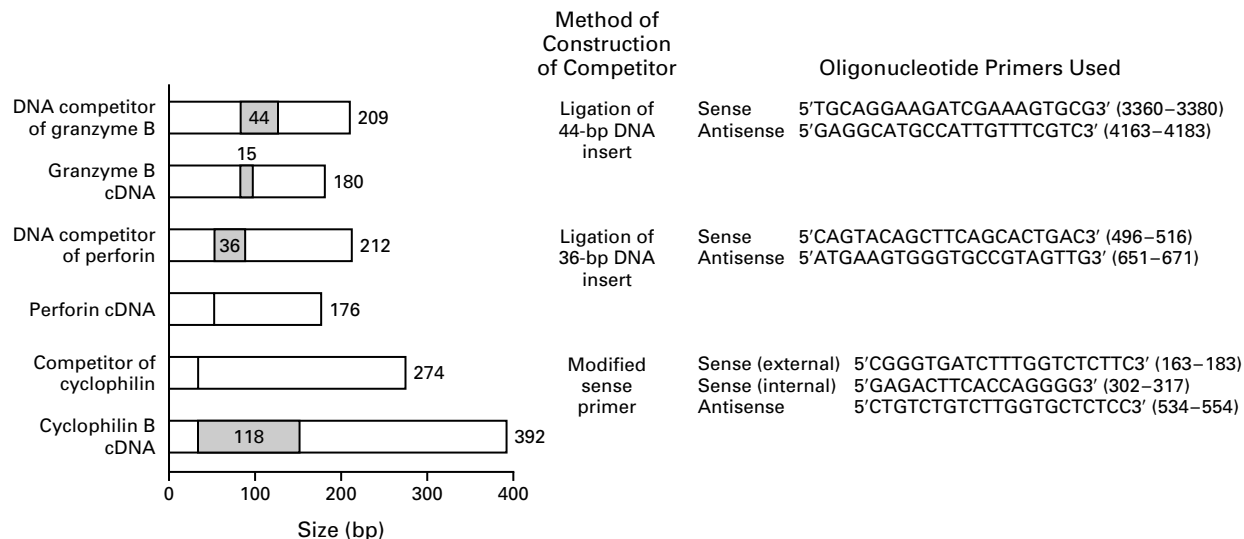


Figure 1. Design and Construction of DNA Competitors.

A DNA competitor of granzyme B cDNA was prepared by digestion of the 180-bp naturally occurring product of PCR (GenBank accession number M28879) with *Mse*I and ligation of the subfragments with a 44-bp DNA insert with appropriate cohesive ends at the 5' and 3' ends. A DNA competitor of perforin cDNA was prepared by digestion of the 176-bp naturally occurring product of PCR (GenBank accession number M28393) with *Nla*III and ligation of the subfragments with a 36-bp DNA insert. The 274-bp cDNA competitor of cyclophilin was amplified with use of a modified sense primer that contains the external sense primer at its 5' end and a 16-bp subfragment internal sense primer at its 3' end corresponding to sequences 302 to 317 within the naturally occurring product of PCR (GenBank accession number M60857).

athy), and the other had moderate (grade II) changes. Among the five biopsy specimens classified as showing chronic allograft nephropathy, three had grade II chronic allograft nephropathy and two had grade I changes.

Levels of mRNA in Urinary Cells

The levels of perforin and granzyme B mRNA, but not those of constitutively expressed cyclophilin B mRNA, were higher in urinary cells from patients with an episode of acute rejection than in those without such an episode (Fig. 2 and Table 1). The log-transformed mean (\pm SE) level of perforin mRNA was 1.4 ± 0.3 fg per microgram of total RNA in the patients with an episode of acute rejection (24 samples from 22 patients) and -0.6 ± 0.2 fg per microgram of total RNA in the patients without an episode of acute rejection (127 samples from 63 patients) ($P < 0.001$). (A negative value for the log-transformed data corresponds to an untransformed value of mRNA that is less than 1 fg per microgram of total RNA but greater than zero.) The levels of perforin mRNA in urinary cells obtained from patients with an episode of acute rejection were significantly higher than the levels in patients with stable graft function after transplantation ($P < 0.001$), patients with other findings ($P < 0.001$), or patients with chronic allograft nephropathy ($P = 0.03$).

The levels of granzyme B mRNA were 1.2 ± 0.3 fg per microgram of total RNA in the patients with an episode of acute rejection and -0.9 ± 0.2 fg per microgram of total RNA in the patients without an episode of acute rejection ($P < 0.001$). The levels of granzyme B mRNA in urinary cells obtained from patients with an episode of acute rejection were significantly higher than those in patients with stable graft function after transplantation ($P < 0.001$) and patients with other findings ($P = 0.001$), but not in patients with chronic allograft nephropathy ($P = 0.12$). The levels of cyclophilin B mRNA did not vary significantly among the four groups of patients ($P = 0.90$) (Table 1 and Fig. 2).

Sixteen of the 24 biopsy specimens showing acute rejection were obtained within three months after transplantation. The mRNA levels of cytotoxic genes in the urinary cells from these 16 biopsy specimens were similar to the levels in the urinary cells from the 8 biopsy specimens that were obtained more than three months after transplantation (perforin, 1.4 ± 0.4 vs. 1.5 ± 0.5 fg per microgram of total RNA, $P = 0.76$; and granzyme B, 1.0 ± 0.3 vs. 1.6 ± 0.4 fg per microgram of total RNA, $P = 0.23$).

Sixteen of 24 biopsy specimens showing acute rejection had had histologic changes of grade IB or less. The mean level of perforin mRNA was 1.6 ± 0.3 fg per microgram of total RNA in urine samples from these 15 patients, and it was 1.0 ± 0.5 fg per microgram of

total RNA in the urine samples from the 7 patients with changes of grade II or grade III ($P=0.29$). The mean level of granzyme B mRNA was 1.6 ± 0.3 fg per microgram of total RNA in the patients with changes of grade IB or less and 0.6 ± 0.4 fg per microgram of total RNA in the patients with changes of grade II or grade III ($P=0.05$).

ROC-Curve Analysis of mRNA Levels

The ROC curves (Fig. 3) show the fraction of true positive results (sensitivity) and false positive results ($1 - \text{specificity}$) for various cutoff levels of perforin mRNA, granzyme B mRNA, and cyclophilin B mRNA. The log-transformed threshold that gave the maximal sensitivity and specificity for perforin mRNA was 0.9 fg per microgram of total RNA; at this threshold, the sensitivity was 83 percent and the specificity was 83 percent ($P < 0.001$) (Fig. 3). The log-transformed threshold was 0.4 fg per microgram of total RNA for granzyme B mRNA; at this threshold, the sensitivity was 79 percent and the specificity was 77 percent ($P < 0.001$) (Fig. 3). The levels of cyclophilin B mRNA were not useful in identifying allografts that would show acute rejection.

The ROC-curve analysis included all 151 urine specimens in which transcript levels were measured. Forty-four samples were from patients who had undergone renal-allograft biopsy, and 107 were from patients classified on the basis of clinical criteria as having a stable course after transplantation. Whereas the presence or absence of acute rejection is known with a high degree of certainty in patients who have undergone allograft biopsy, some patients classified on the basis of clinical criteria as having a stable course after transplantation might have histologic changes of acute rejection.²⁹ In order to eliminate this possibility, we repeated the ROC-curve analysis using only the results from the patients who had undergone allograft biopsy. This evaluation revealed that the log-transformed perforin mRNA level of 0.9 fg per microgram of total RNA had a sensitivity of 83 percent and a specificity of 83 percent ($P < 0.001$) and that a granzyme B mRNA level of 0.4 fg per microgram of total RNA

had a sensitivity of 79 percent and a specificity of 64 percent ($P=0.006$) for the diagnosis of acute rejection (Table 2).

Renal-Allograft Recipients with Delayed Graft Function

Of 11 biopsy specimens from patients with delayed graft function, 2 showed acute tubular necrosis, 7 had

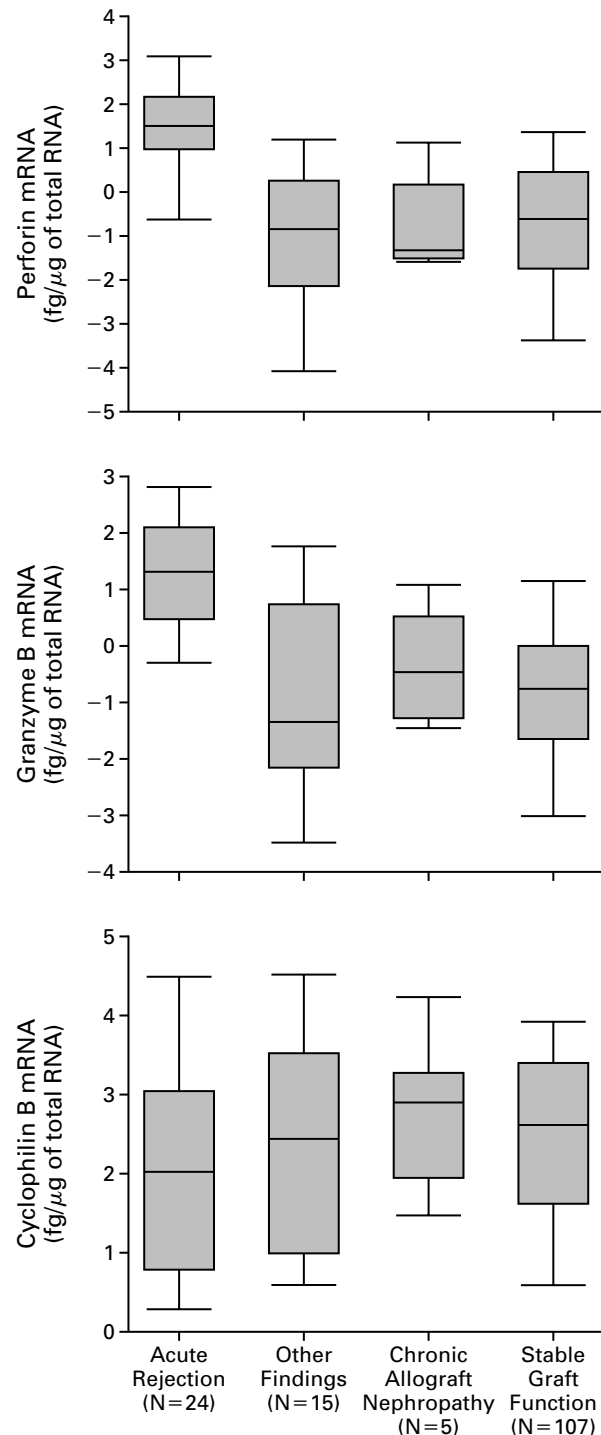


Figure 2. Levels of mRNA in Urinary Cells.

Box and whisker plots show the 10th, 25th, 50th (median), 75th, and 90th percentile values for perforin mRNA, granzyme B mRNA, and cyclophilin B mRNA in urine samples from patients classified as having an episode of acute rejection, other findings on allograft biopsy (acute tubular necrosis, toxic tubulopathy, or nonspecific changes), chronic allograft nephropathy, or a stable course after transplantation. The levels of perforin and granzyme B mRNA, but not those of cyclophilin B, were significantly higher in the patients with an episode of acute rejection than in the patients in the other groups ($P=0.001$ by one-way mixed-level analysis of variance). Values in parentheses are the numbers of urine samples. In all cases log-transformed values are shown.

TABLE 1. LEVELS OF mRNA IN URINARY CELLS FROM PATIENTS WITH AN EPISODE OF ACUTE REJECTION AND PATIENTS WITH OTHER FINDINGS ON ALLOGRAFT BIOPSY, PATIENTS WITH BIOPSY EVIDENCE OF CHRONIC ALLOGRAFT NEPHROPATHY, AND PATIENTS WITH STABLE GRAFT FUNCTION AFTER TRANSPLANTATION.*

TYPE OF mRNA	ACUTE REJECTION (N=24)	OTHER FINDINGS (N=15)	CHRONIC ALLOGRAFT NEPHROPATHY (N=5)	STABLE GRAFT FUNCTION (N=107)	P VALUE†
	fg of mRNA/μg of total RNA				
Perforin	1.4±0.3	-0.8±0.5‡	-0.7±0.8‡	-0.6±0.2‡	<0.001
Granzyme B	1.2±0.3	-0.7±0.5‡	-0.3±0.7‡	-0.9±0.2‡	<0.001
Cyclophilin B	2.3±0.3	2.4±0.3	2.7±0.6	2.5±0.1	0.90

*Levels of mRNA were measured with the use of gene-specific competitor templates in competitive, quantitative PCR. Plus-minus values are means (±SE) of the natural logarithm of mRNA levels. The diagnoses of acute rejection, other findings, and chronic allograft nephropathy were made by histologic evaluation of renal-allograft-biopsy specimens. The clinical diagnosis of stable graft function was based on the finding that serum creatinine levels either had decreased or had not changed by more than 0.2 mg per deciliter during the seven days before and the seven days after the urine samples were collected. Values in parentheses are the numbers of urine samples.

†P values were calculated with the use of log-transformed mRNA levels as the dependent variable in one-way (mixed-level) analysis of variance. Dunnett's procedure was then used to compare the mRNA levels in samples showing acute rejection with the mRNA levels in each of the three other groups of samples, and it showed that levels of perforin mRNA in urinary cells obtained during an episode of acute rejection were significantly higher than those in specimens with other findings (P<0.001), chronic allograft nephropathy (P=0.03), or stable function (P<0.001). Dunnett's procedure also showed that levels of granzyme B mRNA in urinary cells obtained during an episode of acute rejection were significantly higher than the levels in specimens with other findings (P=0.001) or stable function (P<0.001) but not in specimens with chronic allograft nephropathy (P=0.12). None of the pairwise comparisons of cyclophilin B mRNA levels were significant.

‡A negative value for the log-transformed data corresponds to an untransformed value that is less than 1 fg of mRNA per microgram of total RNA.

evidence of toxic tubulopathy, 1 had nonspecific changes, and 1 had evidence of both acute tubular necrosis and acute rejection. The levels of perforin mRNA and granzyme B mRNA were significantly lower in the 19 urine samples from the 10 patients with delayed graft function from nonimmunologic causes than in the 24 samples from the 22 patients with an episode of acute rejection (perforin mRNA, -0.8±0.5 vs. 1.4±0.3 fg per microgram of total RNA, P<0.001; and granzyme B mRNA, -0.4±0.5 vs. 1.2±0.3 fg per microgram of total RNA, P=0.004). The levels of perforin mRNA and granzyme B mRNA in the only patient with a clinical diagnosis of delayed graft function and a histologic diagnosis of acute rejection were 1.0 and 1.2 fg per microgram of total RNA, respectively, and were similar to those in the patients with an episode of acute rejection.

Serial Studies in the Early Post-Transplantation Period

Sequential urine samples were obtained from 37 patients during the first nine days after transplantation. A mixed-level two-way analysis of variance was used to estimate and compare the mean levels of perforin mRNA, granzyme B mRNA, and cyclophilin B mRNA during days 1 through 3, 4 through 6, and 7 through 9 in 8 patients in whom acute rejection

developed within 10 days after transplantation and in 29 patients in whom acute rejection did not develop within the first 10 days. The levels of perforin mRNA and granzyme B mRNA, but not those of cyclophilin B mRNA, were higher in urine samples obtained on days 4 through 6 and 7 through 9 from patients in whom acute rejection developed than in samples from those without acute rejection (Fig. 4).

DISCUSSION

We found that an episode of acute rejection of a renal allograft, an important and treatable risk factor for allograft failure, can be diagnosed accurately and non-invasively by measurements of perforin and granzyme B mRNA in urinary cells. Perforin, which is stored in and secreted by the granules of cytotoxic effector cells, forms pores in target-cell membranes and causes cell death.¹⁶ Granzyme B, which is expressed primarily by activated cytotoxic cells, is an integral member of the lytic machinery of cytotoxic cells.¹⁷ In the granule-exocytosis model of cytotoxicity, perforin creates holes in the membrane of the target cell and facilitates the entry of granzyme B into the cell.¹⁸⁻²³ Granzyme B then induces DNA fragmentation and cell death through the activation of caspase 3.³⁰

Studies in animals and patients have implicated per-

forin and granzyme B in allograft rejection. Perforin-deficient mice have impaired cytotoxic effector cells and weakly reject cardiac allografts.³¹ Granzyme B-deficient mice have reduced cytolytic activity.²⁰ Clinical studies suggest that acute rejection is characterized by the heightened expression of cytotoxic genes within the allograft.³²⁻³⁸ The functional attributes of perforin and granzyme B provided the rationale for the evaluation of levels of perforin mRNA and granzyme B mRNA in urinary cells as markers of acute rejection.

An increase in the serum creatinine level is often the first clinical indicator of acute rejection and is currently the best surrogate marker of it. However, it lacks sensitivity and specificity. The limitations associated with monitoring allograft rejection by measurements of serum creatinine have been forcefully brought to light by the observation that 30 percent of allograft biopsies performed in patients with stable renal function or in patients who were considered to have been successfully treated for rejection reveal histologic features of acute rejection.^{29,39} These occult rejections appear biologically relevant, since treatment better preserves the structure and function of renal allografts.⁴⁰

The accurate diagnosis of acute rejection is contingent on the invasive procedure of needle biopsy of allografts. Repetitive biopsies, although ideal from a diagnostic perspective, are constrained by several practical considerations, including the complications associated with the procedure and sampling errors.⁹⁻¹⁵ Thus, the availability of a noninvasive indication of rejection would be clinically useful.

We have demonstrated the feasibility of accurate noninvasive diagnosis of acute rejection by measurements of perforin mRNA and granzyme B mRNA in urinary cells, and we think that this diagnostic test also has the potential to predict the development of acute rejection. In this regard, the diagnostic accuracy and mechanistic insights might be further improved by quantification of additional genes such as the fas ligand gene.³³

Allograft recipients with delayed graft function have low rates of graft survival and are at higher risk for acute rejection than are patients with immediate graft function.^{3,8,41} Delayed graft function can result from nonimmunologic causes, immunologic causes, or a

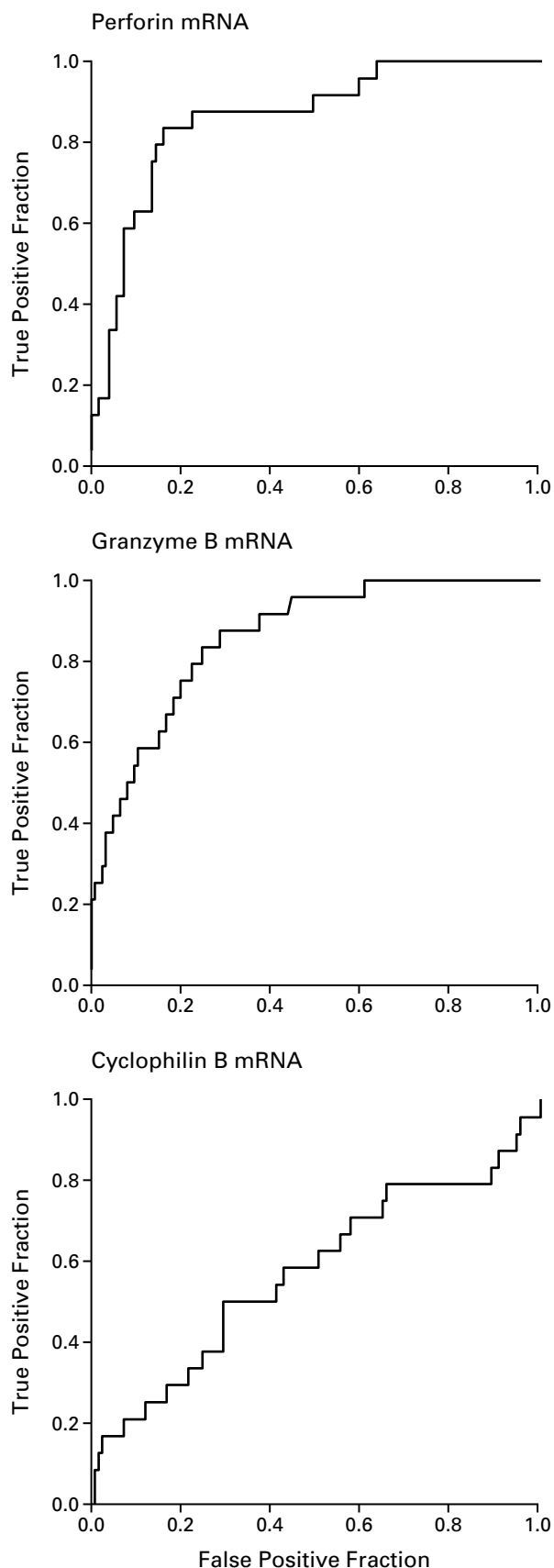


Figure 3. Receiver-Operating-Characteristic Curve of mRNA Levels.

The fraction of true positive results (sensitivity) and false positive results ($1 - \text{specificity}$) for perforin mRNA levels, granzyme B mRNA levels, and cyclophilin B mRNA levels as markers of acute rejection are shown. The calculated area under the curve was 0.86 for perforin mRNA levels, 0.86 for granzyme B mRNA levels, and 0.58 for cyclophilin B mRNA levels. A value of 0.5 is no better than expected by chance, and a value of 1.0 reflects a perfect indicator.

TABLE 2. LEVELS OF PERFORIN mRNA AND GRANZYME B mRNA IN PATIENTS WITH ACUTE REJECTION AND IN THOSE WITHOUT ACUTE REJECTION.*

VARIABLE	ACUTE REJECTION PRESENT (N=24)	ACUTE REJECTION ABSENT (N=20)	P VALUE†
	no. of urine samples		
Perforin mRNA			<0.001
≥0.9 fg/μg of total RNA	20	3	
<0.9 fg/μg of total RNA	4	17	
Granzyme B mRNA			0.006
≥0.4 fg/μg of total RNA	19	7	
<0.4 fg/μg of total RNA	5	13	

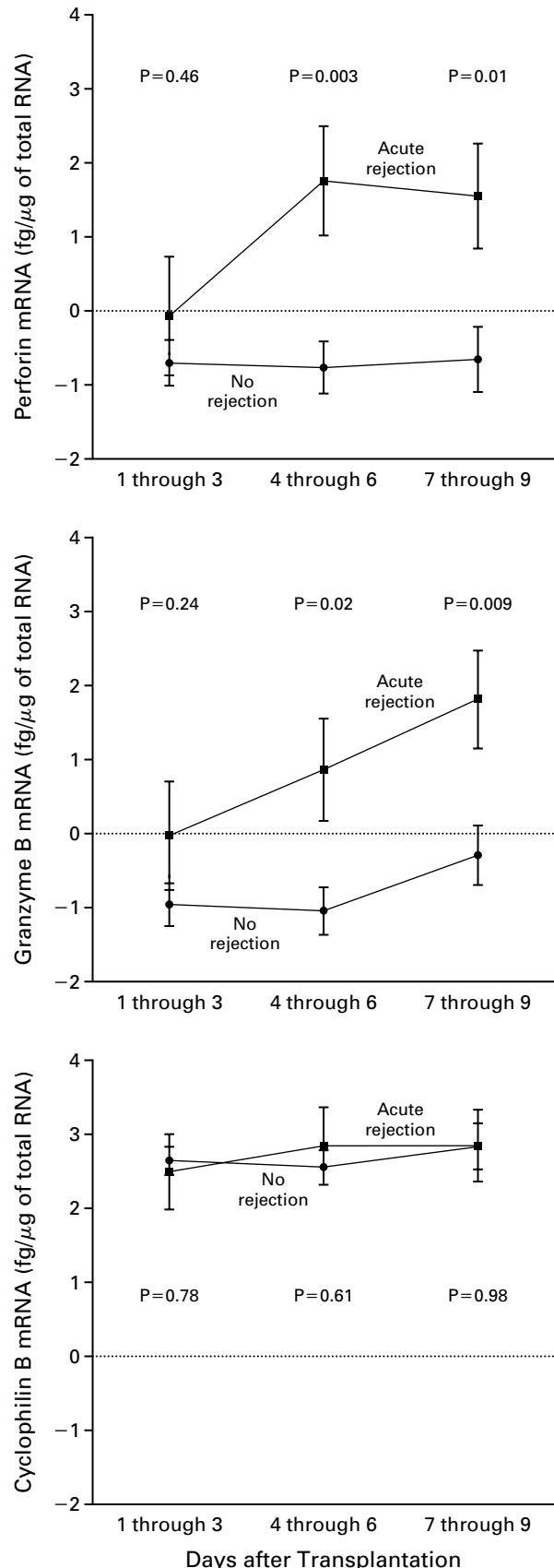
*A receiver-operating-characteristic curve was used to select the best cut-off points. The presence or absence of acute rejection was established by renal-allograft biopsy. Values in parentheses are the numbers of urine samples.

†P values are based on the generalized-estimating-equation analysis of the association of acute-rejection status with dichotomized measures of mRNA.

combination of both. Serum creatinine values are uninformative, and biopsy is currently mandatory to establish the cause. Our data showing that patients with delayed graft function owing to nonimmunologic causes can be distinguished from patients with acute rejection offer a method for clarifying the mechanism and providing a specific therapy for patients with impaired renal function in the period immediately after transplantation.

Our studies using gene-specific constructs of competitor DNA in quantitative PCR demonstrate that acute rejection of renal allografts can be diagnosed accurately and noninvasively by quantification of perforin mRNA and granzyme B mRNA in urinary cells. In addition to functioning as surrogates for allograft biopsy, mRNA phenotyping of urinary cells may lead to the molecular classification of rejection and to the identification of suitable therapeutic targets.

Figure 4. Mean (±SE) Levels of Perforin mRNA, Granzyme B mRNA, and Cyclophilin B mRNA in Sequential Urine Samples. Perforin mRNA, granzyme B mRNA, and cyclophilin B mRNA were measured in urine samples obtained during the first nine days after transplantation. The levels of perforin mRNA and granzyme B mRNA but not those of cyclophilin B mRNA were higher in the 8 patients in whom acute rejection developed within the first 10 days after transplantation than in the 29 patients in whom acute rejection did not develop within the first 10 days after transplantation. The respective numbers of urine samples obtained from the patients with an episode of acute rejection and those without such an episode were as follows: 6 and 43 on day 1, 2, or 3 after transplantation; 5 and 26 on day 4, 5, or 6; and 6 and 14 on day 7, 8, or 9. Means, standard errors, and P values were estimated with use of a mixed-level two-way analysis of variance. In all cases, log-transformed values are shown.



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A U.S. patent entitled "Methods of Evaluating Transplant Rejection (6187543) was issued on February 13, 2001; Dr. Suthanthiran is one of the inventors. The patent is owned jointly by Harvard, Cornell, and the Beth Israel Deaconess Medical Center.

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